Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 25

Projects with a primary purpose of: Basic Research – Sensory Organs (skin, eyes and ears)

Project Titles and keywords

1. **Therapy in mitochondrial optic neuropathy**
    - Optic neuropathy, mitochondria, retinal ganglion cells

2. **Corneal Stromal Stem Cells for Corneal Regeneration**
    - Cornea, Amniotic Membrane, Stem Cells, Ocular Surface, Wound Healing

3. **Maximising the success and effects of glaucoma filtration surgery**
    - Glaucoma, wound healing, transplant, angiogenesis

4. **Protection and repair of the retina & optic nerve**
    - Glaucoma, macular degeneration, optic nerve

5. **Novel therapeutic targets in ocular disease**
    - VEGF, diabetes, AMD, neuroprotection, neovascularisation

6. **Regulation of keratinocyte proliferation and differentiation**
    - Skin, mouth, cancer, inflammation, stem cells

7. **The contribution of mast cells and their mediators in inflammatory skin diseases**
    - Mast cells, psoriasis, atopic dermatitis, allergy

8. **Protecting and repairing the injured eye**
    - Eye, nerves, trauma, neuron, death

9. **Protecting and repairing injured retinal cells in glaucoma**
    - Glaucoma, retinal ganglion cell, trabecular meshwork, scarring

10. **The impact of context on rat whisking behaviour**
    - Vibrissa, whisking, tactile, attention, neuroethology

11. **Control of vascular behaviour in the retina**
    - Retinal vasculature, angiogenesis, inflammation

12. **Retinal development and degeneration in zebrafish**
    - Zebrafish, retinal degeneration, disease mechanisms, treatment

13. **Investigating epithelial maintenance, pathogenesis and wound healing**
    - Epithelium, stem cell, cancer, infection, wound healing

14. Early Diagnosis and Therapy through the Eye

- Retina, neurodegeneration, neuroprotection, imaging, treatment

15. Functions of novel lipids in wounded skin

- Wound healing, lipids, skin disease

16. Novel biologics for treating ocular disease

- Binding domains, auto-immune eye disease

Project 1	**Therapy in mitochondrial optic neuropathy**	
Key Words (max. 5 words)	Optic neuropathy, mitochondria, retinal ganglion cells	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	No	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	No	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Inherited optic nerve atrophy (or optic neuropathy) is one of the commonest causes of human irreversible visual loss in the UK. There are currently no medical or surgical treatments available for any genetic or inherited optic nerve diseases. The diseases lead to visual loss through the disruption in retinal ganglion cell function or loss of these cells. Genes that control the shape and configuration of organelles called mitochondria, which are present in each cell of the body, have been found to be mutated in patients with inherited optic atrophy. By modelling the disease process in mice we can study the effects of the mutations, the mechanisms of the disease and assess the effectives of novel therapeutic interventions.	
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)?	The work is focussed on developing treatments for the prevention of vision loss in human patients with genetic and mitochondrial optic neuropathies. The benefits from such research would be potential ground-breaking and important new treatments for patients with genetic or inherited diseases of the optic nerve.	
What species and approximate numbers of animals do you expect to use	Mice will be used. Animal numbers will be kept to a minimum. We anticipate breeding a maximum of 6000 mice over a period of 5 years. However, this figures represents an upper limit, and we anticipate	

over what period of time?	using 70-80% of these animal totals during this time. The upper figure given takes into account the fact that intercrosses will need to be carried out and 25% of animals thus generated will be non-mutant, 50% heterozygous and 25% homozygous for the mutation.
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 mutation of eye disease genes in mice creates minimal suffering, since mice are nocturnal animals with excellent senses other than vision, and they do not rely on good vision to find food or water. The clinical assessment of the animals before and after treatment is also very well tolerated. The treatments to be used will mostly be agents with prior published pre-clinical and cellular data, so that doses and toxicity are likely to be avoidable or minimal. The adverse effects could include ocular discomfort and inflammation in the eye. The likely/expected maximum overall severity is moderate. All mice will be culled at the end.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Investigating the complex processes involved in inherited optic neuropathy, which consists of many different cellular and tissue interactions in the retina and optic nerve, requires animals to establish the outcome of these processes in living animals. Non-animal alternatives cannot reveal the complex responses generated as a result of the administration of therapeutic interventions, and can only take us part of the way towards understanding the ramifications of any new treatment. Animals have to be used because it is not possible to remove tissue from the eyes of living patients who may be tested with new therapeutic agents without any data on their toxicity or effectives. The whole animal model provides the only way of assessing distant neurological effects of mutations in the causative genes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The research requires the use of both wild type and heterozygous mice and hence no mice are wasted as a result of our breeding strategy. The number of animals used will be minimised by careful design of experiments using contemporaneous control groups and statistically appropriate group sizes, which includes between 4-5 mice in each group. To minimise animal usage we will investigate the cellular effects of therapeutic interventions *in vitro* by establishing cell lines before using these agents in the mice. The use of tissues for organ and cell culture from genetically modified and wild type control

	animals will allow complex molecular and biochemical experiments to be undertaken and will minimise the use of live animals.
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.	Mice are suitable as the chosen species because they have a well-characterised genome, considerable data is available about their normal visual function and physiology and it is possible to generate mice with specific genetic modifications to study. The time taken to generate the cohort of mice required in the experiments is relatively short. The mouse eye, especially the retina and optic nerve, has considerable homology to the human eye. All animals will be closely monitored for distress and signs of ocular inflammation and appropriate steps to minimise harm to the animals will be taken following discussion with the Named Animal Care and Welfare Officer (NACWO) / Named veterinary Surgeon (NVS).

Project 2	**Corneal Stromal Stem Cells for Corneal Regeneration**	
Key Words (max. 5 words)	Cornea, Amniotic Membrane, Stem Cells, Ocular Surface, Wound Healing	
Expected duration of the project (yrs)	1 year	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
	✓	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Corneal blindness is one of the major causes of visual impairment globally. Being the outermost surface of the eye the cornea is subject to many sources of injury or infection causing the transparent window to become opaque. We plan to test a novel source of corneal stem cells for the treatment of acute ocular trauma and inflammation to help regenerate the corneal and save sight. These cells are known to possess potent beneficial properties which promote wound healing. We will use a highly standardised ocular surface injury model, which involves destroying the corneal epithelium with a controlled alkali burn injury. We are testing our preparation for efficacy and safety in an established model and combine our stem cell technology with amniotic membrane, a wound dressing known to provide immediate pain relief to injured corneas.	
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)?	The treatments currently available for corneal scarring include '*Ex Vivo* Expanded Limbal Stem Cell Transplantation' which requires considerable cost, expertise and infrastructure. These therapies have considerable limitations including not being able to be used in acute injury situations. Access is also greatly restricted to highly specialised centres. Our project aims to provide proof of concept evidence that an innovative corneal stromal stem cell source applied in	

