Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 18

Projects with a primary purpose of: Translational Applied Research – Human Cardiovascular Disorders

Project Titles and keywords

1. **Analysing signalling gene function in the heart**
    - heart, electrophysiology, hypertrophy, arrhythmias

2. **Mechanisms of Innate Immunity in Cardiovascular Disease**
    - Heart Disease, Immune System

3. **Prevention of chronic heart failure**
    - Heart failure, myocardial infarction, diabetes, hypercholesterinaemia

4. **Genetic and Bioenergetic Determinants of Cardiac Arrhythmias**
    - Arrhythmias, heart disease, Sudden death, Genetic**s**

5. **Autonomic control of the Cardiovascular System**
    - Cardiovascular disease, autonomic control

6. **The effects of myocardial infarction**
    - myocardial infarction

7. **Preclinical therapies for pulmonary hypertension**
    - Cardiovascular, pulmonary, genetics, therapy

8. **Pathophysiology of the Cardiovascular System**
    - Metabolic syndrome; obesity; heart function; atherosclerosis

9. **Comparative Cardiovascular Homeostasis**
    - Transgenics; Hypertension; metabolism; kidney

10. **Mechanisms of Progressive Cardiac Dysfunction**
    - Heart failure, hypertrophy, cardiac remodelling

11. **Pathophysiology of Heart Failure**
    - Heart Failure, Calcium, β-adrenergic, Ultrastructure

12. **Cardiac injury, repair, regeneration & remodelling**
    - Heart attack, drug therapy, heart failure

13. **Dietary vascular protection in utero and stroke**
    - stroke, obesity, pregnancy, risk, diabetes

14. **Mechanisms of thrombosis and atherosclerosis**
    - Macrophages, endothelial cells, inflammation, atherosclerosis, thrombosis, cardiovascular disease

15. **Investigating the genetics of cardiovascular disease**
    - Hypertension, organ-damage, rats, mice, genetics

16. **Investigating a novel treatment for Heart failure**
    - Perhexiline, metabolism, hypertrophy, heart failure

17. **Small animal models of cardiovascular disease**
    - Ischaemia, therapy, cardiovascular disease

18. **Regulation of thrombosis and haemostasis and its influence on cardiovascular diseases**
    - Clot, Thrombosis, Stroke, Atherosclerosis, Vessel wall

19. **Pathogenesis and therapy of vascular diseases**
    - Atherosclerosis; Aneurysm; Angiogenesis; Cell therapy; Cardiovascular diseases intervention.

20. **Rodent models of arteriosclerosis**
    - Arteriosclerosis, stem cells, endothelial cells, vessel graft

21. **Role of nitric oxide and reactive oxygen species in cardiac function**
    - Nitric oxide, Reactive oxygen species, Heart failure, Diabetes, Atrial fibrillation

22. **Rodent models of pulmonary hypertension and associated co-morbidities**
    - Pulmonary hypertension, pulmonary vascular remodelling, right heart failure

23. **Vascular protective genes in angiogenesis**
    - Vascular, preeclampsia, hydrogen sulphide, Heme oxygenase, pregnancy

24. **Myocardial infarction and heart failure**
    - Heart, failure, infarction, protection, injury

25. **Angiogenesis, microcirculation and microenvironment**
    - Angiogenesis, microcirculation, tumour growth, wound-healing, vascular-targeted

26. Protecting and repairing the diseased heart

- Heart attack, cardiac surgery, ischemia reperfusion injury

27. Factors affecting valve development and disease

- Veins, lymphatics, heart

28. Studies of complex genetic traits in rats

- Rat, cardiovascular, metabolic, kidney genetics

29. Mechanistic studies in pre-clinical stroke/small vessel disease

- Stroke, small vessel disease, hypertension, rats, mice

30. Understanding and Treating Cardiovascular Disease

- cardiovascular development, heart repair

31. The molecular basis of cardio-metabolic disease

- Diabetes, cardiovascular disease, insulin resistance, insulin-like growth factors, atherosclerosis

32. Myocardial Infarction

- Heart attack, vascular dysfunction, nitrite, nitric oxide, perhexiline

33. Calcium-permeable channels and their associated mechanisms and therapeutic potential

- Blood vessel, Cardiovascular disease, Cancer, Diabetes, Calcium channels

34. Improving minimally invasive treatment of coronary artery disease

- Coronary, artery, atherosclerosis, angioplasty, stents

35. Targeting the immune response in cardiovascular diseases

- Atherosclerosis; restenosis; aneurysm; myocardial infarction; stroke

36. Innovative Treatments for Heart Failure

- Heart failure, Stem cell therapy, Gene therapy, Drug therapy

37. Ischaemia-Reperfusion Injury and Shock

- Ischaemia, Shock, Kidney, Haemorrhage, Heart Research, Trauma

38. The long-term effects of prenatal hypoxia on cardiomyocyte function

- Programming, pre-natal hypoxia, cardiac, mitochondria, myocyte

Project 1	**Analysing signalling gene function in the heart**	
Key Words (max. 5 words)	heart, electrophysiology, hypertrophy, arrhythmias	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	In this project mice or rats carrying a genetic modification implicated in cardiac health and disease will undergo physiological analysis to determine the function of the gene in the heart. Echocardiography and ECG will be carried out to determine the structure and electrical function of the heart; these are non-invasive procedures that will be performed under general anaesthesia and can be safely carried out on several occasions in the same animal over time (not more than once per day) to assess the development of disease or efficacy of treatment, therefore reducing the number of animals needed for each experiment. Electrical pacing will be used to monitor cardiac pacing and arrhythmic susceptibility; these techniques are carried out under general anaesthetic from which the animal will not recover, during the process the animal will not feel any pain.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit	The information is likely to be of interest to clinicians, physiologists with an interest in ion channel function and their roles in heart diseases. The secondary potential benefit (medium or long term) relates to the application	

from the project)?	and value of the results, which may at some later stage be of value in the identification of novel molecular targets at which new pharmaceutical products could be aimed. Ion channels and their regulatory proteins are already well recognized as important therapeutic targets for treating a number of different pathophysiologies, in particular heart diseases. A further secondary benefit relates to the production of genetically altered animals with altered regulation of ion channels, not only for our own use but also for the use of preclinical scientists elsewhere.
What species and approximate numbers of animals do you expect to use over what period of time?	4000 mice, 1000 rats
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?	**1. Possible adverse effect associated with modification of specific gene (severity: mild to moderate):** Depending on the specific gene to be modified, adverse effect associated with such gene manipulation may occur. Animals exhibiting any unexpected harmful phenotypes such as poor growth, difficulty breathing or prolonged lethargy will be killed by a Schedule 1 method or if scientifically valuable and compatible with a moderate severity transferred to protocol 2 and the Home Office inspector informed. Manipulation of genes which may cause widespread tissue effects will be limited to cardiac-specific manipulations in order to reduce the likelihood of these adverse effects. All adverse effects will be documented and periodically assessed in order to detect sporadic unexpected events. Animals are routinely maintained in a barrier environment and group housed whenever possible. 2. **Decompensated hypertrophy (i.e. heart failure)) associated Protocols 3 and 4 (severity: moderate).** Less than 5% in Protocol 3 and less than 10 % in Protocol 4 based on our experience, moderate. Animals that show signs of heart failure, particularly respiratory distress, will be killed immediately by a schedule 1 method. Animals that show signs of distress or ill-health such as significant weight loss (more than 20% over a 28 day period) or prolonged lethargy will be

	immediately killed by a schedule 1 method. 3. **Adverse effect associated with surgery – Peri-surgical mortality (severity: moderate).** Typically <10% in TAC (Protocol 4) and less than 5% in protocol 4 for an experienced operator. Such deaths are usually immediate and under general anaesthesia (e.g. due to aortic or pulmonary artery rupture). Recovery is normally rapid with animals routinely given analgesia, fluid replacement as necessary and heat therapy. Animals will be inspected regularly in the 2-3 hours post-surgery and again the following morning, with additional analgesia, fluids and monitoring provided as required. Animals that do not thrive, for instance experiencing mild to moderate weight loss, will receive, if deemed appropriate in consultation with experienced animal care and veterinary staff, increased care such as making available more palatable foodstuffs, wound repair and/or extra analgesia and killed by schedule 1 method if they continue to struggle. Animals that show signs of overt distress, e.g. weight loss of more than 20%, will be killed immediately by schedule 1 method. 4. **There will be forced exercise by swimming and running on a treadmill, with some of the mice experiencing exhaustion as the end point.** Any animal that appears to show continued stress in water, exhaustion, or is unable to swim comfortably will be immediately removed. Any animal that appears to show continued stress after 3 swimming sessions will be excluded from the study, killed by schedule 1 method after and appropriate period of recovery to return to baseline state and the tissue harvested used for in vitro work for which a licence is not required.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	An important aspect in determining the genetic component of a disease is the generation of animal models that can be used to test hypotheses about the pathogenesis of human disease (i.e. the cellular and pathophysiological

	processes leading from genotype to phenotype). Overall, the mouse (and to some extent the rat) is the only viable model from which to determine crucial information regarding pathophysiology, and hence future treatment strategies which are presently needed to make progress in the field.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have used power analysis, and a power of 80%, to determine the sample sizes required in each experimental group. To determine differences that we may encounter between experimental groups we initially took values from the published data of renowned research groups. Over the past few years, we have obtained substantial experience in electrophysiogical and haemodynamic analysis and induction of hypertrophy, as well as molecular and cellular techniques and we have been able to routinely reduce the number of animals required per experimental group.
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.	Overall, mice and rats, the species used in this project, are viable models from which to determine crucial information regarding pathophysiology, and hence future treatment strategies which are presently needed to make progress in the field. In particular, mice are universally used for work involving genetic alterations. The standard protocols, methods and reagents have been optimised for this species and there are acknowledged benefits from their use. While the rat has been the main model of choice for decades. Experimental procedures were developed to generate cardiovascular disease states in this species, such as systemic and pulmonary hypertension, cardiac hypertrophy and failure, myocardial infarction, and stroke. They became extremely valuable models to understand the genetics of these diseases, since powerful genomic tools are now available for the rat. Previous and ongoing work, within the group, using molecular, cellular, bioinformatics, as well as transgenic mouse technology has provided much data as to the role the role of genes involved in cardiovascular disease, particularly left ventricular dysfunction,

	hypertrophy, heart failure and diabetic related cardiovascular disease. However, to comprehensively assess the function and physiological significance of these genes in the myocardium, it is necessary to conduct *in vivo* experiments. Extensive use of bioinformatics and *in vitro* cell biology will complement the proposed animal work. Cellular interaction studies will elucidate potential interaction partners involved in calcium signalling, which will have a wide impact on the understanding of cellular signal transduction within the myocardium, as well as in other signalling pathways important in the heart.

Project 2	**Mechanisms of Innate Immunity in Cardiovascular Disease**	
Key Words (max. 5 words)	Heart Disease, Immune System	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The work covered within the remit of this licence aims to further our understanding of the role that the immune system plays in cardiovascular disease. This is important because despite the advent of statins and other lipid lowering drugs the incidence of atherosclerosis remains very high and cries out for the development of further treatments. Our objectives can be summarised as follows: 1. To further our understanding of the mechanisms relating to the role of the immune system in Atherosclerosis. 2. Identify potential targets for therapeutic intervention. 3. To test potential therapeutic agents in the context of a standard animal model of atherosclerosis.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The project covers several areas of immune involvement in cardiovascular disease. These include developing a greater understanding of how immune signalling molecules called cytokines control and direct white blood cells to the sites of atherosclerosis,	

project)?	understanding the mechanisms and consequences of how the part of the immune system known as “complement” interacts with fat metabolism and finally developing and testing possible therapeutic agents to block the adverse effects of immune involvement in atherosclerosis.
What species and approximate numbers of animals do you expect to use over what period of time?	Only mice will be used during this project. We have been granted permission to use up to 2500 animals to understand the mechanisms involved and up to 1000 for the testing of potential drugs. The licence covers work over five 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 end?	We expect that the vast majority of our mice (70-85%) which have been fed a high fat diet will experience no adverse effects whatsoever. In fact these animals will fall below the “mild” category. Over prolonged periods of high fat feeding (4-6 months) up to 25% of the mice may develop itchy skin causing them to scratch, resulting in sores. These animals we would classify as falling into the “moderate” category of severity. Approximately 5-7% of mice placed on a high fat diet for 12 weeks will die suddenly and without any prior warning. These deaths are almost certainly due to heart attacks or strokes. Because they almost always occur unobserved we cannot say for certain what level of distress the animal experiences before death therefore all these occurrences are graded “severe”.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The development of heart disease is a very complex process involving a host of different systems and processes within the human body. Some of these can be studied in vitro in the laboratory and within the human population. However while these approaches can provide many extremely important insights and are critical in informing all work carried out with animals, they remain largely incapable of mimicking the interactions which occur during the evolution of the disease within the body. Thus it remains essential to use animals in the study of heart disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Through the use of good experimental design, appropriate statistical analyses and sharing of control groups wherever possible, we will ensure the minimum number of 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.	The apoE and LDLr knockout mouse strains are well established (~20years) models of human cardiovascular disease. We will use both these models in combination with various other knockout strains to investigate the role of genes from the immune system in heart disease. We now have several years experience in using these models and this together with knowledge shared from our collaborators and gleaned from published work has allowed us to refine our use such that we expose mice to high fat diet for considerably less time than the licenced period of 12 months. Indeed the vast majority of our experiments are limited to 12 weeks duration. This refinement keeps both the incidence of skin sores (see adverse effects above) well below the expected 25% present after 4-6 months of high fat diet and also restricts the incidence of sudden cardiac death to 5-7%. Experimental animals are monitored closely to ensure that any which show any signs of cardiovascular problems are humanely and rapidly euthanized.

Project 3	**Prevention of chronic heart failure**	
Key Words (max. 5 words)	Heart failure, myocardial infarction, diabetes, hypercholesterinaemia	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of our project is to protect heart tissue against injury which occurs during either an acute heart attack (myocardial infarction, MI) and the subsequent cardiac remodelling or a chronic diabetic state leading to chronic heart failure (CHF). CHF is one of the major causes of death in the UK. There is an increasing incidence with age and, hence, heart failure will contribute more and more to mortality in the future. There are several causes of CHF: (1) myocardial damage due to an acute MI and subsequent loss of heart muscle cells, (2) chronic arterial hypertension leading to cardiac hypertrophy and finally left ventricular dilatation and failure, (3) infection of the heart directly damaging the heart muscle cells, and (4) chronic diabetic state, (5) genetic and rare causes. In this project we intend to address the cause and development of heart failure using two models	

	(ischemia caused coronary artery ligation which is a model of acute MI or diabetes). We will use the information gathered and our models to identify protective drugs or behavioural and nutritional changes which are able to prevent the development of CHF. If successful we intend to progress candidate treatments to humans under these conditions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefit of the present project will be to test the feasibility of drugs to be used and their potential to significantly reduce the development of heart failure. This information will enable us to translate the use of these drugs into the use in patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use about 933 mice for this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	It is expected that 60-70 % of animals are entering these protocols will show no more than mild clinical signs and 20-30 % of animals will show moderate clinical signs such as lethargy and weight loss. At the end of the protocols, the animals will either be transferred to a different protocol or humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	If cardiac cells undergo a period of reduced or absent oxygen supply during an acute heart attack, they rapidly undergo irreversible cell death. The amount of cells affected is dependent on cell-to-cell communication within the cardiac tissue. These effects cannot be mimicked in cell culture. All efforts to establish a reproducible and reliable cell model of infarction have failed so far. Only very basic information about signalling and receptor interaction can be obtained from cell based (in vitro) experiments or experiments using isolated organs. Furthermore, effects of given drugs on cardiac rhythm, blood pressure or cardiac function cannot be assessed using in vitro methods alone. In addition the development of heart failure requires circulation and a certain work load for the heart which cannot be mimicked in isolated organs or cells.
2. Reduction Explain how you will assure the use of minimum numbers	We will use the non-invasive state-of-the-art technology of magnetic resonance imaging (MRI) and echocardiography of the heart. With these non-

of animals	invasive techniques we can minimize the variability of the data and, hence, animal numbers needed to reach significance. I received very valuable statistical advice from the statistical advisor of the 3R committee, Dr Gavin Jarvis.
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 smallest species possible for a ligation of a heart vessel. Furthermore, the availability of suitable genetically modified animals adds substantial advantages to the use of mice. While the surgery itself will be done under deep anaesthesia, the MRI measurements only require light anaesthesia, and the final catheterisation will be done under terminal anaesthesia. Currently, there is no other model available to mimic the effects of chronic treatment after an acute heart attack. Although the severity level can be as high as severe in few rare cases due to the developing heart failure, it is nevertheless not expected and if it occurs it will be for only of very short duration after surgery. Compared to other established methods of heart failure induction (e.g. treatment with anthracyclins), the present method is by far the less severe.

