Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 22

Projects with a primary purpose of: Basic Research into the Gastrointestinal System Including Liver

Project titles and keywords

1. **Mouse models of intestinal disease**
    - Stem cells, epithelium, inflammation

2. **Chararcterisation of the enteroendocrine axis**
    - Enteroendocrine, diabetes, obesity, GLP-1

3. **Developing methods to treat liver disease**
    - Novel, treatment, bile, production, cells

4. **Targeted therapeutic interventions for liver disease**
    - Liver, Inflammation, Fibrosis, Therapy

5. **Molecular mechanisms of stem cell activation**
    - Stem cells, epithelial, activation

6. **Podoplanin-CLEC-2 in inflammatory bowel disease**
    - CLEC-2, Podoplanin, inflammatory bowel disease

7. **Mouse Models of Hepatic Fibrosis and Therapeutics Investigation**
    - Mouse, Hepatic, Fibrosis

8. **Modification of gut function by dietary micronutrient intake**
    - Diet, micronutrient, metabolism

9. **The microenvironment in organ homeostasis and cancer**
    - Liver, skin, cancer, stem cell

10. **Imaging of Stem Cells In Organ Fibrosis Models of Tissue Regeneration**
    - Liver, kidney, stem cell, regeneration, imaging

11. **Study and treatment in liver ischaemia reperfusion**
    - Ischaemia:reperfusion; mesenchymal stromal cells; liver

<table>
<tr><td>PROJECT 1</td><td>Mouse models of intestinal disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Stem cells, epithelium, inflammation</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)</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</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>This research aims to investigate the role of intestinal stem cells and their daughter epithelial cells in chronic inflammatory disease of the intestine, such as ulcerative colitis and Crohn’s disease.</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>Greater understanding of the mechanisms of chronic inflammatory diseases of the intestine and the possibility of new therapeutic approaches to their treatment, by facilitating epithelial repair and regeneration.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice, approximately 170 per year will be used.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>Adverse effects are not expected to exceed the moderate severity limit. Effects may include</td></tr>
</table>

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	diarrhoea, weight loss and stress due to restraint.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Investigations in mice enable integrated response of the intact tissue, which cannot be modelled *in vitro* or *in silico*. A "whole body system" is required because inflammatory responses entail recruitment of cells from the circulation to the intestinal mucosa. Moreover, cells that sample luminal contents in one part of the intestine migrate to the systemic circulation, to subsequently travel to distant regions of the gastrointestinal tract.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Consideration of appropriate experimental strategies, design of specific experiments and relevant statistical analyses (as outlined in http://www.3rs-reduction.co.uk) will enable minimum numbers of mice to be used to address specific scientific objectives. Important issues include clearly defined objectives, planning specific types of experiments (e.g. pilot, exploratory, confirmatory), avoidance of bias, power analysis, control of variability and use of suitable statistical software.
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 because: (i) stem cells have been best characterized in these animals, (ii) they are the lowest susceptible vertebrate group, (iii) the inflammatory and physiological responses are sufficiently similar to those seen in humans, (iv) more reagents are available for the study of the biological and physiological responses in mice than for any other species and (v) a variety of genetically modified mice are available. Our previous studies using different protocols have enabled the identification of experimental conditions in which defined, predictable and consistent spectrum of relevant inflammatory and stem cell-related responses occur with the least welfare cost

	to the mice. Specific control measures and endpoints will be used in case of any unpredictable adverse effects or responses.

PROJECT 2	**Chararcterisation of the enteroendocrine axis**	
Key Words (max. 5 words)	Enteroendocrine, diabetes, obesity, GLP-1	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	Y	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Hormones from the gut control how the body responds to meals. They signal to the brain, to indicate what has been eaten. , and to the pancreas to prepare it to produce more insulin. How the gut hormone system detects food intake, however, and how the hormones act, are only partially resolved. The aims of this project are to provide basic biological knowledge about this gut-brain-pancreatic are sensed in the gut, how the hormone producing cells develop and function, and how they function at their target tissues. Through increased biological knowledge, we aim to identify new genes and pathways that control gut hormone levels or action.	
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)?	It is increasingly believed that altering the levels of gut hormones in the body will provide new treatments for diabetes and obesity. One strategy under development is to trick the body into releasing more of its own gut hormones, by altered diet or drugs. This approach requires us to know which signalling pathways are critical for hormone release. New biological knowledge is used by groups in academia and industry who are focussing on developing these	