	acute injury, stage can help to prevent the extensive damage caused by destructive inflammation. This technology holds great promise for future sight saving therapies in humans and in animals. The technique of using cells that are able to be banked and their numbers expanded, meaning rapid creation of transplants. By creating a manufacturing base we will be able to distribute to all ophthalmic centres in a highly responsive manner.
What species and approximate numbers of animals do you expect to use over what period of time?	8 rabbits will be used. Subject to successful funding and a PPL amendment or new application, this pilot study will be extended to a full trial, numbers of animals will be determined in consultation with a statistician in order to generate clinically significant data.
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?	Though we plan to create a potentially painful ocule surface burn injury in the animals, the injury is highly standardised to create the minimum injury possible to recreate a synergistic epithelial and stromal cells response. The injury will be created inder GA and will have the therapy applied before recovery. The presence of amniotic membrane will dramatically reduce pain, further managed by analgesia and antibiotics. The expected severity is therefore moderate. We have anticipated adverse effects due to the anaesthesia, such as stress and inappropriate depth. We also anticipate the injury causing some discomfort, but this can be controlled with pain relief. The animals will be euthanised at the end of the study, so that we can use the tissue for *ex vivo* analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The majority of in vivo assays are irritation models, which are not similar to, or suited for a wound healing study. Those that are designed for wound healing, mimic only the outer cellular layers of the eye, not the entire cornea. This means it is not a good representation, as you cannot see the interplay of the entire eye, or outside effects such as blinking. Animal movement could affect the surgical attachment of the substrate bandage treatment; and blinking is a common reaction that causes mechanical stresses and strains.
2. Reduction Explain how you will assure	This pilot study will use the minimum number of animals to provide safety and efficacy data, as well as procedure optimisation. If a main study is done,

the use of minimum numbers of animals	we will consult with a statistician to find the lowest number of animals necessary to provide statistical data.
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.	The rabbit eye has been used previously in many ocular studies, and is the accepted animal model for evaluating ophthalmic surgeries. It provides a large area to create more standardised and reproducible defects, as well as better clinical assessments. The size of the rabbit eye will also provide more material for our *ex vivo* analysis. A previous successful study has been performed on terminally anaesthetised rabbits. This study has moved to recovery animals as it is required to more accurately resemble normal human treatment and healing progression.

Project 3	**Maximising the success and effects of glaucoma filtration surgery**	
Key Words (max. 5 words)	Glaucoma, wound healing, transplant, angiogenesis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	☒	Basic research
	☒	Translational and applied research
	☒	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	☒	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objective of this project is to develop new treatments to prevent scarring and optic nerve damage in glaucoma and new enhanced animal models of raised intraocular pressure. This will include new wound healing modulating agents and new devices in glaucoma filtration surgery, the use of novel progenitor cells in transplant studies, and improved drug delivery techniques in the eye. There is a huge clinical need being addressed here, with glaucoma being the leading cause of irreversible blindness and affecting over 70 million people worldwide. Cytotoxic anti-metabolites are currently the only anti-scarring drugs available in glaucoma filtration surgery but these carry the risk of potentially blinding complications like tissue breakdown and infection. There are also no available treatments to reverse the optic nerve damage that occurs in glaucoma. Moreover, there are still a lot of unknowns about the basic biological processes of optic nerve damage and wound healing in glaucoma. This project thus seeks to increase our understanding of the disease mechanisms in glaucomatous optic neuropathy and scarring in glaucoma filtration surgery.	
What are the potential benefits	This project offers the prospect of developing new	

likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	treatments to prevent scarring and to regenerate the damaged optic nerve in patients with the blinding disease glaucoma. In addition, new improved animal models will further increase our understanding of the basic biology and disease mechanisms of complex wound healing and retinal ganglion cell death that occurs in glaucoma. Our project will help to identify new potential drug targets and to develop new drug delivery devices and techniques that could benefit thousands of patients in both developed and developing countries.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to study up to 2,000 mice, 2,000 rats and 1,000 rabbits over the duration of the project licence.
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 experimental glaucoma filtration surgery that we intend to perform on the animals is well established and we anticipate few problems in terms of adverse effects. We will monitor the animals closely both during surgery, ensuring adequate anaesthesia, and afterwards, looking for any signs of adverse events, such as infection, bleeding, unusual weight gain/loss or unusual behaviour. Any adverse effects will be treated where possible, and if the animal appears in distress for more than the period of time specified in the protocol, it will be killed by a Schedule 1 method. If adverse effects do occur, they are likely to be moderate in severity. If this occurs, the protocol in question will be modified to prevent the future occurrence of such effects. At the end of this study, all animals will be killed by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we make extensive use of *in vitro* (laboratory-based, non-animal) methodology in this project, there is currently no effective way to model pathological scarring and retinal ganglion cell death in glaucoma outside the living animal. As such, we have to use animals in this project in order to reliably determine if new wound modulating agents, new glaucoma devices, and transplant of novel progenitor cells can actually work. The animal models chosen have been designed to closely reflect human disease. The new treatments must be able to prevent scarring and regenerate optic nerve function *in vivo* (i.e. in living animals) to be successful in the clinic and the use of animals in this project is therefore essential to

	achieve this aim.
2. Reduction Explain how you will assure the use of minimum numbers of animals	During this project, we will assure that the minimum number of animals is used in order to achieve our scientific aims. This will be done by conducting statistical power analysis prior to initiating experiments in order to ensure that we only use the minimum number of animals required to produce valid statistical comparisons between experimental groups. Our decision to use wound modulating agents or devices or progenitor cells will also be guided by extensive *in vitro* functional assays. This methodology will reduce the likelihood of us testing *in vivo* potential treatments that are functionally ineffective.
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.	Where possible, mice or rats will be used in this project but due to their small size, certain techniques of surgery and examination are extremely difficult, and in such cases, the rabbit will be used instead. The rabbit model is an aggressive model of eye scarring and an established model that has previously helped in the translation of cytotoxic anti-metabolites in glaucoma filtration surgery. We will make every effort to minimise welfare costs throughout this project by conducting the minimum number of procedures on individual animals required to meet our scientific objectives. In order to minimise suffering, we will administer appropriate analgesia for every surgical intervention and monitor experimental animals carefully for signs of distress or abnormal behaviour. All animals will be killed by a Schedule 1 method at the end of the experiment.

Project 4	**Protection and repair of the retina & optic nerve**	
Key Words (max. 5 words)	Glaucoma, macular degeneration, optic nerve	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Glaucoma and macuiar degeneration remain the leading causes of irreversible blindness worldwide and are becoming more prevalent as populations age. Much progress has been made in treating both of these conditions, but there remain a large number of patients who continue to deteriorate despite best currently available treatment. The focus of our work is to understand the mechanisms of retinal degeneration in conditions such as glaucoma and macular degeneration, and to apply new therapeutic strategies including stem cells, gene therapy and other novel therapeutics to try to improve the treatment of human diseases in the future. A particular focus of our work over the next 5 years will be to develop new strategies to enhance optic nerve regeration.	
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)?	These experiments will provide new information on the effect of stem cell transplantation, growth factors and other drugs in animal models of glaucoma that replicate important aspects of the human disease. Our long term goal is to develop successful therapies for progressive diseases like glaucoma, and to enhance functional recovery after optic nerve injury. Our planned studies aim to deliver proof of principle that functional regeneration can be achieved in animal models using the strategies we describe. Successful completion of this work will lead to the identification of	