Project 4	**Genetic and Bioenergetic Determinants of Cardiac Arrhythmias**	
Key Words (max. 5 words)	Arrhythmias, heart disease, Sudden death, Genetics	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our objectives are to improve the assessment and treatment of cardiac arrhythmia that cause much suffering, disease and death in people. Our work has initially focussed on single large effect genetic causes of risk that are usually manifest in young people and may result in the sudden death of an individual due to arrhythmia. This aspect continues but we would now like to extend the range of our experimental programme in order to know more about the burgeoning impact of metabolic disease on cardiac arrhythmias. We already know from much clinical observation that those with obesity and who are metabolically 'unhealthy' get more cardiac arrhythmias than would be otherwise anticipated. The principal objective of the next 5-year project is to understand how the genetic controls of metabolism cause arrhythmias in mice.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	The potential benefits are probably best considered under two headings: first, we will be better able to understand risk factors for arrhythmias and who is at risk so that we can take appropriate preëmptive actions; second, our findings are likely to provide	

project)?	leads for the identification of new drug approaches for the treatment of patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 2500 mice.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We expect few adverse effects based on the lack of adverse events seen over the last several years in the work of our group. Most of the work is completed on tissues from animals that have already been humanely killed. A new development in the current project is that some mice will have an implant of a monitoring device under the skin or in the peritoneal cavity to monitor heart rate and rhythm, and the placement of such a device has a small likelihood of surgical risk e.g. infection. The surgical procedures are procedurally well established and will be conducted by experienced medically trained doctors but if any adverse events are observed then unless readily resolved mice will be humanely killed. In addition some mice may receive drugs for short periods (of around one week maximum) in order to look for new approaches to the correction of arrhythmias. The drugs used are likely to have been used before clinically in patients and also be reported as being used in mice in the scientific literature without any significant reported adverse effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mice have an established position in heart research as they allow us to identify the way genetic alterations lead to clinically relevant outcomes and improve how we treat patients. We can also study human patients taking heart samples at the time of surgery to address some of our questions. The problem is that our patients usually have complex disease and will be treated by many drugs that will tend to confound obtaining robust scientific answers. In addition the development of new drugs for the treatment of patients need to use mice to most efficiently advance drugs to the clinic through identifying drug targets and then assessing drug responses. Genetically engineered fish have been used by other workers and provide complementary data but whilst they are vertebrates they are much removed from

	human physiology. In addition stem cell models offer promise but have unresolved technical issues .The work of this project cannot be addressed scientifically therefore without the use of mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We design our experiments very carefully with statistical guidance so that we use the least number of animals to give us a robust and scientifically useful set of answers. In addition we are very experienced in the use of animals allowing us to obtain the maximum amount of scientific data possible from animal tissues to aid in answering our scientific research questions.
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.	Our work is designed to address how genetic make-up determines the risk of arrhythmia. We can obtain much information from patients in the clinic but using mice we can work out in a very robust way how genes affect arrhythmia risk in mammals and this has significant clinical importance. The mouse has been established as the mammal most readily open to genetic manipulation and is ideal in this role. nd as much work as possible is done *ex vivo* rather than in the live animal. Further refinement will be achieved through limiting drug administration to animals in cases where likely promising outcomes in live animals are anticipated. Accordingly only after thorough trials have been completed in isolated hearts and cells will drugs be administered to animals.

Project 5	**Autonomic control of the Cardiovascular System**	
Key Words (max. 5 words)	Cardiovascular disease, autonomic control	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cardiovascular disease is the leading cause of hospitalisation and morbidity in the western world. We know that abnormal neural control originating in the brain that regulates blood pressure and heart performance make a large contribution to heart disease. We have some insights into how the central control contributes to heart disease and there are drugs that target this aspect. However, we lack information about how cardiovascular function is “sensed’ and how this contributes to heart disease. This is crucial if we are to fully understand how heart disease develops and progression.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We will gain insights into the integrative processes regulating cardiovascular function and how these become altered in cardiovascular disease. We will be in a better position to develop new drugs and therapies for people with cardiovascular disease as well as for animals who themselves suffer cardiovascular problems. These basic studies will make a significant contribution to the health of the population as a whole.	
What species and approximate numbers of animals do you expect to use	Over 5 years Rats 950	

over what period of time?	Mice 300 Rabbits 70 Hamster 70 Zebra fish 100
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 experiments will require surgical manipulation of the cardiovascular system or brain under recovery anaesthesia. This allows us to record data following a surgical intervention. Once data collection is complete the animal is terminally anaesthetised and tissue such as the heart and brain removed for further experimentation. These experiments may result in adverse effects such as movement disorders following brain surgery or cardiovascular problems following heart surgery. These are rare and if occur are managed by judicious pain relief and antibiotics. One procedure in the project is classed as severe, the rest are moderate. Animals are closely monitored and if the severity of the adverse effect is considered excessive then the experiment is terminated.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are looking at systems interaction and behaviours and how these are altered in complex diseases. Previous research has shown that rodents can give insights into human diseases and can lead to novel therapeutic development. Current computer modelling tools lack the level of complexity to give meaningful data. *Ex vivo* tissue sampling can be used experimentation but tissue still has to be derived from an animal. Tissue culture can provide data on how a neurotransmitter might function however, how that function is integrated into a complex behaviour cannot be determined.
2. Reduction Explain how you will assure the use of minimum numbers of animals	From experience power calculations are not best suited for neuroanatomical labelling and subsequent tissue processing experiments because the number of sites where error can be introduced is numerous. For example, precise injection of tracer into the CNS is required. Biological variation in animals can mean the injection does not quiet hit its target. For tissue immunohistochemistry and immunoblot requiring antibodies there can be considerable variation in antibody efficacy from commercial suppliers, coupled with the incubation steps required to utilise the antibody introduce possible places of error.

	Therefore to minimise against these variables, animals are used in groups of 4-6 thereby reducing biological variation for injection site, antibodies from known batches and sources are utilised and tissue from each animal can be processed independently to minimise failure. This type of experimental approach enables the scientific outcomes to be achieved. Power calculations will be used to determine minimum numbers of animals for functional studies. For example, for the experiments proposed in Objective 1 part C, say 9 drugs will be tested with analysis of nerve activity before and after drug administration on each animal. Power calculation predicts 8 animals per group based on an expectation of 80% success rate. The value of 8 comes from the formula n=16* (Coefficient of variation/effect size)^2, at 80% power (beta) and alpha of 0.05. We expect the size of effect to be approximately 1.5 times the background noise of signal (ie coefficient of variation). A further addition to reduce numbers is the fact that the effect of some of the drugs may be reversible so that it may be possible to wash them out and use multiple drugs on the same preparation. This will be done wherever feasible.
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 are trying to understand cardiovascular control in healthy and sick humans. This involves knowing how the heart, blood vessel and brain all work together. Animals allow us to see how these systems do this. The studies will help us prevent and/or develop new treatments to help humans with cardiovascular disease. The treatments may also be applicable to pets such as dogs and cats. Rats are chosen as a widely used cardiovascular model to investigate cardiovascular regulation in humans. A lot is understood about the control of the cardiovascular system in the rat, meaning any new information can be understood without having to repeat experiments in new species. Occasionally, other species eg fish, rabbits and mice are useful to use as alternative models for cardiovascular studies because some work cannot be carried out in the rat. Again, the new information from these alternative species is applicable to human cardiovascular regulation. Sometimes we have to make the animals have

	cardiovascular disease. These animals undergo techniques that mimic human cardiovascular disease at the least severe level as possible for the animal but will still give new information. All surgical procedures are carried out by trained individuals and are carried out in sterile conditions and using techniques to minimise the time in surgery, reducing the risk of infection.

Project 6	**The effects of myocardial infarction**	
Key Words (max. 5 words)	myocardial infarction	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This projects aims are to investigate the electrical and mechanical alterations that occur during the different stages of recovery following a heart attack in both the surviving and damaged regions of the heart. In man, heart disease is common, with a poor prognosis. The underlying mechanisms are complex; in some cases there is a steady deterioration and in others the terminal event is sudden and unexpected. Current treatments are unpredictable in their effectiveness and often disappointing in their ability to reduce mortality. There are examples of studies that have had to be terminated early due to an increase in mortality in the treatment groups. As these treatments were primarily studied in healthy hearts there is a possibility their effects are different in diseased hearts. Many patients with diseased hearts have arrhythmias (abnormal heartbeats) and some treatments may have secondary effects at a cellular level that may further compromise the heart's ability to function properly. By far the most common cause of heart failure is myocardial infarction, i.e. ischaemia to myocardium caused by caused by chronic arterial artery disease. This project will use animal models of myocardial ischaemic damage. In our laboratory, a coronary artery will be permanently tied off giving a small area	

	of damaged tissue. The heart will then be studied whole or single cells will be used to examine mechanical and electrical changes. In our collaborators' laboratory, ischaemia will be induced by placing a frozen probe on the surface of the guinea pig heart to create a similar infarct area. The heart will then be studied whole or single cells will be used to examine mechanical and electrical changes. Studies on human hearts are difficult for a number of reasons including difficulty in accessing both diseased and normal tissue. Variations in age, medication and other health problems make it difficult to reliably investigate the processes at well defined time points after a single incidence of damage. The research proposed using this model is designed to provide new insights into the electrical events that trigger and sustain arrhythmias in cardiac muscle. An improved understanding of what causes these arrhythmias and their relationship with reduced function in a damaged heart is necessary to develop successful treatments that maximise heart performance while minimising the risk of life threatening arrhythmias.
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 project will provide some information about the basic physiology and pathophysiology that occur post infarct and will inform future research and may suggest alternative therapeutic approaches. In the clinic, the currently available pharmacological regimens, although rational, have been unpredictable in their clinical effects and have often been particularly disappointing in their ability to reduce mortality. Indeed, there are numerous examples of trials that have had to be terminated early as a result of evidence of increases in mortality in the treatment groups. One possibility for these adverse effects reflects the difficulty in predicting the underlying primary cause of the arrhythmias at the different stages post-infarct. For example, there is increasing evidence that many antiarrhythmic drugs have different electrophysiological effects in abnormal myocardium that could indeed exacerbate the propensity for arrhythmogenesis. These agents have primarily been studied in isolated normal tissues whereas arrhythmias presumably usually arise in damaged myocardium. It is therefore hoped that the findings of this study will inform subsequent work designed to tailor therapeutic regimens to the various stages of scar formation and remodelling seen post-

	infarct. In the event that a new potential therapeutic target or that a currently available drug may have beneficial effects at a given stage post infarct, subsequent studies will incorporate appropriate treatment groups in order to examine that particular rationale. The results from these studies will provide information concerning the structural and molecular basis for the changing predisposition to arrhythmias and sudden death post-MI, as well as information concerning contractile dysfunction. This research will aid in the modification of current treatment strategies to tailor treatments to the stage of the remodelling process.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use around 500 rabbits and 50 guinea pigs.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The animals will experience moderate post-operative pain which will be controlled with pain killers. A small number of experimental animals (less than 10%) will experience sudden and fatal arrhythmias. This is an unavoidable consequence of the model used and we cannot predict which animals will be effected. Due to good surgical and husbandry techniques other adverse effects are very rare.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studies on human hearts are difficult for a number of reasons including difficulty in accessing viable diseased and normal myocardium. Variation in age, medication and underlying pathology of any obtainable tissue and the inability to investigate the processes at well defined time points after a single incidence of damage means that human studies are not viable Rabbits and guinea pigs are the lowest vertebrate groups with electrical and functional characteristics and an infarct structure similar to humans. Smaller hearts cannot fully develop the abnormal heart beats seen in humans. There is however no alternative to using an infarct model to examine the time course of changes that occur post heart attack.
2. Reduction	The minimum numbers of animals will be used in each of the sub-projects involved in this study. These

Explain how you will assure the use of minimum numbers of animals	numbers are determined by the nature of the measurements and the variability between animals. Power calculations have been made to establish the minimum number of animals required to obtain statistical significance. Where possible, standardisation of the experimental conditions will minimise variation between measurements. We continually analyse and test statistical significance so the minimum number of animals are used for each test.
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.	Rabbit and guinea pig hearts and those of larger mammals have clear regional electrical variation that closely resembles that seen in the human myocardium. Furthermore the structure of mature (healed) human infarcts and those seen in the rabbit are largely comparable making this species suitable for this study. Previous experience with this model indicates that the expected adverse effects can be controlled and minimised. Experienced licensees will perform the surgical techniques under aseptic conditions and appropriate pain relief will always be administered. Best practice post operative care will be employed to ensure any discomfort is minimised. Regular examination by veterinary surgeons and experienced technicians will ensure that any untoward effects that do develop are detected early and steps taken to minimise any distress or discomfort.

Project 7	**Preclinical therapies for pulmonary hypertension**		
Key Words (max. 5 words)	Cardiovascular, pulmonary, genetics, therapy		
Expected duration of the project (yrs)	5 years		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x		Basic research
	x		Translational and applied research
			Regulatory use and routine production
			Protection of the natural environment in the interests of the health or welfare of humans or animals
			Preservation of species
			Higher education or training
			Forensic enquiries
	x		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objective of this project is to find and test new treatments for a rare condition known as pulmonary arterial hypertension (PAH), which is characterised by severe high blood pressure in the arteries supplying the lungs. Patients with PAH have a very limited life expectancy despite existing drug treatments. A major breakthrough in understanding and treating this condition was the identification of genetic mutations in certain genes. Our research aims to understand how these mutations cause disease and, based on this knowledge, to design more effective therapies to treat the condition.		
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 major potential benefit from this project is the identification of new drug targets and new drugs to treat or prevent PAH in patients and their relatives. In addition, our research increases knowledge of how PAH occurs and progresses.		
What species and approximate numbers of	This project will use rats and mice, including genetically modified mice. We anticipate the use		

animals do you expect to use over what period of time?	of a maximum of 23,500 mice and 11,200 rats over a 5 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 end?	The majority of our protocols involve the use of genetically modified mice that develop PAH, or require the exposure to certain agents or environmental factors that stimulate the development of PAH. We then use drugs or other substances to try to prevent or reverse the disease. Most of our measurements are carried out at the end of any protocol once the animal is anaesthetised and the animal is not allowed to recover from the anaesthetic. In some protocols we make measurements in anaesthetised animals using non-invasive imaging techniques so that we can follow the course of PAH in each animal. Very occasionally we use a severe protocol that determines the effect of a drug on survival of rats with PAH. Ultimately in patients we want to improve survival, so we sometimes need to show that a drug improves survival in animals. Overall the expected level of severity in our protocols is mild-moderate. At the end of each protocol animals are humanely killed, usually under terminal anaesthesia.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use information from human genetic studies and from tissues and cells from patients with PAH to identify the most promising ways of treating PAH. We also use tissue culture of human cells in the laboratory to provide important information before embarking on animal experiments. Ultimately, before deciding whether to take a new treatment forward into patients, we need to test the approach in an animal model of PAH to see whether it is capable of preventing or reversing the key aspects of the disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	First, we only test treatments in animals for which there is a sound scientific basis, based on our finding patients with PAH and human tissues and cells. When we embark on a study in animals we calculate the minimum number of animals required to provide an answer, for example whether a drug reverses PAH. In addition, because we can measure the development of PAH in individual animals using non-invasive imaging techniques, this reduces the number of experimental groups required to determine

	whether a treatment works.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Rats and mice are the lowest vertebrate group in which models of PAH have been developed and are thus the most appropriate species to study. We require the use of both rats and mice because rats, but not mice, develop pulmonary hypertension in response to certain stimuli and develop more severe PAH (more similar to the human pathology) in response to certain stimuli. On the other hand, mice provide better genetic models of disease. The introduction of sophisticated imaging techniques and measurements similar to those used in patients with PAH means that the number of groups of animals can be reduced. Animals are monitored at least daily to check for any signs of distress. If an animal shows any features of reaching moderate severity it will be humanely killed. We work closely with the veterinary surgeon who advises on methods to reduce welfare costs in our experiments.

Project 8	**Pathophysiology of the Cardiovascular System**	
Key Words (max. 5 words)	Metabolic syndrome; obesity; heart function; atherosclerosis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Coronary Heart Disease (CHD) is a chronic disease that causes more than 7 million deaths per annum worldwide, while non-aicholic steatohepatitis (NASH) is a chronic liver disease that is on the rise. The main risk factors (comorbidities) for both include poor diet resulting in high blood cholesterol, Type 2 diabetes and obesity, which together are termed Metabolic Syndrome (MetS). The complexity of these factors working together presents a significant need to identify new approaches to both prevention and treatment of this CHD and HASH in order to reduce the global burden of disease. The key scientific objectives of the project are to identify new biochemical markers in the blood that will indicate the level of risk of people with metabolic syndrome developing CHD and NASH and to identify new drug targets that can be used to prevent the development or progression of these diseases.	
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 results from these studies will lead to a greater understanding of how MetS contributes to the development and progression of atherosclerosis, worsens the outcome of a heart attack, and leads to the development of NASH. This will be of benefit to researchers within the field of cardiovascular science, researchers working in the field of NASH and liver cancer, and to the pharmaceutical industry undertaking	

	R&D programmes to develop novel ligands targeting the receptor systems under study. In addition, identification of biomarkers that are associated with MetS-related disease development could inform clinical trials that could ultimately inform personalised treatment strategies for individuals with MetS.
What species and approximate numbers of animals do you expect to use over what period of time?	All experiments will be carried out using either rats (approximately 750) or mice (approximately 2,750) over a 5 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 end?	Most of the dietary interventions are classified as mild severity and have little or no adverse effect on the health of the animals. However in animals that are given zinc free diet there is loss of appetite and reduced weight gain, although the animals do not show signs of ill health. In some (very few) experiments there may be a need to restrict food intake in normal fed rats to match that in Zn deficient rats to ensure that the results can be directly attributed to Zn deficiency; this will mean that these animals will also experience slowed weight gain. These experiments would be of moderate severity. In studies where prolonged drug administration that cannot be given via the food or water is required, some animals may have osmotic mini pumps implanted under the skin under anaesthesia; animals normally make a rapid recovery from this procedure due to care measures that include analgesia and heat loss prevention. These experiments would be of moderate severity. At the end of any dietary or drug intervention the animals will be either euthanized by a Schedule I method, or will undergo procedures under terminal anaesthesia, from which they will not regain consciousness; this is an unclassified level of severity. Genetically modified animals bred and used under this licence are similarly of mild severity
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The overall objective is to identify (I) biomarkers and (ii) novel interventions that prevent progression of predisposing dietary risk factors for CVD. A significant part of the programme of work will employ a range of dietary Interventions in mice or rats, each of which IS used to induce a different pathological state. Since the aim is to study the impact of these interventions on whole body physiology there is no alternative to using live animals.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Procedures involving animals will not be carried out if the data obtained is already available, with the exception of replication of work in order to validate the study under our own conditions or if we have reasonable doubts as to the veracity of the data. Measures taken to avoid unjustified duplication of procedures will include close monitoring of the literature and conference attendance. Exact numbers of animals required for any study will be determined by the experimental design which will, wherever possible, allow assessment of a combination of interventions against the same contemporary controls. To ensure sufficient statistical power estimates, power calculations will be made with the advice of the university statistician.
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 of the animal models to be employed in the project are long established and well characterised. For example rodent (rat and mouse) models of MetS have been published widely in the literature (rats- 2,936 original articles; mice 3,337 ar-tides). The choice of species will depend upon the nature of each individual study and will be informed by the wider literature. General measures to minimise welfare costs will be to closely monitor body weight, food intake and check general health on a daily basis. For the majority (<95%) of studies animals will be group housed and for any surgical procedures pen-operative and anaesthesia care measures will be taken following consultation with the Named Veterinary Surgeon.

