Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 3

Projects with a primary purpose of: Translational and Applied research – Other Human Disorders including:

- Respiratory Disorders
- Gastrointestinal Disorders including Liver
- Immune Disorders
- Urogenital/Reproductive Disorders

Project Titles and Keywords

1. **Gene transfer to small animals**
    - Gene therapy, genetic disease

2. **Development of assays to quantify gluten content in gluten-free foods**
    - Coeliac disease, gluten, monoclonal antibodies

3. **Zebrafish as a model of inherited renal disease**
    - Cystic kidney, cilial, treatment, pronephros

4. **Neurodevelopmental disorders: Causes & treatment**
    - Autism, Schizophrenia, Genes, Environmental risk factors, phenotyping

5. **Development of diagnostics and therapeutics for pancreatitis**
    - Pancreatitis; acute; chronic; diagnostics; therapeutics

6. **Models of Airway inflammation**
    - Respiratory, Inflammation, In-vivo, Infection, PK/PD

7. **Novel Therapies to Treat Autoimmune Diseases**
    - Diabetes, Multiple Sclerosis, EAE, Lupus, Psoriasis

8. **Immunology studies to support drug discovery**
    - Immunology, Inflammation

9. **Genetic models to study inflammatory diseases**
    - Inflammation, arthritis, infection, PAR-2, MKP-2

10. **Interventions against Chlamydia.**
    - Chlamydia, macaque, vaccine, efficacy, immunogenicity.

11. **Mechanisms and Treatments of Renal Transplant-related Injury in Mouse Models**
    - ischemia/reperfusion (I/R) injury, immunosuppressant-cyclosporine A (CsA), erythropoietin (EPO) derived new cyclic helix B surface peptide (CHBP), caspase-3 small interfering RNA (siRNA) and genetic modified mice

12. **Finding treatments for chronic kidney disease**
    - Kidney, fibrosis, CKD

PROJECT 1	**Gene transfer to small animals**	
Key Words (max. 5 words)	Gene therapy, genetic 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[1]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aims of this project are threefold: i) To improve the technology underlying gene therapy ii) To use existing technology, and that developed during this license, to apply gene therapy to mouse models of diseases, particularly inherited genetic diseases, with the intention of bringing these treatments to the clinic and iii) To develop these technologies to make somatic transgenic biosensing mouse models. These would allow continual non-invasive measurement of disease (and potential treatments) in mice, with the intention of greatly reducing the numbers used by conventional analysis (where mice are culled at many time points)	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	From aims (i) and (ii) we hope to develop cures for diseases, particularly inherited genetic diseases, which are incurable and often completely untreatable. From aims (i) and (iii) we hope to greatly reduce the number of mice used in conventional disease modelling while providing more accurate data for individual animals.	

What species and approximate numbers of animals do you expect to use over what period of time?	We will use mainly mice (4000 over the five year project) and some rats (100 over the five year 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?	The diseases we are studying are fatal genetic diseases of children so we are using many mouse models of these diseases. These mice can often fail to thrive, lose weight and become poorly. Some with neurological disease may start to show symptoms of paralysis. However we aim to be vigilant for such signs and to euthanase them if they do. The expected level of severity is moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Genetic diseases often affect multiple organ and tissue systems; we need to ensure that the gene therapy vectors (the particles used to deliver a corrected version of the gene) are able to correct the gene in all the affected tissues. On saying that, we test all the gene therapy vectors in tissue culture before they are used in mice, and we are working with collaborators to develop testing procedures in human induced pluripotent stem cells (IPSC5) which would partly-replace some animal experiments. Not only are we trying to evaluate correction of the defective gene in all tissues, we also want to examine the impact on health and behaviour, which can only be tested in a whole animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	With reference to aim (ii), we have found that gene therapy has become effective enough that mice can be cured sufficiently to live a healthy life to breeding age. Therefore, where possible, we intend to maintain colonies as “cured knockouts”. This means that we can reduce the number of mice maintained in the colonies around fourfold, and eliminate un-necessary injections completely, thus reducing total number of injections 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.	Aim (iii) is specifically directed at reducing mouse numbers used more broadly in rodent experimentation by developing continual, non-invasive bioimaging technology. Therefore, rather than studying, for example, thirty-six mice, and culling six each at six separate time points, the biosensing technology could allow the same experiment to be performed on only six mice, monitoring these individuals over the whole time We intend to use only rodents, mainly mice, during this project, since a major aim of this project is to develop treatments for inherited genetic disease and there are many mouse models which are relatively good models of human genetic disease. They have been designed to carry defects in the same respective genes, and show very similar pathology. Importantly, exactly the same gene therapy vector that we use to treat a mouse can then be used to treat a human being, As mentioned above, we are now finding that gene therapy technology means we can cure these models sufficiently to allow them to breed and remain quite healthy. Therefore we do not need to routinely perform ear punches and blood collections to find out the genotype of each mouse; this minimises the overall harm to all mice in the colony.

PROJECT 2	**Development of assays to quantify gluten content in gluten-free foods**	
Key Words (max. 5 words)	coeliac disease, gluten, monoclonal antibodies	
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
	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[2]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aims are to develop improved assays to quantify gluten in gluten-free foods. The antibodies will be used in dot blot and ELISA assays to identify coeliac toxic prolamins in various cereals and to quantify the amount of coeliac toxic prolamins in foods for individuals with coeliac disease. We will use the new antibodies to determine whether novel cereals contain coeliac toxic prolamins and if they are present, the levels. This is to add identification and testing of newly introduced foods for individuals with CD to ensure they comply with the regulation that they are either gluten-free (<20 ppm) or gluten reduced (21-100ppm). This is of individuals with CD.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Production of additional monoclonal antibodies to coeliac toxic prolamins will enable quantitation of these proteins in dedicated foods for individuals with CD. This will include new gluten-free foods that are being developed by baking companies to generate gluten-free foods with improved sensorial	

project)?	characteristics than those currently available.
What species and approximate numbers of animals do you expect to use over what period of time?	Baib C strain mice sixty/year that represents three hundred over five 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 animals are provided with a gluten-free diet that has not been observed to generate any side effects or complications. The animals will be immunised initially by subcutaneous injection with gluten protein and finally with an intravenous immunisation. This may cause a mild reaction at the injection site, although none have been observed in our previous similar experiments. All the animals will be killed at the end of the individual experiments or at the end of the experimental period.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Coeliac disease (CD, gluten-sensitive enteropathy) that affects 1% of individuals in the UK is treated with a gluten-free diet. Prior to this discovery affected children had a 20% mortality. Methods are required to identify and quantify the amount of CD toxic proteins in gluten-free foods. Animals in this case mice are required to generate monoclonal antibodies that can be used to quantify coeliac toxic proteins in gluten-free foods. This enables affected individuals to assess the gluten content of gluten- free foods. No other simple methods are available to measure the amount of CD toxic proteins in gluten-free foods for individuals with CD.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We need several animals for each immunogen as some mice do not achieve an adequate immune response. We will use only three animals per immunisation to keep numbers as low as possible.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Baib C strain mice have been shown to be the best choice of animal to generate monoclonal antibodies to gluten proteins and peptides The volumes of adjuvant to be used are expected to be below current practice on review of the most recent methods of generation of monoclonal antibodies. It is necessary the produce

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	antibodies known as lgG rather than 1gM antibodies. In order to do this the use of an adjuvant known as Freund's Complete Adjuvant (FCA) is needed. This is known to cause greater adverse effects Freund's Incomplete Adjuvant .In order to minimise the adverse effects, it will be used once only. It is anticipated that the severity experience by the animals will be within the mild severity limit.

<table>
<tr><td>PROJECT 3</td><td>Zebrafish as a model of inherited renal disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cystic kidney, cilial, treatment, pronephros</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[3]</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[4]</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>We will assess the role and function of genes implicated in human cystic kidney disease and related conditions. The exact functional role of such genes and their encoded proteins remains poorly understood and there is a fundamental and clinical need to know more about these genes. In addition, this project will determine the response of cystic kidney disease to various proposed treatments.</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>This work will generate novel insights into the disease processes underlying cystic kidney disease. A better understanding of cystic kidney disease may ultimately lead to novel therapies for human patients with these diseases.</td></tr>
<tr><td>What species and approximate numbers of</td><td>We will use the tropical fish called Zebrafish. Adult fish will be housed in tanks and be mated to</td></tr>
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animals do you expect to use over what period of time?	generate zebrafish eggs on a weekly basis. Adult fish of 3 months to 18 months of age will be used to generate eggs. Typically each pair of adult fish will produce 100 eggs per mating. Each zebrafish egg produces a single fish embryo, which grows to almost full maturity over the next 5 to 7 days. We plan to use around 10,000 eggs in this project which will be studied for up to 5 days post fertilization. Around 1000 embryos will be used between 5-7 days post fertilization..
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?	Adult fish will not be experimented on directly. Rather the eggs will be involved in experiments. Most of the fish eggs will be treated with compounds from birth to up to 7 days Some fish will undergo a small injection of dye at 3 days post fertilisation to allow measurement of kidney function. For any procedure which might be associated with any discomfort, all animals will undergo general anaesthesia appropriate for the species. We expect a mild level of severity for all these experiments and at the end of the procedure animals will be humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to understand the disease process in cystic kidney disease we need to study model systems, since ethical concerns limit the studies that can be done directly in people. The zebrafish provides a useful model which is an alternative to using mice. It offers considerable advantages over other animal models, for example it has transparent embryos allowing development to be studied easily, and it is also possible to modify gene expression by injection into the fertilized egg. Zebrafish are considered to be of lower sentience than mice and since our experiments use zebrafish at a very early stage in development, these experiments are much less likely to cause any suffering. For these reasons we aim to perform these studies in zebrafish in preference to mouse or other model systems. We

	have successfully used cell models to test drug treatments and mechanisms of disease. This has allowed a targeted approach to planning in vivo experiments. The present cell culture systems do not allow us to explore the mechanisms of kidney development and their response to mutations and treatments. For this the zebrafish is the ideal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Healthy adult fish will be maintained at a minimal number in order to produce sufficient eggs for experiments. Fish will be grown up in the nursery to replace adult fish that are no longer productive, allowing maintenance of the smallest number of healthy adults in the aquarium.
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 zebrafish is a useful animal in which to explore kidney disease as it has a simple kidney with features remarkably similar to the human kidney. We aim to understand the events that lead up to a kidney cyst developing by mimicking cystic kidney disease in the zebrafish and examining the consequences in detail. This level of information could not be obtained from patients and would take huge numbers of mice to achieve a similar result. We wish to test drugs which change the activity of kidney cyst formation in zebrafish. We will also test the ability of drugs to alleviate developmental kidney failure and find out which drugs are most effective. The proposed treatments will cause minimal harm to the animals as they will not be prolonged beyond the initial developmental stages.

<table>
<tr><td>PROJECT 4</td><td>Neurodevelopmental disorders: Causes & treatment</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Autism, Schizophrenia, Genes, Environmental risk factors, phenotyping</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)[5]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></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[6]</td><td>Yes</td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Neurodevelopmental psychiatric disorders are prevalence and life-long conditions which cause significant burden to the individuals affected, their families and society. Genetic and environmental risk factors have been linked to these disorders, but a direct test of how such risks act to cause brain and behavioural differences is not possible in humans. In addition, testing new treatments for disorders which may start very early in life and persist life-long is often not feasible in humans. Therefore rodent studies are vital. The objectives of this body of work are:

1. To investigate the genetic and environmental risk factors for neurodevelopmental psychiatric</td></tr>
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	disorders such as schizophrenia and autism. 2. To test treatments which may prevent or reverse the rodent equivalent of traits of these 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)?	This work is much needed because neurodevelopmental disorders such as schizophrenia and autism are leading causes of disability, affecting a range of behaviours including mood, cognitive function and social ability. Schizophrenia affects approximately 1 in 100 people, at an estimated cost of over £6 billion in the UK alone. ASD affects approximately 1:110 people, with annual costs in the U.K. of more than £34 billion. The causes of these conditions are poorly understood, therefore prevention is extremely challenging. Current treatment options for schizophrenia are far from ideal. Medications are accompanied by significant adverse effects and many people do not respond. There are no treatments for the core symptoms of ASD. Thus there is an urgent need to establish (i) the causes of these severely debilitating disorders and (ii) effective treatments, and this is the focus of the proposed work.
What species and approximate numbers of animals do you expect to use over what period of time?	Rodents (rats and mice). Approximately 2000 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 genetic changes we propose are those which are linked to disorders like schizophrenia and autism. They are not expected to produce a severe abnormality - in fact the outcome may be relatively subtle changes in behaviours and brain. Similarly, the environmental exposures are relatively mild and not expected to produce a gross abnormality. Never-the-less, for manipulations such as exposing the animals to stressors linked to schizophrenia and

	autism will elicit some discomfort, but this is Expected to be moderate and brief. Examining the brain and behaviour of animals exposed to genetic and/or environmental risk factors is generally mild, however, for tests which involve looking at how the animals learn about stimuli which predict unpleasant exposures such as a very brief foot-shock, there may be a brief period of discomfort. These tests will be limited in our experiments. For imaging procedures the animals will be anaesthetized to ensure minimal adverse effects. When testing drugs in these animals, the aim is not to examine 'toxicity' of the drugs, but rather to use drugs to probe a possible chemical pathway at work in conditions such as autism and schizophrenia, or to improve behaviour and brain features. All animals will be humanely sacrificed at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Neurodevelopmental psychiatric disorders such as autism and schizophrenia are highly complex. The brain and behavioural traits cannot be studied in the test tube. There is no single brain tissue damage that explains these conditions, rather, they are the result of widespread subtle differences across may brain regions which give rise to complex behaviours. The affected animal is not obviously 'abnormal' and therefore, only by studying the 'whole' living animal, can a fuller understanding of the possible causes and treatments of such traits be achieved. Moreover, it is not ethical to manipulate either the genetics or the environment, especially during early development, in humans. Therefore, no feasible alternative would entirely replace animals.
2. Reduction	We will plan experiments carefully to ensure the use of sufficient but minimum numbers of animals

Explain how you will assure the use of minimum numbers of animals	are used to provide a statistically sound result. In our experience 10-12 animals per group in experiments is often reasonable, but we will consider each step of the study carefully to ensure the number of animals used is appropriate. Although the number of animals used in the experiment is large, the study the design allows genetic and environmental factors as well as their interaction to be studied, and yields much more information per animal than would separate studies. In behavioural tests a number of tests can be conducted in the same animal with appropriate 'rest' to minimize the number of animals used. Similarly, it may be possible to conduct behaviour and scanning in the same animal (but only where the experience of the behaviour does not itself impact upon the results of the imaging).
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 are the most appropriate animal models for this project. Mice and rats share approximately 97% or more of the same genes as humans. The field has over many years developed reliable test batteries in rodents to examine complex behaviour, including tasks that assess social behaviour and memory, maximizing refinement of the behavioural assessment of the rodents. The rodent brain and its similarities to the human brain is well understood, again optimizing refinement of imaging techniques used in of our studies.

<table>
<tr><td>PROJECT 5</td><td>Development of diagnostics and therapeutics for pancreatitis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Pancreatitis; acute; chronic; diagnostics; therapeutics</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[7]</td><td>
<table>
<tr><td>Basic research</td><td><u>Yes</u></td><td></td></tr>
<tr><td>Translational and applied research</td><td><u>Yes</u></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[8]</td><td><u>Yes</u></td><td></td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Methods for establishing the diagnosis and prognosis of acute and chronic pancreatitis in patients are imprecise. Despite the high incidence, morbidity and mortality of acute and chronic pancreatitis there is no licensed specific drug therapy. This project is designed to advance our understanding, develop new diagnostics and treatments for acute and chronic pancreatitis.</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>Advance in our understanding of acute and chronic pancreatitis, development of new diagnostics and methods to predict disease course and response to therapies, development of new treatments for acute and chronic pancreatitis.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mouse 2,000 over 5 years and rat 200 over 5 years.</td></tr>
<tr><td>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</td><td>The adverse effects are the induction of acute and chronic pancreatitis, the most significant symptom of which is pain that will be treated with appropriate pain killing medicine. Because acute pancreatitis can be severe and when severe causes death of one out of every four people, occasional</td></tr>
</table>

end?	experiments testing the most promising drugs in development have to be undertaken that use mortality as an end point, to determine whether the drug reduces mortality.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Every means possible in patients and using isolated cells is used alongside the work proposed in this project to (i) advance our understanding of acute and chronic pancreatitis (ii) develop new diagnostics (iii) develop new treatments. Nevertheless there are many new approaches that have to be tried in animals before trying out new diagnostics or new treatments in patients, to determine efficacy and safety. Although we undertake as many experiments as possible in isolated cells, we have to use live animals. There is no other way to do this, and new diagnostics and new treatments will not be developed otherwise.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every experiment will be done in a logical manner, in an ordered sequence with go-no go points. Whenever possible, methods other than live animal experiments will be adopted. Each new diagnostic or new treatment under test will only be tested in more than one experiment if at each stage clear promise is evident. While allowing for exploration of different doses, when a treatment is shown not to work in protocols that do not depend on mortality as an end point, the treatment will not be tested using mortality as an end point. Similarly, if treatments fail in two different disease models any treatment will not be tested in further disease models unless there is compelling scientific reason for this, e.g. there is a specific mechanism that will be addressed which has not been tested previously.
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 models of pancreatitis that are proposed have been studied the most intensively and characterised the most reliably. These models are also those that have the most obvious clinical parallels and therefore will be predictive of likely clinical success. The general measures to minimise welfare costs include (i) using the fewest number of animals compatible with high quality scientific investigation (ii) logical sequence of investigation with appropriate and realistic go-no go points at all stages (iii) appropriate use of anaesthesia and analgesia as and when required (iv) optimal husbandry practices to care for the animals in state-of-the-art facilities (v) overall approach integrated

	with cell-based and clinically-based investigation that ensures live animal experiments are entirely in tune with the front line advance of pancreatic research and only used when there is no alternative approach to answer critical questions.

<table>
<tr><td>PROJECT 6</td><td>Models of Airway inflammation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Respiratory, Inflammation, In-vivo, Infection, PK/PD</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[9]</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[10]</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of this programme of work is to allow the company to provide a service to assist other companies to identify, discover and develop new medicines that are relevant to respiratory diseases.

We will achieve this by advising which animal studies are required, undertake these studies and analyse and interpret the data obtained to enable selection of substances with the best chance of going on to be developed into a new medicine.</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 expected benefit of this project is to provide, where there is scientific justification, high quality animal model data to enable decisions on the progression, of new medicines for the treatment of respiratory diseases.</td></tr>
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What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of this five year project it is expected that up to 1500 mice and 1500 rats will be used.
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?	Typical studies can last from 1 day up to 7 weeks and comprise dosing with inflammatory substances, compound dosing and blood sampling. The output from these studies will measure airway inflammation, lung function and levels of compound within the blood and tissues. The majority of animals on this project licence will only experience mild severity and no adverse effects are expected. A small minority of animals may experience moderate severity in response to inflammatory substances or compound effects or when surgical preparation of animals is undertaken to implant mini-pumps or insert intravenous catheters to facilitate dosing and sampling. Surgery will always be carried out using appropriate anaesthesia and pain relief. All studies will be closely managed so the minimum number of animals experience pain and distress. Animals will be observed regularly to monitor changes in appearance and behaviour and action will be taken to alleviate any pain and distress, such as withdrawal of the animal from further dosing, or euthanasia. At the end of each study animals will be humanely killed and tissues will be taken for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This data cannot be generated without using animals because of the many and complex interactions that occur between inflammatory responses and the function of the lungs make it essential that novel targets are evaluated in animals. While a wide range of *in vitro* and *ex vivo* data can be used to increase our

	understanding of a target in an isolated biological system, understanding the integrated response in a whole animal is vital to guide medicine development.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical advice will be sought to ensure the least number of animals are used and the maximum value can be gained from the data they generate. Compound levels in blood and other tissues will also be measured often within the same study to help understand what the medicine does to the body and the body does to the medicine. Imaging (MRI) maybe used in some study designs to further reduce animal numbers by measuring in-life lung inflammation in the same animals at key points throughout the study.
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 animals models used for this program of work have been chosen and developed to represent the least severe way of measuring lung inflammation and lung function. Mice will be used when we are investigating the effects on the immune system due to availability of biological test assays. Rats will be used where a direct comparison is required with toxicological data to support a human dose calculation. All animal work will be subject to ethical review and conducted by highly trained, competent scientists. These scientists also have access to a Named Veterinary Surgeon and Named Animal Care and Welfare Officers at all times for advice on study design and animal welfare matters.

<table>
<tr><td>PROJECT 7</td><td>Novel Therapies to Treat Autoimmune Diseases</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Diabetes, Multiple Sclerosis, EAE, Lupus, Psoriasis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[11]</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>Yes</td><td></td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[12]</td><td>Yes</td><td></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>This project aims to develop novel therapies to treat autoimmune diseases.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Autoimmune diseases affect patients causing pain, impaired function and diminished quality of life. By contributing to the development of new candidate drugs, our project will benefit the patients improving their quality of life and reducing pain. By providing high quality services and scientific expertise, we are able to make the testing of such drugs more cost effective, more informative and reduce the need for companies to set up the models in house.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>The estimated number of animals to be used over the duration of the project is 15 820. Animals to be used include a majority of mice (85%), some rats</td></tr>
</table>

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over what period of time?	(15%) and a minority of rabbits (<1%). They are the least sentient species which allow the objectives to be met.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	*Expected adverse events: (i) a*dverse reaction to a lead candidate (< 25% incidence), (ii) diabetes, obesity, hyperglycaemia, hyperketonemia, diabetic neuropathy, diabetic nephropathy, (iii) EAE, bodyweight loss, limb and tail paresis, limb and tail paralysis, (iv) skin thickening, flaking, scabbing, erythema, (v) albuminuria (vi) dry eye, eye inflammation. Expected level of severity: Moderate except EAE models (severe). Measures taken to limit harms: frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events, moderate signs tolerated for no more than 24 hours, severe signs not tolerated. Humane endpoints are applied to minimise harm and include humane culling prior to the development of severe clinical signs. At the end of an experiment, all animals will be culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune system's response to the presence of antigens, involves multiple systems, multiple organs and multiple cell types. The complexity of the immune responses cannot be reproduced *in vitro*. In addition, the symptoms of autoimmune conditions –hyperglycaemia, production of autoantibodies, paresis, paralysis, skin inflammation, dry eyes and pain- cannot be modelled *in vitro*. *In vitro* experiments on cell lines and *ex vivo* experiments on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.
2. Reduction Explain how you will assure the use of minimum numbers	Power analysis will be performed to establish the total sample size required to generate meaningful data. Typically, power value will be set at 80% in

of animals	order to reduce the number of animals used in the studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Animal suffering will be limited by choosing mild over moderate severity models and by choosing acute over chronic models. A typical experiment will involve a local or systemic administration of a disease-inducing agent. Lead compounds will be administered prior to or from of signs of disease (prophylactic and therapeutic regimen, respectively). Animals will be monitored frequently and scored for clinical signs of disease. Blood, cells and/or tissue samples will be collected at the end of the experiment for ex vivo analyses.

<table>
<tr><td>PROJECT 8</td><td>Immunology studies to support drug discovery</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunology Inflammation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[13]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[14]</td><td><s>Yes</s></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>There are many human diseases in which the immune system plays a key role, including arthritis, asthma, atopic dermatitis (eczema) and lupus. Although there have been significant advances in biomedical research in recent years, we still do not have adequate therapies for all of these diseases, and we do not fully understand the immune mechanisms that drive them. Breaking down complex immune mediated diseases into simple mechanistic immunology models enables us to gain new knowledge of underlying biology and identify new ways of treating disease. This Licence enables us to use simple models of immune function to study these new mechanisms and conduct studies to determine if they are likely to be critical in human disease, and therefore worth pursuing as new drug targets.</td></tr>
</table>

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)?	We believe it is likely that we will obtain new knowledge about the immune system and how it contributes to disease pathology in experimental animals, and ultimately in man. We will use this knowledge to help us discover new potential therapeutics in a range of immune diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the five year duration of the licence, we estimate that 5000 animals (4160 mice and 840 rats) will be used.
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?	Most of these studies will involve immunising animals, so that they develop a specific ('allergic') response to a protein or substance they have not encountered before. The animals will then be exposed to that substance again and the inflammatory response measured either in the animal (e.g. looking at swelling) or more commonly by culling the animal and examining the cells using in vitro techniques. We may also look at more complicated immune responses such as anaphylaxis, a rapid allergic response to a protein such as peanut which causes a substantial immune reaction and in some cases cardiovascular collapse. In addition some experiments will study how immune mechanisms contribute to the closure of wounds and the healing of skin. To do this animals are anesthetised and a surgical wound is generated and covered by a sterile dressing and monitored over a period of days as it heals. All animals are killed at the end of the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We will always consider in vitro studies in place of in vivo experiments, and it is highly unlikely that any work will be done without prior in vitro exploration of the hypothesis. Complex immunology cannot yet be reproduced in a cell only or computer based system.
2. Reduction Explain how you will assure	We will use statistical techniques to ensure that we use the right number of animals in our studies – too many would waste animals, but too few and we

the use of minimum numbers of animals	may need to perform additional experiments to be able to interpret the results correctly.
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 mice in particular, have a well characterised immune system, and there are established techniques and reagents for working with these animals, which will enable us to meet our objectives. We will use the most refined techniques available to meet our experimental goals by optimising the protocols to minimise the impact on animal welfare. Analgesia and anaesthesia will be used as standard unless there is a significant reason not to e.g. it is known to interfere with the immune response.

PROJECT 9	**Genetic models to study inflammatory diseases**	
Key Words (max. 5 words)	Inflammation, arthritis, infection, PAR-2, MKP-2	
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
	x	Maintenance of colonies of genetically altered animals[15]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Inflammation underpins a large number of chronic diseases such as arthritis, asthma, multiple sclerosis, inflammatory bowels disease and others. Whilst the many of these conditions are managed clinically using drugs, there are no cures currently available and millions of people continually suffer from these and other related conditions. Therefore, understanding the role of different cellular enzymes underpinning inflammation is essential for the future development of new medicines. The objectives of this project is to create a better understanding of the role of two protein molecules, PAR-2 and MKP-2, in the regulation of a number of inflammatory diseases including arthritis multiple sclerosis (MS) and parasite infection. The role of PAR-2 in arthritis has been previously established but it has not been identified which of the many cells of the joint play a role. By contrast the involvement of MKP-2 in a number of inflammatory diseases is not as well defined. The purpose of the project is to establish models in which	

	the PAR-2 and MKP-2 genes can be deleted in specific cell types and tissue involved in the disease, for example specific white blood cells. These models can then be used in specific tests of arthritis, MS and other diseases to determine if each gene plays a role.
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)?	By understanding the role of PAR-2 and MKP-2 in the regulation of such inflammatory diseases we can consider the potential to block each protein with drugs and to develop a new medicine. For PAR-2, this is more advanced with a number of prototype molecules being developed which require testing. The role of MKP-2 in disease and the potential for MKP-2 to be a bonafide target for the development of new medicines is not known therefore this need to be established in this project
What species and approximate numbers of animals do you expect to use over what period of time?	This project we will use mice as these are the most suitable species for gene deletion studies. We will use approximately 10,000 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?	As this project just involves generating mice with the PAR-2 and MKP-2 gene deleted in specific tissues then the level of severity is mild. There is a small degree of discomfort and pain associated with testing the mice to ensure that the gene deletion is the correct one. The mice will be sacrificed and tissues used for analysis whilst others will be used on other licences.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Human diseases such as arthritis are highly complex and involve the interaction of many proteins within different cells and tissues. Therefore to understand the contribution a single protein can make to disease at a cellular and tissue level genetically altered mice are required in which the gene for a given protein is deleted. Genetically altered mice also provide the best models relative to the human disease condition when examined at the whole animal level. Whilst using cells from human provides important data they cannot be readily used to identify the role of a given

	gene nor replicate the complexity of the disease, thus the quality of the science will be enhanced using genetically altered mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of mice is required for backcrossing in order to get mice onto the appropriate genetic background. However, we will immortalise some cells derived from the mice in order to do the biochemical studies which require substantial numbers of mice to get small amounts of tissue.
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 will be used as they are the best species for gene deletion. Also they are the species in which a large number of disease models have been established including both osteo- and rheumatoid arthritis, MS, pathogen infection and others. Animals will be group housed in cages which permit free movement and contain environmental enrichment appropriate to their species. Husbandry and care procedures are based on best practice, and regular monitoring will be conducted by highly trained staff. In all cases, the endpoints of the experiments will be measurements acquired from tests which are considered minimally traumatic to the animals and are of short duration.

