Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 1

Projects with a primary purpose of: Translational and Applied research – Cardiovascular Disorders

Project Titles and Keywords

1. **Preclinical evaluation of ex vivo generated blood cells**
    - Stem cell expansion, cultured blood

2. **Understanding maladaptation in the failing heart**
    - Heart failure, signalling

3. **Heart transplantation using Circulatory Determined Death donors (DCD)**
    - Heart, Transplant, Circulatory Determined Death

4. **Inflammation and Arterial Disease**
    - Inflammation, heart, artery, diet

5. **Microbiological and immunological aspects of equine periodontitis**
    - Horse, periodontitis, tooth loss, bacterial gene sequencing, immune response.

<table>
<tr><td>PROJECT 1</td><td colspan="3">Preclinical evaluation of ex vivo generated blood cells</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Stem cell expansion, cultured blood</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td colspan="3"><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[2]</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">The UK blood services play key roles in treating patients with blood disorders, serving more than 30 million individuals. Unfortunately, for some patients the transplants are ineffective, often due to insufficient numbers of stem cells in the transplanted material. In addition, there are supply shortages of red blood cell for transfusions in many countries. Individuals who require regular blood cell transfusions are at risk from transfusion transmitted infections and adverse reactions from receiving mismatched blood. The ability to expand the number of haemopoietic stem cells that are present in readily available sources such as cord blood and manufacture cultured red blood cells from stem cells are important steps towards solving these</td></tr>
</table>

	problems. Cultured blood cell products are likely to contain higher proportions of stem cells than those from blood and stem cell donors. They may, therefore, provide clinical advantages by surviving longer and performing better. We have been developing methods for clinical grade expansion of stem cells and manufacture of red blood cells. This study proposes to perfect these procedures and assess the effectiveness of the cultured blood cell products in an animal model, in order to gain regulatory approval for use in human volunteers.
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)?	Increasing the number of stem cells transplanted should result in replacement of malignant cells with normal blood cells and improve survival and quality of life for these patients. Likewise transfusions of artificial red blood cells for patients who are dependent on regular blood cell transfusions will reduce the risks associated with mismatched blood and those of transfusion transmitted infections.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use immune deficient mouse species that are the most suitable for evaluation of human blood cells. Over the 5 years of the project the proposed work will use no more than 3300 mice including those animals used for the purposes of breeding.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We will inject expanded human blood cells into mice to determine whether these cells can establish and mature into fully functional blood cells. We will monitor progress by examining animals and by removing blood samples in order to identify the presence of human cells. At the end of the study, animals will be killed and we will undertake a post mortem examination in order to find out which tissues/organs the transplanted human cells established in.
Application of the 3Rs	
1. Replacement State why you need to use	Although assessing stem cells in cell culture can provide a lot of useful information, we need to study how they behave in the complex environment of a

animals and why you cannot use non-animal alternatives	living animal. It will be important to determine how long these cells survive in a living animal and whether they can mature into fully functioning blood cells that become established in this environment. As a result it is necessary to take studies in living animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Much of our work is undertaken in the laboratory to generate cells that can be used for stem cell transplantation and for blood cell transfusions. The ability of the cells to divide and mature into functioning blood cells will be investigated by cell culture. Consequently, only products that can expand and mature in the cell culture systems will be used in the animal models. The laboratory-based experiments will provide essential information as to the suitability of the expanded cells and the best techniques used to generate them. This will allow us to significantly reduce the number of animals used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use mice that have an underactive immune system, so that when we inject human cells into them, the mice do not recognise them as foreign and the cells become established and grow. The species chosen are the most compatible for evaluation of human cells and their growth. We make use of good experimental techniques with minimal intervention to avoid distressing the animals, expert preparation of samples for investigation, strict adherence to protocols and keeping the time for which an animal is under experimentation as short as possible.

<table>
<tr><td>PROJECT 2</td><td>Understanding maladaptation in the failing heart</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart failure, signalling</td></tr>
<tr><td>Expected duration of project</td><td>5</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></td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To understand the reason that heart failure develops after an initial event like a heart attack or high blood pressure</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>Nearly 1 million people in the UK have heart failure and half of these will die within 5 years after diagnosis. At the moment there is no cure for heart failure because we do not understand how the disease progresses. The aim of this work is to describe the way that the sub-cellular organisation of the cardiac muscle cell changes in heart failure with a view to pinpointing a target that we could manipulate to reverse these detrimental changes</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>Rats: 450 in total