Project 9	**Comparative Cardiovascular Homeostasis**	
Key Words (max. 5 words)	Transgenics; Hypertension; metabolism; kidney	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Essential hypertension Ts defined as high blood pressure is often a complication of diabetes and is affected by genes and by factors like dietary salt and calorie intake, both of which are high in most developed countries. Often there is little clue to the underlying cause(s) of the hypertension — why are some people more affected by salt than others; what are the effects of diet during pregnancy or as a baby on blood pressure as an adult; what key changes at the gene or cell level lead to gross changes of blood pressure at the whole body level? This project covers a range of animal models designed to increase our basic understanding of the complex interactions between genetic and environmental factors, which contribute to normal blood pressure control, and how imbalance leads to hypertension. The kidney is a critical organ for salt handling and will be a key focus of this project. Novel data and insights will be obtained regarding blood pressure regulation, salt balance, and the mechanisms underlying kidney damage and repair.	
What are the potential benefits likely to derive from this project (how science could be advanced or	Scientists will benefit from our discoveries because our results will be published widely, allowing other scientists to build on our results. Health care providers, such as doctors, will immediately benefit from insights	

humans or animals could benefit from the project)?	into the disease process, which may ultimately lead to improved treatment. Pharmaceutical companies may benefit from the identification of new targets for drug therapy. Ultimately, the patient will benefit from better understanding of the disease, and improved treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 18500 zebrafish, 5000 Rats and 6000 mice over the 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?	Animal welfare is of the highest priority. All procedures and experiments are approved by the local Ethics Committee. All surgical procedures are carried out under deep anaesthesia, with animals monitored for any signs of stress both during and after surgery. Wound healing is monitored closely. Any animal that does not recover from surgery, or shows signs of distress/adverse effects (such as excessive weight loss, hunched posture, reduced activity and loss of condition) will be humanely culled. Experimental zebrafish will be monitored closely for signs of distress, such as feeding or shoaling changes, loss of colouration, gasping for air or bleeding, and will be humanely culled if the severity limit is reached. Where multiple rounds of live imaging are required (rodents or zebrafish) animals will be allowed to completely recover from each anaesthetic, and will be observed for normal feeding and behaviour between sessions. Single-housing of animals may be stressful, but where possible, environmental enrichment is provided. At the end of the experiments, all animals are humanely culled. For rodent work, most procedures are predicted to be mild to moderate, while for zebrafish, approximately 90% are mild to moderate and the remaining 10% severe. Close monitoring means that only a fraction of animals would reach this level of severity before being humanely culled.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have a strong track record of using alternative models, such as isolated organ preparations, cell culture and zebrafish larvae where possible. For example, 3D printing of cells will inform our studies on kidney tubule function. We use this reductionist approach to inform our animal experiments and

	thereby reduce overall numbers of animals used. However, blood pressure control is the result of complex physiological processes involving interactions between multiple organs, hormones and the nervous system and understanding the role of specific genes within this network necessitates the use of animals, because this complex physiology cannot be fully modelled in vitro.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use statistical analysis to estimate the minimum number of animals required to achieve meaningful results. We routinely monitor experimental results allowing us to adjust numbers pro-actively and use the minimum required to achieve robust statistical analyses. Careful experimental designs are used to maximise the information obtained from the experiment. Quantification of gene expression levels in small numbers of control and test animals at key stages in disease development gives us very useful information about potentially important genes for further study. We use, when appropriate, long-term (longitudinal), rather than single time point, study design, e.g. for imaging or following blood pressure changes. These procedures are statistically powerful and importantly direct other studies to informative time points. Longitudinal techniques considerably reduce the number of animals needed for time-point evaluation in, e.g. disease progression and remission, and repeated imaging technologies maximize the data obtained from each animal.
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.	Comparative studies in the zebrafish allow us to identify and characterise equivalent gene functions in a simpler vertebrate, to investigate gene effects on cardiovascular and kidney development and function, and ultimately to apply this knowledge back to more complex rodent models. Both rat and mouse transgenic strains are used to model clinical conditions, and are critical for comparative purposes regarding cardiovascular, metabolic and kidney function. Technology for genetic manipulation is advanced for these species. We use highly efficient, state-of-the-art, gene targeting techniques, to maximise information obtained from genetically modified models. Where possible, we will turn gene expression on or off experimentally in order

	to minimise exposure to disease symptoms. Where more refined approaches become available, during the course of this licence, we will aim to incorporate them.

Project 10	**Mechanisms of Progressive Cardiac Dysfunction**	
Key Words (max. 5 words)	Heart failure, hypertrophy, cardiac remodelling	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will investigate the changes that occur within the cardiovascular system during the development and progression of heart failure in order to identify new therapeutic targets and treatments for this condition. Chronic heart failure (CHF) affects up to 2% of adults. It is caused by diseases such as high blood pressure, diseases of the valves in the heart and damage to the heart following a heart attack. These diseases increase the amount of work that the heart has to perform. Different diseases affect the heart in different ways. Diabetes is also a risk factor for development of CHF and about 19% of CHF patients have diabetes. CHF carries an unacceptably high rate of death and complications despite recent treatment advances. A better knowledge of the mechanisms that underlie the development and progression of CHF is essential to develop new therapies	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	This work should substantially increase our understanding of the cardiovascular changes which are critical in the development and progression of CHF. By identifying mechanisms underlying development of CHF and by undertaking initial experimental studies in animals, this research may	

project)?	provide the basis for devising novel strategies and medicines for treating cardiac disease. In the long term this will benefit patients stricken with this serious and common disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 6,000 mice per year and up to 600 rats per year will be used for each year during the 5 year course of this project
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice or rats will be used to model CHF. Four complementary models will be used which mimic the main causes of human CHF. Surgical models involve constriction of a blood vessel (the aorta) or making a connection between the aorta and a vein. High blood pressure may be induced by the administration of various agents that increase blood pressure. In some studies, diabetes will be induced either by altered diet or by injection of agents that decrease insulin production. The four models differ in the way that they stress the heart, and will provide information that will vary depending on the stress. Different stresses may require different treatments. We will also test new treatments in these models. The development of CHF may take 6-8 weeks, during which time animals will be closely monitored. They will undergo echocardiography and MRI imaging techniques; they may have devices implanted to measure blood pressure and ECG in order to obtain data on heart function. Surgery can result in complications in the first 24 hours and animals will be closely monitored during this period and humane end points applied. Heart failure disease states can lead to significant harm. Animals will be humanely killed prior to development of heart failure to minimise adverse effects and any suffering that the animals may experience.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Because CHF is a complex disorder involving many organs in the body, there is no feasible alternative to the use of animal models.
2. Reduction Explain how you will assure the use of minimum numbers	Principles of good experimental design will be followed to ensure clear answers to questions being addressed while using the minimum number of animals. For many studies, non-invasive techniques

of animals	that allow serial assessment of cardiac function will be used, allowing reduction in numbers. This is especially valuable when assessing the impact of medicines aimed at preventing or slowing CHF. Where possible, additional information will be obtained from studies in cultured cells.
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 will be performed using mice and rats because all relevant methods and techniques are successfully established in these species and because of the availability of genetic alterations. Such genetically altered animals allow the study of specific biochemical pathways in the animal with a view to understanding their role in the disease, and interfering with them to provide new treatments. All surgeries will be performed under aseptic conditions and animals treated with appropriate anaesthetics and pain relief. Since CHF develops slowly, disease progression needs to be followed for several weeks. The development of heart failure may be associated with loss of weight, listlessness and rapid breathing in the late stages. However, animals will rarely be allowed to progress to such a stage. They will be closely and regularly monitored during the study. Any clinical problems will be dealt with in consultation with the veterinary surgeon. Animals will be humanely killed at a pre-determined endpoint or at the end of the study, whichever happens first.

Project 11	**Pathophysiology of Heart Failure**	
Key Words (max. 5 words)	Heart Failure, Calcium, β-adrenergic, Ultrastructure	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Diseases affecting the heart still occur at very high rates and generally have extremely poor outcomes. For example, most people diagnosed with heart failure survive less than 5 years, often dying from contractile failure or cardiac arrhythmias, and those with atrial fibrillation have a 5-fold increase in the risk of suffering a stroke. Current treatment options are limited and, for example, following a heart attack some people either require a heart transplant or are implanted with artificial heart pumps; both of which present significant problems for the patient and health care providers. Importantly, the presence of one cardiac disease such as heart failure often predisposes an individual to another such as atrial fibrillation. There are some common characteristic changes occurring in the properties of these intricately inter-linked diseases of the heart such as heart failure, myocardial infarction or atrial fibrillation. These changes then conspire to lead to a decrease in the hearts ability to pump blood (reduced contractility) and render it more susceptible to life threatening disturbances in the rhythm of the heart (arrhythmias). Our previous work has shown that there are important roles for; i) how calcium is controlled in the cells of the heart and, ii) the structure of heart cells	

	that contribute to changes in the hearts ability to contract and its susceptibility to arrhythmias. Importantly the changes in calcium and cellular structure are clearly both involved in the reduced pump function and the increased propensity for arrhythmias observed in various heart diseases and may be causally linked to one another. In this project we aim to further our understanding of these factors and to determine the molecular, cellular and tissue mechanisms that are responsible for the hearts reduced contractility and increased susceptibility to arrhythmias in conditions such as heart failure, atrial fibrillation and following a heart attack (myocardial infarction). A second aim of this project is to use explanted hearts to understand the mechanisms that cause some patients who receive a heart transplant to reject the donated heart. This will be achieved by mapping the content of cells in the heart that cause rejection and how this changes when explanted hearts are preserved with different solutions.
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)?	• Academic knowledge furthering understanding of the mechanisms causing heart failure and associated medical problems • Potential to identify new targets for drugs or devices to treat people with heart failure • Understanding the factors that lead to rejection of hearts used for transplantation could modify clinical practice and reduce the need for immunosuppressant drugs in transplant recipients
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep, approximately 1000 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 majority of animals will however undergo moderate procedures with the expected adverse effect to mainly be the development of heart failure. Similarly the majority of animals will also undergo minimally invasive surgical procedures that are employed during routine diagnostic investigations and interventions used to treat patients with heart diseases e.g. pacemaker implantation. Animals will be closely monitored for the onset of the signs of heart failure and once these become evident they will be humanely killed and tissues harvested for *in vitro*

	experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	• The muscle cells within the heart are terminally differentiated and are not amenable to long term cell culture • Diseases such as heart failure are systemic and can not be fully reproduced using tissue culture approaches • Computer models are not 'clever' enough to predict the outcomes of the systemic influences occurring in conditions such as heart failure on the function of the heart • Diseased human heart tissue is of limited availability and also suffers from being derived from highly variable disease causes and subject to multiple, again varying, treatment strategies. • Healthy human heart tissue is even less available than diseased tissue.
2. Reduction Explain how you will assure the use of minimum numbers of animals	• Tissues derived from each animal are used across multiple projects thus reducing numbers required • Experimental design is key to performing this programme of work • Where it is possible we will use non animal alternatives such as cell lines or computer models to test the validity of hypotheses before undertaking animal experiments
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	• Procedures are refined to be as minimally invasive as is possible • We employ as clinically relevant approaches as possible • Animals are housed in social groups where possible so that animals undergo procedures that are as similar as possible to those performed on humans • Animals are housed in social groups wherever possible or, if they have to be singly housed for example following recovery from surgery, this is within sight of / proximity to group housed animals in the same room and for as short as time as possible and for less than 24 hours.

Project 12	**Cardiac injury, repair, regeneration & remodelling**	
Key Words (max. 5 words)	Heart attack, drug therapy, heart failure	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cardiovascular disease (CVD) is the main cause of death in the UK (27% of deaths in 2015). Heart attack occurs when blood supply to the heart muscle is blocked by the formation of blood clots in diseased arteries. Short-term survival after heart attack is increasing thanks to improved intervention to restore blood supply to the heart. However, this means that more people now survive with damage to their heart muscle and have an increased chance of developing heart failure, a chronic, debilitating disease. Over 1.4 million adults in the UK currently live with heart attack associated conditions. The aim of this project is to develop new means of (i) reducing loss of heart muscle (ii) replacing heart muscle that is lost, and (iii) reducing the development of heart failure after heart attack.	
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)?	There is a current need to reduce the likelihood of debilitating chronic heart failure developing after heart attack. This project will improve fundamental understanding of heart injury, repair, regeneration and remodeling and will identify targets that might be useful in the development of new medicines for management of patients with cardiovascular disease.	
What species and approximate numbers of	The license will involve mainly mice (6-8000 over 5 years to allow for breeding of mice with genetic	

animals do you expect to use over what period of time?	modification of pathways of interest to the pathology of heart attack, as well as use in procedures) and also rats (<1000).
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?	Heart attack will be induced by ligating one of the main coronary arteries that supplies blood to the heart. A small minority of animals (<5%) will die under anaesthesia due to heart attack. Further mice may die suddenly in the days after heart attack (5-10%) due to cardiac rupture, this occurs when the repair process is impaired and is not associated with clinical signs of suffering. The % of mice in this category can be higher in some strains or where genetic modification impairs healing. The majority of mice recover with a maximum of moderate severity (short term weight loss), gain weight and move around the cage normally until the end of the experiment when they are culled under terminal anaesthesia after collecting images showing heart structure and function. Tissues are collected for analysis of pathological changes e.g. changes in heart size and stiffness. Occasionally (<5%) mice will become more severely ill in the hours after surgery. Careful monitoring during this period usually allows these animals to be culled before they display clinical signs of substantial severity.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the heart, repair and remodelling takes place under conditions where there is still a requirement for blood to be pumped around the body. While some elements can be modelled outside of the body e.g. formation of new blood vessels it is not possible to reproduce either the forces involved in heart pumping or the complex repair processes that occur when the heart is *in situ*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals to be used will be minimized by applying advanced imaging techniques, and removal of small blood samples for analysis e.g. by flow cytometry. These allow us to track heart cell loss and repair as well as structure and function over time in the same animals gaining the maximum information and avoiding the need to generate different animals for each time-point of interest.
3. Refinement Explain the choice of species and why the animal model(s)	Mice will be the main species to be used for this project, primarily because this allows us to use genetic modification as a means of probing pathways of interest. Suffering will be reduced by use of pain

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.	relief and fluid replacement after surgery and by good animal husbandry. Ligation of arteries 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 and avoidance of certain strains of mice.

Project 13	**Dietary vascular protection in utero and stroke**	
Key Words (max. 5 words)	stroke, obesity, pregnancy, risk, diabetes	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	✓	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We have spent several years researching a natural dietary compound named sulforaphane (contained in cruciferous vegetables such as broccoli) that appears to improve health in a wide range of diseases of the blood and heart. While sulforaphane is known to strengthen the body's own defence systems, it also leads to types of protection that are not yet fully understood. Several large clinical trials of sulforaphane are ongoing, e.g. targeting people with specific conditions such as asthma. We seek to provide justification to extend those trials to two further conditions, which we have shown are related in their effects on blood vessel dysfunction. The first condition is observed in babies whose mothers suffered pregnancy-associated diabetes, i.e. gestational diabetes. These children are at greater risk of cardiovascular disease and Type 2 diabetes in early and/or later life. The second condition is stroke and occurs primarily in the elderly and is a primary cause of death and disability in the UK. Stroke is a	

	condition caused by a blockage of blood flow to the brain, and currently there are very few treatments for stroke patients. We have already performed initial experiments, the results of which support our theory. These experiments were performed using cells isolated from umbilical cords obtained with informed, written consent from mothers on a maternity ward, and with data derived from a rodent model of stroke under previously approved work, to support the premise that dietary sulforaphane provides health benefits in these two medical conditions. At present, pregnant women are excluded from all sulforaphane trials on a 'better safe than sorry' basis, and no trial has been initiated to assess the health benefits of sulforaphane in stroke. This project is designed to fill gaps in our scientific knowledge of sulforaphane that must be closed before human clinical trials for these disorders can begin. Questions that require answers are: (i) are doses of sulforaphane that protect isolated blood vessel cells from diet-associated stress safe to administer during pregnancy? (ii) can sulforaphane reverse heart and blood vessel damage occurring during pregnancy that predisposes offspring to heart disease and stroke in later life? (iii) can a simple change in diet in elderly patients reduce the risk of a stroke, reduce the damage following a stroke, or even improve recovery after a stroke has occurred? The work completed under this Project Licence is intended to answer these questions.
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 is explicitly designed to address clinical needs. Stroke is the third largest killer in the UK, but there is only one effective treatment, which must be given within hours of the onset of symptoms, as it otherwise runs the risk of doing damage, rather than helping. This time limit means that less than 5% of all stroke patients receive the treatment. There is a desperate need for new stroke therapies, but little work is being conducted by drug companies due to past failures. Evidence from animal models applied to humans most at risk of damage from stroke (e.g.

	elderly, especially women after menopause) could lead to immediate changes in socioeconomic behaviour, as dietary recommendations can be released much faster than a drug can come to market. While lack of stroke therapies is a constant clinical issue, the obesity epidemic is a growing one. A pregnant mother's high-fat diet may cause long-term elevated risk of heart disease and/or stroke in her children. Based on current trends, this risk will only increase in coming decades. Our research provides the possibility of a natural dietary remedy, which would bypass the slow process of drug approval. Even more importantly, we hope to demonstrate that the increased risk suffered by children who have already been born could be reversed with appropriate natural dietary interventions.
What species and approximate numbers of animals do you expect to use over what period of time?	In total, we expect to use approximately 3,000 mice, and 50 rats over a 5 year project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We have explicitly designed all experiments and protocols to allow us to answer scientific questions with the minimal discomfort to experimental animals, as described below. The most common intervention will be modification of diet, either to a high-fat Western-style diet, or to include supplementation with sulforaphane, a broccoli-derived compound, with no expected adverse effects. Some procedures will require surgery with anaesthetic and pain relief, such as removal of the spleen or ovaries to simulate the effects of aging on the immune system and hormone levels, vascular injury and implantation of 'radio-telemeters', small devices which allow assessment of heart-rate, blood pressure and activity. These will cause moderate discomfort while the surgical wound heals, but only for a few days. The fact that this discomfort is brief is underlined by the fact that heart rate and blood pressure in mice are less elevated by the process of radio-telemetry than by non-surgical

	methods of measurement, similar to the blood-pressure cuffs used by doctors in patients. All other common procedures to obtain scientific data involve only brief discomfort, such as withdrawing blood, or are conducted under general anaesthetic without recovery, to avoid any sensation of pain. Stroke in humans is a severe disease, and the leading cause of adult disability in patients in the UK. As such, modelling stroke is a severe procedure, and may cause weight-loss and stroke-related disability, such as limb weakness and lack of mobility in animals for several days, leading to moderate stress for the animal. When measuring recovery following a stroke with behavioural testing, this is unavoidable. However, our lab has developed methods to assess the development of a stroke whilst an animal is under non-recovery anaesthesia, allowing us to answer several scientific questions without the animal experiencing post-stroke distress. Wherever possible, we will perform this non-recovery surgery, thus subjecting animals to moderate, rather than severe procedures. All animals will be humanely killed at the end of experimentation, or in the case of breeding stock, at the age of one year.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have performed many experiments using isolated cells derived from patients and animals, rather than animals themselves, which have led us to propose a potential dietary therapy for patients suffering stroke and pregnancy-associated diabetes. However, the complex interplay between different organs seen in the disorders we are investigating are not currently possible to model using either cells or computer systems. Both diet-associated cardiovascular risk during pregnancy, and brain damage, inflammation and recovery following stroke, are complex disorders that can only be ethically studied in an intact animal model and must, from an ethical perspective, be shown to have value in a comparable animal model prior to any consideration being given to trials in people.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We have performed statistical tests based on work performed in our and other laboratories to model the minimum number of animals and experiments necessary that will be required to detect a statistically relevant difference/benefit. We will also make maximal use of animal tissue from animals that have been humanely killed following experiments, so that wherever possible, multiple questions can be answered from a single procedure or Protocol and the data from animal use maximised.
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 and rats are the least sentient animals we can use to model the clinical conditions we are investigating, while still remaining relevant to humans. Our pregnancy-related disorder models use the least invasive and stressful methods of measurement, to allow us to pick up subtle changes in blood-pressure. Our stroke research has been refined by modifying the surgery in accordance with the latest literature to reduce death and distress following recovery, and, wherever possible, to answer experimental questions under general anaesthesia without recovery, to avoid the stresses associated with post-stroke disability.

