Risk Assessment made under the Genetically Modified Organisms (Contained Use) Regulations 2000

(Form GMM – for genetically modified micro-organisms and eukaryotic cell and tissue culture systems)

Department: NDCLS | **Supervisor: Dr Stephen Hyde** **Ref. No: CBGM17**

Project Title: Minimal Lentiviral Vectors

Overview of Project:
(include aims and objectives)

The aim of this project is to use lentiviral gene transfer vectors to introduce transgene and/or RNA interference expression cassettes into mammalian cells. A variety of non-harmful transgenes / RNAi targets will be investigated. In each case, the objective will be to modulate expression of the gene under investigation by the expression of full length and/or truncated forms and/or RNAi mediated silencing.

Lentiviral Vector Systems

Third-generation lentiviral vector packaging systems will be used, lacking all lentiviral accessory (vif, vpr, vpu and nef) and trans-activating (tat) proteins. The 5' LTR region in such systems lacks the tat-responsive lentiviral U3 region, which is replaced with an heterologous promoter – typically the commonly used RSV or CMV promoter.

In the lentiviral vectors to be used, the 3' LTR sequences contain a large deletion in the U3 region removing enhancer and promoter activity. Thus all vectors contain self inactivating (SIN) LTR regions.

The lentiviral packaging system used may divide the expression of lentiviral gag, pol and rev across two or more expression cassettes (Dull *et al*., 1998 *Journal of Virology* 72:8463).

The lentiviral vectors to be used may contain a mutant form of the woodchuck hepatitis B virus (WHV) post-transcriptional regulatory element (WPRE). The wild-type WPRE region contains a truncated form of the WHV X protein that may have oncogenic properties (Kingsman *et al.,* 2005 *Gene Therapy* 12:3). The mutant form of the WPRE used in these studies precludes expression of the truncated X protein by the inclusion of five point mutations within the putative X protein promoter region and one point mutation within the X protein start codon. Such mutant WPRE sequences appear not to have oncogenic properties (Themis *et al.,* 2005 *Molecular Therapy* 12:763).

Envelopes that facilitate transduction of a wide range of cell types will be used including the commonly used VSV-G envelope (Emi *et al*., 1991 *Journal of Virology* 65:1202), the SeV-HN/SeV-F envelope system that has similar tropism to VSV-G (Kobayashi *et al*., 2003 *Journal of Virology* 77:2607) and baculovirus GP64 envelope that has similar tropism to VSV-G (Kumar *et al*., 2003 *Human Gene Therapy* 14:67).

The lentiviral vectors to be used may be rendered non-integrating by ablating the activity of the lentiviral integrase protein (Yanez *et al.,* 2006 *Nature Medicine* 12:348).

Viral packaging will typically be performed by multiple plasmid transfection, though stable producer cell lines may also be developed. The theoretical risk of generating replication competent virus is greatly minimised by the use of multiple expression cassettes containing minimal regions of lentiviral sequence homology.

Collectively, the safety features incorporated into the lentiviral vector systems to be used: 3rd generation packaging, deletion of accessory proteins, deletion of trans-activating tat protein, the use of SIN LTR's, the use of mutant WPRE, the use of conventional well-studied envelope pseudotypes and the use of non-harmful inserts supports the designation at Hazard Class I modified by elimination of the use of sharps (SACGM Compendium Of Guidance, 2007 Part 2:124; www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/).

Inserts

The lentiviral vectors to be used will contain non-harmful inserts.

Commonly used sequences to initiate and terminate transcription (enhancers / promoters / polyadenylation / transcriptional termination signals) may be used. Examples include viral enhancer/promoter elements such as the immediate-early enhancer/promoter from CMV, the RSV LTR, the SV40 promoter; mammalian enhancer/promoter elements such the elongation factor 1 alpha promoter, the ß-actin promoter, the phosphoglycerate kinase promoter, the ubiquitin B and C promoters, the U6 promoter, the H1 promoter; and mammalian and viral polyadenylation signals such as the SV40 and bovine growth hormone polyadenylation signals, the U6 and H1 terminator sequences.

Commonly used, simple to measure reporter genes may be utilised. Examples include firefly luciferase and similar transgenes, jellyfish green fluorescent protein and similar transgenes, *E.coli LacZ*, mammalian serum, blood clotting and red cell production factors such as alpha-fetoprotein, FIX and EPO.