	new treatments for optic nerve diseases and macular degeneration that can be tested in human clinical trials.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats and mice provide a useful model for human retinal and optic nerve degeneration while avoiding the difficulties of using larger animals such as dogs and primates. The rat eye is bigger and easier to study with an optic nerve head structure more similar to humans than the mouse eye. Inbred mouse strains, however, are potentially a very powerful system in which to study the effect of individual genetic changes on the effect of elevated intraocular pressure. As the two species provide complimentary but different information, both will be used in the proposed Programme of Research. We expect to use up to 3000 rats and 2800 mice 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?	From our previous experience using our glaucoma models and the other procedures described in this licence over the last 13 years, animals are unlikely to lose more than 20% of their body weight (most continue to gain weight normally) and they are unlikely to become unresponsive or to exhibit any other systemic signs of distress such as failure to groom or withdrawal. A small proportion of animals may experience eye complications related to procedures performed and if these cause, or are likely to cause, rupture of the eye then animals will be killed. All animals will be humanely killed on completion of the experiments and the tissues will be processed to maximize the amount of useful data obtained from each animal. Stored tissues may be used in future experiments where possible to reduce the numbers of additional animal experiments required.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We do as much of our work as possible without using animal procedures, using special retinal cell and tissue culture techniques we have developed. However retinal and optic nerve degeneration are complex processes and some aspects can only be explored in the intact living eye. We are now at the stage where only in vivo models, including models of glaucoma, macular degeneration and optic nerve injury, can achieve our objectives. After extensive literature review we can find no ex vivo method that can be used to answer our specific and important research questions.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample size calculations using data from previous experiments and pilot studies will be used to minimise the group sizes required to demonstrate clinically relevant (as opposed to simply statistically significant) effect sizes. Statistical advice is readily available on our campus. As an additional measure, we are developing longitudinal in vivo retinal imaging techniques that can allow us to follow degeneration and therapy in the same animal over time. These techniques should help us reduce the number of groups required compared to using conventional histological endpoints alone.
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.	Animal suffering will be minimised by paying careful attention to experimental design and surgical technique. As a practicing eye surgeon I perform procedures similar to those detailed in this application on humans every day, including injections into the eye, laser treatment to the retina and surgery to protect the optic nerve. I will use my knowledge of these procedures to make sure that effective analgesia is used, follow up is appropriate, complications are minimised and any unexpected outcomes are dealt with in a way which prioritises animal welfare.

Project 5	**Novel therapeutic targets in ocular disease**	
Key Words (max. 5 words)	VEGF, diabetes, AMD, neuroprotection, neovascularisation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The broad aims of this group of projects are to understand the disease mechanisms of the major blinding diseases, including age related macular degeneration (AMD) and diabetic retinopathy (DR), to evaluate the risks/benefit relationship of the current treatments, and to develop novel and more effective therapeutics for these pathologies. Because these ocular diseases are characterized by abnormal growth of blood vessels, inflammation and neurodegeneration in the eye, we sought to use animal models, including genetically modified rodents to achieve our aims, which should allow quicker translation of our findings into clinical application. We are partnered with bio-pharmaceutical companies to help us rapidly translate the animal findings into potential new human treatments. The specific aims of the project include: **1) Determine the specific role of VEGF-A in nerve cell function in the retina and its protective action** Vascular endothelial growth factor (VEGF) blocking drugs are currently being used in the clinic to treat the abnormal blood vessel growth and leakiness in AMD and DR. However, work has shown that VEGF is an important survival factor for the nerve cells in the eye. Thus, taking away VEGF may cause damage, at the	

	same time as treating eye disease. Therefore, we want to find out a more precise role for VEGF in nerve cells in the eye, to better evaluate the risks to patients being treated with VEGF blockers. We will need animal models of diabetes, AMD and glaucoma to help us find this out. **2) Determine if neuroprotectants can protect against the loss of retinal nerve cells following use of VEGF blockers in models of eye disease** Following on from aim 1), we want to see if it is possible to reverse the adverse effects of VEGF blockers, using "neuroprotective" drugs, either those already existing, or novel ones supplied by our industry partners in combination with the VEGF block. We need animal models to test the effectiveness of the drugs. **3) Identification of new drug targets for AMD/DR.** We will use animal models of AMD, DR and glaucoma to investigate potentially new pathways that contribute to these diseases, and in doing so find new drug targets that could be used to treat them. **4) Assess and manipulate chorio-retinal transport function as a means of creating novel models of AMD and DR** Disruption of transport of nutrients from the choriocapillaris, the blood supply at the back of the eye, has been implicated as a factor in causing AMD and DR pathology. We will do experiments in animals to monitor this transport, then manipulate it to create new models of AMD and DR.
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)?	The potential benefits likely to derive from this project are: 1) To make important contributions to the basic understanding of the pathology of eye diseases 2) To develop new and better animal models of eye disease, to help discover novel drugs for patient testing To develop novel therapeutics for patients with our academic and industry collaborators. Within the timeframe of the current PPL, my research team has performed proof-of-concept rodent studies that have directly led to initiation of 4 neovascular AMD clinical trials of novel therapeutics
What species and	We will use mice and rats, with 2500 mice, and 250

approximate numbers of animals do you expect to use over what period of time?	rats used approximately per 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?	Expected specific adverse effects related to the protocols will include loss of vision in the glaucoma models, systemic inflammation in inflammation models, and weight loss, dehydration and the effects of hyperglycaemia in diabetes models. In our animal experiments there will also be adverse effects related to general techniques, such as anaesthesia, injections and blood sampling. The overall severity of the project will be moderate. The vast majority of the animals will be killed by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Though cellular and organ culture models are useful and will be utilized for proof of concept, the rigorous examination of diseases requires research focused on interactions between different cells in the eye (i.e., endothelial cells, neurons, glia, epithelia and inflammatory cells), which cannot yet be modelled outside the context of a living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We already and will continue to reduce our animal numbers by doing as much cell culture work as possible. For example by growing retinal nerve cells, and endothelium to test potential drug candidates before they are introduced into the animal. We will also use sensitive measurements for changes in function and nerve cell survival that also allow us to follow a single animal over a period of time rather than using multiple animals.
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 will use rats and mice because they are the most easily genetically modifiable mammals that share a similar eye with man. The majority of work will be done in mice, but rats can be more suitable for diabetes models for example, because the changes in the eye happen more quickly. We are able to administer drugs to the eye directly by injection, to minimise risk to other organs. We will also use well-defined humane end points to minimise any harm to the animals.

Project 6	**Regulation of keratinocyte proliferation and differentiation**	
Key Words (max. 5 words)	Skin, mouth, cancer, inflammation, stem cells	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of the project is to discover how the properties of cells called keratinocytes are controlled via the genes they express and by signals that they receive from other cells. Keratinocytes are the cells that form multilayered epithelia, such as the outer covering of the skin and the lining of the mouth. Multilayered epithelia act as a protective barrier between our bodies and our environment and are frequent disease targets. By investigating the ways in which cell division and maturation are controlled, we aim to obtain new ways of preventing or treating benign skin conditions such as psoriasis and eczema, and also cancers of the skin and oral cavity.	
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)?	Tumours of the epidermis, called basal cell carcinomas and squamous cell carcinomas, are the most common tumours in humans. Psoriasis affects 2% of the population and eczema is also very common. Tumours of the oral cavity are less common but have a 5 year survival rate of only 50%. By gaining new understanding of the properties of keratinocytes we can potentially prevent or treat these and other diseases of multilayered epithelia.	

What species and approximate numbers of animals do you expect to use over what period of time?	89,000 mice 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?	The expected adverse effects are the development of skin wounds, inflammation and cancer. In most cases the severity will be mild. However, in some situations, such as tumour development, the severity will be moderate. At the end of each experiment mice will be humanely killed and their tissues will be subject to analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible we replace mice with studies of cells that are grown in culture in the laboratory. However, there are three situations in which cultured cells cannot be used: (1) when the properties of the tissue need to be studied for over 3 weeks, which is the limit for maintaining keratinocytes in culture; (2) when communication between keratinocytes and the multiple different cell types within the tissue, such as nerves and blood, needs to be analysed; (3) when the effectiveness of drugs that might act indirectly on keratinocytes needs to be tested.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To prevent unnecessary breeding we keep stocks of frozen mouse sperm and embryos. In planning our experiments we perform statistical analysis of the minimum number of mice required to observe a clear outcome. We share necropsy samples with other research groups so that they can obtain data without having to breed their own mice. It is anticipated that advances in non-invasive imaging technology will potentially reduce the number of animals used in this licence.
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.	Mice are the lowest form of mammal that can be used to study normal and diseased tissue and are the only mammal in which genetic modification works reliably. Whenever possible, we minimise harm to mice by carrying out procedures for the shortest time periods. When a new procedure is involved, training is first carried out on dead animals. We perform pilot experiments with the minimum number of mice and mildest conditions predicted to have an effect.