Project 14	**Mechanisms of thrombosis and atherosclerosis**	
Key Words (max. 5 words)	Macrophages, endothelial cells, inflammation, atherosclerosis, thrombosis, cardiovascular disease	
Expected duration of the project (yrs)	Five years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Atherosclerosis is the most important cause of cardiovascular disease, being responsible for very substantial mortality and morbidity. It is a chronic inflammatory condition, in which fats and inflammatory cells accumulate in walls of arteries. This can lead to blood clotting (thrombosis) and occlusion of blood flow in coronary and carotid arteries, resulting in heart attack or stroke. Although much progress has been made in understanding the causes of atherosclerosis and arterial thrombosis, we still know relatively little about how the disease progresses over a period of years. There is a need for more refined diagnostic techniques and preventative treatments. We have the following objectives: • To understand the molecular events within the walls of arteries that result in thrombosis, particularly with respect to expression by a type of white blood cell, called a macrophage, of a substance called Tissue Factor that stimulates blood clotting. • To understand how differences in blood flow within arteries alter the biology of the endothelial cells that form the inner lining of arteries, and how	

	this accounts for the patchy localisation of atherosclerotic lesions. We are particularly interested in ways in which the sensation of blood flow by endothelial cells leads to the turning on of genes that maintain cell health. • To understand how antibodies in the blood (for example reacting with proteins that carry cholesterol) influence the progression of atherosclerosis. We have observed that antibody levels tend to be increased in people who remain free of heart attacks, and we need to know why this is. We plan to test whether antibodies have a role in safely disposing of tissue debris that gets trapped in the walls of arteries and which can stimulate chronic inflammation and atherosclerosis. • To develop "molecular imaging" techniques that may be taken into clinical studies for evaluating the behaviour of atherosclerotic lesions and thereby determining the risk that they will rupture and cause thrombosis. We are particularly interested to develop substances that are labelled with light-emitting factors and which can target specific biological processes within atherosclerotic lesions. Once evaluated in preclinical models, localization of such substances may be detected with special catheters placed in coronary arteries during clinical diagnostic angiography procedures.
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)?	• Understanding the molecules involved in allowing macrophages within atherosclerotic lesions to express Tissue Factor could identify targets for the development of drugs that prevent thrombosis. If the molecular pathways regulating Tissue Factor expression in macrophages are different to those in cells outside blood vessel, this could be a way to prevent thrombosis without the risk of bleeding that currently accompanies anti-coagulant therapy. • Identifying ways in which beneficial genes in blood vessels are stimulated by blood flow could result in new ways to maintain vascular health, for example through diet, exercise or drug treatment. • A greater understanding of how antibodies affect atherosclerosis could lead to immunological vaccine-type approaches to preventative

	treatment. • Currently the ability of cardiologists to diagnose atherosclerotic lesions that are at risk of rupture and causing a heart attack is quite low (~40% predictive accuracy). The introduction of molecular optical imaging into the catherisation armamentarium could add a new dimension to diagnosis and transform the accuracy of treatment decisions.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice – ~2620 /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?	Our protocols have been designed to reach a moderate level of severity at the maximum. Adverse effects are not expected, but could include infection as a result of irradiation or allergy to injection of substances. Every animal undergoing recovery surgery will receive adequate and timely painkillers to reduce pain or discomfort after the procedure. Behavioural signs will be checked for on a regular basis, with a frequency appropriate to and detailed in the individual protocols. We will especially look for any evidence of rough fur, poor eating or drinking, inability to groom, immobility or poor balance despite appropriate treatment will be killed. Any animal in which pain is uncontrolled, or which has significant complications, or whose general health deteriorates significantly will be killed by a Schedule 1 method. Animals will be humanly killed using approved methods at the end of the protocol.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of animals is essential for our work, since it is only by study of the intact animal that we will be able to work out how atherosclerosis is caused, how it can be most effectively and accurately diagnosed, and what therapeutic approaches might be of benefit. Cell culture experiments, using cells derived from genetically-modified mice bred in the project, form a major part of our work. Experiments in the whole

	animal are however also necessary as in vitro methodology cannot mimic the complexity of the whole animal. Nevertheless, we will make maximum use of preliminary in vitro experiments to refine questions and protocols prior to conducting specific experiments in vivo.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The models of inflammatory disease which we have developed are generally extremely reliable and reproducible, so we can obtain scientifically significant results from small groups of animals. As stated above, numbers are also reduced by using *in vitro* methods where applicable. Statistical power studies will be used ahead of experiments to ensure that the numbers of animals are neither too low to obtain statistically significant results, nor higher than is necessary. Bias will be avoided, and hence reproducibility assured, by randomisation of mice to treatment groups and by operatives being unaware ("blind") of their identity. Animal numbers may also be reduced by the application of whole body imaging techniques that allow study of animals longitudinally and thereby avoid killing different groups of mice at each time-point of an 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.	Mice are most suitable for our project due to: • the availability of a wide range of genetically modified animals which can be used to determine the effect of specific proteins on vascular inflammation and atherosclerosis. • A well-researched and understood immune system, which is an advantage for our study on the role of antibodies. • The availability of modern imaging equipment that allows an evaluation of molecular targeting in small animals. All mice will be housed in groups where possible with appropriate environmental enrichment and fed according to current institutional 'best practice'. Any animal which becomes unwell during the course of the experiments, for any reason, will be humanely killed.

Project 15	**Investigating the genetics of cardiovascular disease**	
Key Words (max. 5 words)	Hypertension, organ-damage, rats, mice, genetics	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Hypertension is the most prevalent modifiable risk factor for heart disease, chronic kidney disease and stroke, and remains the leading cause of morbidity and mortality world-wide, accounting for ~12.8% of all annual deaths. The purpose of this project is to better understand the molecular genetic processes underlying the development of hypertension and its associated end-organ damage. These studies will identify novel therapeutic targets.	
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)?	During this project we will utilise relevant rodent models of cardiovascular disease in order to examine the mechanisms responsible for disease causation and progression. These models will include animals that have been genetically modified to alter specific genes or proteins that may play an important role in the disease process. These studies will lead to improved understanding of the cardiovascular disease process and identification of new therapeutic targets, which will ultimately lead to better treatment and prevention of cardiovascular disease in humans.	

What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate that we will use approximately 8,500 rats and 5,500 mice over a 5 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 end?	The overall level of severity for this project licence is moderate. Animals used in this project are mice or rats with altered cardiovascular phenotypes, with mutations in genes or transgenic for genes that may function in cardiovascular biology. However, with the exception of Stroke prone spontaneously hypertensive (SHRSP) rats, these models show no outward signs of adverse effects, are viable, fertile and are a normal size. During the previous 20 years of breeding and maintaining the SHRSP colony we have observed approximately 4-5% incidence of strokes in male rats. The majority of these sustained strokes from 4 months of age, which is the time of established hypertension. Spontaneous strokes are almost never encountered in female SHRSP rats. Symptoms of stroke include inactivity and lethargy, hunched appearance, piloerection (bristling of fur), uncoordinated movements, loss of weight and appetite. Any animal showing these signs will be euthanised promptly. All animals undergoing procedures on this licence will be carefully monitored on a daily basis and any animals that exceed the clinical signs associated with moderate severity will be promptly and humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nature and complexity of the cardiovascular disease process makes finding alternatives to live animal models extremely difficult. However, wherever possible we will use non-animal alternatives (e.g. cell-based assays) for our investigations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We obtain expert statistical advice before commencing any new studies and perform power calculations which allow us to identify the lowest appropriate group sizes for each procedure. In addition, the use of techniques such as MRI and echocardiography allow non-invasive serial measurements in the same animal thereby

	significantly reducing the number of animals required for most studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The rat and mouse models used in these studies have been carefully selected based on their unique genetic profiles. These animal models will allow us to determine the direct contribution of specific genes and proteins to the progression of hypertension and cardiovascular disease. Our *in vivo* protocols have been designed to provide the maximum detailed characterisation of the cardiovascular system whilst at the same time ensuring that the animals under investigation experience the least pain, suffering, distress or lasting harm. For example, analgesia is given for all surgical procedures and disease development is monitored by non-invasive imaging that allows early endpoints to be instigated prior to animals showing significant clinical signs.

Project 16	**Investigating a novel treatment for Heart failure**
Key Words	Perhexiline, metabolism, hypertrophy, heart failure
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

To identify the mechanisms underlying the development of left ventricular hypertrophy and heart failure.

To assess if and how perhexiline and the novel derivatives delay the progression from left ventricular hypertrophy to heart failure.

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)?

Current clinical interventions available for the treatment of diastolic heart failure are minimal. This project provides an insight into the cardioprotective effects of novel drugs (perhexiline and its derivative) and their ability to delay/ prevent the development of heart failure. This project will also provide an understanding of how these novel drugs work within the myocardium in a more physiological and clinically relevant set up.

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

Mice will be used over the course of the next 5 years (approximately 1000 in the course of 5 years).

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

Upon completion of the non-invasive in vivo assessments, all mice will be humanely killed If animals show signs of pain, discomfort or distress during the measurement period, the experiment will be immediately terminated and the animal returned to its

cage. If animals continue to exhibit pain, stress or discomfort, the Named Veterinary Surgeon will be consulted, and the animals humanely killed, as appropriate.

Application of the 3Rs

Replacement

Cardiac hypertrophy and heart failure are complex multifactorial syndromes which involve the interaction of numerous body systems over a prolonged period of time. As such it is impossible to assess the novel drugs in a single cell isolated set-up.

Reduction

When possible, mice used for in vivo cardiac function assessment will also be used for tissue collection for molecular analysis.

Refinement

The mouse genome can be easily manipulated thus allowing for various genetic models to be developed which are of relevance to investigate cardiovascular diseases and drug targets. Furthermore, heart failure in humans is characterised by features such as enlarged heart and contractile dysfunction which can only be replicated in an animal model.

All animals will be closely monitored following surgery and provided with analgesia prior to and after surgery. Those who are deemed unhealthy 3 days following surgery (drop of more than 10% body weight) will be euthanized via schedule 1. Animals undergoing drug treatment will also be monitored for food and water intake to ensure that this is not disrupted

Project 17	**Small animal models of cardiovascular disease**
Key Words	Ischaemia, therapy, cardiovascular disease
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

This programme of work has 2 main objectives:

1. to determine whether a novel compound which blocks the function of a specific heart cell membrane protein (ion channel) has a beneficial effect during a heart attack and
2. to determine whether a non-invasive protocol of temporarily disrupting blood flow to the limbs can modify re-modelling of the heart caused by high blood pressure.

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 aims to improve our understanding of how the heart is damaged during a heart attack, and also to assess the efficacy of a novel group of compounds to treat the immediate muscle damage. It will also assess the potential for a non-invasive procedure (remote ischaemic conditioning – achieved by repeated temporary interruption of blood flow to a large muscle bed, e.g. a limb, using a tourniquet) to modify the enlargement of the heart due to high blood pressure or diabetes, which are among the most significant risks for heart disease.

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

1000 rats and 650 mice over 5 years

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

Models of myocardial infarction (heart attack) and persistent hypertension (high blood pressure) are prepared surgically with full recovery of consciousness; therefore there are both anaesthetic and post-op pain risks to the animals. Anaesthesia will be carefully monitored until full recovery occurs, and post-op pain will be treated appropriately for as long as necessary. There is a small risk of intra-operative or immediate post-operative death in the animals undergoing the heart attack procedure, however these animals will likely die under full general anaesthesia therefore no suffering is expected to occur.

Severity is graded in the ‘severe’ range for the heart attack model and ‘moderate’ for the high blood pressure models. Longer-term consequences, including the development of heart failure, will be routinely assessed by clinical signs and imaging where appropriate. Remote ischaemic conditioning (temporary interruption of blood flow to a muscle bed, e.g. a hind limb) should have no risk other than repeated sedation, and would normally be considered to be of mild/moderate severity. However animals transferred from the surgical protocols may be at increased risk of death during this procedure, therefore under these circumstances, this protocol has been classified as 'severe'. Induction of diabetes is expected to be of ‘moderate’ severity and will be used for additional studies. It may be combined with the models already described (heart attack, high blood pressure, remote ischaemic conditioning) conferring some additional risk, but this will be mitigated by increased monitoring whenever necessary. All animals will be humanely euthanased at the end of the experiments by trained staff.

Application of the 3Rs

Replacement

Diseases of the heart and circulatory system are multi-faceted and rely on interaction between several body systems. These include complex biochemistry, hormone changes and blood pressure effects and cannot be replicated in vitro.

Reduction

Appropriate statistical tests will be applied to all experimental procedures. Once the proposed models are validated (pilot data) power calculations will be used to determine group sizes.

Refinement

The techniques described are widely used in rats and I have substantial experience in this species. They are established in the scientific community as excellent clinical models and data generated are considered applicable to human disease. Welfare costs to the animals are significant but will be mitigated by extensive use of pain relief and by the use of sedation/anaesthesia whenever it is required.

Project 18	**Regulation of thrombosis and haemostasis and its influence on cardiovascular diseases**
Key Words	Clot, Thrombosis, Stroke, Atherosclerosis, Vessel wall
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

The main objective of the project is to determine whether proteins expressed in blood cells (e.g. platelets), plasma or in our vessel walls play an important role in thrombosis and blood clotting and could potentially represent a therapeutic target for the treatment of cardiovascular diseases (CVD).

CVD represent still the leading cause of mortality in the western world. Platelets together with coagulation proteins in our blood and the cells lining up our blood vessels (endothelial cells) are important in preventing excessive bleeding and in other vascular events such as wound repair. Understanding how this delicate balance is regulated is crucial to prevent unwanted bleeding or excessive clotting (thrombosis). Platelets are involved in arterial disease and thrombus formation in blood vessels, which can potentially lead to heart attack and stroke. Current antiplatelet agents including aspirin and clopidogrel are not 100% efficient in blocking platelet function, and can be associated with severe side effects such as excessive bleeding. Similarly, current therapies (e.g. statins – modulating blood cholesterol levels) aiming to prevent excessive narrowing of our blood vessels (atherosclerosis) can still be improved.

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)?

Every year 4 million people in Europe die of CVD (180,000 in the UK alone). Therefore, a better understanding of the vessel wall, platelets and coagulation factors biology will enable to identify new targets for treating/preventing thrombosis & atherosclerosis. As part of this effort, using gene expression profiling, and our expertise in biochemistry we have previously identified platelet/endothelial cell/plasma proteins that are important for blood clotting. We wish in the present project to characterize their role in platelet/endothelial cell function, thrombus formation and also in platelet formation and how these could influence diseases such as atherosclerosis, deep vein thrombosis (DVT) or stroke. We have been publishing our findings in academic journals in addition to present our work at international conferences and will continue to do so.

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

To provide a better understanding of the underlying causes and mechanism involved in CVD, this project will use over a five year period a maximal number of 6,050 mice.

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?

When ex vivo results are encouraging, we will undertake in vivo studies to monitor platelet formation/clearance, bleeding time, thrombus formation, and disease states (e.g. atherosclerosis, DVT, stroke). No adverse effects are expected with most of these models as they are performed under terminal anaesthesia. For one thrombosis model (protocol #9), although these animals are subjected to recovery surgery, they are expected to survive the procedure. In humans, deep vein thrombosis (DVT) symptoms vary greatly and in at least 50% of cases there are no reported symptoms - it is expected it will be the same for the mouse DVT model. In addition, we will evaluate the role of the proteins of interest in stroke: this involves the temporary/permanent occlusion of a blood vessel in the brain (the middle cerebral artery) that lead to brain damage and therefore has a substantial severity limit. Stroke signs may include moderate degrees of paralysis, hemiplegia, sensory loss in face & limbs (at opposite side of the vessel obstruction in the brain), and in some cases the brain damage may cause mortality. Because of the severity of this model, animals will be provided with highest levels of post-operative care and will be checked at least three times daily after they have recovered. Whenever an animal show a poor recovery after surgery, advice will be sought from the NVS & NACWO. A good communication between scientists, NVS, NACWO & CBS staff will be established to provide highest levels of care. Any animal showing constant circling behaviour, having >3 brief seizures (<3 min), unable to eat (>20% weight loss) or with paralysis of all extremities (quadriplegia or tetraplegia) in addition to

piloerection, hunched back or other signs of distress or pain that cannot be relieved with analgesics will be killed humanely and immediately using a schedule 1 method to keep the animal suffering to a minimum. In all cases, we will use a protocol that causes the least suffering to the animals to achieve our scientific objectives. For all surgical procedures, the animal is under general anaesthesia which is sufficiently deep and stable to ensure the animal is insentient throughout.

