2017/2018 Summer Studentship Project Application Form

Send to: Research Office, University of Otago Christchurch, PO Box 4345, Christchurch, by 5pm on 3 July 2017

Supervisor Information (First named supervisor will be the contact):	
First Supervisor's Name and Title: Dr. Nadia Mitchell	
Department - UOC &/or CDHB (if applicable): Radiology	
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First Supervisors Mailing Address: Level 5, 2 Riccarton Ave, PO Box 4345, Christchurch Mail Centre, Christchurch 8140	
Co-Supervisors Name and Title(s):Prof. David Palmer (Lincoln University and adjunct Professor in Radiology, UOC)	

Research Category (Choose one category only **– to be used for judging the students' presentations):**		
Clinical √	Laboratory	Community

Project Title (20 words MAXIMUM):
Sheep as a pre-clinical model for human gene therapy

Project Description:

Introduction:

Gene therapy involves the replacement of a defective gene with a functional one. For hereditary diseases, viruses are modified to deliver a corrective copy of a mutated gene into host cells. The viruses, termed viral vectors, hijack the host cell machinery to make a functional therapeutic protein to either stabilise or reverse a clinical disease state. Most brain-directed gene therapies have been developed in mice, but the lissencephalic rodent brain lacks major neuroanatomical structures prominent in the human brain and is very much smaller, 0.4 g vs 1.4kg, as is the whole animal. Instead sheep represent ideal candidates for these investigations. They are similarly sized to humans, weighing 3.5 - 4.5 kg at birth and grow to 80 – 110 kg adults. The gyrencephalic ovine brain weighs ~140 g in adulthood and is similar in physical organisation to the human brain. Thus sheep models can provide excellent translational data on vector titres, doses, delivery routes and target sites and are often predictive of actual clinical outcomes.

The lysosome is an intracellular organelle which breaks down and recycles larger molecules to smaller constituents for cellular function. If a protein involved either in lysosomal processing or directly as a lysosomal enzyme is defective, this results in the accumulation of these large molecules in the lysosome, causing a lysosomal storage disease (LSD). Over 50 different LSDs exist, and most are currently incurable. Progressive neurodegeneration, seizures, loss of vision and premature death are often listed as defining common features. Gene therapy constitutes an attractive strategy to correct these diseases; viral vectors carrying a functional copy of the defective gene can be directed to the main target site, namely the brain. However given the neuropathology in the LSDs is widespread, it is important to determine the best delivery mechanism to achieve the greatest spread of the gene through the brain to best optimize successful correction.

Aim:

Here, viral vectors carrying a reporter gene (GFP; green fluorescent protein) will be injected into the cisterna magnum of two normal sheep, to see how effective this delivery route is at spreading the gene through the brain.

Four weeks post-injection, sheep will be sacrificed and the vector distribution will be tracked by GFP expression, using immunohistochemistry and fluorescent microscopy on *post mortem* tissue sections. Results will be directly compared with previous GFP expression studies in the normal sheep brain (described below) to determine the best delivery route to achieve the greatest spread of GFP through the sheep central nervous system.

Possible impact (in lay terms):

Gene therapy represents an attractive treatment strategy for many LSDs, which currently lack treatment. Preliminary gene therapy studies in mouse models of LSDs have been encouraging but large animals, such as sheep, provide a valuable tool in which to test different delivery routes and expedite these approaches to the clinic.

The most direct gene therapy stratagem is via direct injection of viral vectors into the brain tissue (parenchyma) (Fig. 1). However, when tested in the sheep brain, there was relatively restricted diffusion of the vector, which limited GFP expression to the vicinity of the injection sites (Mitchell, 2016). It has been estimated that 50 to 350 separate injections would be required for complete coverage of the entire human infant brain, with each injection risking infection, inflammation or toxicity. Nevertheless, intraparenchymal delivery has been used in LSD patients affected by Canavan disease (Janson *et al*., 2002; Leone *et al*., 2012), late infantile Batten disease (Crystal *et al*., 2004; Worgall *et al*., 2008), metachromatic leukodystrophy (Zerah *et al*., 2015) and mucopolysaccharidoses type IIIA and B (Tardieu *et al*., 2014).

As an alternative, we recently explored convection-enhanced parenchymal delivery in the sheep brain earlier this year with collaborators from the University of Manchester, UK. This method delivers a continuous pressure-driven infusion of viral vector, enabling convective distribution over large volumes of the brain. The final analyses are still pending and although the method should result in greater vector spread, it is still very invasive.