Project 7	**The contribution of mast cells and their mediators in inflammatory skin diseases**	
Key Words (max. 5 words)	Mast cells, psoriasis, atopic dermatitis, allergy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of the project is to investigate the role and mechanisms of a type of tissue resident cell, mast cells, in inflammatory skin diseases. Mast cells are mainly thought of in relation to allergy; however, new studies have highlighted their importance in inflammation. Currently there are no effective therapies to block mast cell involvement in inflammation. This project will investigate the factors which can block mast cell mediated inflammation in skin conditions (e.g. atopic dermatitis, psoriasis).	
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)?	Mast cells contribute to both acute and chronic inflammatory skin diseases for which effective treatment is currently limited. A deeper understanding of mast cell function in these conditions promises to help us not only optimize current treatments but also to propose new therapeutic targets for the future.	
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used for all experiments and we expect to use up to 2000 mice for Protocol 1, namely the breeding and maintenance of genetically modified mice, of which we currently have 5 strains, and up to 2000 mice for protocol 2, Murine models of skin inflammation: passive cutaneous anaphylaxis, atopic dermatitis, psoriasis. These experiments will be	

	carried out over the course of 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?	All experimental protocols will produce some degree of localised skin inflammation. In general this will be confined to a small patch of skin around the treatment area (<6cm^2) which will appear red and slightly swollen. The proposed experiments should have little effect on the animal's behaviour. However, the skin patch may become itchy and we will therefore induce inflammation on the back to minimise scratching. Collectively, the proposed experiments will not exceed moderate severity. The animals will be sacrificed when the experimental protocol is completed and the research end points are achieved. However, if animal suffering is deemed to exceed the set severity limit, and suffering cannot be alleviated, the animals will be humanely euthanized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The skin is a complex organ, which unfortunately cannot be studied in isolation. Blood supply and neurological input play key roles in directing skin responses. Perfused and innervated human skin equivalents would be an ideal replacement as animal experimentation should be limited to the unavoidable, however, at present these systems are still under development. Furthermore, primary mast cells generated from bone marrow cells of mice or human blood progenitors and the few cell lines (human and mouse) available do not fully mimic the maturity, plasticity and mediator repertoire found in tissue resident mast cells. In using a small number of mice to look at skin inflammation *in situ* we may be able to identify potential therapeutic interventions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use professional statistical advice and always use the minimal number of animals, which allows for solid statistically relevant results (maximised power), but will allow for us to reach our scientific objectives successfully. We will be mostly using a two-way ANOVA statistical tests for the analysis and type I errors will be controlled by choosing the significance level of 5%, with the power level set between 80 and 90%. We will make use of pilot studies to assess efficacy/doses/set up optimisation prior to a full-scale study. We will collect and store tissue and cell samples which we will make available to other scientists in the field so that these specimens can be used for further experiments in the future without the

	unnecessary repeat of animal experiments.
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.	Laboratory mice strains are susceptible to experimentally induced skin inflammation, which reflects human skin inflammatory conditions. Animal welfare will be a priority across this project and will be taken seriously by all members of the research group. We will monitor animals on procedures daily to ensure there are no unexpected or adverse reactions to the procedures they are subjected to. Any animal seen to be in discomfort or distress, which, upon taking advice from the facility vet, cannot be relieved, will be removed from the study and euthanized.

<table>
<tr><td>Project 8</td><td colspan="3">Protecting and repairing the injured eye</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Eye, nerves, trauma, neuron, death</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><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></td><td>No</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</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 colspan="3">The objectives of this project are to determine changes that occur after injury to the optic nerve that connects the eye to the brain and relays information about vision. We are particularly interested in learning how neurons deal with the injury that makes them vulnerable to death, the scar tissue that forms after injury and the lack of axonal regrowth that follows nerve injuries.

This will allow for a better understanding of the mechanisms of nerve injury and will help us to identify therapeutic drugs that will be used to protect nerve cells from death, dissolve scar tissue and promote nerve to re-grow.</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="3">The project will provide important data that will improve our understanding of the changes that occur after nerve injury and provide an insight into what is required to promote nerve cell survival, removal of scar tissue and promote axonal regeneration.

This will underpin the discovery of novel therapeutic drugs that will be used to promote nerve cell survival, scar tissue removal and axonal re-growth.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Rats: 1,900
Mice: 1,200
Over a period of 5 years</td></tr>
</table>

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?	Potential harm results from optic nerve, optic chiasm and retinal injury, which will be created under general anaesthesia. In the vast majority of cases, there is no adverse response to the loss of sight since rats/mice do not use vision as a primary sense. The maximum likely severity for all procedures is moderate, although some of the procedures will be mild. There are clear guidelines in place in our facility to ensure that suffering in animals is minimised by either administration of pain-killers or termination of experiments. Intravitreal injections/topical eye drops may cause irritation at the site of injection/application but in our extensive experience this has not been the case. As with the injury, we will remain vigilant for any adverse effects and will promptly provide pain relief or treatment if appropriate, or humanely kill the animal. Schedule 1 methods will be used to kill all animals or animals will be perfused with 4% paraformaldehyde under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no adequate substitute for using the *in vivo* models described in this application. Establishment of potential clinical relevance of regulatory molecules interacting in a dynamically changing central nervous system injury site can only be achieved in an animal model. The rat/mouse is our prototypic laboratory animal and has been rigorously characterised by ourselves for the optic nerve injury paradigm and shown to be representative of the human condition by others. The tools for the project have all been prepared in relation to the models described herein and continuity of the study in this species will be essential for significant progress to be made in a timely and efficient manner.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Some of the end-point measurements (e.g. axon regrowth, scar formation etc) may be essentially qualitative and for these we use 3-6 animals per treatment group. In most experiments with quantitative end-points, 6 animals are randomly assigned to each treatment group, a number calculated as the minimum required to provide

	statistically significant results. This has been determined on the basis of our previous experience with procedures, the methods of analysis and after consultation with statisticians to calculate power. Since blinded rats are not apparently debilitated, bilateral optic nerve lesions are used where possible to enable the size of treatment groups to be reduced by 50%.
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.	The model selected closely resembles the features seen in humans after injury. Most therapeutic agents are evaluated and optimised *in vitro* prior to *in vivo* application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are done on harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. These refinement steps significantly reduce animal usage and severity.

<table>
<tr><td>Project 9</td><td>Protecting and repairing injured retinal cells in glaucoma</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>glaucoma, retinal ganglion cell, trabecular meshwork, scarring</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</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></td><td>No</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</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>The objectives of this project are to determine changes that occur after induction of glaucoma, which causes a rise in pressure in the eye that leads to neuronal death. We are particularly interested in learning how pressure rises as a result of scarring that occurs to fluid drainage sites in the eye and how this rise in pressure kills neurons and compromises the function of the visual system. In particular, we are interested in removing the scar tissue build-up that blocks draining points and rescuing neurons from death.</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>The project will provide important data that will improve our understanding of the changes that occur after glaucoma and provide an insight into what is required to reduce a rise in pressure within the eye, remove scar tissue, promote nerve cell survival and promote axonal regeneration.