Application of the 3Rs

Replacement

We will first perform in vitro experiments including platelet function tests, coagulation and other biochemical tests from blood samples taken from human or mice under anaesthesia to obtain initial data of the function of the protein of interest before proceeding to in vivo work. Although valuable information will be obtained, these do not reflect physiologic conditions and the complexity involved in arrest of bleeding, or pathological conditions arising in a blood vessel (e.g. atherosclerosis, DVT, stroke) and therefore render the use of a whole animal model unavoidable. Indeed the vessel wall has a complex architecture and interactions between EC and other cells/tissues as well as the immune system cannot be reproduced in vitro. Thus, to establish the role of a protein in thrombus formation and/or the development of a pathological conditions, the use of genetically-altered mice is the gold standard model. There are indeed a large number of mutants available and many published articles in the field using this species.

Reduction

The numbers of animals will be minimised by careful experimental design and appropriate statistical analysis with seeking advice to Statistical Services Unit and following the ARRIVES guidelines. Techniques will always be refined to aim to reduce the number of animals, including the use of non-invasive novel imaging technologies. This will allow to visualise how the disease (e.g. atherosclerosis, DVT, stroke) develop over time without needing to sacrifice the mice and without surgery. Finally, careful experimental plan will ensure that several tests can be performed using blood samples/tissues from the same mice.

Refinement

The mouse is the ‘gold-standard’ model for studies on thrombosis and blood clotting. Mice also are a good model of human disease and physiology. This is because of the large number of genetically-modified mutants that are available and the extensive amount of work that has already been performed and published. Animals will receive pain relief to treat any apparent discomfort and before any recovery surgery. When animals will be given a soft high fat diet cage environment will be enriched by providing additional tunnels and spatula to prevent animals to develop overgrown teeth. For all procedures, we will continually monitor the literature for

methods of refinement and consider whether the use of animals is necessary to address the experimental question under investigation.

Animal welfare costs will be minimised by application of rigorous and comprehensive humane endpoints.

Project 19	**Pathogenesis and therapy of vascular diseases**
Key Words	Atherosclerosis; Aneurysm; Angiogenesis; Cell therapy; Cardiovascular diseases intervention.
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Arteriosclerosis is a vascular disease that occurs in the middle and large sized arteries (where the major artery wall becomes harder and narrow), which can block the vessel lumen resulting in reduced or ceased blood supply to the organ. This disease is responsible for major cardiovascular diseases and is the number one killer for humans. Although much progress has been made in the past decades about the mechanisms and pathogenesis of atherosclerosis, there remains a pressing need to identify the key regulatory factors in the development/progression of atherosclerosis for future therapeutic intervention of cardiovascular diseases. Furthermore, the underpinning problems of blood vessel re-narrowing after angiography (a medical imaging technique used to visualize the inside or lumen of blood vessels and organs of the body) need to be identified since such findings would aid us to design and develop new therapeutic drugs to improve the long term outcomes of bypass graft and angioplasty/stenting procedures. Therefore, the major aims of this present project are 1) to study the problems underlying the development and progression of arteriosclerosis and angiographic restenosis; 2) to discover new therapeutic methods, e.g. stem cell therapy and gene therapy for controlling and/or preventing of this disease.

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)?

To aid our research we have recently established several mouse models for different type of arteriosclerosis, in which we have showed that stem cells contribute to the formation of disease in the vessel wall. Based on this, we intend to research ways in which we can gain a better understanding of how arteriosclerosis develops and how it can be treated using novel methods, which might act by altering the biology of stem cells or other relevant cells in atherosclerotic plaques. Since many of these

mechanisms and molecules are conserved between vertebrate species, the work proposed in this project will have direct relevance for analogous process and disease states in humans. This project will therefore advance knowledge and understanding of important processes underlying human health and disease, and will allow us to learn more about the problems and to be able to devise new medicines and treatments for these diseases.

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

Mice. Our proposal requires a maximum of 2340 adult mice in the life of the license.

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?

In a typical experiment, mice will receive an altered diet (e.g. high cholesterol), or an injection of a substance/agent that modify the blood cholesterol levels or can stimulate the development of vessel changes. The animals will or will not receive a surgery (e.g. injuring the inner layer of blood vessel, blocking the blood supply to tissues, or blood vessel transplantation). Some animals may be given further treatments with cells, genes, special growth factors, drugs, other agents, etc. during the surgery or at later time. Based on our experience, adverse effects caused by treatments are anticipated to be very limited in the most of our protocols and where they do occur to be very brief in duration. Although it is anticipated to be very rare, following adverse effects may occur in some animal after procedure: hunched posture, loss of appetite, weight loss, dehydration, diarrhoea, inflammation and infection, ulceration, difficulty of breathing, or sudden death (due to aortic rupture). The potential adverse effects associated with these studies will be minimized by the use of anaesthesia, aseptic surgical procedures and use of painkillers. High standards of housing and care also minimize the stress associated with the procedures. At the end of these experiments and/or when signs of discomfort or pain are manifested, the animals will be humanely killed and tissues collect for biochemical and histological analysis.

Application of the 3Rs

Replacement

The major aims of the present project are to study the problems behind arteriosclerosis development and to discover new therapeutic methods for controlling and preventing this disease. There is no suitable non-animal alternative to the approach to be used, as the development of atherosclerosis (where the medium/large size of artery wall becomes harder and narrow) or aneurysm (a localized, blood-filled balloon-like bulge in the wall of a blood vessel) involves multiple cell types as well as many circulating factors that regulate the accumulation of these cells in atherosclerotic lesions, and there is no non-animal model available that properly mimics this complex system. Currently, the best method available to address the potential effects of any gene on atherosclerosis is to compare atherosclerotic lesions from given gene deficient and wild-type animals.

Reduction

The number of animals used in this project will be minimised by careful experimental design according to extensive previous experience in the models, and determined according to pre-defined and appropriate statistical power calculations and subsequent analyses. Specifically, we have consulted with our animal care and welfare officer, named veterinary surgeon and statistician prior to choosing the mouse models. Animals will be assigned into groups randomly with the similar age, gender, body weight, housing environment and other procedures to avoid bias. In order to reduce the number of animals needed, all surgical procedures will be carried out using optimized equipment and techniques, by well-trained person. The size of each groups required for analysis and quantification for the influences of treatments on diseases will be determined based on previous studies, preliminary data and power calculations. The minimum number of animals will be used to obtain statistically conclusion.

Refinement

Mice are the species of animal with the lowest capacity to experience pain, suffering, distress or lasting harm, which have a relatively well-defined genetic map and can relatively easily be genetically altered. ApoE is an apolipoprotein responsible for transporting fats from blood cells to the liver, and thus removes blood fats. ApoE-deficient mice fed with the normal diet have spontaneous high level of blood fatand atherosclerosis (where the medium/large size of artery wall becomes harder and narrow) in the arterial wall, which are similar to those seen in humans. The animal models chosen are the least invasive and simplest of those available, which have been reproduced by many other laboratories and are well described in the literature. Our group also have excellent work experience on these models. The protocol has been designed to minimise animal suffering by using suitable anaesthesia and analgesia during operation. Particularly, the animals will be humanely killed before tissues are removed for experiments or if the animals fail to suitably recover after surgery. It is expected that the degree of distress/suffering will be between mild and moderate for most of the treatments, except that the male mice subjected to the higher concentration (>1000ng/kg/min) of angiotension II (a nature hormone in the body that can increase blood pressure) infusion for 4 weeks, which will lead to sudden death for some animals due to artery broken. All the operations will be performed by well-trained personal licence holders in the group. Furthermore, we will constantly envisage for procedure refinements and replacements.

Project 20	**Rodent models of arteriosclerosis**	
Key Words (max. 5 words)	Arteriosclerosis, stem cells, endothelial cells, vessel graft	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Hardening and narrowing of the arteries (arteriosclerosis) cause heart disease and stroke, which is the main cause for death in humans. At the present the therapeutic approaches used in clinic are not optimal. Searching for new drugs and therapeutic methods is needed. Stem cells have the capacity to become any tissue in the body as instructed, eg. cells on blood vessels. Recent years, many studies confirmed that stem cells are related to blood vessel and cardiovascular diseases. This project will elucidate the molecular mechanisms underlying cardiovascular diseases, especially stem cells and arteriosclerosis, mainly focus on the study of vascular stem cell in arteriosclerosis, the investigation of stem cell migration from both the vessel wall and blood and evaluation of the effect of drugs, genes and stem cell therapy on vascular disease, providing a hope for new treatment.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	We planned a series of experiments to investigate the molecular mechanisms of blood vessel cell death, proliferation, differentiation and vascular repair. These studies will provide us potential targets to design new drugs to enhance cell survival and vascular regeneration. Because we have known the	

project)?	contribution of stem cells to arteriosclerosis, we can find out a way to promote stem cell differentiation toward vascular cells to repair damaged vessels. Finally, we have initially tested a laboratory created blood vessel using stem cells in mouse model, and we will further improve in the second generation of blood vessel and then test-in animal models. Thus, our results will provide basic knowledge useful for both scientific research and clinic application in the future in cardiovascular diseases, e.g. new drug discovery, preventing re-closure of vessel and generating laboratory created blood vessels for clinical use.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to 20,000 mice and up to 350 rats will be used during the 5 year course of this project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mice or rats will be used as models of atherosclerosis which have similarity to clinical diseases in human. To create high blood cholesterol level, mice will be fed with high fat diet. At the same time cholesterol lowering agent or other agents may be fed as control. Mouse vein, artery and heart will be respectively transplanted from donor mouse to a recipient mouse in different models which create the various situations of vessel and organ transplantations similar to the patients. We will also test new treatments in these models. The repairing process of the vessel and development of atherosclerosis after surgery will be studied from the time when the surgery start and up to 2 months after the surgery. Some animals may undergo MRI scanning or laser Doppler image analysis to detect the lesion development. Imaging will be completed under general anaesthesia to minimise the distress to the animals. All surgeries, eg. vessel grafting, injuries, heart transplantations are carried out under anaesthesia and aseptic condition. Pain relief is provided pre-, during and after the procedures. Surgery may result in complications in the first 24 hours and animals will be closely monitored during this period of time and humane end points applied to minimise adverse effects and any suffering that the animals may experience. The severity level of the project will be minimised as far as possible.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Arterisosclerotic lesion formation is a complex process in a living animals. There is no feasible way to replicate this process in a lab setting without use of whole animal interactive body system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have built up sound knowledge from literatures and thorough in vitro studies in our lab. We will make good experimental design, consult the named veterinary surgeon, named animal care and welfare officer and statistician and employ well trained technical team, to reduce the number of animals used to the minimum.
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 will be carried out using mice and rats, for several reason. These species have well-defined genetic map, availability of genetically alteration, established relevant methods and techniques and are the least sentient animals capable of delivering science of project. The animal models chosen are the least invasive and simplest of those available. The vein and artery graft methods chosen has easier techniques to re-join the vessels which shorten the operation time and increase the success rate. The heart transplantation model used is much simplified by transplanting donor heart to neck of recipient animal instead of replacing its own heart .This does not adversely affect the circulation of recipient mouse and is less invasive to the animal, easy to perform and less stressful to the animal during follow-up checks as the heart beating is easily visible under neck skin.

Project 21	**Role of nitric oxide and reactive oxygen species in cardiac function**	
Key Words (max. 5 words)	Nitric oxide, Reactive oxygen species, Heart failure, Diabetes, Atrial fibrillation	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The main objective of this project is to study how nitric oxide (NO) and reactive oxygen species (ROS) regulate cardiac function. Our previous work and that of others have already identified changes in the production of these messenger molecules in situations where adverse left ventricular remodelling takes place leading to heart failure or heart rhythm disturbances. Based on these results we will: 1. Test the beneficial effects of modifying NO and ROS signalling pathways on hypertrophy, inflammation, atrial fibrillation and diabetes. 2. Study other genes that will serve to identify novel molecular targets for future therapeutic interventions. As these conditions are common (1 in 4 life-time risk of development) and often requiring long, and often acute hospital stays, the cost of caring for patients with heart failure (HF) and/or atrial fibrillation (AF-most common heart rhythm disorder) has been estimated to consume *ca.* 4% of the annual NHS	

	budget in the UK. These data underscore the major public health burden posed by HF and AF in our society and the need for a better understanding of the mechanisms that control the evolution from myocardial remodelling to pump failure and rhythm disturbances. The Framingham Heart Study was the first to demonstrate that diabetes (DM) and obesity are independent risk factors for developing AF.AF is associated with considerable morbidity, decreased quality of life, and increased mortality as a consequence of heart failure and thromboembolic events. AF accounts for 25-33% of all ischemic strokes. Diabetes is also a common condition and growing healthcare burden, particularly in developing countries. The total number of people with diabetes is estimated to rise from 366 million in 2011 to 552 million by 2030. A high proportion of patients (50-75%) will develop diabetic cardiomyopathy. An important need remains to further delineate the basic mechanisms of diabetic cardiomyopathy and to translate promising therapies in preclinical models to humans.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By elucidating the mechanisms leading to an increased production/bioavailability of reactive oxygen species in the diseased myocardium, our reaserch will increase our understanding of myocardial NO-redox biology, identify new biomarkers of disease evolution and enable us to test whether specific interventions targeted to restore a normal myocardial NO-redox balance will prevent or retard the evolution towards HF and AF in chronically stressed hearts.
What species and approximate numbers of animals do you expect to use over what period of time?	We have estimated that a maximum of 10,900 mice will be used over a period of 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We expect that the level of severity will be moderate for the majority of mice. The experiments we are proposing include phenotyping new genetically modified mouse models as well as generating mouse models of human disease, notably diabetes and cardiac hypertrophy secondary to pressure overload (e.g., a situation that can be observed in patients with high arterial

	pressure or aortic valve stenosis) on which we could also test new therapeutic strategies. Characterisation of these models would typically include non-invasive (*e.g.*, echocardiography and/or magnetic resonance imaging) under anaesthesia and/or invasive (*e.g.* Left Ventricular haemodynamics or transoesophageal pacing) evaluation of cardiac function under terminal anaesthesia and *ex-vivo* investigations in myocardial tissue and cells. Type 1 Diabetes will be induced by the administration of streptozotocin that is toxic to pancreatic beta cells, type 2 Diabetes by dietary modification or the use of transgenic models. These mice will experience high blood glucose, which will be monitored, and polyuria/polydipsia, therefore, they will have free access to water and absorbent bedding will be added to the cage whenever necessary. Cardiac hypertrophy will be induced by aortic banding or by administration of angiotensin II or isoproterenol via an osmotic minipump implanted subcutaneously. We routinely apply pre- and post-operative care to the mice that undergo procedures (anaesthesia/analgesia). After they recover from surgery most mice will be free from symptoms for the duration of the study. Occasionally animals will sudddenly develop deep abdominal breathing, which is the first sign that they have developed heart failure. Suffering is minimised by using this as an immediate humane end-point. These animals are likely to experience a severity level that is severe. However, the majority of animals are killed humanely at the scientific endpoint without exhibiting any symptoms of heart failure. At the end of these experiments the animals will be humanely killed and tissues collect for biochemical and histological analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we are actively engaged in the process of reduction, we recognise that at present, it would be impossible to model the behaviour of such a complex system in silico or using cell lines. We are carrying out a number of complementary experiments in humans and surplus human tissue from patients undergoing cardiac surgery as well as developing a computational model of the healthy and diseased

	myocardium to guide our experiments and minimise the use of animals and *in vivo* experimentation. In order to characterise the function of a specific gene on NO-redox balance we will initially make use of isolated cardiomyocytes and perfused heart preparations from mice, as these experiments are the least severe. To complete these first investigations and gain insights of the function of these genes in disease. We will need to use models of human disease (diabetes, hypertrophy).
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum numbers of animals required have been carefully reviewed by the funding agencies and have been based on power calculations. Numbers have been reduced in single cell based experiments (cell shortening, calcium measurements and patch clamp, immunohistochemistry and molecular studies) due to the availability of three different cellular electrophysiology set ups as well as other equipment (e.g. confocal microscope/FRET system) on site, allowing us to have several scientists working on cardiomyocytes isolated from one heart. To maximise the use of animals, we share our surplus tissue with other groups with non-cardiac research interests. As part of the strategy to reduce the number of animals we are developing the optical mapping of the atria. This technique will allow us to assess cardiac electrical properties in perfused hearts instead of using live animals, thereby reducing the number of *in vivo* experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Surgical and imaging techniques are constantly refined and used by all the groups in our Department. Any step forward on the refinement of these procedures that leads to minimise welfare cost to the animals is actively saught and shared in our regular animal welfare meetings. We have modified the protocol to induce type 1 diabetes in mice. This allows us to generate a less severe model where we can study early changes in the myocardium. The severity and variability of previous protocols have been significantly reduced. Diabetic mice are subject to regular screening (blood glucose measurements and body weight monitoring). Careful attention is given to excess consumption of water or frequent wetting of bedding material.

	Absorbent bedding is added whenever necessary. Refinements have also reached other techniques such as the reduction in volume of blood required for glucose measurements or the delivery of drugs by using implants instead of multiple injections. Strict humane end-points, evaluated by Vets, will be applied to minimise suffering of the animals. We routinely apply pre- and post-operative care to the mice that undergo procedures, ie, analgesia, heat support, access to water-softened chow, subcutaneous fluids and oxygen. Mice are allowed to recover in a heated chamber and checked after recovery. Recovery surgery is performed earlier in the day to allow sufficient monitoring within normal working hours.