Mice: 750 in total</td></tr>
</table>

over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Most animals will undergo surgery but this will be associated with minimum discomfort; animals will be anaesthetised throughout and given pain relief during the post-operative period. As heart failure develops, the animals may become a little lethargic and breathless, but we monitor heart function by echocardiography to ensure that heart failure does not because too severe. All animals will be humanely killed before they reach a stage of severe heart failure. Many different types of data will be obtained from each animal (heart and cardiac cell function, muscle biochemistry and structure).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Heart failure is a complicated process that involves hormones and nerves. It is not possible to properly replicate the disease process in a cultured cell in the dish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will perform calculations that tell us the minimum number of animals required in order to be able to test our hypothesis. For every animal we will derive the maximum amount of data that we can from it. We will also share tissue from these animals with other scientists who are interested in skeletal muscle and the brain/nervous system.
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.	Mammalian species are required to properly replicate the process of heart failure seen in man. Rats and mice have similar genes and similar cardiovascular function to man. Much work has been previously performed in these species to support the hypothesis for our research. Suffering of the animals will be minimised. All surgery is performed under anaesthesia. Pain relief will be given during the post operative period. Animals will be monitored very closely and our local veterinary officer will advise about acceptable levels of symptoms. If any animals show symptoms beyond this level they will be humanely killed.

<table>
<tr><td>PROJECT 3</td><td>Heart transplantation using Circulatory Determined Death donors (DCD)</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Heart, Transplant, Circulatory Determined Death</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>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</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>The aim of the project is to study the short term function of the transplanted Donation after Circulatory Determined Death (DCD) heart. Currently the scientific unknown is whether the DCD heart is capable of supporting function within the first week of being transplanted.</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 number of heart transplants performed in the UK has declined significantly over the last ten years due to a shortage of brains stem dead donors (BSD). Despite this decline the number of patients on the waiting list continues to grow. This increasing supply and demand mismatch results in approximately 10% of patients dying whilst waiting for a heart with only 43% of listed patients ever being transplanted. We are currently investigating a relatively new type of donor called the Circulatory Determined Death donor (DCD). These are donors who’s hearts have stopped before their organs have been procured. This new type of donor has successfully been used in liver, kidney and lung transplants. Our previous work reveals that some of these donors may also be suitable as heart donors. We suspect that if we manage to use 10% of the current number of DCD donors as heart donors then the number of heart transplants in the UK will double.</td></tr>
<tr><td>What species and approximate numbers of</td><td>Approximately 150 pigs over a one-year period.</td></tr>
</table>

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The donor animals will be created under general anaesthesia from which the animals will not be allowed to recover. The few recipient animals will be under anaesthetic for the first 12 hours following transplantation and will be monitored of complications such as bleeding, stroke, rejection and poor cardiac function. Only animals that exhibit no signs of these complications will be allowed to recover. These animals will be monitored intensely for one week and then euthanised by a Schedule 1 technique. Due to complications that can arise within the 12 hours following transplantation whilst the animal is under anaesthetic a severe licence has been applied for.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to prove that it is possible to transplant and use DCD hearts we must show that they have restored sufficient function to be able to support the circulation. Due to the complexity of the circulatory system live animals need to be used.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have previously transplanted pig DCD hearts in Canada and have expertise related to this. We routinely perform heart transplantation in humans and therefore plan to minimise the number of animals lost due to technical error.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen the pig model as they are very similar in size, anatomy and physiology to the human. The method of resuscitating the donor heart, transporting and assessing the donor organ has already been optimised in the small animal rodent model. Welfare costs have been minimised by keeping the transplanted recipient animal anesthetised for the first 12 hours. In human heart transplants this window is when the majority of complications arise. Only animals that show no signs of complications during this time period will be allowed to recover.

PROJECT 4	**Inflammation and Arterial Disease**
Key Words (max. 5 words)	inflammation, heart, artery, diet
Expected duration of the project (yrs)	
Purpose of the project (as in section 5C(3)[7]	Basic research Yes ~~No~~ Translational and applied research Yes ~~No~~ Regulatory use and routine production ~~Yes~~ No Protection of the natural environment in the interests of the health or welfare of humans or animals ~~Yes~~ No Preservation of species ~~Yes~~ No Higher education or training ~~Yes~~ No Forensic enquiries ~~Yes~~ No Maintenance of colonies of genetically altered animals[8] Yes ~~No~~
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of the project is to understand the biological mechanisms occurring in artery walls that ultimately lead to heart attacks. The focus of these studies will be on molecules that drive inflammatory processes in artery walls. Inflammation is thought to play a key role at all stages of disease but there are no specific treatments available yet that target these in man.
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 research will lead to a greater understanding of the key molecules that control inflammation in diseased artery walls. From this research we will be able to pinpoint the pathways and individual molecules that could be targeted directly or with repurposed or new drugs/treatments as a prelude to first in man studies.
What species and	We will use mouse preparations of atherosclerosis