<table>
<tr><td>PROJECT 10</td><td>Interventions against Chlamydia.</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Chlamydia, macaque, vaccine, efficacy, immunogenicity.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[16]</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[17]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Genital Chlamydia trachomatis is the most commonly reported bacterial sexually transmitted infection (STI) worldwide. The World Health Organization estimates 92 million cases of Chlamydia STI, together with over 85 million cases of ocular Chlamydia infection (trachoma) per year. In England, over 186,000 new cases were diagnosed in 2011, with sexually active young adults at highest risk of infection. Chlamydia is asymptomatic in approx. 50% of infected men and 70% of infected women and can lead to a wide range of complications, including pelvic inflammatory disease (PID), ectopic pregnancy and tubal factor infertility (TFI) in women and epididymitis in men. It represents a substantial public health problem, estimated to cost the UK</td></tr>
</table>

	NHS in excess of £100M per year. Although effective antimicrobial therapy is available to treat *Chlamydia* infections, it is unlikely to result in the control or eradication of *C. trachomatis* disease since individuals may be asymptomatic, may not respond to therapy or may be treated too late to prevent the development of long-term consequences. A vaccination program is considered to be the best approach to reduce the prevalence of *Chlamydia* infections
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will result in a well characterised and robust model of *Chlamydia* infection resulting from lower genital tract challenge. Using this model we will investigate the efficacy of potential *Chlamydia* vaccines. The development of a vaccine will greatly improve the health and wellbeing of millions of people worldwide. If this protection extended to prevention of Trachoma then the problems of childhood blindness could also be addressed. It has been estimated that even a vaccine that only offers partial protection could eradicate *Chlamydia trachomatis* disease within 20 years.
What species and approximate numbers of animals do you expect to use over what period of time?	Macaques will be used for this study. Humans and macaques have similar genetics, physiology, anatomy, metabolism and immunology - all key components of the host-pathogen interaction. It is believed that the more human-like the host, the more likely it is to respond to pathogens in a human-like manner, and therefore be more representative of the human response to infection. We plan to use no more than 176 animals over the 5 year duration of this licence. Using animals obtained from a UK Breeding colony where animals are of a similar genetic background, will allow us to minimise the number of animals will be minimised by controlling experimental variability. This will allow for smaller group sizes and will reduce the need to repeat studies. We will employ well established procedures and use highly trained and experienced staff. Intrinsic inter-sample variation will be kept to a minimum as animals will be

	maintained in consistent controlled environments and sample collections will be performed at the same time of day on each occasion and where possible with standard operators. Statistical advice will be sought on the design of studies to ensure the use of the minimum number of animals per group that will provide meaningful 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?	Animals will be anaesthetised for all procedures. A liquid culture of *Chlamydia* trachomatis will be applied to the ectocervix and vagina. If the course of infection is similar to that in humans, inflammation of the cervix may occur which manifests as a discharge or bleeding from the vagina. Other symptoms may include abdominal discomfort. Animals will be routinely examined endoscopically for signs of inflammation, swabs will be collected to allow determination of pathogen shedding from the vagina mucosa, and blood samples will be taken to allow analysis of immune responses. Pet CT scanning will be employed to allow non-invasive monitoring of infection. At the end of the study animals will be euthanized and tissues examined for the presence of chlamydia and pathological changes induced by infection.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst the isolation, infectivity and preparation and characterisation of challenge stocks can be performed using cell cultures, there are complex interactions between pathogens such as *Chlamydia* and the host which involve the target cells, the innate defence mechanisms of the mucosa and genital tract and the innate and acquired responses of the immune system which cannot be reproduced in a cell or tissue culture environment. Currently the only way to investigate this complexity and to define the dynamics of what is occurring in real-time, is to use an animal model.
2. Reduction Explain how you will assure the use of minimum numbers	We will use animals obtained from a UK Breeding colony where animals are of a similar genetic background to minimise the number of animals required by controlling experimental variability. This

of animals	will allow for smaller group sizes and will reduce the need to repeat studies. We will employ well established procedures and use highly trained and experienced staff. Intrinsic inter-sample variation will be kept to a minimum as animals will be maintained in consistent controlled environments and sample collections will be performed at the same time of day on each occasion and where possible with standard operators. Statistical advice will be sought on the design of studies to ensure the use of the minimum number of animals per group that will provide meaningful 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.	Humans and macaques have similar genetics, physiology, anatomy, metabolism and immunology - all key components of the host-pathogen interaction. It is believed that the more human-like the host, the more likely it is to respond to pathogens in a human-like manner, and therefore be more representative of the human response to infection. Macaques for these studies will be sourced from UK breeding colonies and housed in socially compatible groups (compatibility can be assessed whilst animals are still housed within the breeding colony) with various enrichment strategies available throughout these experiments. Animals will be vaccinated and / or challenged with *Chlamydia* and blood samples and information on clinical parameters collected generally at weekly to monthly intervals. Wherever appropriate for the type of procedure, animals will be sedated to minimise stress. Wherever possible pre-challenge samples will be taken and vaccinations given whilst the animals are still within the colony environment. Considerable experience has now been gained in working with macaques and this experience has led to the establishment of robust clinical, behavioural and immunological data that can be used to define individuals that will progress to disease and allows early intervention using humane end-points.

<table>
<tr><td>PROJECT 11</td><td>Mechanisms and Treatments of Renal Transplant-related Injury in Mouse Models</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>ischemia/reperfusion (I/R) injury, immunosuppressant-cyclosporine A (CsA), erythropoietin (EPO) derived new cyclic helix B surface peptide (CHBP), caspase-3 small interfering RNA (siRNA) and genetic modified mice</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[18]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</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[19]</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>Transplantation is the best treatment for end-stage renal failure patients and completely changes the quality of their life. However, transplanted kidneys, subjected to initial injury casused by blood discontinuation/reconnection (I/R), followed by a gradual renal damage induced by immunosuppressants and other complications such as rejection episodes, will potentially develop renal dysfunction. However, there is lack of timely diagnosis and effective treatment so far.