Project 22	**Rodent models of pulmonary hypertension and associated co-morbidities**	
Key Words (max. 5 words)	Pulmonary hypertension, pulmonary vascular remodelling, right heart failure	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	1) To evaluate the role of identified molecules and miRNAs in rodent models of pulmonary hypertension and pulmonary artery banding models. 2) To test agents for their ability to prevent progression / induce regression of pulmonary vascular disease. 3) To develop novel rodent models of pulmonary vascular disease and associated co-morbidities based on our better understanding of disease pathogenesis.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	We have already identified several potential novel drug targets and as our research progresses we will validate these and identify which of them are key disease regulators associated with both Pulmonary Hypertension and other co-morbidities. By integrating our studies on human disease we can modify and manipulate our current rodent models to make them even better and more accurately reproduce aspects of the human disease phenotype. Through our current translational programme we have established	

	a robust platform on which to test novel treatments including for example novel human monoclonal antibodies to OPG, TRAIL, small molecule inhibitors to SMURF1, and their downstream signalling kinases e.g. FAK.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice – 4200 over 5 years Rats – 950 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 most common adverse effect will be associated with the development of pulmonary hypertension. Regardless of the model used and much like human disease these present as a general malaise, loss of appetite, breathlessness and weight loss. The expected severity of these studies is Moderate. Since this license is underpinned by research with a strong translational drive, In the majority of instances we will be administering agents that we hope will alleviate disease and therefore symptoms. In animals where we are studying disease mechanisms so animals will be irradiated and undergo a bone marrow transplant to elucidate the contribution of a specific gene in tissue compared to bone marrow derived (inflammatory) cells. To assess the disease progression and determine the efficacy of any treatments some animals may require the implantation of telemetry devices to allow for the continuous measurement of blood pressure and /or undergo non-invasive imaging, in both cases anaesthesia will be required, most commonly isoflurane which is very rarely associated with any adverse effects. Animals undergoing surgery may incur surgical complication in which case they will be humanely killed. All animals will be humanely killed at the end of the study, or if they are deemed to be likely to go experience effects of more than moderate severity.
Application of the 3Rs	
1. Replacement State why you need to use	Due to the complexity of the cardiopulmonary system with the interaction of multiple cell types and organs it is not possible to model all aspects of this disease by

animals and why you cannot use non-animal alternatives	using in vitro cell based models and human genetics alone.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use a combination of cells and patient material to perform a large number of studies to examine expression and function of molecules *in vitro* models prior to initiating any *in vivo* studies. Through well developed protocols that have been fine tuned over the past 10 years we collect high fidelity data that allow for as few animals as is absolutely necessary to 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 and Rats are the lowest form of species that have a 'human like' cardiopulmonary system. The models proposed are widely used throughout the scientific community and we are recognised as being one of the best labs at performing them. Mice represent the best model to dissect the role of the gene in disease development due to the increasing availability of 'floxed' knock out mice. Rats represent the best rodent model in terms of disease complexity and are therefore the model of choice to perform therapeutic studies on established disease. All rodents are monitored daily and assessed within the severity limits set out within this license. Adverse effects are written up on protocols and monitored. All animals will be humanely killed at the end of the study, or if they are deemed to be likely to experience effects of more than moderate severity. We work closely with the Named Veterinary Surgeon and animal care staff to ensure that the procedures and animal husbandry conditions are as refined as possible.

Project 23	**Vascular protective genes in angiogenesis**	
Key Words (max. 5 words)	Vascular, preeclampsia, hydrogen sulphide, Heme oxygenase, pregnancy	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	☒	Basic research
	☒	Translational and applied research
	☐	Regulatory use and routine production
	☐	Protection of the natural environment in the interests of the health or welfare of humans or animals
	☐	Preservation of species
	☐	Higher education or training
	☐	Forensic enquiries
	☒	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The projects aims are to determine whether natural protective factors can reduce the severity of preeclampsia and atherosclerosis and to establish the mechanisms involved in these disorders. These discoveries will lead to refinement in the management of these diseases and aid development of novel therapeutic targets. The project plan is to utilise multiple murine models of preeclampsia and use already established models of atherosclerosis to test the role of protective genes, such as heme oxygenase, in the development of these two disorders. Firstly, we will use established models of preeclampsia and atherosclerosis to test the role of genes involved in promoting vessel growth in alleviating the symptoms of the two diseases. Secondly, we will utilise two new models of preeclampsia: i) Surgical model that mimics preeclampsia but free from genetic manipulation and ii) placental cell specific genetic manipulation to identify genes important in preeclampsia. We plan to use the two diverse approaches to test novel	

	therapies identified from in vitro experiments. [161 words]
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 aim is to publish the findings in academic journals. The information is likely to be of interest to pre-clinical scientists interested in development of diseases of pregnancy and of related disorders. The secondary benefit relates to the value of the results to clinicians and to the possibility that new molecular targets may be identified, for which new pharmaceutical products could be developed. [63 words]
What species and approximate numbers of animals do you expect to use over what period of time?	Our project requires the use of both mice and rats. Previous experience and statistical knowledge will be used to determine the minimum number of animals necessary, although the existence of biological variation and the varied nature of the work make it difficult to give precise numbers. Since we work in very close collaboration with national and international researchers within the field of angiogenesis, it will be easy to avoid repetition of experiments, minimizing numbers further. We will also aim to perform multiple analyses on tissue derived from each animal. The laboratory has good experience of working together in this way and maximizing the use of experimental tissue. Experience and statistical expertise (detailed above) will be used to determine minimal usable number of animals; by the very nature of scientific research and the existence of biological variation, it is difficult to give detailed numbers. We attempt to maintain a constant body weight so that we can use one set of controls for one series of experiments; given that capillary supply is size-dependent failure to maintain constant weight between animals supplied at different times will invalidate this approach, and more controls will be needed. We are also committed to ensuring reduction of animal numbers through the appropriate design of experiments, i.e. the organization of an experiment properly to ensure that the right type of data, and enough of it, is available to answer the questions of interest. Data will be subject to statistical

	analysis using an appropriate statistical methodology. Where applicable, data will be assessed for normality and equal variance to determine whether parametric or non- parametric tests should be employed before performing tests of significance, or whether arc-sine or logarithmic transformation is required. Unpaired and paired Students t-test, signed rank, Mann-Whitney U and Kruskall-Wallis one way ANOVA are example of tests that will be performed as appropriate.
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 basic design of all the experiments performed in this programme will be to induce vascular remodeling by surgical manipulation of the vascular system combined, in some instances, with a high-fat diet, in rats or mice. The process of remodeling will be followed by suitable analysis in live (anaesthetized) animals (for instance using magnetic resonance imaging or ultrasound). At the end of the experiment the animals will be killed to provide tissue for laboratory analysis. In some instances the process of remodeling will be manipulated (for example by changing blood pressure, altering vascular factor concentrations, or by administering pharmacological agents). Design and implementation of investigations in animals will be based on previous experience in our laboratories and reported in the scientific literature and will be complemented by laboratory experiments using, for example, cell cultures or biological samples from humans. All animals will be kept according to best husbandry practices and checked regularly by suitably qualified staff. Surgical techniques will be performed using appropriate anesthetic and analgesia to minimize distress and discomfort. In the vast majority of techniques used the discomfort caused to the animals is low. For procedures that have a high impact on the animals, potential adverse effects are well understood and will be monitored and treated accordingly to reduce suffering. These procedures will only be used for translational proof-of- concept experiments. The minimum appropriate number of animals for use in each experiment is based on careful statistical

	calculations.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In our opinion, alternatives to animals cannot be used to meet the aims and objectives of this work because *in vitro* models of angiogenesis differ fundamentally from the *in vivo* process. Many of the characteristics of endothelial are altered in tissue culture systems, the contextual significance of extra-cellular matrix molecules and interstitial cell types is lacking, and the time course of adaptations cannot be mimicked realistically. The different factors initiating or controlling angiogenesis *in vivo* have also been proved to be different, based on our previous work, from those *in vitro*. The integrative nature of *in situ* tissue function is thus of paramount importance to the problems being addressed and the scientific rationale of the work is best served by use of animal models. However, we are also attempting to replicate some of these studies utilising *in vitro* methods, if successful, will lead to a degree of replacement of animals in future research. Therefore, in our opinion, alternatives to animals cannot be used to meet most of the aims and objectives of this work at the present time. Although it is encouraging that our data may be translated into the clinical setting, fundamental research is still needed in order to understand the mechanisms involved. Such investigations are impossible without the use of animals, all alternatives having been shown to be of limited value. Our laboratory is currently unique internationally, in its studies on the above factors *in vivo*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where appropriate the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%. Otherwise, we will use the least number of animals to provide an adequate description, generally on the basis of previous experience (ours, or from the literature). In terms of the numbers of animals required, we expect that 6-8 animals per treatment group should be sufficient to obtain the required results. Furthermore, as part of good laboratory

	practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material; and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and the treatment differences that are to be estimated). In some instances and in order to minimize the number of animals used, repeated blood sampling will be taken in the same animal to assess circulating factors such as proteins and or dose of therapeutics.
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 experimental models we have selected to use for our studies are currently the most refined, least severe and the most routinely used for the relevant pathologies. In addition to this, we plan to utilise new methodologies using a surgery based, non-transgenic model (RUPP) and a lentiviral-mediated transgene expression exclusively in the placenta and not the fetus (Placenta specific gene transfer). The key advantage of the RUPP model is the experimental induction of chronic uteroplacental ischemia that closely resembles preeclampsia in women without the need for additional genetic manipulation. Thus these new complementing models will aid in giving a detailed mechanistic insight in the pathogenesis of preeclampsia and pregnancy based disorders. Adenoviral mediated gene transfer for the study of atherosclerosis, angiogenesis and pregnancy based vascular remodelling is the least severe and efficient method for induction of disease. These experiments will be carried out using rats or mice as appropriate mammalian models to develop knowledge, as a prelude to veterinary application and human experimentation. Our previous experience in the use of these models has enabled us to refine the procedures in order to obtain reproducible angiogenesis in a relatively short duration. This facilitates the study and interpretation of mechanisms

	in a minimum number of animals. Mice will be the major targets because the use of transgenic and knockout technology can be used to investigate further the molecular basis of observations made in the rat. In addition to local expertise in surgical techniques and angiogenesis research, we have long-term established collaborations with others who have experience of this particular model. Dose setting studies will be undertaken, at a low dose in no more than two animals initially. Pharmacological dose will be selected on the basis of previous animal studies with the type of compound, or by extrapolation from *in vitro* work or other appropriate data.

Project 24	**MYOCARDIAL INFARCTION AND HEART FAILURE**	
Key Words (max. 5 words)	Heart, failure, infarction, protection, injury	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The purpose of this project is to ultimately benefit patients by reducing myocardial infarction and its main consequence, heart failure. Although there have been major advances in the prevention and treatment of myocardial infarction (MI) it still remains the most common cause of death in developed countries and is becoming more common in developing countries. Myocardial infarction is caused by a sudden blockage of the blood vessels supplying the heart muscle also known as the myocardium. Patients can die within minutes to hours of the blockage due to direct injury to heart muscle. However many patients survive these first few hours only to die days or even years later due to heart failure. The processes that cause initial heart muscle death and then late failure are not completely understood but they involve changes occurring in the various cell types that make up the heart itself as well as in the hormones and nerve impulses that influence the heart but arise from outside it. These nerve impulses and hormones arise from organs such as the brain, kidney and gut but also	

	from glands such as the adrenals, near the kidneys. The complexity of the cell types within the heart in combination with the effects of nerves and hormones means that simpler models in cultured cells or in isolated hearts cannot completely replace experiments in intact animals. The majority of experiments will be done on isolated cells or hearts to model conditions during MI. These experiments involve the administration of a high dose of anaesthetic to achieve a very deep level of anaesthesia. Once this is achieved the heart is removed. For the reasons described above we cannot avoid experiments in living whole animals to look at the late changes occurring in the heart that cause it to progress towards heart failure. Studies we will carry out in animals include coronary artery obstruction induced by tying a ligature around a coronary artery under general anaesthesia. Animals will then be allowed to wake up and changes in the heart monitored for up to 6 months through the intact chest using echocardiography (sound waves), magnetic resonance imaging, X-ray computed tomography and other “non-invasive” imaging techniques that are also used in patients to monitor heart failure after MI and allow multiple measurement to be taken from a single animal. These investigations will be done under anaesthesia as described in our protocols and may also involve the administration of agents to improve image quality (so called contrast agents and tracers). At the end of the period of observation, which maybe up to 6 months, animals will be subject to a final deep anaesthetic for further measurements before the heart is removed and analysed. Based on previous work we will need up to 200 mice and 50 rats per year for experiments of this type. In this way we hope to identify factors that reduce MI and heart failure.
What are the potential benefits likely to derive from this project (how science could be advanced or	Signalling proteins, or Kinases, are known to be crucially important to heart function during and after myocardial infarction (MI) and during the swelling of the

humans or animals could benefit from the project)?	heart that leads to heart failure. Our own research, to date, has also shown that there are kinases that become active during MI and post-MI remodelling that determine injury. The proposed benefit of this project of work is to identify and manipulate these kinases in order to change the way the heart responds to stress. If this were possible it would add to the therapies currently available to reduce MI, post-MI remodelling and their associated mortality
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to work mostly with mice, using a small number of rats to verify key findings or to use techniques that do not give clear results in the mouse because of their small size. In total, we expect to use approximately 12,000 mice, and 700 rats.
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 have explicitly designed all experiments and protocols to allow us to answer scientific questions with the minimal discomfort to experimental animals, as described below. The most common intervention will be manipulation of isolated hearts obtained from animals that have been humanely killed. Myocardial infarction (Ml) and the cardiac remodelling which can ensue in humans is a severe disease, and is one of the leading cause of adult mortality and morbidity in the UK adult population. As such, modelling Ml and cardiac remodelling is a severe procedure, and may cause weight-loss and moderate distress in animals for several days following surgery. These effects will be minimised by close monitoring and administration of pain killers. However, our lab has methods to assess the development of injury during the surgical procedure, and without recovering from general anaesthetic, allowing us to answer several scientific questions without the animal experiencing post-MI distress. Wherever possible, we will perform

	this non-recovery surgery, thus subjecting animals to mild, rather than severe procedures. All animals will be humanely killed at the end of experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have performed, and continue to perform, many experiments using celPs derived from patients and animals, rather than animals themselves. However, the complex interplay between different organs seen in the disorders we are investigating means that the processes cannot, therefore, be completely modelled in cell culture or in the isolated heart. Both cell culture models and the isolated heart are however used extensively to screen for relevant signals. Within our research we use a variety of these models in which Ml is simulated using chemicals and oxygen starvation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have performed statistical tests based on previous work performed in our and other laboratories to model the minimum number of animals and experiments necessary to demonstrate benefit, if it can be seen. We will also make maximal use of tissue from individual animals following experiments, so that wherever possible, multiple questions can be answered from a single animal.
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 and rats are the least sentient animals we can use to model the clinical conditions we are investigating, while still remaining relevant to humans. Our cardiac remodelling work has been refined by modifying the surgery in accordance with the latest literature to reduce death and distress following recovery, and, wherever possible, to answer experimental questions under general anaesthesia without recovery, to avoid the stresses associated with post-MI morbidity and minimise any associated suffering.

Project 25	**Angiogenesis, microcirculation and microenvironment**	
Key Words (max. 5 words)	Angiogenesis, microcirculation, tumour growth, wound-healing, vascular-targeted agents	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	**X**	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Despite recent advances in treatment, deaths from diseases that show abnormal vessel control such as cancer and non-healing wounds are common. The overall project objectives are to understand how blood vessels, existing and new are controlled in health and disease. We know blood vessels are required for the development of diseases such as cancer, but because the cancer continues to grow, blood vessel growth is not switched off, and this is in part because the vessels do not mature correctly. By determining the signals which are used to control blood vessels maturing in the normal situation this will help us identify which signals and cells are not functioning correctly in disease. In addition therapies for cancer and non-healing wounds which target the blood vessels directly and also those which have indirect effects on the vessels by effecting other cells and/or molecules in the surrounding	

	microenvironment will be evaluated. A proportion of patients respond to blood vessel-directed therapies but some do not, probably due to both the structure and nature of the blood vessels produced, so model systems will be used to improve our understanding of which vessel signature is most likely to respond to such therapy.
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)?	Cancer and abnormal wound-healing, both diseases exhibiting abnormal blood-vessel activity, impose significant socio-economic costs in the UK, with cancer treatment consuming approximately 5% of the NHS budget. Similar to many therapeutics, only a small proportion of patients respond to vascular-targeted agents alone or in combination. An improved understanding of the key pathways and molecules, particularly in the way the vessels mature is crucial. This, combined with a systematic approach to understand the likely blood vessel signature of a specific tumour should allow improved selection of patients likely to respond to these therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	5700 mice will be used 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?	All models have been used and refined during previous studies, with the severity not exceeding moderate for any procedure or protocol. Approximately 80% of the animals will undergo procedures to induce either a wound or growth of cancer. Animals will be given pain relief to minimise discomfort and close monitoring will minimise distress. We will also follow established guidelines to study tumour models that ensure animal welfare is paramount and tumours are not allowed to exceed the minimum size or adverse effect necessary for the study. All procedures will continue to be refined where earlier time points may be used, and longitudinal evaluation in a single animal, therefore minimising any distress to the animal. All animals that either have

	wounds or tumours induced will be monitored closely and humanely killed at the end of the experiment. Animals will be housed in appropriate social groups in cages that are environmentally enriched in a manner appropriate for the species. Husbandry and care will be based on best practice and veterinary advice and will be performed by highly trained and competent staff. All animals under procedure will be frequently and closely monitored and if health problems are observed veterinary advice will be sought.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Modelling blood vessel development and the interactions with the complex surrounding microenvironment in wound healing and during cancer growth, is not possible to fully recapitulate using non-animal alternatives. However cells in culture will be used to assist our studies. Colleagues in Physics and Maths using computer-based approaches help us model the likely blood vessel profiles and outcomes of a certain treatment.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The generic experimental design to be used in all three objectives of the project is based on power calculations, our previous quantitative published data and experience. If large differences are detected for specific interventions, animal numbers will be reduced in subsequent experiments. The use of fluorescently labelled cells and/or genetically modified mice will reduce the number of animals required/study as angiogenesis will be imaged in one animal during the disease progression.
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 established and refined mouse models to study normal, tumour and wound angiogenesis during previous project licences. Cell-lines for implantation and biological reagents for processing the tissues to understand mechanisms are available or have been created. We have a wealth of experience using these *in vivo* models, have generated and optimised tools for imaging, monitoring and downstream analysis and have published our findings in the scientific literature. Wound healing – the two models will determine the

	how the later phases of angiogenesis with complete skin closure occur and allow high resolution imaging of the cells involved in the process (window chamber model), with the time course determined during the previous licence. Tumour models – the implantation cell number, growth kinetics and time-course of blood vessel development has been defined. The window chamber model allows longitudinal evaluation of tumour blood vessel growth in high resolution and is the most refined model to determine why the blood vessels do not stop growing in a tumour and how they respond to therapy. Tumour cells introduced into the circulation has been refined to model secondary tumour growth particularly to the bone as occurs in breast and prostate cancer. The human bone model has been refined to allow smaller fragments of bone to be implanted under the skin with the tumour cells already in the bone. We know that administration of human tumour cells will home to the human bone rather than the murine bone which will reduce pain and suffering to the animal. Genetically altered animals are the most refined to study any involvement of a specific molecule in blood vessel growth and maturation. We will continue to refine models and measurements, allowing intervention at earlier time-points, thereby minimising stress to the animal. Suffering will be reduced by close monitoring, using antibiotics and/or analgesia as appropriate in consultation with the NVS and through regular husbandary.