This will underpin the discovery of novel therapeutic drugs that will be used to dissolve scar tissue and protect nerve cells from death, thus prevent vision loss.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Rats: 2,000
Mice: 500
Over a period of 5 years</td></tr>
</table>

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?	Potential harm results from establishing a high pressure within the eye. In the vast majority of cases, pressure rises will be kept to the lowest level possible to reach our scientific goals – a level that has been carefully determined. Animals exhibit a normal baseline intraocular pressure (IOP) of 10-20mmHg, which is comparable to baseline levels in humans. In humans, anything above an IOP of 21mmHg is defined as hypertension. However, in animals experimental IOP is generally raised to 30-40mmHg. Although IOP may be high, animals just like humans exhibit no discernible adverse effects. Any animal that shows unusually high pressure will be killed humanely. There are clear guidelines in place in our facility to ensure that suffering in animals is minimised by either administration of pain-killers or termination of experiments. Intravitreal/intracameral injections/topical eye drops may cause irritation at the site of injection/application but in our extensive experience this has not been the case. As with the induction of glaucoma, we will remain vigilant for any adverse effects and will follow unit guidelines in the event they occur and will promptly provide pain relief or treatment if appropriate, or humanely kill the animal. Schedule 1 methods will be used to kill some animals, whilst others will either be perfused with 4% paraformaldehyde or killed by cervical dislocation under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no adequate substitute for using the *in vivo* models described in this application. Establishment of potential clinical relevance of regulatory molecules interacting in a dynamically changing CNS injury site can only be achieved in an animal model. Humans cannot be used since it is unethical to remove the whole eye for histological assessment after induction of glaucoma and its treatment using our experimental therapeutics. Other less sentient animals such as fish cannot be used since fish regenerate their retina and show no RGC death after induction of experimental glaucoma.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Some of the end-point measurements (e.g. cell survival, axon regrowth, scar formation etc) may be essentially qualitative and for these we use 3-6 animals per treatment group. In most experiments with quantitative end-points, 6 animals are randomly assigned to each treatment group, a number calculated as the minimum required to provide statistically significant results. This has been determined on the basis of our previous experience with procedures, the methods of analysis and after consultation with statisticians to calculate power. Since blinded rats/mice are not apparently debilitated, bilateral induction of glaucoma is used where possible to enable the size of treatment groups to be reduced by 50%.
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.	The model selected closely resembles the features seen in humans after glaucoma. The rat/mouse is our prototypic laboratory animal and has been rigorously characterised by ourselves for the glaucoma paradigm and shown to be representative of the human condition by others. The tools for the project have all been prepared in relation to the models described herein and continuity of the study in these species will be essential for significant progress to be made in a timely and efficient manner. Most therapeutic agents are evaluated and optimised *in vitro* prior to *in vivo* application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are done on harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug delivery strategies. These refinement steps significantly reduce animal usage and severity.

Project 10	**The impact of context on rat whisking behaviour**	
Key Words (max. 5 words)	vibrissa, whisking, tactile, attention, neuroethology	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Rats and other small mammals move their long whiskers forwards and backwards in front of their snout many times a second. Using high-speed video recordings, researchers are able to observe these fast-moving sensory hairs. Until recently, it was thought that the movements of these whiskers was largely patterned and symmetrical, however, we now know that animals like the rat are able to modify the movements of their whiskers in order to touch and sample important parts of their environment, much like how we humans use our hands to analyse an object, or move our eyes to see important parts of our environment. This ability is called active sensing, and the aim of this project is to advance understanding of the active control of sensing and the sensory apparatus in mammals using the model system of rodent whisking behaviour. Specifically, we intend to examine **1)** the role of context (e.g. locomotor mode, presence of other members of the same species) on the control of whisker movements, **2)** how animals coordinate the movement of their whiskers and eyes during tasks aimed to elicit their attention, and finally, **3)** how being reared in an enriching environment (one that has a lot of room to move around, many objects, plentiful and varied food, and the presence of litter mates) influences the way in which whiskers (and the	

	area of the brain that controls the whiskers) develops.
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)?	The research will benefit the substantial neuroscientific community studying the rat whisker system as a model of mammalian sensory and motor processing. In particular, it will inform these researchers as to the type of signals processed by the whisker system during normal and enriched behaviour, and will enhance our understanding of how contextual changes effect active sensing systems. The research will also be of direct benefit to engineers investigating artificial tactile sensing systems, such that the behavioural whisking strategies adopted by rats can be 'plugged-in' to rodent-like whiskered robots who require a reliable sense of touch for navigation and exploration, such as in disaster zones where search and rescue methods cannot rely on visual cues alone. Importantly, the work proposed in this project will also investigate the role of environmental enrichment on behavioural and brain development, such that its findings may have significant impact for the measurement and assessment of animal welfare and policy written to inform the treatment of animals more generally.
What species and approximate numbers of animals do you expect to use over what period of time?	This project uses only the rat, a whisker specialist. Around 50 animals a year, over a 5 year period will be used (total <=250).
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 only scientific technique being carried out using these animals is behavioural observation. Specifically, animals will be filmed intact and awake using high-speed video cameras, predominantly behaving freely and naturally. In some cases (the most severe of procedure in this project license), animals will have a head-plate fixed onto their heads under general anaesthesia in order to attach a miniaturised head-mounted eye-tracking camera that may either allow free movement, or will require them to be head-fixed. Animals will always be allowed to recover fully, with the appropriate analgesia administered, and at all times animals will be handled by the experimenter and habituated to the testing environment before testing begins. The majority of the work carried out under this project license will have little or no impact on the health and welfare of the animals and would be classified as of mild severity. However some animals may become stressed by the tests used and some will undergo

	surgical procedures to fix a plate to the head and so will be classified as having reached moderate severity. At the end of experiments animals will be re-used in other studies where possible, but will be killed if they have undergone a surgical procedure or develop an adverse effect such as an infection following the surgical procedure for example.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Behavioural investigations of whisker sensing require an operating, intact whisker sensory system. At the current time such systems only exist in mammals with a complete nervous system. It should be noted that we currently use artificial (robotic) models of the whisker system that can be used to replace some experiments involving animals, nevertheless, some animal experiments are required to help develop these robotics models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power analyses will be carried out in G*Power in order to plan the animal numbers required for our desired effect size. For freely-moving behavioural experiments, we can obtain enough data from each animal to allow for a relatively small number per study (usually 5-20 depending upon the amount of experimental conditions). We also have a large expertise of statistics in-house to call upon if we require it. All studies will be planned and carried out ethically and with integrity. All data obtained is archived in a high-capacity computer server and carefully catalogued. This allows some new hypotheses to be tested against existing datasets rather than by collecting new data. Wherever possible animals will be tested in several behavioural paradigms, and data acquired will be used to test multiple hypotheses.
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.	Rats have been selected as these animals have an active and highly-developed whisker sensory system that has already been the subject of intensive study in many laboratories. By advancing the understanding of the whisker system as a rodent model of mammalian motor control there is also the possibility of reducing the amount of related work performed with non-human primates. In order to reduce welfare costs to our animals: **1**) the majority of experiments will involve techniques that have been piloted over a number of years such that likely sources of variability in the data have been identified, **2**) most experiments will involve only observation of

	unrestrained intact animals performing natural behaviours, **3**) animals will be handled prior to testing to minimise distress, **4**) animals will be fed as often as they require except where food or water need to be restricted for learning paradigms, **5**) animals will be housed with only members of the same species, and in the same room with only the same species where possible, **6**) in experiments with juvenile rats, animals will kept away from their mother and littermates for the minimum period necessary for testing. Heated pads or similar will be used to ensure that juveniles maintain body temperature and **7**) head-mounted equipment or tracking aids will not be used with juveniles of less than 4 weeks in age.