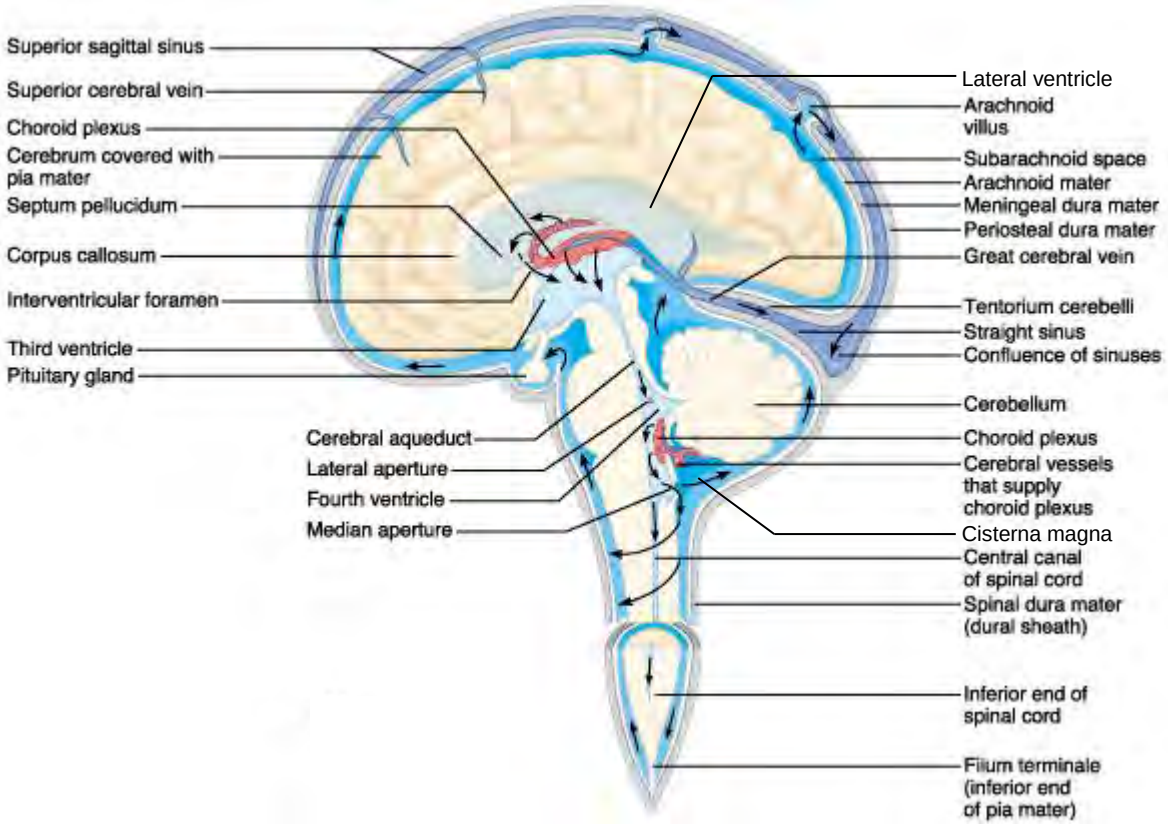
Intracerebroventricular gene therapy has recently proven effective in correcting LSD (neuro)pathology in diverse animal models (Rafi *et al*., 2012; Haurigot *et al*., 2013; Katz *et al*., 2015; Ribera *et al*., 2015), including sheep models of the lysosomal storage disorder, Batten disease, at Lincoln University (Mitchell, 2016). The vector is delivered into the fluid filled spaces (ventricles) of the brain (Fig. 1). The cerebrospinal fluid (CSF) here circulates through the brain, bathing it and the spinal cord, hence providing an easy way of distributing the vector and resulting in a more global delivery. GFP-positive cells were observed throughout the normal sheep brain and along the length of the spinal cord, with no evidence of inflammation or toxicity to the brain cells. Access to the ventricles is routinely used to relieve intracranial pressure (Rosenbaum *et al*., 2014) however there was some concern about the 'safety' of this route in children and so alternative CSF-directed delivery routes are being considered.

Intrathecal (into the CSF in the lumbar spinal cord) is one such route and will be explored in sheep at Lincoln University later this year. However this method was not particularly successful in pigs (Federici *et al*., 2012); the vector must travel from the base of the spine to the brain hence the spinal cord was well transduced but GFP expression was only seen in the rear end of the brain.

In this proposed summer research project, the intracisternal delivery route in normal sheep will be tested. In a small pilot study, vectors carrying GFP will be delivered into the cisterna magnum – another fluid filled space in the brain which is readily accessed. CSF flows through the cistern and then across the brain. Four weeks later, the two sheep will be sacrificed, the brain and spinal cord analysed for GFP expression and compared to the distribution patterns seen by the other delivery routes described above.

This study will directly compare intracisternal delivery to other delivery routes in the sheep brain and providing information on whether it is a potential route to be further explored in humans.

Figure 1. Gene therapy routes to target the brain and cerebrospinal fluid (CSF) flow
Viral vectors can be injected directly into the brain tissue (intraparenchymal); into the CSF of the lateral ventricles (intraventricular); into CSF at the cisterna magna (intracisternal) or into CSF at the lumbar or inferior end of the spinal cord (intrathecal lumbar).



