NON-TECHNICAL SUMMARY

Treating a range of pathophysiological retinal insults with gene therapies

Project duration
5 years 0 months

Project purpose
- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words
No answer provided

<table>
<thead>
<tr>
<th>Animal types</th>
<th>Life stages</th>
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<td>Mice</td>
<td>adult</td>
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<tr>
<td>Rats</td>
<td>adult</td>
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Retrospective assessment
The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits
Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What is the aim of this project?

The overall aim of this Project Licence (PPL) is to design and test novel gene therapies to treat a variety of eye diseases. Both cell culture systems and rodent disease models will be used to measure gene therapy effectiveness and safety. We have made significant progress in ways to treat glaucoma, a disease of the retina, and will now expand our treatments to other retinal diseases that can lead to blindness. These include diabetic retinopathy/diabetic macular oedema and both dry and wet-form age-related macular degeneration (AMD). Over the tenure of the licence we expect to patent a further two products. The expectation is that one or more of these gene therapies can be progressed into clinical studies to treat unmet medical needs and maintain patient vision.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In 2010, the global cost of vision care was estimated to be £2.5 trillion for the 733 million people living with low vision and blindness. Eye disorders constitute one of the costliest health considerations worldwide and there remains a high unmet medical need to treat common eye diseases. Many current therapies require frequent daily application, such as eye drops to lower eye pressure in glaucoma, or monthly back of the eye injections of ranibizumab for AMD. In both of these cases, there are issues, either related to compliance, discomfort, correct placing of the eye drops over the cornea or the inconvenience of monthly eye injections. In addition, repeated injections into the eye can lead to corneal and retinal scarring. Gene therapies offer treatments which require only a single administration thereby generating clinical benefit over many years whilst minimising side effects.

What outputs do you think you will see at the end of this project?

The team are hoping to publish all notable results in high-quality peer-reviewed journals to inform peers of the successes. In addition, data may be incorporated into product patents and used in documents for regulatory filing which will support novel products being advanced into clinical trials.

What will be the impact of this proposed work on humans / animals / the environment in the short-term (within the duration of the project), in the medium-term and the long-term (which may accrue after the project is finished)?

The scientific community will benefit from scientific publications (expectation of 2/year) over the course of the project. The team are also confident of generating two novel product concepts within the timeline of the project. It is expected that these products can outperform current market leading products. We would then be able to progress these programmes towards non-clinical (safety) assessments in preparation for clinical development. Longer term outputs would include clinical trials and approval for patients. Initially, the gene therapies would benefit more affluent countries (due to initial high costs, but
also due to the higher proportion of patients suffering from diabetic and age-related eye pathologies). Over time, costs will considerably reduce, and treatments will benefit less affluent countries. Stable storage, ease of injection (not needing a surgery suite or surgeon), minimal complications and long-term effectiveness should benefit hard to reach communities with poor healthcare infrastructure.

**How will you maximise the outputs of your work?**

We will be publishing results originating from this project in notable peer reviewed journals and presenting the data at research conferences. It is also a major aim to patent constructs and novel models which may have commercial benefit and may be incorporated into products which could be progressed into clinical trials.

**Species and numbers of animals expected to be used**

- Mice: 3000
- Rats: 1100

**Predicted harms**

**Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.**

Describe, in general terms, the procedures animals will undergo, eg injections, surgical procedures. Include the typical number of procedures individual animals will undergo and the likely duration of suffering.

All experiments involve injecting a viral vector into the eyes of mice or rats, usually on one occasion only whilst the animal is anaesthetised. The animals are then left for several weeks so that the viral vector can express throughout the retina and increase the expression of desired proteins.

After this time, an injury to the eye is performed to damage the retina. This can be achieved via genetics, through addition of a chemical, or through a surgical procedure to the eye. Some injuries occur rapidly (within a week), others can take months for damage to occur. Occasionally the injury needs to be repeated although it is expected the majority of animals would only need a one-off or double injury procedure. Again, these injuries occur with appropriate anaesthetic and pain relief.

During the disease progression various in life examinations are performed to measure the animal's vision and judge the success of the treatment. These tests are similar to what take place in an eye clinic or at an optician. The equipment used is smaller and tailored for animals but produces the same outcomes. To keep the animals still, general anaesthesia is required for some of the assessments, but none are painful.

Sometimes, animals need to be anaesthetised on several occasions if multiple tests are to be performed. This usually takes place over several weeks and for established gene therapies which are
working effectively. Animals might also have small blood samples taken during the disease progression and after treatment. Blood sampling is more common in the diabetic models to confirm the pathology and ensure sugars in the blood are elevated to a level that can cause visual loss. Sometimes tears or small ocular fluids are collected to support blood results, but these would be in less than 5% of cases and often collected on one occasion.

At the end of the experiment, animals are killed humanely, and ocular tissues collected and processed.