A variety of manipulations have been studied to minimise transplant-related injuries, including</td></tr>
</table>

	immunosuppression using CsA that also are toxic to kidney in long-term; the novel peptide CHBP derived from EPO protecting renal tissues without increase red blood cells; RNA interference (destroying existing harmful RNA), especially siRNA targeting different key molecules such as caspase-3, an enzyme involving in inflammation and apoptosis (cell suicide) linked to injury. The I/R injury mouse model with or without CsA reflects the clinical setting post transplantation and plays important roles in the scientific translational research. The well-established genome database of mouse species provides a great platform for the development of deficient mice such as properdin or MASP-2 deficiency, as well as gene therapy. The mouse model could be applied to investigate not only the mechanism of disease, but also systemic responses and compensative effects to certain treatments, which is a necessary step before any therapeutic measures could be used in humans. **The general project plan**: **Phase 1:** Studying the dynamic change at early and later stages in both I/R injury and CsA renal toxicity and understanding the mechanism involved using wild-type and genetic deficient mice. **Phase 2:** Observing the effects of CHBP and/or caspase-3 siRNA treatment in transplant-related injury and disclosing the underlying mechanism. **Phase 3:** Defining biomarkers for diagnosis of transplant-related injury, and confirm the obtained data in order to design new siRNAs targeting biomarkers for further treatment.
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)?	By examining the interaction between I/R injury, CsA, CHBP, caspase-3 siRNA in wild type and genetic deficient mice, we hope to disclose the mechanisms of injury and involved signalling pathways that can be recognised, monitored and targeted. The ultimate aim is to develop treatments to slow or even stop the process of these injuries in patients, preventing patients with transplanted kidney dysfunction reaching end stage failure

	again. We will also aim to identify biomarkers that would be used by clinician for timely diagnosis and tailoring individual personalized siRNA treatment in order to improve allograph survival and patients' quality of life, and subsequently reduce the financial burden of society.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use **up to 350 C57BL/6 mice** (about half wild-type and half genetically modified) for this project. The same strain of genetic deficient mice is available locally for this study. In order to further minimize the number of experimental animals, required sample sizes were estimated according to preliminary data and power calculations with statistician's support. The "sham I/R + saline" group will be only used at 24-h and 12-week time points. In addition, if the caspase-3 siRNA groups will be used, the existing control groups for the CHBS groups will also be shared. Mice will be randomly divided into different experimental groups including sham operation and vehicle control, I/R injury with or without CsA, and/or CHBP, and/or caspase-3 siRNA in both wild-type and deficient mice (such as properdin or MASP-2). For the renal I/R, mice will be anesthetised with the inhalation of isoflurane via oxygen carries and bilateral renal vascular pedicle occlusion for 30 min will be performed using non-traumatic microvascular clips. Gavage, intraperitoneal injection and intravenous injection will be used for administering CsA, CHBP and caspase-3 siRNA respectively. Using these mice subjected to transplant-related renal injury will also allow us to exploit the molecular basis of damage an increased range of time points, which cannot be detected by any other measures including living image system.
In the context of what you propose to do to the animals, what are the expected adverse	Pain killers will be given to the animal to ensure that any pain following the operation is minimised. The experiment will be terminated if there are

effects and the likely/expected level of severity? What will happen to the animals at the end?	unexpected adverse effects, such as unseen surgical damage caused by operation or accidental physical injury caused by gavage. According to previous studies, the degree of I/R injury and **CsA toxicity is from mild to medium, which should not be severe enough to cause symptoms** making animal suffering. However if any animal does display any symptoms related to kidney failure, i.e. anorexia, severe weight loss or dehydration, less likely, it will be killed to prevent suffering. **There are also no reported obviouse adverse effects of CHBP and caspase-3 siRNA.**
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have done in vitro and ex vivo experiments to select the most effective sequence of caspase-3 siRNA. A “pre-clinical” *in vivo* model is particularly crucial before any treatment could be translated from the bench to the clinic. The proposed studies will include novel therapeutic approaches such as CHBP derived from EPO and siRNAs targeting specific gene, which need to be fully assessed, not only their efficacy, but also side effects and system compensative responses. These examinations cannot be achieved by using neither *in vitro* nor *ex vivo* models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Pilot studies have been performed to obtain preliminary data from small numbers of wild-type animals. The power calculation was also used to minimise the number of experimental animals. Wild-type C57BL/6 mice will act as controls for the targeted genetically deficient mice. Two treatments of CHBP and caspase-3 siRNA will share the same set of control groups; and sham operation will be used for only 2 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	The mouse model of I/R and CsA renal toxicity to be used in this project is the one of most refined models of kidney injury related to kidney transplantation currently available. It has been widely used by investigators around the world and results in a predictable disease phenotype. The

measures you will take to minimise welfare costs (harms) to the animals.	pilot study of similar experimental design has been performed in wild-type mice elsewhere. Therefore, preliminary data already obtained such as the dosage of treatment, delivery route and interval. This proposed project will explore the effects in genetic deficient mice with more broad time points 24 and 72 h, 4, 8 and 12 weeks. The degree of I/R injury and CsA toxicity is from mild to medium in histology, which will not cause physical suffering for animals. However, mice will be closely observed for symptoms of renal failure; if there is any, the animal will be killed to prevent further suffering. The surgical procedures will be carried out by well-trained experienced operators, and animals will receive analgesia to prevent post-operative pain. *The* **Laboratory Animal Science Association** *guidelines on administration routes, frequencies and volumes will be adhered.*