	novel treatments. Understanding how the gut responds to food intake could also influence how we give dietary advice in the context of obesity and diabetes.
What species and approximate numbers of animals do you expect to use over what period of time?	All experiments will be performed in mice. The greatest usage (15,000 over 5 years) is the consequence of breeding and maintaining a number of transgenic and genetically modified mouse lines. This number includes mice produced as part of the breeding program, but that do not have usable genotypes. These are culled after genotyping, or used in procedures that do not require a specific genotype. Up to 700 mice will be injected with hormones to make them produce more egg cells, for generating, freezing and transferring embryos. Up to 200 mice may have embryos implanted so they can give birth to new mouse strains. Metabolic analysis of living mice will be performed on group sizes of 10-20, but is only performed for selected genes or targets that we strongly believe will affect the gut hormone axis. We estimate that 1500 mice will be required for this work.
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?	Breeding and maintenance of genetically modified mice should have no adverse effects on health, as most of our mouse lines carry only harmless markers. Imported strains that lack specific genes of interest will only be useful for our studies if the mice are otherwise healthy, so we will not be aiming to import mice that have a known health issue. We will, however, closely monitor the health of newly imported and generated mouse strains. Nearly all the mice produced by breeding the transgenic lines are used for tissue harvesting after killing by a schedule I method. Most in vivo analysis will be performed in terminally anaesthetised mice to prevent suffering. Some animals will be administered substances we predict will alter the secretion or action of gut hormones, but the effects on metabolism are likely to be subtle and evident only after challenging mice with a test meal or other similar procedure. Some metabolic assessments will be performed in free-living mice, that may be subjected to 2 different metabolic challenges with subsequent collection of sequential very small blood samples. As the same mice may also have other mild interventions during their lifespan, the overall severity of the protocols for these

	groups has been classed as moderate. All animals will be killed when they have completed all necessary procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We work with cell lines where possible, but have data showing that they do not always show the same behaviour as native cells. Some experiments must therefore be performed in native tissues. Many of our experiments are performed using tissue samples maintained in the lab, but for some experiments it is necessary to mimic the normal gut, in which food is placed on one side and the other is exposed to the bloodstream. This is not yet possible in the laboratory, and requires intact mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Mice will be bred at the lowest numbers possible to keep the different strains alive. Harvested tissues will be shared between different group members where possible, and maintained in culture for up to a week to enable the use of tissues from one mouse to be used for several experiments. Pilot studies will be performed to assess variability and time courses of effects, to optimise and minimise the final group sizes for metabolic assessments of live mice.
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.	There are many genetically modified mouse strains carrying markers in cells involving the gut hormone system. These are ideal for characterising the gut hormone system in tissue samples. When it is necessary to assess the gut hormone system in the intact intestine or animal, procedures will be performed under terminal anaesthesia whenever possible.

PROJECT 3	**Developing methods to treat liver disease**	
Key Words (max. 5 words)	Novel, treatment, bile, production, cells	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)	Y	Basic research
	N	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals

- **Summarise your project (1-2 sentences)**

In this project we aim to investigate the role of extracellular matrix in the development, cell differentiation and function of the liver. To use this we will use different types of natural and synthetic scaffold material for generating differentiated liver cells in order to assess whether functional liver tissue can be generated in culture.

- **Objectives: Explain why you are doing this project. Describe the scientific unknown(s) or clinical or service need you are addressing. Give a brief scientific background or other explanation of why the work is needed.**

Currently many liver diseases can only be treated by organ transplantation and there is an acute shortage of donor organs. Thus the object of research is to investigate whether special types of scaffold material can facilitate liver regeneration without organ transplantation. The function of the regenerated tissue will be assessed in the mice that mimics the human liver disease caused by the inability to secrete bile. Thus the main function assessed will be the ability of the regenerated tissue to secrete bile.

- **Outline the general project plan.**

Non-animal studies will be performed in order to develop the best methods for generating liver tissue in culture. Once the most adequate *liver* tissue is generated and tested in vitro, it will be tested in deficient mice, with engraftment being carried out in several locations before moving on to studies in humans.

- **Predicted harms: Give a brief description of the procedures to be applied to the animals used in this project and describe the expected adverse effects.**

Harms predicted are mostly surgery related, such as bleeding, infection and liver failure. Adverse effects may include infection or graft rejection, resulting in weight loss. Wild type mice will also be required for production of liver matrix and donor cells.

- **Predicted benefits: Outline in a few sentences how science will advance, or people or animals will benefit from this project.**

1 his project will advance understanding of how liver can be generated to replace organ donation in some of the patients with severe liver disease. It will also provide a method for researchers to test future cultured liver organoids in a physiologically relevant location within the mouse and thus provide translatable results.

- **Estimate the numbers of animals of each species to be used; explain what types of animal will be used and why you chose the particular types of animal. Explain how you will ensure that you use the minimum number of animals.**

1200 mice will be used. We have chosen to use mice for this project. This is because they are mammals and thus their basic biology is similar to humans — they contain the organ system of interest, the liver which is amenable to transplant and developing techniques that are easily translatable to humans. Mouse models of inherited cholestatic liver disease will provide a good platform for assessing the ability of a cultured liver organoid to help alleviate the symptoms of these disorders.