Genes associated with the pathophysiology of the common inherited disease cystic fibrosis may be utilised. Examples include modulators of epithelial fluid balance such as CFTR an epithelial chloride channel associated with cystic fibrosis and ENaC the epithelial sodium channel; and modulators of lung function such as alpha-1-antitrypsin.

Genes associated with the common causes of blindness may be utilised. Examples include RPE65 involved in Leber's Congenital Amaurosis, ABCA4 involved in Stargardt's disease; and USH2A and myosin VIIA involved in Usher syndromes.

In each case, the objective will be to modulate expression of the gene under investigation by the expression of full length and/or truncated forms and/or RNAi mediated silencing.

Give details of Recipient/Host(s): *(specify if wild type or disabled)*

Disabled E. coli, K12 and B derivatives, and BL21 and similar.

Mammalian cell lines.

Experimental Animal Model Systems.

Vector(s):

Standard bacteria vectors
(eg plasmid, phage etc).

Replication defective third-generation lentiviral vectors (EIAV, FIV, HIV, SIV).

Normal/expected biological action of inserted DNA/RNA or transcribed/translated gene product:

Non-harmful inserts described above.

Technique used to introduce insert or vector into host:

Bacterial Hosts: Standard laboratory methods including transformation and/or electroporation.

Lentiviral Producer Cells: Standard laboratory methods including transfection, electroporation and/or transduction.

Experimental Mammalian Cell Lines & Animal Models: Lentiviral Transduction.

Assessed By:

Date: 9th July 2010

Signature: 

Risk Assessment approved by Genetic Modification Safety Committee

Date: 21st July 2010

Signature: 

(Biological Safety Officer)

Permission granted by Head of Department for project to be undertaken

Date: 21st July 2010

Signature: 

(Head of Department)

RISK ASSESSMENT FOR HUMAN HEALTH AND SAFETY

Human health hazard identification – (Identify any potential harmful properties of:)

i) the recipient micro-organism *(for micro-organisms also give ACDP hazard group)*

ACDP1 for all bacterial recipients. E.coli strains are disabled and cannot colonise the human gut.

Minimal hazard for murine and human cell lines obtained from commercial sources that are well characterised and authenticated – containment level 1.
Primary human cells and cell lines that are not fully authenticated and characterised may carry contaminating infectious agents – containment level 2 required under the COSHH Regulations. None high risk for blood borne pathogens will be used.

ii) the inserted (donated) genetic material

Inserts code for normal mammalian genes or selective alterations of those genes. Also standard marker genes such as lac Z, GFP, etc. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host or normal human defence mechanisms. Gene transfer is expected but unlikely to be hazardous.

iii) the donor micro-organisms *(where used/appropriate)*

N/A – inserts are from mammalian sources

iv) the vector

Non-hazardous standard plasmid or phage vector systems will be used in bacterial hosts.

Third-generation, disabled, replication defective, self-inactivating lentiviral vectors will be used. The vectors will contain non-harmful inserts.

Insert expression form the virus is directed by internal heterologous enhancer/promoter elements that do not transcribe the regions necessary for viral mRNA incorporation into viral virions.

Some viral vectors may include a **mutant** version of the woodchuck hepatitis B virus post-transcriptional regulatory element (WPRE). In the mutant WPRE sequences included, the woodchuck hepatitis X protein expression is abolished by oblation of promoter activity and mutating the ATG initating codon. Some intermediary bacterial plasmids used in the construction of the final vector genomes may contain wild-type versions of the WPRE sequence. Plasmids containing wild-type version of the WPRE will not be introduced into mammalian cells and will not be used to generate lentiviral vectors.