CSF is produced by the choroid plexus of each ventricle. It flows through the ventricles and into the subarachnoid space via the median and lateral apertures, bathing the external surface of the brain. Some flows through the central canal of the spinal cord. At the arachnoid villi, CSF is reabsorbed into the venous blood of the dural venous sinuses.

Method:

This proposed project is a pilot study, involving only two sheep. Intracisternal injection is relatively straightforward and, in fact, it is through the cisterna magna (cerebellomedullary cistern) that we routinely aspirate CSF samples in sheep. Pre-operatively animals will be generally assessed (weight, temperature, pulse, respiratory rate). This will occur prior to commencement of the summer student.

Either immediately at the start of the project, or preferably on a day prior to the start of the project, two normal control sheep will receive a common gene therapy vector, adeno-associated virus (AAV) expressing a reporter gene, green fluorescent protein (GFP) through the cisterna magnum. Vector is available at Lincoln for this purpose and the surgery will only take at most 1 hour per animal.

Under general anaesthesia (diazepam/ketamine, 1:1, i.v.), the head will be shaved and held in position as for CSF collection. A 22g needle fitted with a 3-way tap will be inserted into the cisterna magnum and the correct depth verified by easy withdrawal of CSF. The tap will be switched and the suspension of vector particles injected in a small volume of saline. Sheep will receive 500 μL of scAAV9.CB.GFP (total dose 3 x 1013 viral genomes, an equivalent dose to that given in the previous sheep studies described above. Once the full dose has been deposited, the needle will be withdrawn and the animal monitored closely post-surgery. For the first two weeks following surgery, neurological status and rectal temperature will be recorded by the summer student. Pulse, respiratory rate, temperature and weight will be measured weekly.

During this time, under the tutelage of the first supervisor and a technician, the summer student will learn brain sectioning and immunohistochemistry techniques on historical stored samples at Lincoln. At 4 weeks post-surgery, sheep will be euthanised by captive-bolt and exsanguination. Brains will be collected and perfusion-fixed *in situ* by established methods routinely used at Lincoln University (Oswald *et a*l., 2005; Mitchell, 2016). Spinal cord samples will be collected and fixed in formalin. The brains will be bisected at the sagittal midline and left in fixative (10% formalin) for a further 7 days. Fixed brains will then be equilibrated in cryoprotective solution (10% ethylene glycol, 20% sucrose in 0.9% NaCl) at 4°C for 5 days and stored frozen at -80°C until they were sectioned. This should co-incide with the 2017/18 Christmas break.

Into the New Year, sequential 50 µm sagittal brain sections will be cut through the medio-lateral extent of both hemispheres using a freezing sliding microtome. Every 40th sections will be stained for GFP as described previously (Linterman *et al*., 2011). If time permits, parallel series of sections will be double-labelled with fluorescent GFP and either an astrocytic marker (GFAP) or a fluorescent neuronal marker (NeuroTrace) as established methods (Linterman *et al*., 2011) to identify the key cell populations transduced. Results will be directly compared with historical slides from the previous GFP expression studies in the normal sheep brain described above.

References:

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Student Prerequisites (eg. Medical Student) if applicable:

Administration Details

1. Is ethical approval required? Yes/No

   If Yes: please circle or tick one of the following:

   a) Applied for (provide application #) Lincoln University #614

   b) Approved (attach a copy of the letter of approval from the ethics committee or application #) Lincoln University #614

   c) To be done

2. Are you able to provide the funding for this project (ie. $5,000 for the student, incidental expenses should be met from departmental or research funds) Yes/No

   If Yes: Please provide name of the funder _____

   If No: Please provide ideas of possible funding sources, including past funding agents and topics often associated with this research area, for the Research Office to contact.

   I have a CureKids post-doctoral research fellowship which should cover the incidentals, surgical and consumable costs associated with this project but not the student stipend.

   _____

3. Medical Records or Decision Support accessed Yes/No

4. Health Connect South or other DHB records Yes/No

5. Signatures:

   - I have read the 2017/2018 Summer Studentship programme handbook.
   - I am prepared to supervise the project and will be available to the student during the studentship (including Christmas/New Year break if the student is working during this time).
   - I agree to assume responsibility for the submission of the student's reports to the Research Office by the due date 29 January 2018.
   - I agree that the project lay report may be available to local media for publicity purposes.

Signature of Project Supervisor(s):	Date: 3.7.17

- I understand that I am responsible for hosting the Summer Student chosen for this project and will meet any costs incurred. I agree that incidental expenses will be met from departmental or research funds.

Signature of Head of Department: (Print Name)	Date:
Signature of Clinical Director: (if applicable) (Print Name)	Date: