Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 5

Projects with a primary purpose of: Translational and Applied research – Human Sensory Organ Disorders (skin, eyes and ears)

Project Titles and Keywords

1. **Novel Therapies to Treat Inflammatory Diseases**
    - Arthritis, Colitis, Dermatitis, Nephritis, COPD

2. **Tissue repair**
    - Connexin, antisense, wound-healing, diabetes

3. **Ocular gene and stem cell therapy**
    - Retina, eye, gene-therapy, cell-therapy

4. **Improved treatments for ocular surface disease**
    - Corneal blinding, transplant, stem cell, aniridia, interstitial keratitis (corneal *scarring)*

<table>
<tr><td>PROJECT 1</td><td>Novel Therapies to Treat Inflammatory Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Arthritis, Colitis, Dermatitis, Nephritis, COPD</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project aims to develop novel therapies to treat inflammatory diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Inflammatory diseases affect patients causing pain, impaired function and diminished quality of life. By contributing to the development of new candidate anti-inflammatory drugs, our project will benefit the patients improving their quality of life and reducing pain. By providing high quality services and scientific expertise, we are able to make the testing of such drugs more cost effective, more informative and reduce the need for companies to set up the models in house.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>The estimated number of animals to be used over the duration of the project is 14 200. Animals to be used include a majority of mice (80%) and some rats (20%).</td></tr>
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over what period of time?	They are the least sentient species which allow the objectives to be met.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Expected adverse events: (i) adverse reaction to a lead candidate (< 25% incidence), (ii) arthritis, joint swelling, reduced mobility, (iii) colitis, bodyweight loss, diarrhoea, intestinal bleeding, abdominal discomfort, change in appearance or behaviour, (iv) skin thickening, flaking, scabbing, erythema, (v) emphysema, (vi) liver dysfunction, (vii) peritonitis, (viii) kidney inflammation. Expected level of severity: Moderate. Measures taken to limit harms: frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events, moderate signs tolerated for no more than 24 hours, severe signs not tolerated. At the end of an experiment, all animals will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Inflammation, the immune system's response to the presence of antigens, involves multiple systems, multiple organs and multiple cell types. The complexity of the inflammatory response cannot be reproduced *in vitro*. In addition, the symptoms of inflammation -heat, redness, swelling and pain- cannot be modelled *in vitro*. *In vitro* experiments on cell lines and *ex vivo* experiments on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power analysis will be performed to establish the total sample size required to generate meaningful data. Typically, power value will be set at 80% in order to reduce the number of animals used in the studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Animal suffering will be limited by choosing mild over moderate severity models and by choosing acute over chronic models. A typical experiment will involve a local or systemic administration of a pro-inflammatory agent. Lead

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	compounds will be administered prior to or from of signs of disease (prophylactic and therapeutic regimen, respectively). Animals will be monitored frequently and scored for clinical signs of disease. Blood, cells and/or tissue samples will be collected at the end of the experiment for ex vivo analyses.

PROJECT 2	**Tissue repair**	
Key Words (max. 5 words)	Connexin, antisense, wound-healing, diabetes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	Y	Higher education or training
	N	Forensic enquiries
	Y	Maintenance of colonies of genetically altered animals[3]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To find ways to improve the wound healing process. To understand how chronic wounds form and why they do not heal with the aim of identifying therapeutic targets.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	From our previous work we already have one drug in clinical trials to promote the healing of venous leg ulcers (about to start phase 3) and diabetic foot ulcers (phase 2) with orphan drug status for persistent epithelial defects in the eye. We are now exploring new targets and types of wound which should provide significant benefit to mankind.	
What species and approximate numbers of animals do you expect to use over what period of time?	Maximum of Mice 1500 and 900 rats 1200 transgenic mice.	

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The proposed wound healing models are of moderate severity, Induction of diabetes can be detrimental to the animals and their weight and well-being will be closely monitored. All animals will be culled at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are studying the wound healing response which has an inflammatory component that can not be replicated in culture. it also involved many different cell types which cannot be reproduced in culture.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We do test our drugs as much as possible on cultured fibroblasts and keratinocytes before moving on to animal models.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We are using well established models of wound healing in mice and rats. The STZ diabetic rat is the FDA approved model for diabetic wound healing. We will monitor the animals after surgery and score their well-being.

<table>
<tr><td>PROJECT 3</td><td colspan="2">Ocular gene and stem cell therapy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Retina, eye, gene-therapy, cell-therapy</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[4]</td><td colspan="2"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td></td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
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<tr><td>Maintenance of colonies of genetically altered animals[5]</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">The overall aim of this project is to develop new methods to treat various blinding eye diseases by using gene therapy or stem cell therapy.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="2">The eye diseases we are targeting are currently either poorly treated or there are no effective treatments for them at all. Although many of these diseases are relatively rare, their impact on the individual patient and the community is substantial. The work in the previous project period has already led to clinical trials of gene therapy for retinal dystrophy and we will initiate several more clinical trials on inherited and acquired disease in the coming years based on the work proposed here. The research for some of the other objectives, especially the stem cell research, is still in a relatively early stage and it is unlikely to lead to clinical application in the duration of this project. However, successful completion of these objectives will be an essential first step in the development of a treatment for these untreatable, blinding diseases.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="2">Most studies will be using mice, either normal animals, or animals that have an inherited disease. Occasionally, rats may be used as the larger rat eye will allow a more precise manipulation of ocular tissues. Prior to application in patients, new treatments need to be tested in a second (larger) animal; to this end, we intend to use rabbits.</td></tr>
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	We are currently investigating novel treatment strategies for approximately 10 different ocular diseases, a program of work for which we use about 5000 rodents and 30 rabbits each year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A variety of natural and artificial mouse strains with inherited retinal disease exist. As rodents are nocturnal animals who primarily use other senses, the retinal degeneration has little impact on the animals' welfare. We administer the putative therapeutic agent into one eye of the animal, while it is under anaesthetic. When the treatment has taken effect, we can determine how well the retina in the animal is functioning and how it looks. These functional assessments do not require any surgery and are designed to have minimal impact on the wellbeing of the animal. At various time points after treatment, animals will be humanely killed to determine with greater precision the progression of the disease and the effects of that the treatment had.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although therapeutic vectors are tested in cultured cells prior to use in animals, treatment efficacy can only be proven in animals, as the diseases we aim to treat (like retinal degeneration) are complex disorders, involving interactions between multiple cell types, release of endogenous survival factors, the bioavailability of the vector, transgene expression level achieved and, for some therapies, penetration of secreted gene products through the retina. Assessments of cell transplantation techniques involve immune and wound healing responses, migration into the retina and integration into the existing neural network. Current knowledge and techniques are insufficient to model all these interactions, either in a culture system or using computers, well enough to reliably predict treatment outcomes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The stem cell transplantation project has recently developed methods that enable us to grow 3D retinal structures in culture. This will allow us to test the direct effect of retinal gene therapy on the photoreceptor cells in cultured cells, thereby reducing the number of animals used. New *in vivo* assessments of the health of the retina will allow us to monitor how a disease is progressing in living animals rather than in post-mortem retinal tissue. Similarly, a new method of disease scoring for retinal inflammation can follow progression in individual animals *in vivo.*

3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	As therapeutic studies for inherited diseases require an animal model of that disease, most studies will be performed in transgenic or mutant mice. Additionally, mice are the lower vertebrate group with a fully post-mitotic retina, and they will answer all scientific questions as adequately as larger animals. Occasionally, rats may be used as the larger rat eye will allow a more precise manipulation of ocular tissues. Other the last licence periods, we have continually refined our procedures. Recent refinements include performing multiple assessments under single anaesthesia to limit the number of events the animals undergo, and improvements in inhalation anaesthesia equipment that will allow its use (for procedures that require light sedation, such as fundus imaging) without obstructing access to the eye. We have introduced new methods to model the unwanted growth of blood vessels after corneal transplantation that do not require the animal to undergo a lengthy and potentially more harmful transplantation procedure. Furthermore, we have developed improved gene therapy vector production techniques, which give far better vector purity and thus will further decrease the (already low) risk of inflammation after treatment.

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<tr><td>PROJECT 4</td><td>Improved treatments for ocular surface disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>corneal blinding, transplant, stem cell, aniridia, interstitial keratitis (corneal scarring)</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[7]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>In healthy eyes the clear window at the front, the cornea, is covered with a thin layer, the epithelium. Like skin, the epithelium is constantly replaced by new cells that grow from stem cells that live at the edge of the cornea where the clear window meets the white border. There are diseases where these stem cells are missing and the patient loses sight as the window clouds over and the eye is very painful. This can be because of a genetic problem, or can be caused by chemical damage or injury. To treat these patients the surface of the cornea must be repaired and donor stem cells provided to replace the thin surface layer. Donor stem cells are prepared on a membrane that is attached to the front of the eye.

Another condition where sight is reduced or lost is</td></tr>
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	interstitial keratitis (stromal scarring) where the cornea becomes cloudy when the clear tissue under the epithelium is damaged. RAFT containing replacement stromal cells could be used to replace the damaged stroma and improve clarity and vision.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will test a product that is expected to provide novel treatments for patients with limbal epithelial stem cell deficiency and interstitial keratitis. The product that can be reproduced to GMP standards avoiding the huge variability seen in the current options and will better address the clinical needs.
What species and approximate numbers of animals do you expect to use over what period of time?	Rabbits, up to 100 over 5 years
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Each rabbit will have one eye that is kept closed after the operation and this will be sore or itchy. The eye will be treated with anti-inflammatories and pain killers incorporated into an eye drop thus reducing itching and pain. The severity level for this study is moderate. The animals will be culled at the end point of the experiment. Post mortem of the treated eye will follow. The subsequent results will demonstrate the level of integration of the graft in to the front of the eye.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Over the last 4 years the research on tissue equivalents has evolved. We have reached the point where translational research is essential. It is now necessary to demonstrate that there are no adverse reactions in an animal model before first in man studies can begin.
2. Reduction Explain how you will assure	We took advice from a statistician and calculated how we can determine the smallest number of

the use of minimum numbers of animals	animals needed to give a clear and reliable result.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen rabbit for these tests. In spite of the rabbit's small body, rabbit and human cornea are of similar size and the rabbit eye is commonly used in ocular surgery research. Surgical techniques pioneered using rabbit cornea tend to give comparable results when later developed for human therapy.