Our use of in vitro work stated above will limit the number of animals required for in vivo work. For each and every experiment, as part of good laboratory practice, we write an experimental protocol. This will include an outline of the method of analysis of the results in order to allow us to assess the minimum use of animals whilst obtaining statistically significant results. The experimental design and methods of analysis of the results for the proposed methods have been discussed with the Statistical Advice Service provided at UCL. Some of the measures we will be making are qualitative. For the qualitative experiments, the amount of material required will be the minimum necessary to provide an adequate description.

- **Demonstration of compliance with the 3Rs: State why you have to use animals and cannot use non-animal alternatives. Where appropriate, say how**

you will use non- animal studies in parallel with the project.

The objective is to develop an alternative to liver transplant for cholestatic liver diseases. Whilst a lot of preliminary work will be done in vitro, the cultured organoid must be tested for functionality in vivo before patient work is considered. This must therefore be carried out in animals, and therefore no alternative is suitable.
In vitro work will include differentiation of hIPSCs into liver cells, and to achieve accurate targeting of the differentiating cells onto the scaffolds.
Keeping the 3Rs in our mind, we have also included a special protocol to create an artificial liver scaffold, which if works properly then could be an alternative to biological liver scaffold and will significantly reduce the necessity of animal works.
If our project becomes successful then the isolated perfused cultured liver organoids would serve as a valuable tool for the ex-vivo toxicological and therapeutic studies and could be an alternative to the in vivo works.

- **Explain why the protocols and the way they are carried out should involve the least suffering.**

In the protocols, only up to 70% of the liver mass is removed, as the remaining 30%. is able to provide basic liver function in order to involve the least suffering.

<table>
<tr><td>PROJECT 4</td><td>Targeted therapeutic interventions for liver disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Liver, Inflammation, Fibrosis, Therapy</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)</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>Liver disease is the fifth most common cause of death in the UK and the only situation where death rates continue to rise annually. Thus a huge number of patients are currently awaiting liver transplantation. There are too few donor organs to fill the demand and new treatments are urgently needed. Our program of work is designed to understand the mechanisms which underpin liver disease and to test whether our new therapies can reduce disease.
Specifically our key achievable objectives are:
1. To investigate the contribution of white blood cell populations and platelets to the development and resolution of inflammation and fibrosis in solid organs
2. To define the molecular contribution of key adhesion molecules, chemokines and other proteins to disease pathogenesis and resolution.
3. To test the efficacy of targeted therapeutic interventions on inflammatory disease and fibrosis</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>Our data will help patients with liver disease and basic scientists and clinical scientists studying and treating disease. In particular we are testing drugs and treatments which could be used to target inflammation and fibrosis which underpin most chronic human liver disease. Thus the likely benefits are an advancement in our understanding of the molecular mechanisms of the most common types of liver disease, and more importantly</td></tr>
</table>

	preclinical testing of new anti-inflammatory and antifibrotic drugs which could be used in human patients. Data originating from these studies will be published in high impact scientific journals and presented at national and international symposia and conferences. Thus benefits from our work include transfer of knowledge, training opportunities for future clinicians and academic scientists as well as benefits in terms of new drugs for patients in the UK whose only option is currently transplantation.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse – up to 20K 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?	In the vast majority of cases when generating liver injury it is important to state that, similar to the situation in humans, this does not cause obvious external symptoms or suffering in the mice. Thus all of our protocols (with the exception of mouse breeding) are of moderate severity. In general we will need to measure serum biomarkers and look at liver tissue to detect injury. However significant weight loss (in the absence of any other signs of ill health or behavioural change) will be seen with some of the dietary models. It is possible that animals may experience discomfort from handling and administration of cells, anaesthesia or drugs. It is also possible that a small number of animals (less than 5%) may acquire infections post irradiation or surgery. All animals will be sacrificed by schedule 1 methods upon completion of the experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex disease pathways we are interested in involve the interaction of several cell types and regulatory signals that are hard to recreate *in vitro*. They can also cause simultaneous damage to organs other than the liver. Mice share the main components of their immune systems with humans, established liver disease models are available, and a wide range of genetically manipulated strains and therapeutic reagents are available. Thus they are the best model for us.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimize our use of animals we perform human studies where possible, and design our animal studies based on this experience. For testing drugs, we perform pilot studies allowing refinement of group size, and experiments don't progress if

	statistically significant results are not evident. We design experiments to run serially with outcomes from initial groups informing the design of subsequent experiments. Importantly, many of the molecular pathways we investigate operate in more than one organ and similarly the models we use (eg dietary fatty liver injury) can result in wider systemic disease (eg atherosclerosis). Therefore to maximise the useful information we can collect from each animal, we will collect blood, liver and other solid organs. These samples are used to investigate the wider significance of our pathway or therapeutic intervention.
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.	All experiments are carried out in accordance with best practise guidelines. We use well established protocols selected to accurately recreate the varied clinical scenarios, histological changes and biochemical picture seen in patients. The most common causes of liver disease in people that we wish to recreate in our murine systems are toxic injury, infection, obesity and diabetes and autoimmunity – all of which cause disease by different molecular mechanisms. Thus we have chosen several mouse models which most faithfully recreate these diseases. These models are either currently available in house or are commonly used and are deemed representative by the scientific and regulatory community. Scientists visit collaborators to learn best practise and keep up to date with best practise in the literature. Mice are the best model because: (i) the main components of their immune systems are shared by humans; (ii) a wide range of wild type and genetically manipulated strains are available; (iii) an extensive range of reagents is available for analysis of the cellular and molecular interactions All experiments will run in conjunction with humane endpoints to ensure animals do not experience discomfort or adverse events such as excessive weight loss or infection. We utilise body condition scoring and daily observation by both investigators and BMSU staff, and seek advice from our local vet as necessary. Animals giving cause for concern are humanely culled.