Virus will be generated by either:
i) Transient transfection of multiple plasmid DNAs into a highly transfectable mammalian cell line such as HEK293T. The vector genome plasmid provides vector genome mRNA that is generated in a tat independent fashion by replacing the U3 portion of the lentiviral 5' LTR with an heterologous promoter. The lentiviral 3' LTR is modified by deletion of enhancer and promoter elements from the U3 region resulting in self-inactivating LTRs that are unable to generate full length viral mRNA. For packaging, gag, pol and rev products are supplied in trans by one or more plasmid DNAs. Lentiviral accessory products such as vif, vpr, vpu and nef, found in first-generation packaging systems are not produced by the packaging pDNAs. Lentiviral trans-activating tat protein found in first- and second-generation packaging systems is not produced by the packaging pDNAs. Sequence homology between various elements is minimised (eg by use of multiple heterologous promoter systems and/or codon optimisation of gag and/or pol regions) to inhibit the generation of replication competent virus. Commonly used, non-lentiviral, envelope products (eg VSV-G, Baculovirus GP64, Sendai virus F & HN etc) are supplied by one or more additional plasmid DNAs. Such envelopes allow concentration of viral particles and facilitate viral transduction of a broad range of mammalian cell types.

GUIDANCE

Potentially harmful effects include:

disease to humans – consider all properties which may give rise to harm eg infection, toxins, cytokines, allergens, hormones etc

alteration of existing pathogenic traits – consider alteration of tissue tropism or host range, alteration in susceptibility to human defence mechanisms etc

adverse effects resulting from inability to treat disease or offer effective prophylaxis

possibilities for any disablement or attenuation to be overcome by recombination or complementation

adverse effects resulting from the potential for transfer of inserted genetic material to another micro-organism

ii) Producer cell lines. Cell lines may be created by conventional mammalian transfection/transduction and selection processes that express some or all of the third-generation packaging elements described above. Typically, transcriptional control elements (eg Tet-On or off, cumate switch etc) are incorporated to eliminate constitutive viral production. If only some of the required elements are included, viral production may require transient transfection with the missing components.

Replication competent lentivirus has not been observed with such packaging systems (Escarpe *et al*., 2003 *Molecular Therapy* 8:332; Miskin *et al*., 2006 *Gene Therapy* 13:196).

Collectively, the safety features incorporated into the lentiviral vector systems to be used: 3rd generation packaging, deletion of accessory proteins, deletion of trans-activating tat protein, the use of SIN LTR's, the use of mutant WPRE, the use of conventional well-studied envelope pseudotypes and the use of non-harmful inserts supports the designation at Hazard Class I modified to eliminate the use of sharps (SACGM Compendium Of Guidance, 2007 Part 2:124).

v) the resulting genetically modified micro-organism

No significant hazards identified above, the resulting GMOs are therefore not expected to carry any additional risks to that of the un-modified recipients.

E.coli strains used are disabled.

Cell lines would be recognised as non-self by the immune system and be removed.

The integration of the viral genome into a recipient animal may cause an insertional mutagenic event, but this is unlikely to be deleterious to human health.

Lentiviral vectors described have altered broad host specificity but are replication defective and contain no harmful gene inserts.

Brenner Scheme values (*COMPLETION OPTIONAL and in any case for disabled E. coli only*)
Access Expression Damage Overall

Control measures – Assign provisional containment level:

Containment Level: 1

with Good Microbiological Practice and Good Occupational Safety and Hygiene

Note: **under COSHH Regulations some cell lines require Containment Level 2 plus microbiological safety cabinet**

Assign a provisional containment to control the hazards identified above taking account of severity of any consequence and likelihood of harm occurring. Select from 1,2,3 or 4

NATURE OF WORK TO BE UNDERTAKEN
Give brief description of types of laboratory procedures including maximum culture volumes at any time (show as multiples of unit volumes):

For E.coli work
The procedures are standard laboratory practice for gene cloning and manipulation. Individual culture volumes will typically be ≤500mL.

For Mammalian Cell And Tissue Culture Work
The procedures are standard laboratory practice for mammalian cell and tissue culture. Individual culture volumes will typically be ≤100mL.

For Animal Work
The procedures are standard laboratory practice for animal research.
The proposed experiments will typically involve viral volumes of ≤10mL.

Lentiviral particles will only be administered to animal models via the nasal route. Such procedures do not involve any sharps, and thus there is minimal percutaneous injection risk.

