G: NON-TECHNICAL SUMMARY (NTS)

Please attach the Non-technical Summary as generated by your application in ASPeL.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

This project will identify genes involved in cardiovascular disease and will determine their role in both the healthy and diseased cardiovascular system. In particular, there is a need to understand the genes involved in the development heart failure because current treatments are not effective.

Where our knowledge of the fundamental role of particular genes in the cardiovascular system is more advanced we will provide new insights in developing treatment options for heart failure by testing whether drugs which affect the action of these genes are able to halt/reduce the development of heart failure and its associated diseases. We will also determine whether treatment with genetically modified stem cells can improve cardiac function following a myocardial infarction

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)?

Since we are directly studying the relevance of these genes in established animal models which mimic the most prevalent human cardiovascular diseases it is anticipated that our studies will provide novel information regarding such genes and reveal potential new targets for the treatment of heart failure and associated diseases such as high blood pressure, arrhythmias, and heart attacks. The ultimate aim is to develop more effective treatments for heart failure and its associated diseases such as high blood pressure, arrhythmias, and heart attacks. By characterising the role of genes in the healthy and diseased cardiovascular system this project will provide an essential link between basic scientific research and future medical treatment because once we know the identity of the proteins and pathways responsible it may be possible to develop treatment options to either reverse/enhance their detrimental/beneficial effects.

What types and approximate numbers of animals do you expect to use and over what period of time?

During this 5 year project we expect to breed approximately 12,500 mice. The majority of these mice will have a genetic modification which will enable us to study the role of our genes of interest in the cardiovascular system. We expect that of these mice 4,150 will be used in regulated procedures, tissue/blood will be collected from an additional 3,500 mice. Mice are used in this project since these remain the most suitable animals for genetic modification experiments. As the main focus of the described work is to study gene function in the cardiovascular system mice have been chosen because the anatomy and physiology of the heart and vasculature is both well documented and physiologically similar to humans.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

To investigate the role of a gene in cardiovascular health and disease we will breed mice carrying mutations in the genes we are studying. Genetically normal mice will be bred to act as control animals for the experiments. To determine the effect of the gene on cardiovascular performance mice will undergo a series of tests which mimic those performed by a cardiologist on human patients eg heart rate and rhythm will be measured by ECG, blood pressure recorded, cardiac ultrasound used to assess the structure and pumping of the heart. This series of tests has a mild severity as there is no significant impact on the well-being and general condition of the animal. At the end of the experiment the animal will be killed and blood, urine and tissue will be collected for biochemical analysis. To determine the effect of the genes in the diseased cardiovascular system we will generate models to mimic human cardiovascular disease. Cardiac hypertrophy, hypertension, and myocardial infarction, all of which potentially lead to heart failure, will be modelled and the effect on cardiovascular function will be assessed using the series of tests described in the paragraph above. Both hypertrophy (cardiac growth) and hypertension can both be induced by surgical implantation of a small device to release drugs to raise blood pressure or lead to hypertrophy. The surgical procedure to implant the device is carried out under general anaesthesia with appropriate analgesia to relieve any post-surgical pain. Animals fully recover from the surgery within 24-48 hours and the hypertrophy/hypertension develops over the following 7-14 days. The animals do not display symptoms of the hypertrophy/hypertension unless it develops into heart failure. The visible symptoms of heart failure are lethargy, lack of interest in food, drink and surroundings and laboured breathing. Mice exhibiting these symptoms will be humanely killed. A surgical procedure to constrict the aorta will also result in hypertrophy as the heart works harder to pump the blood around the body. Again this procedure is carried out under general anaesthesia with analgesia as described above, with the hypertrophy developing over 1-5 weeks. Myocardial infarction (MI), a heart attack will be induced under general anaesthesia with appropriate analgesia by constricting one of the major blood vessels of the heart. As in the human population a number of mice (~10%) will die of acute heart failure within 24 hours of the MI and a further ~13% will die of cardiac rupture 4-5 days after the MI. These deaths are very sudden and are instantaneous. Over the following 1-5 weeks the heart forms a scar where the MI has occurred and cardiac hypertrophy and subsequent heart failure may occur.

Application of the 3Rs

Replacement

State why you need to use animals and why you cannot use non-protected animal alternatives

Replacement

As the main focus of the described work is to study gene function in the cardiovascular system mice have been chosen because the anatomy and physiology of the heart and vasculature is both well documented and physiologically similar to humans . Insights into the underlying mechanisms of

heart development and function can be gained from studying lower organisms such as *drosophila*, fish and worms but they do not possess the four chambered heart and subsequently have a different circulatory system.