Project 11	**Control of vascular behaviour in the retina**	
Key Words (max. 5 words)	Retinal vasculature, angiogenesis, inflammation	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The retina critically depends on healthy blood vessels delivering oxygen and nutrients, and removing waste products. This is crucial for normal vision and complications caused by abnormal blood vessel behaviour in the retina are the main cause of blindness in the Western world. The biggest effects on vision occur when vessels become leaky or start to proliferate (forming new vessels). This happens in many blinding diseases. In order to develop therapies for these diseases it is imperative to properly understand the cellular and molecular mechanisms that control retinal blood vessel behaviour in health and disease. In this project we aim to study the factors and genes that control retinal blood vessel behaviour in the eyes of mice because their retinal vasculature is very similar to the human. Furthermore, we isolate and culture specific cells from specifically bred mice to study cellular functions in vitro.	
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)?	Pathology of blood vessels in the retina is the major cause of blindness in the developed world. Because humans so heavily depend on visual function, any visual impairment has major consequences for the quality of life of affected individuals, but also is a significant burden on society. Understanding the cellular and molecular mechanism that control retinal vascular behaviour in health and disease is critical for	

	the development of novel therapies and has therefore of major importance, not only for the individual, but also for society as a whole.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 1500 adult mice and around 200 litters of mouse pups (on average there are 6 pups per litter) per year for the duration of the project licence.
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 majority of mice bred under this licence will not have any adverse effects as they will either have harmless or silent genetic mutations. A smaller proportion (around 10-20%) of animals may undergo procedures that fall into the moderate category, mainly because they will undergo general anaesthesia with recovery for imaging purposes. But these animals are not expected to suffer any pain. An even smaller proportion (around 2-5%) are expected to undergo procedures with a moderate severity limit such minor surgery, exposure to hyperoxia or chemically induced diabetes. Every effort will be made that no animal exceeds the moderate severity level and all animals will be humanely killed at the end.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Blood vessels are normally perfused with blood and interact with surrounding tissue. These conditions can so far not be recreated in vitro. Therefore it is not possible to study all aspects of vascular behaviour in cell culture experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	For all our studies we carry out power calculations in advance to establish the minimum of mice needed to obtain statistically sound data. However, we generally aim to study phenotypes with large effects. This reduces the statistical need for larger cohorts of experimental animals. Furthermore, we have developed in vivo imaging methods that allow us to follow animals longitudinally, thereby reducing the total number of animals needed to study different time points.
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	The retinal vasculature in mice is similar in humans and can relatively easily be observed and manipulated. It is therefore one of the most useful model system in vascular biology. Furthermore, recent developments in genetic engineering allow the manipulation of specific genes in a time dependent manner in specific cell populations. This means the

minimise welfare costs (harms) to the animals.	systemic effects on the animals by such manipulations are minimal.

Project 12	**Retinal development and degeneration in zebrafish**	
Key Words (max. 5 words)	Zebrafish, retinal degeneration, disease mechanisms, treatment	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to use zebrafish as a model system for understanding of the molecular mechanisms of retinal degeneration, and for the identification of chemicals for potentially treating patients with retinal diseases. Retinal degeneration (RD) due to light-sensitive cell (photoreceptor)-death is the commonest cause of blindness in humans. There are two types of RD, including inherited RD and non-inherited (e.g. age related macular degeneration and diabetic retinopathy). The most frequent-subtype of inherited degeneration is retinitis pigmentosa (RP) with a worldwide prevalence of 1 in 4000 in the general population. RP can be inherited as an autosomal dominant, autosomal recessive, X-linked, mitochondria, or digenic trait. To date, more than 50 mutated genes have been identified. The other major type of non-inherited retinal degeneration is diabetic retinopathy (DR), a common cause of blindness in working people. The disease mechanisms of RP and DR are not fully understood and effective treatments are still not available. Due to rapid and external development, coupled with optical clarity during embryogenesis, and since many of the genes and pathways are conserved in human,	

	zebrafish is increasingly used as a model to study several human diseases. These include cardiovascular disease, cancer, neurodegenerative disease and visual disorder. We have established zebrafish as a model of RP and found that depletion of RP-causing genes: *RPGR* and *RP2,* cause retinal defects. This model provides us with a unique opportunity to directly address the consequence of mutations found in inherited retinal degeneration. The objectives of this project are to understand the functions of retinal disease-causing genes in zebrafish, to characterize human retinal disease-causing mutants in zebrafish, to create zebrafish retinal degeneration models, and to screen small molecule libraries or natural products to identify candidate chemicals with the ability to rescue zebrafish retinal degeneration phenotype. Such compounds may be potentially useful in the future for the treatment of patients with retinal degeneration.
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)?	The primary potential benefit relates to new knowledge about the pathogenesis of retinal degeneration and the discovery of novel therapeutic compounds. The aim is to publish the findings in academic journals. The information is likely to be of interest to pre-clinical scientists working in the area of retinal diseases. The secondary potential benefit relates to the value of the results to clinicians, in particular ophthalmologists, and to the possibility that new molecular targets may be identified, for which new pharmaceutical products could be developed.
What species and approximate numbers of animals do you expect to use over what period of time?	About 6350 zebrafish, including embryos, larvae and adult, will be used for the project.
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?	Some mutant fish and drug-treated fish may experience mild or moderate suffering. All fish and embryos used in the project will be humanely killed with a fatal dose of anaesthetic.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We have done in vitro experiments to analyse the toxic effects caused by different mutants in a mammalian cell system, but full testing requires a

use non-animal alternatives	fully formed functional retina. As it is not feasible to produce an adequate model with functional retina in vitro, we therefore have to use a live animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposed experimental designs and methods of analysis of the results have been discussed with the statistician, the numbers of animals will be minimised by careful experimental design and appropriate statistical analysis.
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.	<u>Choice of species</u> Unlike other vertebrate model systems used in the laboratory, zebrafish embryos are fertilized outside the mother, and are transparent during early embryogenesis. This allows for the exquisite detail of cell and tissue development, changes in swimming behaviour and eye development, in a vertebrate animal to be visualized in living animals, something that is not possible in other vertebrates (e.g. frogs, mouse). The experimental embryos will be monitored until 5 days post-hatching when the fish will be killed with an overdose of anaesthetic. Some of the mutant fish may experience moderate suffering due to visual defect. These fish will also be humanely killed immediately by an overdose of anaesthetic. For developing drug candidates, we will relate the dose of chemicals or natural products to what we already know for embryos; for chemical inducing retinal degeneration, we will relate the dose of chemicals to what other researchers have published. We anticipate that initially some animals will experience mild to moderate severity of pain, suffering, or distress from the chemicals in the water as we optimize the procedure. We will minimize this by careful monitoring of the fish, and using the minimally effective dose of chemical.