GUIDANCE
Consider any activities that may involve risks which require specific additional control measures such as:

inoculation of animals or plants with GMMs

the use of equipment or procedures likely to generate aerosols

large scale work

Bacteriological and cell culture waste will be treated by autoclave on a make safe cycle as specified by BS2646, Part3, 1993; or by exposure to a suitable disinfection solution eg Virkon. Single use sharps will be used and disposed of without re-sheathing via an established clinical waste stream for sharps. Animal carcases will be disposed of via an established clinical waste stream for incineration. Provide details of any non-standard laboratory operations: None **<u>Additional control measures</u>** required for specific risks: Percutaneous infection risk: To minimise risk of infection via percutaneous injection SACGM Guidance recommends the elimination of sharps from use during viral production and use. Disposable plasticware such as the culture plates, flasks, tubes, vials and pipette tips are unlikely to pose any risk and so no other additional precautions are required. After consideration of the procedures to be undertaken, no additional need was identified for additional control measures to protect human health and safety	
RISK ASSESSMENT FOR ENVIRONMENTAL HARM **Environmental hazard identification -** Identify any potentially harmful properties of: i) the recipient micro-organism None. No disease or other harmful effects to humans, other animals or plants. ii) the inserted (donated) genetic material None. Inserts code for normal mammalian genes, or selective alterations of those genes. Also standard marker genes such as lac Z, GFP, etc. Inserts are not expected to have harmful physiological or pharmacological properties or to affect pathogenicity of cloning host. iii) the donor micro-organisms *(where used/appropriate)* N/A – inserts are from mammalian sources iv) the vector None. Similar standard lentiviral vectors are widely available with no noted harmful effects. Emergence of replication competent viruses during production or use has not been observed. The vectors will contain non-harmful inserts. v) the resulting genetically modified micro-organism None. The viral vectors have broad host specificity but are replication defective and contain no harmful gene inserts. Resulting GMOs carry no additional hazards compared with those already present in the environment. Any transfer of genetic material to other organisms would be of minimal hazard. GMOs would not survive outside laboratory conditions: E.coli strains do not colonise the human gut; mammalian cells will not survive outside laboratory conditions; animal models are housed in a designated facility minimising the risk of any viral spread to the environment.	***GUIDANCE*** *Potentially harmful effects include: disease to animals including allergenic and toxic effects* *disease to animals and plants* *adverse effects resulting from inability to treat disease or offer effective prophylaxis* *adverse effects resulting from establishment or dissemination of the GMMs in the environment* *adverse effects resulting from the natural transfer of inserted genetic material to other organisms*
Where potentially harmful effects are identified estimate: i) consequence/severity of effects Negligible ii) likelihood of effects being realised *(taking containment and control measures assigned above into account)*	*select from: Severe/Medium/Low/Negligible* *Select from: High/Medium/LowNegligible*

Negligible iii) overall risk Effectively zero **<u>Additional control measures</u>** required to reduce all risks to low/effectively zero: SACGM Guidance recommends the elimination of sharps from use during viral production and use.	*Select from:* *High/Medium/Low/Effectively zero*
CLASSIFICATION AND ASSIGNMENT OF FINAL CONTROL MEASURES **Consider each item on Table 1a** indicate whether or not it is required taking account of the provisional containment level assigned to protect human health and safety and any additional control measures necessary to control specific activities and environment risks **Consider also Tables 1b and 1c where appropriate** **<u>Classification:</u>** **Class: 1** **<u>Assign corresponding level of containment:</u>** **Containment Level: 1** **Modified to incorporate the elimination of sharps from use during viral production and use.** *Note:* **under COSHH Regulations some cell lines require Containment Level 2 plus microbiological safety cabinet.**	***GUIDANCE*** *Mark up table(s) by circling for each item the first correct answer reading across the table from left to right* *The highest numbered column in which a control measure is required indicates the Class of the activity – circle class on table 1a* *The class number indicates the minimum containment level required*

Table 1a: Containment Measures for Activities involving GMMs in Laboratories

Where an item is listed as "may be required" this indicates the item to be an option at that particular containment level and its requirement should be determined by the risk assessment for the particular activity concerned. Delete no or yes as indicated by risk assessment.

