Notification of a Notifiable Low Risk Dealing (NLRD)

EXAMPLE No: 2

NLRD Type:

PC1(a)

PC2(l)

Part 2: Description of the Dealings and GMO(s)

Briefly describe the purpose of the Dealing (in no more than a few sentences) and proposed methods to be undertaken in the dealing (as dot points).

We seek approval to conduct all types of dealings with the GMO's described here and in the following Table of this application. That is, we wish to create, culture, propagate, grow, import, transport, store, possess, conduct experiments with and dispose of the described GMOs.

Purpose of the Dealing:

1. To determine the functional contribution and mechanism of tissue repair using fetal mesenchymal and endothelial stem cells (MSC).

    - The endogenous contribution of fetal stem cells will be assessed in microchimeric cross bred models of transgenic fetal cells persisting post-pregnancy in wild type mice exposed to a range of injuries.
    - The utility of human fetal stem cell therapy will be determined after perinatal transplantation in mouse models of congenital disease with a survival advantage for normal cells or postnatal transplantation in tissue injury models.
    - The relative contribution of various chemokine/receptor systems (e.g. SCF/c-kit, SDF/CXCR4, Ceramide-1P, HGF/ c-met, PDGF and PDGFRα/β) implicated in stem cell homing to injured tissue will be evaluated by downregulating or knocking out receptors. Candidate receptors will be overexpressed to inform potential therapeutic strategies to facilitate stem cell engraftment.

2. To exploit ontogenic differences in stem cell maturity to derive fetal stem cells with advantageous properties for translation.

    - The contribution of candidate genes from microarrays to desirable fetal stem cell properties (eg proliferative capacity, differentiation potential) will be evaluated by up and/or down-regulation of the gene in mesenchymal and endothelial stem cells in vitro and subsequent assessment using in vitro assays (proliferation, differentiation, etc) and in vivo assays (ectopic bone formation assays and tissue injury models).
    - Maximise the efficiency of reprogramming / deprogramming approaches to derive primitive fetal stem cell populations from less (iPS) and more mature stem/progenitor populations.

Proposed methods:

Dealings with non-GMO relevant to this Application:

- Mating and experimentation on mice that are spontaneous mutants for hereditary disease models (eg "oim/oim" osteogenesis imperfecta mice) not involving transplantation of somatic GM cells.

Exempt dealings relevant to this Application:

- Transformation and growth of bacterial hosts (e.g. E.Coli K12 derivatives such as DH5α) with non-conjugative plasmids
- Production of replication-defective retroviral vectors unable to transduce human cells
- In vitro transduction of somatic murine cells with replication-defective vectors (unable to transduce human cells) for the purpose of upregulating or downregulating chemokine systems implicated in MSC homing (e.g. SCF, SDF, CaSR, HGF, PDGF A/B/AB), candidate genes that may distinguish fetal and adult mesenchymal stem cells and/or marker gene expression (eg GFP, renilla / firefly luciferase, lacZ, rainbow)
- Transplantation of somatic GM cells into non-GMO mice (e.g. spontaneous mutants or inbred strains)
- Primary cell culture of somatic cells derived from GMO mice (e.g. transgenic mice expressing marker genes; e.g.GFP, LacZ, luciferase)

Description of NLRD dealings:

In Vitro Dealings With Replication-Defective Lentiviral Vectors:

- Production of replication-defective, self-inactiviating lentiviral vectors for the purpose of lentiviral transduction of mammalian cells, in vitro, as described below.
- Transduction of human or murine cells with marker genes (eg GFP, renilla / firefly luciferase, lacZ, rainbow)
- Transduction of human or murine cells with siRNA to downregulate chemokine systems implicated in homing (e.g. SCF, SDF, CaSR, HGF, PDGF A/B/AB) or candidate genes that may distinguish fetal and adult stem cells
- Transduction of human or murine cells to upregulate chemokine systems implicated in homing (e.g. SCF, SDF, HGF, PDGF A/B/AB, CaSR) or candidate genes that may distinguish fetal and adult stem cells
- Transduction of human or murine cells to overexpress disease genes of relevance to tissue repair (i.e Col1A, dystrophin, β-glucuronidase, α-L-iduronidase, NPC protein, *tcirg1* etc)