approximate numbers of animals do you expect to use over what period of time?	from approximately 1500 mice over the course of a five years to undertake these studies.
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 atherosclerosis preparations where high fat diets are used have adverse effects of greasy fur and skin irritation which are of mild-moderate severity. The atherosclerosis treatment preparations e.g. use of metal cages called stents are technically challenging procedures so the adverse events relate largely to the surgical procedure and are of moderate severity. In preparations where the impact of treatments upon recovery after heart attack is studied, the adverse effects relate to the technical difficulty of the method used to create the preparation as well as to the efficacy of the treatment leading to likely moderate/substantial severity. All the animals are humanely killed at the end of procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As well as biological mechanisms, we wish to study physiological consequences e.g. blood pressure, ECG and cognitive consequences of atherosclerosis. This is only possible in a whole animal setting.
2. Reduction Explain how you will assure the use of minimum numbers of animals	As much information as possible will be gleaned *in vitro* before proceeding to mouse preparations. We will keep our experimental design and power calculations for group sizes under review to ensure that the minimum number of animals is used. These will be revisited each time a new individual study plan is prepared.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to	The mouse offers the best benefit/cost ratio for these proposed pre-clinical studies of atherosclerosis and its consequences. We continually refine our models and are fortunate to be able to have the technical expertise to deploy the most appropriate of these to address our objectives. General measures to minimise welfare

minimise welfare costs (harms) to the animals.	costs are the use of ventilated caging especially where use of anti-inflammatory treatments is studied, individual study plans and health/welfare recording for each mouse during the more complex procedures.

<table>
<tr><td>PROJECT 5</td><td>Microbiological and immunological aspects of equine periodontitis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Horse, periodontitis, tooth loss, bacterial gene sequencing, immune response.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>3 years</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>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[10]</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>Our knowledge of the causes of gum disease in horses is very poor, even though this is a very common disease and causes severe pain and tooth loss. Research into this important disease has been ignored for decades. It is likely that bacteria play an important role in the disease, as is the case in the human form.

We shall use the most cutting-edge laboratory method available for the genetic analysis of oral bacteria to provide an in-depth understanding of the types of bacteria that cause gum disease in some horses but not others. As well as detecting known types of bacteria, we can also identify bacteria that cannot be grown in the laboratory, including new types which have not been discovered previously</td></tr>
</table>

	and which may contribute to causing the disease. We shall also look at how these bacteria interact with the immune system of the horse.
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 study will improve enormously our knowledge of the microbes which inhabit the healthy and diseased oral cavity of the horse and improve our understanding and treatment of oral disease in the horse. Knowledge of the bacteria associated with gum disease could ultimately lead to the development of improved strategies for the eradication of infecting bacteria in affected and susceptible horses, including systemic antimicrobials, local oral therapies and immunological therapies. If specific bacteria are shown to be involved in the disease, then vaccine development is a real possibility. This will aid in improving the oral health of the horse population, reducing oral pain and tooth loss, with obvious welfare improvements, and this will of course be of significant benefit to horse owners and veterinarians. Another reason it is important to understand how and which bacteria can cause disease in the mouth of horses is because it is highly likely, as has been shown for humans, that these bacteria can spread via the bloodstream to cause serious diseases in other parts of the body.
What species and approximate numbers of animals do you expect to use over what period of time?	We need to use horses since we are investigating a spontaneously occurring disease in this species but by using clinical cases we are avoiding the necessity to create a disease model in normal healthy horses, thus avoiding unnecessary pain and suffering. We need to study 20 horses with dental disease and 20 without, over a 3 year period.
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	One of the studies we need to do in order to understand the immune system's response to gum disease and why it is not able to resolve the disease in many animals, is the collection of a gum biopsy which requires a Home Office licence. Collecting the biopsy which is only 2mm in diameter is a very innocuous procedure and it will be

end?	collected when the horse is anaesthetised for treatment for its primary problem which may be dental or some other problem (many cases of gum disease are not recognised by the owners and only become apparent when the mouth is examined under anaesthesia by the attending veterinarian). We anticipate no adverse effects with collecting the biopsy.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study can only be performed on animals since we are using clinical cases to investigate a complex spontaneously occurring disease. Periodontitis and tooth loss, as in people, involves a complex interaction between the microbial flora within the oral cavity and the patient's immune system and an imbalance between the microbial flora found in the healthy mouth and the diseased mouth. This is further complicated by the effects of diet and management. The unravelling of such a complex disease means we need to study clinical, naturally occurring cases.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of horses we are using is based on a statistical calculation.
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 study can only be performed on horses since we are investigating a spontaneously occurring disease specifically related to this species. By using clinical cases we will avoid having to create a disease model in normal healthy horses. We are also using tissue culture studies to supplement the clinical case studies. These techniques are a substitute for using live animals and will provide the additional information we require to assess the equine immune system. Any horse having a biopsy will receive post-operative analgesia both locally (anaesthetic gel) and systemically as part of normal hospital practice.

	Because we are using clinical cases, they will be subjected to all the protocols that are used in a specialist equine referral hospital, relating to husbandry, care, welfare and pain control.