Containment Measures	**Containment Levels**			
	1	~~**2**~~	~~**3**~~	~~**4**~~
Isolated laboratory suite	**not required**	~~not required~~	~~required~~	~~required~~
Laboratory sealable for fumigation	**not required**	~~not required~~	~~required~~	~~required~~
Surfaces impervious, resistant and easy to clean	**required for bench**	~~required for bench~~	~~required for bench and floor~~	~~required for bench, floor, ceiling and walls~~
Entry to lab via airlock	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Negative pressure relative to the pressure of the immediate surroundings	**not required**	~~may be required no / yes~~	~~required~~	~~required~~
HEPA filtered extract and input air	**not required**	~~not required~~	~~required for extract~~	~~required for input and extract~~
Microbiological safety cabinet/enclosure	**not required**	~~may be required no / yes~~	~~required~~	~~required (class 3)~~
Autoclave	**required on site**	~~required in the building~~	~~required in the lab suite~~	~~required in lab (double ended)~~
Access restricted to authorised personnel	**not required**	~~required~~	~~required~~	~~required~~
Specified measures to control aerosol dissemination	**not required**	~~required so as to minimise~~	~~required so as to prevent~~	~~required so as to prevent~~
Shower	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Protective clothing	**suitable protective clothing required**	~~suitable protective clothing required~~	~~suitable protective clothing required~~	~~complete change of clothing and footwear~~
Gloves	**not required**	~~may be required no / yes~~	~~required~~	~~required~~
Control of disease vectors (eg rodents, insects) which could disseminate GMMs	**may be required no / ~~yes~~**	~~required~~	~~required~~	~~required~~
Specified disinfection procedures in place	**may be required no / ~~yes~~**	~~required~~	~~required~~	~~required~~
Inactivation of GMMs in effluent from handwashing sinks, showers etc	**not required**	~~not required~~	~~may be required no / yes~~	~~required~~
Inactivation of GMMs in contaminated material and waste	**required by validated means**	~~required by validated means~~	~~required by validated means~~	~~required by validated means~~
Laboratory to contain its own equipment	**not required**	~~not required~~	~~required~~	~~required~~
An observation window or alternative so that occupants can be seen	**may be required no / ~~yes~~**	~~may be required no / yes~~	~~required~~	~~required~~
Safe storage of GMMs	**may be required no / ~~yes~~**	~~required~~	~~required~~	~~secure storage required~~
Written records of staff training	**not required**	~~may be required no / yes~~	~~required~~	~~required~~

CLASSIFICATION	**CLASS 1**	~~**CLASS 2**~~	~~**CLASS 3**~~	~~**CLASS 4**~~

Table 3: Additional Containment Measures for Animal Work Where Viral Particles Are Present
Where an item is listed as "may be required" this indicates the item to be an option at that particular containment level and its requirement should be determined by the risk assessment for the particular activity concerned. Delete no or yes as indicated by risk assessment.

Containment Measures	**Containment Levels**				**Addition/ modification**
	1	~~2~~	~~3~~	~~4~~	
Isolation of animal unit (note 1)	**may be required no / ~~yes~~ ***	~~required~~	~~required~~	~~required~~	modification
Animal facilities (note 2) separated by lockable doors	**may be required no / ~~yes~~ ***	~~required~~	~~required~~	~~required~~	addition
Animal facilities (cages etc) designed to facilitate decontamination (waterproof and easily washable material)	**may be required ~~no~~ / yes**	~~may be required no / yes~~	~~required~~	~~required~~	addition
Floor and/or walls and ceiling easily washable	**may be required no / ~~yes~~ ***	~~required for floor~~	~~required for floor and walls~~	~~required for floor, walls and ceiling~~	modification
Appropriate filters on isolators or isolated rooms (note 3)	**not required**	~~may be required no / yes~~	~~required~~	~~required~~	addition
Incinerator for disposal of animal carcasses	**required to be accessible**	~~required to be accessible~~	~~required to be accessible~~	~~required to be on site~~	addition
Appropriate barriers at the room exit, and at drains and ventilation duct work	**required**	~~required~~	~~required~~	~~required~~	addition
Animals kept in appropriate containment facilities, such as cages, pens, tanks or isolator	**may be required ~~no~~ / yes**	~~may be required no / yes~~	~~may be required no / yes~~	~~may be required no / yes~~	addition

CLASSIFICATION	**CLASS 1**	~~CLASS 2~~	~~CLASS 3~~	~~CLASS 4~~

* While not required, this is common standard practice and will be performed

Notes

1. "Animal unit" means a building, or separate area within a building, containing an animal facility and other areas such as changing rooms, showers, autoclaves, food storage areas etc.

2. "Animal facility" means a facility normally used to house stock, breeding or experimental animals or one which is used for the performance of minor surgical procedures on animals.

3. "Isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be more appropriate.