Dealings with GMO Rodents:

- Mating, experimentation, transplantation and imaging of mice that are hereditary disease models generated by genetic modification (e.g. α-L- iduronidase Idua-/- mice). No mice will be transformed using lentiviral vectors.
- Mating, experimentation, transplantation and imaging of mice transgenic for conditional and constitutive reporters (GFP, renilla / firefly luciferase, lacZ)
- Mating, experimentation, transplantation and imaging of mice transgenic for conditionally and constitutively up or down regulated homing receptors (c-kit, CaSR, CXCR4, c-met, and PDGFRα/β).

Table 2.1: is intended to generate a concise, accurate record of all the GMOs to be generated or used and the purpose of the proposed dealings. Attachment 1 provides example reference responses to the description of the GMOs. Attachment 2 provides information relating to the completion of the column headed 'NLRD Type'.

2A	2B	2C	2D	2E	2F	2G
COMMON NAME OF PARENT ORGANISM	SCIENTIFIC NAME OF PARENT ORGANISM	VECTOR(S) & METHOD OF TRANSFER	EXEMPT HOST/ VECTOR SYSTEM ?	IDENTITY & FUNCTION OF NUCLEIC ACID & ORGANISM OF ORIGIN	ORGANISMS OR TISSUES TO BE USED WITH THE GMO(S)	NLRD TYPE
Mouse	Mus musculus	Plasmid microinjected into embryos	No	Cell tracking and expression of green or red florescent protein, renilla or firefly luciferase, Lac Z as marker / reporter genes	Human cells	PC1 (a)
Mouse	Mus musculus	Plasmid microinjected into embryos	No	Gene deficiency models of human hereditary disease (eg. the lysosomal storage diseases, MPS 1 (α-L-iduronidase, Idua-/-).	Human cells	PC1 (a)
Mouse	Mus musculus	Plasmid microinjected into embryos	No	Conditionally and constitutively up or down regulated homing receptors (c- kit, CXCR4, c-met, and PDGFR α/β)	Human cells	PC1 (a)
Human cultured cells	Human cell line or primary cultured cells	Replication defective lentiviral vector (as defined according to OGTR regulations Schedule 3, Part 2, 2.1 l)	No	Cell tracking and expression of green or red florescent protein, renilla or firefly luciferase, Lac Z as marker / reporter genes	Mouse Murine tissue (co-culture)	PC2 (l)
Human cultured cells	Human cell line or primary cultured cells	Replication Replication defective lentiviral vector (as defined according to OGTR regulations Schedule 3, Part 2, 2.1 l)	No	Up or down regulate chemokine systems implicated in homing e.g. SCF, SDF, HGF, PDGF A/B/AB	Mouse Murine tissue (co-culture)	PC2 (l)

Human cultured cells	Human cell line or primary cultured cells	Replication Replication defective lentiviral vector (as defined according to OGTR regulations Schedule 3, Part 2, 2.1 l)	No	Up or down regulate candidate genes identified in microarray comparison of human fetal and adult stem cells	Mouse Murine tissue (co-culture)	PC2 (l)
Murine cultured cells	Murine cell line or primary cultured cells	Replication Replication defective lentiviral vector (as defined according to OGTR regulations Schedule 3, Part 2, 2.1 l)	No	Cell tracking and expression of green or red florescent protein, renilla or firefly luciferase, Lac Z as marker / reporter genes	Mouse Human cells (co-culture)	PC2 (l)
Murine cultured cells	Murine cell line or primary cultured cells	Replication Replication defective lentiviral vector (as defined according to OGTR regulations Schedule 3, Part 2, 2.1 l)	No	Up or down regulate chemokine systems implicated in homing e.g. SCF, SDF, HGF, PDGF AIB/AB	Mouse Human cells (co-culture)	PC2 (l)
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Murine cultured cells	Murine cell line or primary cultured cells	Replication Replication defective lentiviral vector (as defined according to OGTR regulations Schedule 3, Part 2, 2.1 l)	No	Up or down regulate candidate genes identified in microarray comparison of human fetal and adult stem cells	Mouse Human cells (co-culture)	PC2 (l)