There has been a recent increase in the use of stem cells as an experimental model. There is a lack of human cardiovascular tissue available for research which has led to the development of techniques to induce cardiomyocytes from human stem cells. Our own work involves transforming human skin fibroblasts to stem cells (known as induced pluripotent stem cells-iPSC) and from there growing the cells under such conditions to convert them to cardiomyocytes (iPS-cardiomyocytes). These cells have many of the characteristics of human cardiomyocytes and thus we are developing them as a model system in which to characterise the effect of gene modification on hypoxia and hypertrophy and to understand the signalling pathways involved.

This *in vitro* system can complement and enhance our research involving animals but will not act as a replacement for experiments which require the understanding of gene function within the context of the whole organ. These factors can only be investigated *in vivo* within the context of the whole organ because the cardiovascular system responds to factors carried in the blood and from cell to cell, as well as to the physical forces imposed by the beating heart and blood pressure. In combination with gene modification these factors will affect the disease process.

Reduction

Explain how you will ensure the use of minimum numbers of animals

Reduction

The personnel involved in this project have extensive experience of all the key techniques to be used within this project; including small animal surgery, cardiovascular phenotyping and induction of cardiovascular dysfunction. Their experience has and will continue to ensure that procedures are carried out efficiently, thereby minimising the suffering to animals, and the numbers required for reproducible results.

Genetically modified and wild-type litter-mates are analysed in groups of the same age and sex to eliminate variation in the results due to anything other than the genetic modification. This regimen of using animals of the same age, sex and strain is essential, since such differences affect heart rate, blood pressure and indices of contractility.

The series of tests to analyse cardiovascular function under basal and stimulated conditions was designed and refined in order to obtain information on several factors from each animal, whilst not raising the severity level beyond mild, as the procedures have no significant impact on the well-being and general condition of the animal. This includes collection of cardiovascular tissue and blood that will be used extensively in biochemical, molecular and *in vitro* studies.

The use of sham operated animals versus non-treated controls will be considered for each experiment. Where possible control data from previous experiments will be used, negating the use

of further sham controls. Where available data comparing sham and untreated controls will be compared and where possible untreated controls have been used. Where it is considered scientifically valuable to use sham-operated controls we have found that the number of animals in the sham group can be lower than the number of treatment animals as the variation and therefore standard deviation between mice is lower.

Refinement

Explain the choice of animals 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.

Refinement

The work carried out in this project studies the function of a number of genes implicated in cardiovascular disease, particularly heart failure and its co-morbidities including hypertrophy, hypertension, and myocardial infarction. It is essential to understand the role of these genes in normal cell function and to study the consequences of abnormal function. We are directly studying the relevance of these genes in established animal models which mimic the most prevalent human cardiovascular diseases.

To minimise the harm to the animals used in these studies we have selected and refined approaches and techniques which cause the least pain, distress and lasting harm whilst being able to meet our objectives.

The effect of any genetic modification on the health of the animal is unpredictable, as is the effect of a procedure on a genetically modified animal, so all animals are inspected at least once a day. Any animals showing signs of distress are evaluated in consultation with the veterinary surgeon and humanely killed if the distress cannot be averted. The majority of genetically altered strains we are currently studying do not have observable phenotypes that impact on behaviour. One strain results in homozygous embryonic lethality at day 4 of development – but breeding is now maintained by crossing wild type mice with those carrying a heterozygous mutation.

All surgical techniques are carried out under general anaesthetic to ensure that the animal does not feel any pain, and any post-surgical pain is treated with the use of analgesics. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates, is humanely killed.

All surgery is routinely conducted in the morning and rarely on Friday's in order to maximise the time for post-operative close monitoring. Animals will be monitored daily for the duration of the procedure, with additional monitoring where necessary. For example, when a new strain is used the frequency of monitoring will increase and will include out-of-hours monitoring (between 5pm and 8am and at weekends), in the days following surgery. If there are routinely no ill-effects of treatment/strain then the final daily monitor will occur at the end of the working day. If there are

concerns about an animal then the frequency of monitoring will be increased and will also occur out-of-hours. Frequency will be decided on a case by case basis.

Physiological analyses (echo, haemodynamics) are also performed under general anaesthetic, in the majority of cases the animal is under terminal anaesthetic from which it does not recover. ECG may also be performed on conscious animals in a non-regulated procedure; this is a quick procedure which is not stressful and does not require anaesthesia.

Any stress caused by administration of pharmacological agents is momentary as the injection is given. Where applicable mini-osmotic pumps are used to administer hypertrophic, hypertensive and other pharmacological agents. Although their use initially involves minor surgery, the technique in our experience leads to highly reproducible and consistent results requiring fewer animals per experimental group. The use of osmotic pumps also reduces handling and stress in animals.

Coronary artery ligation for induction of myocardial infarction is a severe protocol. This can lead to death if the mice develop acute heart failure or if the heart ruptures but these are minimized by careful placement of ligatures, refined surgical techniques and post-operative care.