PROJECT 5	**Molecular mechanisms of stem cell activation**	
Key Words (max. 5 words)	Stem cells, epithelial, activation	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 purpose of this project is to understand the molecular mechanisms by which stern cells maintain adult tissue homeostasis and repair damaged tissues (e.g. liver). Since during tumour formation, similar mechanisms have to be put in place to activate the resting cells to start proliferating, understanding these mechanisms is crucial to improve our knowledge on the basics of cancer initiation.	
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 expected to provide novel information about the properties on how cells proliferate and its implication in tumour formation. Cell proliferation is essential for maintaining the homeostasis of tissues and for tissue repair. The balance between proliferating to repair a damaged tissue and finalizing that proliferation once the tissue is completely repaired needs to be tightly controlled to prevent the formation of tumours. We aim to understand how tissue repair is regulated and controlled and how deregulation of these processes results in diseases such as cancer. This project will increase our knowledge on tumour initiation and consequently could lead to the discovery of new targets and therapeutic strategies for treating cancers, such as liver or	

	pancreas, among others.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice —9100 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?	To study the role of stem cells during tissue homeostasis we expect *50%* of the animals to experience mild discomfort. This will be related to intraperitoneal injections of inducing agents and will last <1 day. From the studies of tissue regeneration, we expect the adverse effects to be mild. Mainly ~ 50% of the animals will be experiencing mild discomfort due to the induction of a repair response. As we always aim at the tissue to repair, this will last no longer than 48h. To induce tumour formation in the mice, we will be using inducible systems. Tumours will only be induced in adulthood in ~ 10% of the mice. This might result in moderate discomfort to the mice. Animals will be monitored daily and when tumours develop the animals will be humanly killed before the onset of any metastasis. In all cases, animals will be humanly killed after the experiments, either after induction of a tissue repair response or after generation of tumours. We will analyse the presence of particular phenotype by using the molecular, histological or culture techniques available (e.g. appearance of tumours, or tissue repair after damage induction)
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal studies are unavoidable if we seek comprehensive knowledge and understanding of gene function, physiology and pathology. The necessary animal studies in this Project will exclusively involve mice. In that regard, the knowledge and expertise accumulated from the investigation of the mouse is incomparable. Up today, there are no alternative methods to fully understand tissue regeneration and tumour initiation in the context of the whole organism. For the majority of the proposed studies, the mouse is

	the most appropriate animal model because: (i) it is a mammal; (ii) physiology is more extensively characterized in mice than in other mammalian model species; (iii) mice are amenable to transgenic manipulation; (iv) a large number of relevant transgenic and knock out lines are already available; (v) most of our knowledge in liver regeneration has been obtained from studies in mouse. Nevertheless, this Program will make extensive use of the organoid culture technology I have developed, which by itself extensively reduces the animal numbers. The use of this near- native culture system allows this project to comply with the 3Rs (replacement, reduction and refinement) by keeping the mice numbers to be used to minimum.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As mentioned, This Program will make extensive use of in *vitro* culture screenings to underscore potential candidate factors influencing cell proliferation and tumour formation, prior to test these into the animals. Also, as many of the procedures are very well established in the mouse, and don't require of additional experiments to test them in this animal model, using mice allows us to reduce the number of animals that would be required if setting up protocols would be needed. Finally, whenever possible, we will perform pilot studies with the minimum amount of animals possible, an example would be the validation of new alleles, or combinations of genotypes.
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	All studies in this Research Program will involve exclusively inbreed mice from different backgrounds. In that regard, the knowledge and expertise accumulated on resident stem cells from the investigation of the mouse is incomparable. The experiments will involve creating and analyzing transgenic mice and performing liver regeneration studies following chemical injury to the liver. Chemical injury can result in discomfort to

(harms) to the animals.	the animals that will last less than 48h. We will use the minimal doses that give any effect. When available, the drugs will be given orally, either supplemented on the diet or drinking water, to prevent any stress to the mice. Any animal in distress will immediately be euthanized. Finally, the use of chemical injury compared to other more severe forms of injury as, e.g. hepatectomy, allows the animals to be kept in groups, not isolated in cages, which adds to their animal welfare.