Project 13	**Investigating epithelial maintenance, pathogenesis and wound healing**	
Key Words (max. 5 words)	epithelium, stem cell, cancer, infection, wound healing	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
	x	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Most epithelial tissues constantly replace damaged or dead cells throughout adult life. This process of continual cell replacement is carried out by stem cells, which can generate all of the cells within a particular tissue. The behaviour of stem cells must be tightly regulated so that our tissues always have the correct numbers and types of cells. Failure of this system results in medically important problems such as cancer (too many cells) or impaired wound healing (too few cells). The main objective of our research is to understand how stem cells contribute to replacement of normal skin and other epithelia and to understand how these processes are disturbed during wound-healing or diseases such as cancer. We are particularly interested in investigating the role of a family of genes (Polycomb Group Genes) that act as master-regulators controlling stem cells and cancer development in other tissues.	
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)?	A long-term objective of these studies is to characterise the regulation of epithelial stem cells. Our ultimate aim is to identify potential new treatments in humans, which would modify epithelial cell behaviour in circumstances such as infection, ageing, wound healing and cancer.	

What species and approximate numbers of animals do you expect to use over what period of time?	In the course of these experiments, we anticipate that a maximum of 5000 genetically-altered or wild-type mice may be used over a period of five years. However, statistical techniques will be used to ensure that the minimum number of control and experimental animals is used in each experiment to generate the maximum amount of data.
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?	- We expect that the level of severity for these experiments will be no more than moderate. At the end of the study, all mice will be killed by approved methods. Some of the experimental techniques and their expected adverse effects include: - **Generation and gene induction in genetically modified mice.** Possible adverse effects of gene inducing agents include skin inflammation, thickening or flaking, hair loss or altered pigmentation, benign dermal cysts, skin erosion or tumour development. - **Tissue labeling.** Mice may experience transient hair loss. - **Wound-healing studies.** Although strict asepsis will be used, wound-healing studies may rarely lead to infection or bleeding and will cause discomfort in the immediate post-operative period which will be controlled by pain–killers.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal methods will be utilised whenever possible to replace and complement the animal studies. However, cell culture conditions cannot adequately model the complex environment within intact tissues that regulates stem cell behaviour. Furthermore, the contribution of epithelial stem cells to wound-healing, response to infection and development of tissue pathologies (e.g. cancer) can only be studied in the context of the whole animal as these processes involve interactions of multiple cell types and different types of communication between the cells.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise animal numbers, we will use non-animal experiments and analyse tissues obtained from human patients whenever possible. Because we intend to evaluate the consequences of genetic alterations in several epithelial tissues, whenever possible we will coordinate collection time-

	points so that multiple tissues can be isolated from a single mouse, thereby reducing the total number of genetically-modified mice required. We will make every effort to design experiments using statistical predictions, to maximize the information gained from the minimum resource.
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.	The processes that regulate epithelial stem cell behaviour involves complex interactions between a diversity of cell types, which can only truly be present in the context of living organisms. To study gene function in mammals, where findings can be most easily translated into clinically significant findings, we believe that the mouse clearly represents the most effective animal model. There are already multiple genetically modified mouse models available to address key experimental hypotheses in a highly refined manner. The extensive availability of models and well-characterised experimental methods (such as wound-healing protocols) suggest that the mouse is clearly the best animal model available to achieve our experimental objectives. We will continue to keep abreast of literature in order to identify experimental refinements that may be introduced during the five year course of the project.

Project 14	**Early Diagnosis and Therapy through the Eye**	
Key Words (max. 5 words)	Retina, neurodegeneration, neuroprotection, imaging, treatment	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Retinal neurodegeneration is seen worldwide, in a number of conditions in which the structure and function of retinal nerve cells (RGCs) is progressively lost. These RGCs send visual information from the eye to the brain, and their death leads to irreversible blindness. RGC loss is the consequence of not only a number of ocular diseases, such as glaucoma, age-related macular degeneration, and diabetic retinopathy, but also several disorders in the Central Nervous System, including Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) diseases. Vision loss in these diseases often occurs well after significant and irreversible loss of retinal nerve cells has occurred. Treatment of these diseases is a big challenge with enormous unmet medical, social and economic needs. This is largely because we still do not completely understand the mechanisms by which these diseases cause such devastating conditions and so this research is crucial. The prime goals of this project are firstly to establish and validate animal models of diseases resulting in retinal neurodegeneration. Secondly to evaluate and characterize retinal neurodegenerative processes and mechanisms in these models. Finally to	

	investigate the therapeutic strategies targeting the above identified mechanisms, and the methods of drug delivery of these strategies. By evaluating molecular mechanisms of damage in the eye, we hope to identify new strategies which may be used to target these same molecular processes. We will evaluate these strategies with a view to establishing which agents would be potentially best to use in patients with retinal degenerative disorders.
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)?	The proposed project will be conducted by using currently available advances of technologies, including non-invasive real time imaging of RGC degeneration and death, and functional assessments of RGCs. Previous projects have already led to one such development; e.g. an unique imaging technique is used to monitor RGC death, that has just completed a Phase I clinical trial having been well characterised in animal models in previous projects. Using DARC and other techniques, we expect this project will lead to real advances in the early diagnosis and treatment of neurodegenerative conditions. The ultimate goal is the development of preventative procedures and medications for patients and animals experiencing the onset of irreversible blindness associated with the neurodegenerative diseases which we wish to investigate under this project license. As we develop new strategies, we will evaluate these with a view to establishing the best potential therapies to use in patients with retinal degenerative disorders.
What species and approximate numbers of animals do you expect to use over what period of time?	Rat: - 2600 Mouse: 3200 Rabbit: - 220
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?	As all models used in this project will have retinal neurodegeneration and cell death, visual defects are expected. These visual defects are not usually associated with pain and ongoing distress. In some rare cases, surgery-induced models may cause ocular issues, including eye surface dry, haemorrhage, infection, cataract et al. In substance-

	induced models, some animals may have a body weight loss, and develop clinical symptoms, such as weakness and stiffness in diabetic models and Parkinson's disease models, which may cause distress. Mild to moderate levels of severity are expected in the models used in this project, but if the levels of severity go beyond animals will be humanely euthanised. To minimise adverse effects and to ensure animals are treated in the best possible manner: - Good handling, regular monitoring and easily available veterinary advice will be used. - Animals will be observed regularly so that adverse effects can be detected at an early stage. - Interventions will only be conducted on one eye, with the animal under general anaesthetic. - An analgesic will be given to all animals post-operatively to minimise pain. - Body weight will be monitored regularly, administration will be stopped if >10% weight loss occurs; and soaked diet will be supplied. - If any animal appears to be experiencing suffering or appears unwell beyond the moderate level, they will be humanely euthanized. - After procedures, animals will be recovered from the anaesthetic and carefully observed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This work relies on the assessment of processes in the living being – it is not possible to use in vitro culture systems as whole body conditions are essential. There is no alternative to using live animals to answer some of our scientific questions. In vitro methods do not allow direct translational links to be made. It is not possible to reproduce the highly complex anatomical structure of the eye and brain in vitro. Moreover, our studies explore the use of real-time retinal nerve cell death and apoptosis measurements and their correlation with disease and treatment. This cannot be done in tissue culture models where the environment can never be completely replicated.
2. Reduction Explain how you will assure the use of minimum numbers	The proposed project will be conducted by using currently available advances of technologies, including non-invasive real time retinal imaging of

of animals	RGC degeneration and death and functional assessments of RGCs. As these methods can be used in the same animal over time, the number of animals used will be significantly minimised. As a result, the number of animals in this project is significantly reduced, i.e. the number of rats, mice and rabbits has a 60.4%, 41.18% and 33.33% reduction, respectively, compared to the last PPL.
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.	Rodents and rabbits will be used in this project. Rodents have been shown to be suitable for modelling retinal neurodegeneration and cell death, which are similar to human diseases. The rabbit model is chosen because of its close relationship to the human in terms of eye structure, such as corneal thickness and tear film, and its translational nature to humans for topical drug application. Their use will allow answer our scientific questions: 1. Is the eye affected by conditions? 2. Can ocular changes be detected in vivo? And 3. Can the changes be modulated, treated, and monitored using the eye?