Project 26	Protecting and repairing the diseased heart	
Key Words (max. 5 words)	Heart attack, cardiac surgery, ischemia reperfusion injury	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of these studies is to develop and characterize in pig an experimental model of disease resembling what happen in patients suffering heart attack or undergoing open heart surgery. We then aim to use these developed experimental models to test and optimize novel treatments using new drugs or stem cells based therapies that could be used to improve the recovery of patients suffering heart attack or undergoing open heart surgery. During a heart attack or open heart surgery the heart muscle is starved of oxygen due to a restricted blood supply. Further damage also occurs when the blood supply is initially restored. The work conducted under this license will evaluate and refine the use of new drugs or stem cell based therapies to reduce complications and ultimately improve the life expectancy of these patients. These studies are an essential prerequisite to the transfer of such	

	treatments in human clinical practice**.**
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 will be to determine the effectiveness and safety of drugs and therapeutic interventions prior to their use in the clinical practice. The studies undertaken will enable treatments to be optimised in a way that will maximise their beneficial effects and minimise any risks. It is to be expected that all of the proposed treatments will prove safe and effective and that the testing program will significantly speed their introduction and uptake within clinical practice
What species and approximate numbers of animals do you expect to use over what period of time?	These studies will use pigs. Pigs are the most relevant species for these studies because their size, anatomy and physiology closely match that of humans. We estimate that we will use 430 over the 5 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 end?	The animals used in this study will be anaesthetised and either undergo open heart surgery or have heart muscle injury induced that replicates that incurred during a heart attack. For all the animals there will be no suffering during the procedure, as the whole procedure will be conducted while the animal is anaesthetised. For those animals that are allowed to recover following the procedure pain control, consistent with that given to patients in hospital, will be given to minimise any suffering. Based on our previous experience we expect most pigs to return to normal behaviour within 24 hours of the procedure. The heart injury incurred by the pigs will be the minimum needed to evaluate treatments and should not compromise the animal's general wellbeing. All surgical procedures will be performed by experienced surgeons with the highest sensitivity for the animal's wellbeing. Recovery will be managed by expert professionals who will optimise pain control after any surgical procedure. Animals will be housed in normal pens and always grouped with companion animals of the same species. On completion of the study protocol all the animals will undergo a final general anaesthesia to facilitate data acquisition and scanning and then be killed whilst anaesthetised. The

	overall severity of the procedure for pigs allowed to recovery post surgery is moderate but in all cases pigs are expected to show normal behaviour within 24 hours of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	These studies will evaluate treatments, destined for introduction into human surgical practice that are aimed at restoring normal heart function following a heart attack or open heart surgery. It is not possible to undertake this work without using animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Sample size is calculated by expert biomedical statisticians based on very sensitive biochemical markers or in-vivo imaging scans. In addition, we will: 1) Use internal controls for evaluation of end points within the same heart 2) Use heart scans similar to those used in human hospitals for mid-term evaluations, instead of using interim histology/ termination; 3) Refine the sensitivity of our study by combining high-quality imaging standards with sensitive markers of ischaemia-reperfusion injury in blood, urine, and biopsies; 4) Use cryo-preservation of tissue and other specimens from the termination procedures for future analysis.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	All the procedures will be undertaken by experience physicians using the same level of expertise, sensitivity, and care as provided by the human hospitals. An expert anaesthetist will be available during any procedure and during the post-operative stay in ITU and/or recovery. Painkillers will be used according to clinical standards to minimise post-operative pain. We will use absolutely sterile conditions and modern ventilators as well as state of the art heart-lung

	machine, Cath Lab for angiography, and surgical instrumentation. Sterilisation of instruments will be in a NHS-standard autoclave. Antibiotic, anticoagulation and/or antiplatelet treatments will be appropriate to the procedures being undergone, and the intraoperative and post-procedural recovery and housing will be to clinical standards. We routinely use state-of-the-art MRI and echocardiography for serial assessment of endpoints and to monitor any functional decline before the animal shows any clinical signs. In selected experiments telemetry based monitoring of vital signs may be used.

Project 27	Factors affecting valve development and disease	
Key Words (max. 5 words)	Veins, lymphatics, heart	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	x	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Valves present in the vessels ensure the one-way flow of fluids within the vessel. Those in the veins work to reduce blood pressure in the veins of the leg. If these valves do not develop properly or are damaged by conditions such blood clots, then the pressure of blood in the veins of the leg increases (venous hypertension) and over a long period of time causes swelling, pain and ulceration. These conditions are highly debilitating and consume more than 2% of the NHS budget each year. Existing treatments are inefficient and ulcers frequently recur because venous hypertension persists. There are large gaps in our knowledge of how vein valves develop and are maintained. The aim of this project is therefore to study the genetic and physical mechanisms that regulate how and when vein valves form, and how errors in these processes lead to poor valve function and subsequent disease.	
What are the potential benefits likely to derive from this	A better understanding of the factors affecting valve development may allow identification of those at risk	

project (how science could be advanced or humans or animals could benefit from the project)?	of venous hypertension, enable preventive therapy, or lead to new treatments to replace absent or poorly working valves. These would aim to reverse the problem of venous hypertension and prevent ulceration or promote healing in patients with ulcers.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 10,000 mice during course of this 5 year project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	By removing a gene in a mouse we can determine the importance of that gene in the development of a body structure such as a vein valve. We can also mimic human disease caused by mutations in genes by generating mice with the same mutations using new gene editing techniques. Our experience of deleting the genes that we think may regulate vein valve development has to date shown very little adverse effects to the animal, as vein valves are not as critical to normal vein function in a mouse as they are in people. When mice undergo surgery to alter blood flow in leg veins this will be performed under general anaesthesia, with appropriate pain relief to reduce to a minimum the effects that an animal may experience as a result of the surgical procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex dynamic cellular and molecular environment that regulates vein valve development inside a living organism cannot be replicated in the laboratory. Vein valve development occurs from late pregnancy through to the early period following birth. Study of the cellular and molecular processes underlying the development of vein valves would therefore require access to tissue during these stages, which is not ethically possible in man. We will use of a mouse model of vein valve development that we have previously characterised in order to investigate the patterns of expression of key

	factors during valve formation and the effect of alterations in blood flow on valve development and maintenance. The use of genetically modified mice will help us to find out how manipulating the structure and production of factors affects the development and maintenance of a valve.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where possible, we will use alternatives such as cells and computer models to simulate valve growth, while valve tissue and scans from humans will be used for comparative studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the least sentient animals in which we can perform these studies. This study will involve the use of a mouse model of vein valve development that we have previously characterised. We will aim to use improved vein/valve imaging techniques, as they become available, to assess valve development over time, which should dramatically lower the numbers of animals required for our studies. We will keep animal numbers to a minimum by carrying out statistical calculations, prior to commencing experiments, that will tell us how many animals are needed for each experiment. These will be based on our knowledge of the variability in various measures that we wish to use in the model. All animals will receive appropriate anaesthesia during surgical procedures and pain-killers to relieve suffering. Our long experience with this and other models of venous disease in mice is that they tolerate blockage and manipulation of blood flow in their veins very well. Adverse events from procedures carried out in these studies will therefore be minimal and mostly relate to post-surgical discomfort, which is controlled by the use of appropriate pain relief until the animal has recovered.

Project 28	Studies of Complex Genetic Traits in Rats
Key Words	Rat, Cardiovascular, Metabolic, Kidney, Genetics
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Complex diseases, such as heart disease, kidney disease and type 2 diabetes, are major causes of morbidity and mortality worldwide. Importantly, they are modified by both genetic and environmental factors. However, whilst a number of environmental risk factors for these diseases have been discovered (e.g. alcohol consumption, smoking, a sedentary lifestyle, and poor diet), their underlying genetic causes are still poorly understood.

Here, we aim to identify the genes and molecular pathways influencing such diseases in animal models, and in so doing, hope to find ways to prevent/reverse disease processes in humans.

Given their close evolutionary (and, therefore, genetic) relationship to humans, rats are our model system of choice for such studies. More specifically, we aim to use genetically-engineered rat strains already predisposed to cardiovascular, inflammatory and metabolic diseases. This not only helps us better understand the genetics of the disorders, but also helps us describe associated clinical phenotypes.

We anticipate that our studies will lead to translatable discoveries that benefit human health.

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)?

Previously, we developed genome editing methods in rats to derive new knowledge about human cardiovascular disease and insulin resistance. In this licence, we aim

to maintain our leading position by generating novel gene-targeted rat lines. These new models will help us better understand such diseases in a more tissue- and organ-focused manner; greatly refining our understanding of their pathology. Furthermore, by complementing our rat studies with those carried out in human cells, we hope to expedite the discovery of novel therapeutic targets in humans.

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

We plan to use approximately 9,000 rats over the five year course of the licence.

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?

We have developed our protocols to ensure that the maximum level of severity experienced by any animal will be moderate; due to the induction of heart, kidney or liver disease. Animals undergoing surgery will be carefully monitored prior to surgery and given medication to prevent pain. During recovery, all animals will be housed in a warm environment, given additional pain management medication and remain under close observation. Where possible, any animal showing severe adverse signs (due to a surgical procedure or otherwise) will be treated or euthanised after veterinary consultation. Where veterinary advice is not available (i.e. during acute surgical complications), animals will be treated or humanely sacrificed as soon as possible. At the end of each study (or protocol), all animals will be sacrificed using an appropriate humane method, and their tissues and bodily fluids harvested for further analyses.

Application of the 3Rs

Replacement

Due to the invasive nature of many procedures, there is limited scope for fully understanding the molecular basis of multi-organ diseases in humans. This is not helped by our genetic diversity and the wide range of environmental factors to which we are exposed.

For example, to understand the pathological processes leading to high blood pressure or cardiac hypertrophy (both of which contribute to cardiovascular morbidity), an appreciation of cardiac, renal (kidney), adrenal and nervous system function within an intact organism is required; especially as each system is made up of various different cell types that become dysregulated during disease.

Although mammalian cell culture and computer modelling methods have greatly advanced our understanding of disease pathology, neither can accurately replicate the full *in vivo* nature of complex, multi-organ diseases. We will, however, complement our studies with such techniques where possible.

Reduction

Many of the techniques used under our previous licence will be applied during the course of this licence. Therefore, we are already experienced in the minimum number of animals required for an expected outcome to be informative; greatly reducing animal waste.

Furthermore, we will effectively combine techniques and procedures to maximise the utility of each animal whilst minimising the distress caused.

Animal use will also be kept to a minimum by first investigating conditions and techniques *in vitro*, rather than in live rats.

Refinement

The laboratory rat has a long and proven history as a model for the study of complex human diseases.

For example, rats have a similar blood pressure and heart rate to humans, making them useful cardiovascular disease models. Genetic research has also demonstrated that many genes predisposing to cardiovascular disease and type 2 diabetes are shared between rats and humans.

Through our previous work, we have greatly refined our surgical techniques and expertise, thereby minimising adverse events and the length of time animals are anaesthetised. We also implemented the use of absorbable sutures, eliminating the need to remove non-absorbable sutures following wound healing. Furthermore, by using implantable devices for blood pressure, heart conductivity, and glucose level monitoring, we have reduced animal handling requirements. Such devices provide more accurate measurements (compared to conventional monitoring) and improve animal welfare.

By closely monitoring the relevant literature, we have also determined the minimum dose requirements for a number of protocols, reducing the harm to the animal without compromising our understanding of the physiological processes. Various protocols will be carried out under terminal anaesthesia, minimising the distress caused to the animal.

All rats (especially those undergoing treatments/surgery) will be closely monitored for signs of illness and distress on a daily basis by expert technicians and researchers. Veterinary advice will be sought as required.

Project 29	Mechanistic studies in pre-clinical stroke/small vessel disease	
Key Words (max. 5 words)	Stroke, small vessel disease, hypertension, rats, mice	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Stroke is the 3rd leading cause of death and the leading cause of acquired adult disability in the UK. Having had a stroke also doubles the chance of developing vascular dementia. Common to both increased incidence of stroke and vascular dementia is high blood pressure. The purpose of this project is to better understand the mechanisms that lead to damage after stroke and the development of vascular dementia. This will, in turn, lead to the identification of new treatment options as currently, only one drug is available to stroke sufferers.	
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)?	During this project we will utilise relevant rodent models and induce an experimental stroke in order to examine the mechanisms responsible for disease causation and progression. These models will include animals that have been genetically modified to alter specific genes or proteins that may play an important role in the disease process. These studies will lead to improved understanding of	

	the how blood vessel in the brain may be altered by disease (cerebrovascular disease) and identification of new therapeutic targets, which will ultimately lead to better treatment and prevention of cerebrovascular disease (such as stroke and vascular dementia) in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate that we will use approximately 5000 rats and 2500 mice over a 5 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 end?	Animals used in this project are mice or rats with altered cerebrovascular characteristics which we will then subject to an experimental model of stroke or vascular dementia. During the previous 20 years of breeding and maintaining the rat colony which displays an increased blood pressure (hypertension) we have observed approximately 4-5% incidence of spontaneous strokes in male rats. The majority of these sustained strokes from 4 months of age, which is the time of established hypertension. Spontaneous strokes are almost never encountered in female rats with high blood pressure. The mildest insult which produces a reproducible degree of ischaemic damage or functional deficit will be used, to minimise animal suffering and post-operative care (regular monitoring, subcutaneous fluids, maintained body temperature, softened diet, etc.) provided. Any animals that exceed the clinical signs associated with severe severity will be promptly and humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nature and complexity of the cerebrovascular disease process makes finding alternatives to live animal models extremely difficult. However, wherever possible we will use non-animal alternatives (e.g. cell-based assays) for our investigations.
2. Reduction Explain how you will assure the use of minimum numbers	We obtain expert statistical advice before commencing any new studies and perform power calculations which allow us to identify the lowest

of animals	appropriate group sizes for each procedure. In addition, the use of techniques such as MRI and echocardiography allow non-invasive serial measurements in the same animal thereby significantly reducing the number of animals required for most studies.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The rat and mouse models used in these studies have been carefully selected based on their unique genetic profiles. These animal models will allow us to determine the direct contribution of specific genes and proteins to the progression of hypertension and cerebrovascular disease. Our *in vivo* protocols have been designed to provide the maximum detailed characterisation of the cardio- and cerebrovascular systems whilst at the same time ensuring that the animals under investigation experience the least pain, suffering, distress or lasting harm. For example, analgesia is given for all surgical procedures and disease development is monitored by non-invasive imaging that allows early endpoints to be instigated prior to animals showing significant clinical signs.

Project 30	Understanding and Treating Cardiovascular Disease
Key Words	cardiovascular development, heart repair
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

This project will investigate how the heart and blood vessels function in health and disease, and how we can use this knowledge to improve outcomes for patients. These include patients who have an inherited condition that results in blood vessel problems that can also affect heart function. In addition, we aim to develop treatments for patients with a heart attack. Patients now have an increased chance of surviving a heart attack, providing they reach a suitable clinic in a rapid time frame due to improved intervention at the acute stage. However, heart attack patients who are discharged from hospital have an increased risk of developing heart failure over subsequent months and years. This (together with our ageing population) means there is a growing number of patients in the western world who develop heart failure. There are now more than 0.5 million patients living with heart failure in the UK. There is no effective treatment (other than heart transplant) and the disease will get progressively worse. Better treatments for patients at the acute stage of a heart attack will reduce damage to the heart and thereby reduce the risk of later progression to heart failure. The work on this licence aims to address this issue using mice to model 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)?

Studies to further increase our understanding of the disease mechanisms will underpin improved treatments for two groups of patients: (i) those with an inherited vascular disorder that affects approximately 1/5000 people; and (ii) those who survive a heart attack that subsequently progresses to heart failure. Based on our advances in understanding we will use drug treatments that can completely or partially rescue the inherited vascular disorder. We will also use small molecule inhibitors for delivery at an early stage following a heart attack to reduce heart injury. Our goal is to improve long term outcomes for both these patient groups.

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

Mice : approximately 3,000 adults and 300 neonates per year. The majority of the adult mice are used in breeding programmes, and because of the silent nature of the gene alterations, they are indistinguishable from wild type mice

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?