<table>
<tr><td>PROJECT 6</td><td>Podoplanin-CLEC-2 in inflammatory bowel disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>CLEC-2, Podoplanin, inflammatory bowel disease</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)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</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>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</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>To determine whether colitis is regulated by the Podoplanin-CLEC-2-Syk pathway</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Inflammatory bowel diseases including Crohn’s disease and ulcerative colitis have no current treatment. This project aims to investigate whether the Podoplanin-CLEC-2-Syk axis regulates acute or infection induced colitis.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Over 5 years, we would expect to use no more than 3,100 animals in total - 900 animals for scientific protocols and 2,500 to breed the genetically altered strains required.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>Administration of tamoxifen via diet – we expect moderate weight loss (15-20%) and reduced</td></tr>
</table>

what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	activities of the mice within the 2 weeks, which will be reverse upon cessation of this altered diet. Animals will be monitored daily for clinical signs such as ruffled coat and hunched posture. Although we do not anticipate this, if these are observed and prolonged (>24hrs) mice will be killed humanely. Administration of tamoxifen via 5x daily IP injection – we expect weight loss of moderate severity within 6 days. This will be accompanied by reduced activities and a ruffled coat. Animals will be monitored daily and more frequently at the critical days, 4-to-6. If mice that have lost weight (up to 20%) are observed with consistent lethargy and hunched posture (more than 6-8hrs) which cannot be remedied promptly and successfully using minor interventions such a warming, mice will be humanely killed. From our previous experience, we expect to humanely cull approx. 10% of animals due to weight loss of more than 20%. Administration of Dextran Sodium Sulfate (DSS) via drinking water and oral gavage of *Citrobacter rodentium* – we expect adverse effects indicative of intestinal inflammation of a moderate severity such as weight loss, diarrhoea, bleeding within the colon and abdominal discomfort. Mice will be observed and weighed daily and a clinical scoring system used to monitor progression of the intestinal inflammation. If an animal has a cumulative clinical score of > 9 it will be humanely killed. In addition, a pain score based on facial expressions will be used. If alterations are observed of a moderate nature analgesic will be administered by sub. cut. injection. If a severe pain score is observed and cannot be remedied by analgesia within 2-3 hours, mice will humanely killed. At several stages of inflammation (e.g. peak infection, or late infection/clearance) animals will be killed humanely to determine the role of the Podoplanin-CLEC-2-Syk pathway at specific inflammatory stages.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	No *in vitro* techniques are currently available that can fully replicate the complex spatial and temporal interactions within the colon. Currently no *in vitro or ex vivo* methods exist to model a full immune response including intact vascular and lymphatic supplies. In order determine whether the increase in lymphatic Podoplanin and/or platelet abnormalities observed in inflammatory bowel disease (IBD) patients drive the pathology of IDB, animal studies are required.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Statistical analysis to ensure that we use the minimum number of mice per group that will be informative will be performed. Statistical analysis from previous studies suggests group sizes of 10 are required to measure significant differences in clinical outcomes (power = 0.9). To maximise the information gained from a single animal we aim to take samples from the blood under terminal anaesthesia and then from multiple body sites post mortem.
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 immune system of mammals is highly conserved with cell types and mechanisms well-maintained. The mouse has been selected because of established and reliable transgene technology and extensive literature on colitis models in murine strains with established and reproducible protocols due to the reliable reagents available. We have generated transgenic mice that do not display any external adverse signs either before or after candidate gene deletion is induced up to six months of age as compared to their littermate controls. Inducible transgenic strains will be activated by the most refined interventions possible to minimise

	stress and pain. We will use pilot studies containing no more than 20 mice per genetically altered mouse strain to determine optimal dosing of DSS and *C. rodentium* so as to minimise animal suffering. By choosing well established protocols to induce colitis we minimise the unknown effects on the mice and subsequently pain, distress and suffering. We will use clinical scoring indicative of the extent of inflammatory bowel disease to minimise the time symptomatic animals are kept. In addition, a pain score will be used to guide analgesic administration and thus reduce pain. Animals that have modified biological pathways that are uncharacterised in terms of their response to inflammatory bowel disease will be observed more frequently to reduce potential suffering. Animals will be given glucose-saline to reduce diarrhoea-induced dehydration. Any additional substances given will be done in the most refined manner possible to reduce stress and pain to the animal.

<table>
<tr><td>PROJECT 7</td><td>Mouse Models of Hepatic Fibrosis and Therapeutics Investigation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Mouse, Hepatic, Fibrosis</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)</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</td><td>Yes</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>Project aim:
Examine and determine biological factors associated with the initiation and progression of hepatic fibrogenesis.

Specific areas of interest include:

The role of the coagulation cascade in the initiation and progression of hepatic fibrogenesis and the testing of novel or potential therapeutic agents for the treatment of hepatic fibrosis.