**Expected impacts or adverse effects on the animals - for example, pain, weight loss, inactivity or lameness, stress, or abnormal behaviour - and how long those effects are expected to last.**

The majority of procedures are not expected to cause adverse effects and the success rates are high. All eye injections should not cause any complication. Likewise, all in life assessments should not hurt or adversely affect animals. The group have several years’ experience with these techniques and rarely encounter problems.

Some injuries to the eye can cause temporary swelling and tenderness, which should be self-limiting. Increased monitoring will occur during and shortly after these steps and animals displaying adverse effects are treated quickly for pain and/or possible inflammation. Vision in the injured eye should decrease with time. For surgical procedures, the injury is limited to one eye as the vision loss can occur rapidly (1-3 weeks). The genetic strains should lose vision gradually over several months. It is important to note that animals will not become blind from experiments and it is expected a maximum of 50% vision might be lost. Patients often lose 50% of their vision without even noticing so this is not expected to impact animal behaviour. With the gene therapy treatments each animal receives, this vision loss should be reduced and therefore animals should be able to see clearer.

The diabetic models will have the greatest impact in relation to adverse effects because they affect the entire animal and not just the eye. Some unavoidable weight loss and possible abnormal behaviour is expected although these will be reduced as best possible through regular monitoring and intervention steps. To match the clinically conditions, diabetes has to last for a sufficient period of time for retinal damage to occur. I have chosen four different diabetic injury models to mimic the disease and ocular damage, and I will be removing protocols and focussing on the models with the highest benefit to cost ratio.

**Expected severity categories and the proportion of animals in each category, per species.**

**What are the expected severities and the proportion of animals in each category (per species)?**

C57BL/6 (a wildtype mouse strain) – mild (50%) moderate (50%)

B6.Cg-Tg(THY-1 YFP) 16Jrs/J (a non-harmful mouse strain that has some fluorescently labelled retinal cells) – mild (60%) moderate (40%)

db/db (a genetic diabetic mouse strain) – moderate (100%)

Ins2Akita (a genetic diabetic mouse strain) – moderate (100%)

Wistar, Sprague-Dawley, Lister Hooded (wildtype rat strains) – mild (50%) moderate (50%)
mild = pain or suffering experienced by an animal is, at worst, only slight or transitory and minor so that the animal returns to its normal state within a short period of time.

moderate = procedures on animals as a result of which the animals are likely to experience short-term moderate pain, suffering or distress. Animals may experience longer lasting mild suffering or moderate impairment of well-being or general condition.

What will happen to the animals at the end of the study?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The eye is a complex organ in which specialised cells transmit visual information to the brain. No cell culture systems have yet been developed which can accurately model the visual system. Therefore, animals are required to understand the pathophysiology associated with common eye diseases and ways to prevent blindness.

What was your strategy for searching for non-animal alternatives?

Where possible gene therapies are examined in cell culture systems for their ability to transfect and express therapeutic proteins to levels that can influence disease pathways. The use of purified primary retinal cells and retinal organoids (tiny, self-organized three-dimensional tissue cultures) will also be further investigated and external experience is being sought to establish these protocols in the lab.

The group already uses rodent and human retinal explants to study mechanisms relevant to ocular disease. We use these tissues to examine markers and optimise processing protocols. I have also received 3R funding in the past and will apply for further non-animal funding to continue researching non-animal alternative approaches.

Why were they not suitable?

Cell culture systems cannot predict overall efficacy (preventing, attenuating or stopping pathology), or model the complexities of diseases. They are also unable to assess any toxicological or immune responses to the gene therapy.

Reduction
Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Sample size calculations are taken into account from pilot data to minimise group sizes required to demonstrate clinically relevant effects. Pilot studies are always carried out using a small cohort of animals (3-6 animals) before beginning a full study.

Testing to see if the vectors express the protein of interest are typically performed on group sizes of 3 to 4 animals due to the reproducibility of the procedure and results. Controls are needed if there is a possibility that the formulation (or vehicle that the compound is dissolved in) could be toxic, in which case groups of animals will receive control administration of vehicle alone. In the case of gene therapies, either empty viruses or virus expressing green fluorescent protein (GFP) are favoured.

For diabetic studies, we have based the estimated number of animals required from scientific literature to obtain statistically and biologically meaningful data. 10-15 animals per group seems appropriate when doing longitudinal experiments due to genetic variability and disease onset. Glaucoma studies are based on previous studies and experience over the past 8 years.

What steps will you take to reduce animal numbers? Where applicable, what principles will you use to design experiments?

Statistical advice has readily been sought via the Biostatistics Unit on our campus. Experiments will also be conducted to the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines and ensured to comply with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for publication. These guidelines help to minimise unnecessary studies, maximise information published and improve reproducibility.