Project 15	**Functions of novel lipids in wounded skin**	
Key Words (max. 5 words)	Wound healing, lipids, skin disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand the role that particular lipids (fats) play in forming the barrier function of the skin. Work from our group has discovered several new lipids generated by skin enzymes. Our preliminary studies indicate that these may regulate immune responses in vitro of relevance to generation of a barrier and in particular during wound healing and we now wish to determine their in vivo actions.	
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)?	Lipids play a major role in the barrier of skin to stop ingress and egress of water and pathogens. The mechanisms of this are still not fully understood. The potential benefits to of this work could include new treatments to accelerate wound healing and promote healthy skin regeneration. We may also uncover new agents that could be developed for treatment of other skin diseases which exhibit lipid alteration.	
What species and approximate numbers of animals do you expect to use	Up to 1,500 mice over 4 year period	

over what period of time?	
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 skin lesions will be re-epithelialised in approximately 7 days and appear to cause little if any discomfort. For all our wound studies, animals will be carefully monitored for deviations from normal behaviour that might indicate pain or suffering and our NVS consulted if this were the case. With regard to addition of bioactive lipids to the wound, we don't expect any adverse effects over the standard effects of the lesion on its own, unless repair was severely perturbed and infection were to occur in which case these animals would be killed by a schedule 1 procedure. This is an unlikely event and collaborators have not yet experienced any agents that effect repair in this way. Appropriate control measures are in place to ensure that all animals will be monitored closely and appropriate action taken. All animals will be killed at the end, either by terminal anaesthesia or an approved humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animals are required for this work because we cannot test the effects of lipids in wound healing using cell models. Up to now, all of our work on these lipids has been on cells and to further our understanding of their roles in disease we now need to conduct in vivo experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Based on previous work using this wound model (carried out by our collaborators, Martin and Eming), appropriate group sizes that give a defined statistical power will be used. Results will be monitored as they are undertaken to determine whether subsequent experiments could use less if possible.
3. Refinement Explain the choice of species and why the animal model(s)	The mice used will include strains with alterations in the enzyme lipoxygenase (LOX) e.g. 12/15-LOX and platelet 12-LOX and others of relevance to the study of lipids in wound healing / barrier repair. The genetic

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.	alterations to be used do not produce more than mild adverse effects in the phenotype. We have allocated the number of 1,500 mice to this, but we will endeavour to minimise this by using the least adverse model available for the purpose. We will also use the smallest possible wounds to collect the information we need – the largest wounds we make are 4mm diameter wounds in mouse back skin. To minimise harm to the animals, they will be monitored daily (more often when undergoing procedures) and where there is any concern, advice will be sought from the Named Veterinary Surgeon and Named Animal Care and Welfare Officer, before appropriate action taken.

Project 16	**Novel biologics for treating ocular disease**	
Key Words (max. 5 words)	Binding domains, auto-immune eye disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Biologics are successfully treating serious diseases of the eye such as AMD and diabetic retinopathy, preventing blindness and providing quality of life. However, the challenge with current biologics, due to their size, is that they must be injected directly into the patients eye causing discomfort and a high risk of infection in addition to cost of treatment. The overarching aim of this project is to assess the ability of small, shark VNAR domains to penetrate in the eye so that treatment can be provided as eye drops to replace the current invasive approach.	
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)?	The numbers of people suffering from eye disease is increasing as we extend our life spans and change our lifestyles. Existing treatment using steroids are effective but can cause serious damage in the long term. Biologics such as antibodies are demonstrating improved clinical outcomes but can only be injected into the eye. Shark VNAR domains are the smallest antibody-like domains in the animal kingdom and be developed to be as efficacious as antibodies with the	

	potential benefit of being applied as eye drops instead of being injected. This project will determine whether this is possible, re-defining the treatment of certain inflammatory eye diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	We will be using mice for our studies as they develop clinically identical disease to humans. We are planning to use genetically modified/transgeneic mice, which do not always develop the required genotype, hence the estimation of 5000 animals during the duration of the project to ensure sufficient mice of the correct genotype for the study. Experiments are planned to obtain statistically robust data, which would take into consideration also biologic variance. This includes meticulous planning of mouse breeding programme and regular frequent checks of the breeding colonies, in order to meet the experimental requirement with minimal mouse numbers. Once particular experiments have been completed certain genetically modified lines may no longer be required and will be cryopreserved ,
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 main procedures to be used are; 1. The induction of inflammation in the cornea (herpes simplex) followed some time later after corneal healing by healthy donor cornea transplantation. Generation of infective keratitis involves corneal scratching under anaesthesia and application of the infective agent. This would cause moderate distress. The corneal graft procedure involves grafting a donor cornea to a recipient with a single continuous suture under anaesthesia, again involving moderate distress. Minimal distress in relation to the handling of the animals while examining under the operating microscope and when administering immunomodulatory treatments. Some animals will undergo more detailed imaging of comeal vessels by scanning laser ophthalmoscope and these animals will be under anaesthesia. All animals will be humanely killed on the end of experiments while tissues will be further processed for detailed immunology investigations. 2. Uveitis model. There are few significant adverse

	events expected. Mice have normally very low visual acuity (nocturnal animals, mainly sensing by smell and whisker touch) and are not adversely affected by the eye inflammation. In addition, the eye inflammation is intraocular and painless (as in humans) so there is no external evidence of disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Corneal graft non-acceptance (rejection) is a clinical entity for which there is no model in vitro. Understanding the mechanisms of graft rejection process is not possible from humans samples as they are harvested as end stage opaque samples just prior to the surgery for corneal transplantation, and do not show the reveal the process or mechanism of rejection. Therefore only animal models are able to provide necessary information that will determine future possible treatment for affected individuals. Approximately 10% of the work will be in vitro. Understanding the mechanisms of inflammation and translating them into therapeutic opportunities for medical practice has already reaped benefit for patients and our further work with these models should in long term provide safer treatments which also prevent visual loss in affected patients.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments will be planned to incorporate optimal experimental design and to use the minimum number of animals per group consistent while ensuring statistically relevant data to demonstrate sensitivity and reproducibility; and provide data for peer review in published papers. We will plan the mouse breeding meticulously and regularly check the breeding colonies, in order to meet the experimental requirement with minimal mouse numbers. As a result of our collaborator'-s previous experience in our existing project licence we have already achieved the best possible designs for our standard laboratory protocols particularly corneal grafting procedures. Where possible (approximately 10% of work) the in vitro techniques will be used which will reduce number of animals used.

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.	The protocols involve procedures performed mainly under general anaesthesia and involve minimal suffering to the animal. Ocular surgery is routinely performed under the high- quality operating microscope with the finest possible instruments and sutures. The immunomodulatory treatments will cause only mild temporary discomfort. The protocols involve procedures performed mainly under general anaesthesia and involve minimal or no suffering to the animal (skin ulceration may occur as a result of immunisation protocol but mice do not experience any distress or pain related to eye inflammation and uveitis). The immunomodulatory treatments will be administered without anaesthesia; however these do not cause suffering, only temporary discomfort.