The identification and role of signalling pathways of the innate immune system in the initiation and progression of hepatic fibrogenesis and the testing of novel or potential therapeutics agents for the treatment of hepatic fibrosis.</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)?	Models of human liver disorders will facilitate investigation of the pathogenesis of liver disease. Identification of key susceptibility genes for liver disorders and the associated biological pathways (or vice versa). Further our knowledge of liver disease in man and have clinical impacts on the prevention and management of patients with these disorders. Allow testing of potential therapeutic agents for liver fibrosis.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, inbred and genetically altered. 4000 animals 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 overall severity of the protocol is moderate and therefore it is suspected that adverse effects will not exceed the criteria this limit defines (as set out by the Home Office and LASA guidelines). Any animals demonstrating adverse effects will be reviewed in a timely fashion. Animals exceeding the humane endpoints set out in the protocol will be culled by a schedule 1 or non-schedule 1 method as describes in the protocol. Any animal demonstrating adverse effects that do not exceeding the humane endpoints of the protocol will be discussed with a NACWO/NVS and a management plan documented and put in place. For example, the induction of chronic liver injury can lead to the development of liver failure however this will be avoided by careful monitoring of animals for signs of advancing liver disease. Animals that are thought to be developing advanced liver disease will be humanely culled prior to the development of liver failure (signs of which may include ascites, jaundice, bleeding). Animals undergoing whole body irradiation and bone marrow transplantation will be monitored for

	signs that the bone marrow transplant has not worked (persistent lethargy >48hrs, general ill health). This can be verified by peripheral blood sampling and if it is thought that the transplant has failed the animal will be humanely culled. Outside of the immune system the gut is the body system most susceptible to irradiation, as a result mice may develop diarrhoea and/or lose weight due to poor absorption of nutrients from the damaged gut or loss of appetite. To combat this mice will be given wet mash and pellets placed on the cage floor (to encourage eating) in the immediate post-irradiation period and, if needed, cages will be cleaned at more regular intervals if there is heavy soiling. Animals with diarrhoea that persists for >24hrs will be humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Physiological and pharmacological studies related to chronic liver injury are hampered by the absence of a realistic model of the disease process. Cell culture provides a useful system for examining receptor responses and activation parameters in an artificial system. Liver injury, however, develops as a result of complex interactions between multiple cell types, which cannot currently be replicated in vitro. The research group is working in collaboration with groups across the country and internationally that are developing in vitro models aimed at recapitulating the complex multicellular interactions of the liver. The research group has access to a tissue bank containing consented human liver tissue and where possible this is utilised. However, human studies are limited by the lack of control over environmental factors, wide genetic variability and the inability to obtain the required volumes of liver tissue at controlled time points required for accurate scientific experimentation. Animal models thus provide the only effective mode of studying the genetics and physiology of chronic liver injury. In this project mice will be the only

	animals used as they represent the lowest vertebrate able to display phenotypes with physiological similarity and genetic homology with humans.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals will be used in all experimental groups, as determined by comparable published literature and statistical calculation of numbers required for statistical significance testing (power calculations). There will be careful planning of experimental timing to minimise the breeding of excess animals and the use of control animals. Harvest of tissue/samples will be planned to try to ensure that all analytical tests can be carried out, preventing/minimising the need for repetition of procedures/models detailed in the 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.	The mouse is the lowest vertebrate that shares enough genetic, anatomical and physiological homology with the human and is therefore of uses for the purpose of modelling many human disorders. The mouse genome/phenome has also been extensively mapped and a huge number of genetically altered strains have been developed that aid the investigation of human diseases. The mouse displays common phenotypic and pathological features of human chronic liver injury. When subject to experimental procedures the mouse develops patterns of chronic liver injury seen in human disease including induction of chronic inflammation, activation of hepatic stellate cells, formation of fibrosis and liver synthetic dysfunction. Chronic liver injury can ultimately lead to end stage liver failure where animals display signs of marked synthetic dysfunction (e.g, coagulopathy), encephalopathy and multiorgan dysfunction. However the aim of this project is to investigate the initiation and progression of chronic liver injury prior to end stage failure and therefore experimental design will be to induce chronic liver injury but not

	end stage liver failure. If any animals demonstrate signs of end stage liver failure they will be humanely culled and the experimental protocol discussed with the NVS and NACWO to determine further refinements.

<table>
<tr><td>PROJECT 8</td><td colspan="3">Modification of gut function by dietary micronutrient intake</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Diet, micronutrient, metabolism</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3"></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</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">Some nutrients needed in the body for optimal health are required in very small amounts so-called micronutrients such as folates, zinc and selenium (Se). Some evidence suggests that low micronutrient intake is associated with increased risk of diseases such as cancers. There is a need to understand the metabolic roles of these micronutrients and whether genetic variation in the human population leads to differences in metabolism and disease risk.