In the past we have also performed sham (pretend) surgeries to control for unexpected inflammation which may cause an unpredictable response. We are quite confident our surgeries will only have the desired effect and are keen not to use excessive animals where possible to comply with the 3R’s. Using both eyes of an animal, for non-injury procedures and transgenic animals, also greatly reduces animal numbers without adversely impacting animal wellbeing.

Any new surgery, and any new Procedure Individual Licence (PIL) holder will undertake preliminary experiments to generate data relevant to the technique and research question. Similarly, doses will be calculated from effective non-toxic concentrations in tissue culture studies to minimise dose ranges needed to be tested in animals.

For all experiments, we will use the minimum number of control groups that we can in order for the experimental data to be interpretable. We know that phosphate-buffered saline (PBS) injection and control vector injection can be used interchangeably (as results are almost identical) so only one type of control will be included alongside test compounds.
To ensure accuracy of data, we will also use masked experimentation for almost all studies whereby each animal is given a number and the processing scientist does not know what procedures or treatments each animal has received. In life results are then not revealed until after the data has been obtained.

What other measures apart from good experimental design will you use to minimise numbers?

To minimise variability, and therefore total number of animals, the majority of procedures will be performed on animals of the same strain. Additionally, I will continue to maximise the amount of data generated from each animal. To achieve this, I will be combining functional and behavioural tests with anatomical studies (e.g. performing retinal functional recordings whilst the animal is alive, before supporting these changes with histological retinal sections). The group will also use more tissues per animal than before. This includes processing both eyes, and collecting the optic nerves, brains and fluids when possible to further explore different mechanisms of degeneration and protection.

The group will list excess organs, where possible, on the animal facility mailing list. We have shared organs with other groups in the past and will continue to donate extra tissues.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Why are the animals, models and methods you will use the best to meet your objectives? Why will your approach cause the least pain, suffering, distress or lasting harm?

Injury models:

Laser treatment to reduce aqueous outflow - An established model of injury in rats. The model has a high degree of success and is the most relevant model for glaucoma we have experience with, causing an elevation in intraocular pressure (IOP). This model is only successful in rats and performs best in non-pigmented strains. Suffering is minimal and complications are alleviated by keeping the eyes lubricated and performing the procedure with pre-defined and optimised laser settings.

Laser treatment to Bruch’s membrane - A new technique to the group but one of the most established techniques in the literature to induce damage to the retinal vasculature. Laser spots are directed onto the retina at the back of the eye and the rate at which blood vessels reform and repair are measured. Suffering and distress will be minimal as the injury is self-contained and only applied to one eye.
Microbead intraocular injections – The injection of tiny magnetic beads into the front of the eye (known as the anterior chamber) blocks aqueous drainage channels and causes a gradual rise in IOP. This technique is relatively easy to perform, and eyes and animals remain healthy. There is minimal pain using this model and it allows for post intervention treatment with gene therapies, better reflecting treating of patients with glaucoma.

Cannulation of the anterior chamber of the eye – Attaching a syringe to the anterior chamber allows a solution to be administered that can increase the IOP within the eye. This model was developed and taught to us by one of our collaborators. The model causes short-term functional effects and can reproducibly increase IOP to a precise level. The pressure returns to normal when the canula is removed and no pain or adverse effects are caused.

Crushing of the optic nerve - the optic nerve crush procedure is well established in our lab and causes a reproducible level of damage. The optic nerve (which transmits visual information from the eye to the brain) is exposed behind the eye and crushed with forceps for a few seconds. The nerve is then released, the tissues moved back into place and the injury site heals on its own. The technique is relatively challenging, particularly on mice, but the data is essential for assessing vector neuroprotective effects. Animal group sizes are also small for optic nerve crush studies, due to the consistent level of injury, making it a useful model for testing multiple therapies against one another.

Having multiple retinal injury models ensures treatments are more likely to be effective when transitioning to patients.

Diabetic animal models:

Ins2Akita mice are a diabetic strain of mouse that lose vision slowly over 6-9 months due to excessive glucose in their blood. We will try to use these animals at the earlier end of the disease progression when adverse effects are less likely.

db/db mice are another diabetic mouse strain that generally lose vision over a chronic 9-12-month period of time, due to similar changes in blood sugar glucose. Where possible we will purchase from breeders, or transfer from other breeding licences, animals close to retinal pathology onset to minimise time kept on this licence.

Streptozotocin (STZ) is a toxin that damages cells of the pancreas, reducing insulin levels and therefore resulting in increased blood sugar levels. The STZ model induces diabetes as early as 3 days post-
treatment and models. It can be used in both mice and rats making it favourable when progressing gene therapies to other species.

It is likely only two diabetic models will be required to show efficacy. These will be selected after preliminary testing for suitability and the models that display the least adverse effects or complications will be progressed on the licence.