<table>
<tr><td>PROJECT 12</td><td>Finding treatments for chronic kidney disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Kidney, fibrosis, CKD,</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3 months only to effect completion of a study started under a previous licence.</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[20]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td></tr>
<tr><td>Regulatory use and routine production</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>No</td></tr>
<tr><td>Preservation of species</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[21]</td><td>No</td></tr>
</table>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objective of this project licence is to complete characterisation of a new mouse model of kidney fibrosis

End-stage renal failure requiring renal replacement therapy affects 35,000 people in the UK and has a mean five-year survival of 87% in 18-34 year olds, falling to 29% in 55-65 year olds making its prognosis worse than most of the common cancers including breast, bowel and prostate. Although kidney injury can be initiated by a number of unrelated conditions such as diabetes, hypertension and autoimmune disease, once started, it is the independent and self-perpetuating process of fibrosis and scarring that eventually damages the kidney causing chronic disease and ultimately leading to the need for dialysis or transplantation. Fibrosis (scarring) is a particularly</td></tr>
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	difficult process to treat as it has many diverse causes and multiple contributing factors and currently there are no specific therapies for slowing its progression, other than treating co-existent hypertension and reducing protein loss in the urine of those affected. Both dialysis and transplantation, whilst effective, are extremely costly therapies, highly invasive and often distressing for individuals. For this reason part 2 of the UK Government's National Service Framework for Renal Services has highlighted the need to "support NHS organisations in the prevention of chronic kidney disease in people at risk" and to "develop strategies for slowing down the progression of the disease". During the development of chronic kidney disease, specialized cells with in the kidney called fibroblasts and mesangial cells begin to multiply and to produce excessive amounts of scar tissue know as matrix. These scar-forming cells can be identified in kidney tissue with special stains, as can the matrix they produce. This matrix gradually overtakes the normal architecture of the kidney and obliterates the normal, functional cells, eventually leading to kidney failure. As part of our aim to identify new treatment targets we have been studying these cells in non animal-based, in vitro experiments to be able to understand the signalling pathways within them that are switched on when they start to multiply and produce matrix. Using these cell-culture experiments we are able to screen potential targets and new treatments for their ability to stop cell growth or prevent matrix production. In this way we minimise the number of animals we need to use and increase the likelihood of positive results. The process of fibrosis is, however, complicated and dependent upon the interaction between many different cell types including fibroblasts, mesangial cells and cells of the immune system. Non animal-based models, whilst excellent for screening potential treatments for fibrosis, are not adequate to assess final impact or potential unwanted effects

	such as poor wound healing. Humans cannot be used in the first instance as the experiments involve new and novel compounds, not previously given to humans. Whilst we expect a number of them to be effective, not all will be and the potential to cause harm means this form of investigation is unsuitable.
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)?	Our aim is to find new treatments designed to prevent or slow progression of chronic kidney disease. Not only will this reduce the number of patients on dialysis treatment in the future, but will also reduce the total disease burden in the country for other conditions related to chronic kidney disease (CKD). Patients with CKD are also at increased risk of developing cardiovascular disease and patients with CKD most commonly die from heart disease. Reducing the burden of CKD in the population could also therefore reduce the overall burden of cardiovascular disease. Anti-fibrotic agents are also likely to be effective in treating other chronic diseases. Liver failure, heart failure and lung failure are all associated with fibrosis in the effected organs and could benefit from any new treatments that reach clinical practice as a result of this project licence, without the need for further animal experimentationn.
What species and approximate numbers of animals do you expect to use over what period of time?	This short licence application will only contain one protocol for aristolochic acid induced nephropathy (AA). This is to enable us to complete a study started under another licence which was still running when that licence expires. We have already made substantial headway in the refinement and characterisation of this model. We have significantly reduced the dose of AA from that used by previous groups and as a result the fibrosis is developing more slowly than predicted which has resulted in models of disease reaching required data points later than anticipated. We would therefore like to characterise the mice already treated with AA by keeping them for up to 100 days to maximise the scientific data available from the existing study animals. 41 mice have already been treated with

	AA under the previous licence and all will be humanely killed once the 100 day point is reached so that the appropriate data can be collected.
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 overall severity classification for our experiments is 'moderate'. As in humans, up to 80% of the kidney tissue can be scarred resulting in moderate renal failure without the animal exhibiting any signs of ill health. However, should the damage get worse than this the animals will lose their appetite, feel generally unwell and lose weight or possibly develop fluid build up in the abdomen. If we see any signs of this the animals will be killed straight away. At the end of the experiments all animals will be killed and their tissues will be stored for analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Fibrosis is a complicated disease process that cannot be adequately copied in cell culture models. Although the majority of our screening protocols are carried out in cells, the final stage of testing efficacy and unwanted side-effects needs to be done in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have a long track record in animal experimentation and have developed ways of designing experiments that minimises the number of animals used, whilst gaining the most information. We use our previous experience extensively, ensuring experiments do not need to be repeated more than once. We have a clear understanding of what we need to measure and how many animals we need to use to detect a significant effect of any treatment.
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	There are a number of animal models of renal disease in existence. We have refined one of these models to make it less severe and more reproducible. We are experienced in identifying animals who are suffering the effects of renal failure and can therefore kill them as soon as these signs develop.

minimise welfare costs (harms) to the animals.	The model of renal fibrosis that we are using is aristolochic acid-induced nephropathy. The previously published version of this model uses multiple injections of a high dose of aristolochic acid. This induces severe acute kidney injury (AKI) which recovers but is followed by a slowly progressive phase of chronic kidney disease (CKD). Using this protocol, the AKI phase is associated with severe renal impairment which is associated with a high morbidity and mortality rate. We have adapted this model by reducing the dose and frequency of Aristolochic acid, giving 2 doses, 3 days apart. The AKI caused by this is mild and associated with minimal morbidity (5-10% weight loss wich recovers within 5 days) and very low mortality. The consequence of this is that the chronic phase develops more slowly so the model is prolonged making it more akin to the human condition and allowing for clear recovery from the acute phase before the chronic phase becomes established.