The objective will be to focus on the micronutrient selenium and genetic variations which influence selenium metabolism. At present there is little understanding of the effects of these variants, especially in combination with altered dietary intake, on tissue function. The lack of such information is a bottleneck in understanding how</td></tr>
</table>

	dietary Se and genetic differences influence cancer risk; this work aims to provide such information.
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 will provide knowledge of if, and how, genetic factors and dietary selenium intake interact to affect gut function. This knowledge will improve understanding of potential benefits of increased selenium intake in humans. This is important because Se intake is low in UK and Europe. Low selenium intake has been linked to increased cancer and cardiovascular disease risk so knowledge of the effects of metabolically related genetic variations should lead to better disease prevention.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. 200, 3 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?	Genetically altered mice expressing a gene region that affects selenium metabolism will be used in these experiments. No adverse effects are anticipated but animals will be closely monitored in terms of general well being and behaviour, food intake and weight gain. The mice will be fed diets suboptimal in selenium(NOT deficient) and so acute health effects will be minimal. Thus level of severity is mild. Animals will be humanely killed at end of experiment and tissue samples collected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Use of animals allows the study of how genetic factors and dietary micronutrients interact in the context of normal tissue and body physiology and over a physiological range of micronutrient intake. Cell culture systems do not allow studies in a physiological situation of multiple cell types in a tissue and multiple tissues in the body.
2. Reduction Explain how you will assure	The number of animals will be based on previous work using diets containing marginally low selenium levels. In this work it was found that 12 animals per

the use of minimum numbers of animals	group gave sufficient power for effects on gene expression in the colon to be detected at $p<0.01$ level of statistical significance. We will therefore use 12 mice per group; with 2 diets and mice of 2 different genotypes this will mean that 48 animals are used per experiment. We plan a maximum of 4 experiments so that repeats with different dietary levels of selenium can be carried out if necessary. Thus a maximum of 200 mice will be 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.	Mice are the most appropriate species because of the availability of genetically altered mice with the relevant genetic variants. GA Mice will be bred and maintained, in order to provide animals for experimental use. Offspring will be kept and fed diets containing what are regarded as being either optimal or sub-optimal levels of micronutrients such as selenium. These dietary regimes are standard protocols that lead to no known animal suffering We do not anticipate adverse effects from the genetic modifications used. However, during breeding and during the dietary experiments animals will be closely monitored in terms of general well being and behaviour, food intake and weight gain.

<table>
<tr><td>PROJECT 9</td><td>The microenvironment in organ homeostasis and cancer</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Liver, skin, cancer, stem cell</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)</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</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>Many adult organs such as the skin, intestine and liver can regenerate from adult stem cells. These adult stem cells are found in a complex environment which contains other cell types, known as the microenvironment or niche. This microenvironment is important in making sure the stem cells divide appropriately and that they either stay as stem cells or become more specialised cell types, liver cells or skin cells for example. Recently it has been found that stem cells in adult tissues are the origins of cancer, and that they become uncontrolled and divide excessively and rather than forming liver or skin they form tumours. What we don’t understand is whether the microenvironment is important in this process. We intend to look at the stem cell microenvironment and ask whether it can promote stem cells to become cancerous.</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)?	This project will allow us to identify what proteins are produced by the cancer microenvironment and ask how they force stem cells to grow in an uncontrolled way to form a cancer. Using this knowledge we will be able to use existing drugs and also design new drugs to prevent this uncontrolled growth and thereby reducing cancer.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and rats during this study. We expect that we will use a maximum of 20000 mice and 500 rats over five years. The questions we are trying to answer require the use of genetically altered animals in which we can specifically change the DNA of the mouse to change how Cancers grow.
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?	In our hands the mice and rats we use will develop liver or skin cancer. We manage these cancers closely, and normally they are not end stage tumours, so they do not give metastasis. The animals with these cancers can lose weight, but this is closely monitored and managed carefully. The adverse effects in these models are moderate; they do not cause high levels of pain. Once the animals have developed cancer, and have been treated with our drugs they will be humanely euthanized and we will remove the cancers to investigate how our drugs work in order to better understand how cancers grow and how we can improve our drugs in the future for patients. In some cases we will irradiate the mice, which allows us to replace their bone-marrow with that of another mouse. We take precautions to prevent these mice becoming sick by keeping their air supply, food and drinking water pathogen free until they have recovered from the bone marrow transplantation and have a reconstituted immune system.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cancers are very complex and consist of lots of different types of cells. We use animal models of cancer because they accurately reflect this. It is very difficult to model these complexities in the lab

	without using animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We are able to calculate the minimum number of animals required to see whether our experiments are working or not. We always use the minimum number of animals in each study. My lab has also worked-up tissue slice culture. This means we can take tissue like liver and slice it very thinly. We can then test our drugs on these slices to see if they are effective before taking them into animals, this means we are able to reduce the number of animals we use as we have some idea of the effects of our drug.
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 use mice and rats as they are good models of human disease. Both liver and skin cancer arise following lots of damage and repeated injury. Mice and rats follow the same pattern of long term disease and then cancer. To minimise harm to the animals we only use models which give us good reproducible results without causing other, unnecessary illness to the animal. We also strive to refine our protocols, so if a newer version is published by another laboratory which clearly demonstrates refinement then we will adopt this protocol.