To avoid adverse effects, all invasive surgical procedures will be carried out according to the Laboratory Animal Science Association (LASA) Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017).

**Why can’t you use a less sentient animal, (for example at an immature stage, a less sentient species or using terminally anaesthetised animals)?**

Due to the complexity of the eye, and as many of the diseases we are aiming to cure occur in adult and aged patients, we need to use appropriate models. We require the use of mammals due to the similarity in the visual system and the length of time for a treatment to express or a disease to progress means procedures under terminal anaesthesia are not applicable. The injury models to be used and gene therapies being designed are also relevant to human disease and therefore have the greatest likelihood of transitioning to patients.

**What are you going to do to refine the procedures (for example increased monitoring, post-operative care, pain management, training of animals) to minimise the welfare costs (harms) to the animals?**

We are introducing a scoring system (1-10) for each procedure with anything lower than 7 having additional checks post-surgery. These animals will be examined thoroughly throughout the study for evidence of ocular damage and given post-operative antibiotic, lubricating, soothing eye gel and/or topical steroids if required. We will also monitor the weight and behaviour of the animals for 7 days post-surgery and provide treats to animals that undergo the more invasive surgeries. Each researcher will keep hold of these records and use them to improve their techniques. From past experience, over time, researcher scores consistently increase from 5’s and 6’s to 8’s and 9’s.

I have also written a health assessment score sheet to identify complications linked to surgery or diabetic strain to help researchers and animal technicians agree on a correct course of action based on a multitude of scores and criteria.

The group will also have a picture monitoring catalogue detailing what to expect post-surgery and possible adverse expects. Our researchers also train routinely on cadavers which allows them to perfect their surgeries and techniques before beginning their projects. This reduces variability in their studies and helps reduce animal usage through failed procedures and minimise pain and adverse effects.
A shared spreadsheet will be maintained within the group which details the course of all animals through all procedures performed. This spreadsheet will record each animal, every procedure performed on that animal, the actual severity reached, additional comments to the study and how the tissues were used and stored. This system has worked very well in the past and we have improved our recording and monitoring of animal welfare.

Over the tenure of the licence we will also employ an experienced animal technician to oversee procedures and work closely with the animal facility technicians. Maintaining active communication with the facility animal technicians is vital and will allow us to immediately identify problems. In the past, I have also given presentations to the animal facility staff and managers about the nature of our experiments and studies. This helps the facility staff understand what we are doing and why certain adverse effects can arise and why others are not expected.

Animals will also be housed in groups to enable social interaction and grooming. From experience, rarely do our animals fight after surgery and open wounds are not expected. We have experienced minimal signs of stress or discomfort from similar procedures in the past and expect a good quality of life for the animals on this licence.

Dark adapted animals will be group housed in a custom dark cabinet with appropriate subsistence for the duration of the acclimatisation. The cabinet is equipped with an alarm system and measures temperature and air flow, alerting the researcher of any deviations outside of the normal range. A timer will also be set to ensure dark adaption does not exceed 24 hours and the animal unit staff informed when animals are placed in the cabinet.

**What published best practice guidance will be followed to ensure experiments are conducted in most refined way?**

Publications will follow the ARRIVE and PREPARE guidelines using the ‘Animal Research: Reporting In Vivo Experiments’ template. LASA publications will also be referred to in addition to any other best practice guidance articles on surgery and procedures.

**How will you ensure you continue to use the most refined methods during the lifetime of this project?**

We will be applying for 3R funding throughout the tenure of the project which requires constantly keeping up to date with 3R advancements. We also seek to collaborate on several 3R projects allowing us to learn and implement new ways to replace, refine and reduce animal usage.

Information will also be periodically checked from www.ubs.admin.cam.ac.uk/3rs/3rs-search-tool, www.animalcare.ubc.ca/animal-care-committee/sops-policies-and-guidelines and www.arvo.org/About/policies/statement-for-the-use-of-animals-in-ophthalmic-and-vision-research/ that provides detailed guidelines for working with animals for eye related research.

**Explain the choice of species and the related life stages**

This project will use both mice and rats, the majority of which are not genetically altered. The group has extensive experience with both species and all new ocular gene therapy drugs require a viral vector to
be tested in a mammalian system.

These animals were selected because their eye anatomy is very similar to that of the human eye, including the ocular response to injury. For the majority of experiments, initial testing will be carried out in adult mice. Adult rats will be required to demonstrate that a novel gene therapy is protective in more than one species. Certain injuries also work more effectively in rats due to the larger globe size.

Younger animals are not suitable for this project as the retina is less susceptible to damage and the profile of proteins in the eye is very different at an early age. Young animals also have smaller, softer eyes making injections and injuries more challenging. Clinically, the diseases we are investigating do not occur in young patients and therefore need to be investigated in a more developed visual system.