<table>
<tr><td>PROJECT 10</td><td colspan="3">Imaging of Stem Cells In Organ Fibrosis Models of Tissue Regeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Liver, kidney, stem cell, regeneration, imaging</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 section 5C(3)</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">Liver and kidney disease in the UK is increasing in prevalence. Stem cells have the ability to regenerate tissue but need more rigorous testing before being applied to humans. The aim of this work is to use novel non-invasive imaging techniques to find out 1. If imaging can be used to detect organ fibrosis and at what stage, 2. If imaging can identify the distribution and life span of transplanted cells for optimal application to organ injury and identify dosing regimens, 3. Use these imaging developments to understand how non-parenchymal cells contribute to organ regeneration and scarring. By using imaging we can develop translatable methods with better designed trials with the ultimate goal of preventing disease and enhancing repair in the liver and kidney; thus eliminating the requirement for transplantation</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)?	The benefits of this work primarily focus on liver and kidney disease and the treatment of such. The impact of techniques for the development of tracking stem cells by imaging has a far wider benefit as stem cells have the potential to be utilized in curing a vast number of diseases. The translation of the novel imaging methods to assess liver and kidney disease can also be applied to other diseased tissues so our work will contribute to disease progression in a range of organs.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice and over the five years we will use a maximum of 4500 animals.
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 we are modelling disease our animals will progressively demonstrate signs of the disease. However using imaging we can closely manage these signs and generally can define disease at a much earlier stage and therefore we ensure that the animals do not undergo any undue suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The regeneration of organs specifically at different disease stages is complex and involves the interaction of the stem cell with many different cell types within the organ and this is what needs to be understood before use in humans. It is impossible to model such complexity without using animal models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	It is possible to calculate the numbers of animals required for experimentation based on previous data. Imaging lets animals be used as their own control, allowing paired comparisons, and imaging is inherently sequential, increasing statistical power and using fewer animals to achieve the same statistical power as conventional designs. In all cases we ensure that we have calculated the minimum number of animals required for the experiment to give us useful data. This approach also reduces the likelihood that the animal

	experiment would have to be repeated.
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.	Using imaging we regularly refine the disease models we use to reduce animal harm and to improve the effectiveness of our models. We can also stage disease and stop experiments before external clinical signs appear, thus limiting disease severity. We also regularly monitor body weight, body condition, food and fluid intake of animals as a measure of disease; we set strict limits to ensure that there is limited harm to the animals used.

<table>
<tr><td>PROJECT 11</td><td>Study and treatment in liver ischaemia reperfusion</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ischaemia:reperfusion; mesenchymal stromal cells; liver</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)</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 (i) to establish a mouse model which recreates the situation found in patients after Donation after Cardiac Death (DCD) liver transplantation, and (ii) to test mesenchymal stromal cells (MSC) in this model to establish if they reduce the extent of liver damage.

This will allow for a better understanding of the processes occurring in this setting and also establish the effectiveness of MSC as a cell therapy.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>This project will provide important data that improve our understanding of the changes that occur in DCD liver transplantation and also insight into the effect of cell therapy in this setting.

This will underpin new clinical trial submissions to the MHRA. Evidence of action and safety of cell therapy from appropriate mouse models will be a key part of submission to the MHRA.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice
800 over 5 year period</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>Potential harm results from the induction of liver damage. In the vast majority of cases when generating liver injury it is important to state that it does not cause overt symptoms of suffering in the mice.</td></tr>
</table>

end?	There are clear guidelines in place in our facility to ensure that suffering in mice is minimised by either administration of pain-killers, or termination of experiments. Administration of cells has the potential to cause irritation at the injection site mice although in our extensive experience this has not been the case. As with the induction of injury we remain vigilant for any adverse effects and will follow unit guidelines in the event they occur. There is also likelihood of transient pain from laparotomy which will be controlled with appropriate pain-killers. Animals will generally be killed under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The actions of cellular therapies are complex often involving assessment of their homing to the site of injury, as well as their interaction with multiple inflammatory cell types in the body. These interactions cannot be assessed in in-vitro assays.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Use of inbred wild-type mice will reduce variability in clinical outcomes. This serves to reduce the numbers of mice needed. Phase 1 studies and pilot studies in Phase 2 will define the variability in the model which will inform the numbers needed as part of a power calculation. This ensures that the correct number of mice are used, thus both potentially reducing the numbers of mice as well as ensuring the scientific validity of the experiment.
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 will closely resemble the features seen in DCD liver transplantation and thus provides useful information when translating the work into clinical studies. Suffering will be reduced by the continued adoption of BMSU guidelines. These ensure that side-effects are looked for and mice euthanased when defined end-points are met. In general these end-points have seldom been met with mice studied on my other licence. Where at all possible mice will be killed by Schedule 1 methods to reduce suffering.