Inactivation of target genes may lead to appearance of clinical symptoms due to the development of abnormal blood vessels. Delivery of substances to rescue the clinical problems will be given via the least stressful method possible eg via the food or drinking water. Alternatively, where injection is required, multiple doses will be given using a surgically implanted minipump. On rare occasions the drug may be delivered locally in the eye of young mice, and implants may be placed beneath the skin to monitor blood vessel development. Ligating a coronary artery will be used to model a heart attack. The surgery is complex and on some occasions this can lead to respiratory distress or intra-operative bleeding. If this occurs the animals are humanely killed without recovery from anaesthetic. A small proportion of animals may later develop fatal disturbance to the heart rhythm or rupture of the heart which leads to sudden death. Some animals will be imaged using MRI or fluorescent methods, and these imaging methods are not normally associated with adverse effects. We keep within moderate severity limits and all animals are humanely killed at the end of the work.

Application of the 3Rs

Replacement

Unfortunately there are no suitable cell culture systems that can be used to replace in vivo models of cardiovascular development and disease, due to the complexity of the processes involved. Mice are being used in this project because this is the simplest organism that has a similar heart and blood vessels to human, and that can

be used to investigate the roles of different genes in cardiovascular development and disease. Alternative less sentient animals such as Zebrafish are not suitable for this work because they are so evolutionarily distant from human that it would be difficult to translate any of our findings. For example they are cold blooded, they have only two heart chambers instead of four; they have no lungs; and they show endogenous regeneration of the heart following injury, a property that mammals do not possess. In some cases we use mouse embryos for analysis. All the work is complemented by cell culture work, for example when investigating processes that occur within individual cells, the effect of bioactive substances will be tested in culture prior to in vivo work.

Reduction

We use the minimum number of animals required for our experiments and regularly consult a statistician for advice. We do pilot work so that perform statistical analyses we can predict the group sizes needed to detect differences with statistical significance . This key feature of good experimental design makes analyses of the data far more efficient and minimises the risks of wasteful experiments. Group sizes, gender, strain and age are matched for control and experimental groups. Sources of variability will be identified and minimised wherever possible. For example variability in the matrigel plug experiments is minimised by using small syringes to generate equal plug sizes; for example, variability in vascular phenotypes following gene activation with tamoxifen is minimised by ensuring the optimised tamoxifen dose is used.

Refinement

The genetically modified mice that we use generally carry ‘hidden’ mutations, such that almost all of animals in protocol 1 are completely healthy until they are given the inducer (eg tamoxifen) to activate the mutation, reducing any clinical effects to the absolute minimum necessary for the project. Where substances are used to reduce clinical symptoms we will use the oral route wherever possible (eg in drinking water).

As this is a continuation of a project licence that has already been running for over 9 years, protocols are already established for the majority of the work described in this application, and numerous refinements that we have introduced can be seen in the sections marked adverse effects at the end of each protocol. By using appropriate anaesthesia and analgesics in the procedures to alleviate pain and discomfort, the protocols cause the minimum possible discomfort to the animals. However, protocol 8 causing cardiac injury is a major surgery to model a heart attack. In a minority of cases (4%) we observe sudden death (just as occurs in patients) which is due to severe heart problems. This means that although 96% of animals undergoing this procedure are classified as moderate, the minority of cases (currently 4%) are deemed to be severe, and the overall classification of this protocol is therefore assigned as severe. Our plans to change to the closed model of transient cardiac

ischaemia which is a more refined procedure is expected to dramatically reduce this risk of severity and we hope to move to a 100% moderate classification.

Project 31	The molecular basis of cardio-metabolic disease
Key Words	Diabetes, cardiovascular disease, insulin resistance, insulin-like growth factors, atherosclerosis
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Diabetes is associated with a 2-3 fold increased risk of cardiovascular disease. In many cases, this is driven by resistance to the effects of insulin and related hormones. How insulin resistance causes cardiovascular disease remains uncertain. We are investigating how insulin resistance leads to cardiovascular disease at the molecular level in order to identify new mechanisms which could potentially be targeted in future

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)?

Understanding how insulin resistance and related processes affect blood vessel health and repair will provide a valuable insight in to how cardiovascular diseases develop. We hope that the information derived from this project will identify new targets which could be developed to create new ways to prevent and treat these diseases in future

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

Approximately 20 000 mice will be used 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 levels of severity? What will happen to the animals at the end?

In many cases, we will be able to examine blood and tissue samples collected after humane killing of mice. In other cases, we can study mice using scans similar to those in humans (for example ultra-sound or magnetic resonance scans (MRI)) whilst they are under an anaesthetic. In some animals, mice will undergo surgery to create diseases similar to those in humans (for example heart attacks, aneurysms and blood vessel damage). Mice will be under anaesthestic for surgery and will receive pain-killers as they recover. Most animals are then humanely killed within 2-6 weeks to study the effects of disease. However, as in humans, a minority of mice might die suddenly.

Application of the 3Rs

Replacement

Diabetes and cardiovascular disease are complex disorders. Whilst we can conduct much of our work in cells in the laboratory, we cannot fully recreate all the processes which occur in the body to cause diseases like heart attacks or aneurysms. In order to identify new ways of preventing and treating these diseases in humans, it is necessary to carry out some aspects of our research in animals.

Reduction

We are able to minimise the number of animals used by careful design of our studies, combining scans, blood tests and detailed examination of tissues collected after the animals are humanely killed.

Several live imaging techniques, e.g. ultrasound, MRI scanning will be used in experiments described in this application. These techniques can pick up progression of adverse changes in cardiovascular system much earlier in experimental animals and produce reliable and repeatable data which is helpful in the reduction of number of animals used.

Refinement

We have chosen the mouse as it is relatively straightforward to alter the genes of mice in order to study the effects of specific proteins in normal function and disease. The mouse is one of the lowest order mammals in which is it appropriate to study human disease. To induce exercise related cardiac effects where possible we will provide free access running wheel as part of home cage environment that reducing the stress of frequent animal handling and forced exercise.

Sequential imaging of animals in longitudinal studies can pick up pathological changes at an early stage thus implementation of humane end points can be

implemented much more accurately, and consistently resulting in refinement of experiments

Project 32	Myocardial infarction
Key Words	Heart attack, vascular dysfunction, nitrite, nitric oxide, perhexiline
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

To investigate the underlying mechanism of a heart attack (myocardial infarction).

To assess novel drug targets to protect the heart and vascular function following 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)?

Current clinical interventions available for the treatment of heart attack and vascular dysfunction are minimal. This project provides an insight into the protective effects of novel drugs (nitrite, perhexiline and its derivative) and their ability to prevent and/or delay the development of cardiac and vascular injury. This project will also provide an understanding of how these novel drugs work within the heart and vasculature in a more physiological and clinically relevant set up

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

Mice/rats will be used over the course of the next 5 years (approximately 21000 in the course of 5 years).

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

Upon completion of the non-invasive ex vivo assessments, all mice/rats will be humanely killed The majority of the animals used will not experience any procedures prior to administration of general anaesthesia without recovery for collection of tissues. This will be carried out at a suitable depth of anaesthesia so the animal does not experience any pain or suffering. Animals will be humanely killed at the end of the tissue collection without regaining consciousness. A small proportion of animals may be treated with drugs prior to the collection of tissues but these drugs are not expected to cause any welfare concerns.

Application of the 3Rs

Replacement

Myocardial infarction and vascular dysfunction are complex multifactorial syndromes which involve the interaction of numerous body systems over a prolonged period of time. As such it is impossible to assess the novel drugs in a single cell isolated set-up.

Reduction

When possible, mice/rats used for ex vivo cardiac and vascular function assessment will also be used for tissue collection for molecular analysis.

Refinement

The mouse genome can be easily manipulated thus allowing for various genetic models to be developed which are of relevance to investigate cardiovascular diseases and drug targets. Furthermore, myocardial infarction in humans is characterised by features such as contractile dysfunction and cell death (infarction) which can only be replicated in an animal model.

Project 33	Calcium-permeable channels and their associated mechanisms and therapeutic potential
Key Words	Blood vessel, Cardiovascular disease, Cancer, Diabetes, Calcium channels
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

The overall aim of this project licence is to identify molecular mechanisms that could be the basis for new therapies aimed at addressing cardiovascular disease and cancer in the absence or presence of the metabolic syndrome.

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)?

One potential benefit of the work is to provide fundamental biological understanding about calcium ion signalling mechanisms in mammalian biology. A second potential benefit is the foundation for new treatments for cardiovascular diseases and cancers which are common causes of premature death and disability, the most common causes of death world-wide, and the causes of over half of deaths in the western world, despite current-day treatments. A particular concern is the confounding factor of the metabolic syndrome which is characterised by obesity, diabetes, greater cardiovascular disease and cancer risk, and resistance to current therapeutic agents.

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

Over the 5 years duration of the licence it is estimated that 28,000 mice will be used.

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

The experiments will involve making modifications to calcium channel mechanisms using genetics or drug-like substances. It is our hypothesis that modification of the mechanisms could be a basis for potential novel therapeutic agents, so we expect that the modifications will have beneficial rather than adverse effects. In some cases we may unexpectedly observe adverse effects of modification leading to mild or moderate, or perhaps in some cases severe, severity levels. Animals will be promptly killed using a Schedule 1 procedure if there is severe adverse effect. In some cases moderate severity might also be considered to be unacceptable and Schedule 1 will therefore also be applied. We have, for example, found that genetic disruption of one of the target mechanisms is lethal in mice at around embryonic day 10. For studies of this mechanism we will use embryos or conditionally induce the modification only in a specific cell type in the adult animal. In some cases we expect to be able to achieve a subtle modification of Piezo1 which is not lethal and does not lead to a severe phenotype; in these cases we will study adult mice if the severity level is mild or moderate. At the end or if there is unexpected adverse severity level, mice will be killed by Schedule 1 procedure. In some experiments we will mimic human disease in mice using genetic or dietary approaches or substance administration or through a surgical procedure or by injecting cells under the skin or systemically. It is expected that the severity level will be mild or moderate. There is the possibility to reach severe adverse effects with procedures of this type, such as with the induction of aneurysm which can be lethal as it is in humans, but we will minimise the risk of lethality by optimising the dose of the inducing agent. Lethality will not be a deliberate end-point of the studies. As part of our aim to develop novel therapeutic agents we will test novel chemicals to determine the maximum tolerated dose. At the end of the experiments or if there is toxicity, animals will be killed by Schedule 1 procedure

Application of the 3Rs

Replacement

Cardiovascular disease and cancer are complex disorders. Whilst we can conduct much of our work in cells in the laboratory, we cannot fully recreate all the processes which occur in the body to cause diseases like heart attacks or aneurysms. In order to identify new ways of preventing and treating these diseases in humans, it is necessary to carry out some aspects of our research in animals.

Reduction

We are able to minimise the number of animals used by careful design of our studies, combining scans, blood tests and detailed examination of tissues collected after the animals are humanely killed.

Refinement

We have chosen the mouse as it is relatively straightforward to alter the genes of mice in order to study the effects of specific proteins in normal function and disease. The mouse is one of the lowest order mammals in which it is appropriate to study human disease.

Project 34	Improving minimally invasive treatment of coronary artery disease
Key Words	Coronary, artery, atherosclerosis, angioplasty, stents
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

Aim:

To improve treatment for patients with blocked and narrowed arteries using minimally invasive techniques through a tube or catheter by balloon inflation, stent implantation and related techniques.

Objectives

1. Optimise artery interventions (balloon, stent, other).

2. Test new drug treatments for heart attacks.

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)?

Background Coronary artery disease due to atherosclerosis (a build-up of fatty tissue in the artery wall) is the leading cause of death and disability, and is increasingly important, due to ageing, high fat diet, obesity, diabetes and smoking. It causes sudden death, heart attacks, angina and heart failure. Programme of work 1. Optimise artery interventions. The commonest treatment for coronary disease is

balloon angioplasty and stent insertion through a tube placed in an artery. The problems are stent blockage with blood clot, excessive scar tissue or restenosis, and inflammation. New stent designs, coatings and drug applications have been tested. The pig is useful because it is similar in size, shape and physiology to man. We will test better metal stents; assess soluble stents; test circulatory support mechanisms; develop new techniques for 'burrowing through' blockages; promote artery healing with stem cells; and test new techniques to image those procedures. We will do so in undiseased animals to assess purely the effects of the intervention and, when necessary, in diseased animals (the Danish Aarhus pig has atherosclerosis, or arterial disease) to assess the effect of interventions when disease is present. 2. Test new drug treatments for heart attacks. Heart attacks occur when diseased coronary arteries block suddenly. They carry a high death rate and can leave the patient with heart failure. We can model a heart attack by blocking an artery with a balloon. We can then test new treatments to limit the extent of the heart attack. One example is to administer carbon monoxide in minute dose and measuring the size of the heart attack using imaging, pressure measurements and microscopy.

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

Pigs 140 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 levels of severity? What will happen to the animals at the end?

1. Interventions. These are moderate, because the surgery is under general anaesthetic, with instrumentation of the arteries through a small incision in the skin. When the animal wakes up, it may have a day or so of discomfort at the wound. The benefit to man is to improve safety of the techniques, preventing deaths due to stent thrombosis, and reducing suffering due to recurrent scar tissue. Fate: the pigs will be killed for analysis. 2. Heart attacks. We create a model of a heart attack by blocking an artery, and then give an agent (or placebo) to reduce heart damage. Post-operative pain is worse than after a stenting procedure and is controlled with pain relief, and there is a risk of sudden death within the first few days due to the heart attack itself (not the treatment). This is quick and painless (we know this from seeing it in patients), this procedure is therefore classed as moderate. The benefit of our therapies is to reduce death rate, prolong life, and reduce heart failure in man. Fate: the pigs will be killed for analysis.

Application of the 3Rs

Replacement

Only scientific questions which cannot be addressed through cell, tissue or small animal experiments are performed in the pig. Examples are: the development of a new stent ± coating or drug, which must be in an artery for 4 weeks, and is too large

for a mouse or rat; the creation of atherosclerosis in a coronary artery rather than a mouse aorta; and drugs which work in a mouse model of heart attack, but need replicating in a large animal before being tested in man.

Reduction

We design experiments carefully to use the fewest animals possible whilst still answering the question, and will use imaging techniques to increase the information obtained and thus reduce the number of animals needed, and will test multiple devices in the same animal (rather than using more animals) if it is appropriate in scientific and animal welfare terms

Refinement

Working with the NVS and named persons we always seek to apply best practice in anaesthesia, pain relief, avoidance of infections, by selecting the most refined techniques and in monitoring and treating any problems that arise as a result of applying the procedures. In general, serious complications that cannot be quickly and effectively remedied will be dealt with by prompt humane killing of the animal.

Project 35	Targeting the immune response in cardiovascular diseases
Key Words	Atherosclerosis; restenosis; aneurysm; myocardial infarction; stroke
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in paragraph (b);

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

Atherosclerosis is caused by the build-up of fatty plaques in the walls of our arteries, and is the cause of heart attacks and strokes. The accumulation of cholesterol in the arteries leads to subtle chemical modifications making it recognised by our immune system as a strange ‘non-self’ material. This leads to activation of selective white blood cells called lymphocytes, which inflames the vessel. We will try to identify the immune cells that instruct the lymphocytes to react aggressively against the deposited fatty material and make the disease worse. The main goal of this part of the research is to determine the identity of the immune cell subset that initiates activation of our immune system against the deposition of fatty material in our arteries. Aneurysm is an abnormal local dilatation of an artery. The main deleterious consequence of aneurysm formation is vessel rupture due to excessive weakening of the artery wall, which may lead to sudden death. The only treatment involves vessel repair through surgery. There is currently no approved medication for this

disease. My laboratory has recently developed original in vitro and in vivo experimental models with the aim to unravel the mechanisms of aneurysm formation and rupture.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Our previous research has already led to the initiation of proof-of-concept clinical trials in humans to test new treatment strategies in patients with heart attack. The present research will lead to a better understanding of how the immune system reacts against the deposition of cholesterol in our arteries and is expected to lead to more effective strategies to combat atherosclerosis and its complications (heart attack and stroke). We also expect that the work will substantially enhance our understanding of aneurysm formation/rupture and will identify critical targets for treatment.

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

Over 5 years period, we will use: Mice: approximately 42850. This amounts to 1.5 mouse/day/researcher. Rats: 260.

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?

We have devised 25 protocols. The majority of animals (~70% to 80%) are not expected to show signs of adverse effects that impact materially on their general well-being. No more than 30% of animals are expected to show moderate or severe clinical signs (e.g., piloerection, dehydration, aneurysm rupture). Very rarely the severity of these signs may be such that the humane end points may be reached. Animals are monitored on a regular basis to detect any sign of distress or suffering. Analgesic agents will be administered as required. In the event of complications, or at the end of the experiment, animals will be killed by a schedule 1 method.

Application of the 3Rs

Replacement

The number of mice for this project may seem relatively high (although it's around 1.5 mouse/day/researcher). This is due to the lack of reliable in vitro models of the diseases that we are addressing in this project and to the absolute need to validate any novel disease-relevant targets in vivo using appropriate models.

In addition, many high-ranked peer-reviewed journals require that every experiment is repeated several times to ensure reproducibility.

During the last 2 years, we have developed the use of human induced pluripotent stem cell (iPSC)-derived vascular cells to further reduce and try to replace the use of

animals. This has allowed us to replace in some cases one mouse model of cardiovascular disease by iPSC-derived vascular cells (collected and generated from individuals bearing or not the 9p21 risk variant), creating an in vitro model of (one aspect) of the disease. We will pursue this strategy and try to apply it in other CVD settings.

Reduction

When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative.

We always aim to maximize the information that can be recovered from a single animal. For example, the same animal may undergo serial imaging in vivo and when killed at the end of the experiment, samples are collected from multiple sites and cavities to assess the effect of candidate gene mis-expression in multiple tissues.

Refinement

Animals are housed according to the best recommendations in an appropriate and enriched environment. By performing pilot studies and choosing well established protocols based on extensive previous experience, we minimise the unknown effects on the mice and subsequently pain, distress and suffering.

We very frequently monitor animal behaviour and well being to detect any upcoming problem at an early stage.

We have recently refined a model of aneurysm induction using peri-aortic elastase instead of intra-aortic elastase, leading to less invasiveness and much shorter duration of surgery. We will pursue such important efforts to improve our models while maintaining animal distress at the minimal level possible. During the last 5 years of my research, only 1% of animals used under my PPL have shown adverse effects.

In vivo imaging and monitoring of immune cells and atherosclerosis development will be performed on a subset of mice using validated and appropriate methodology. This will allow us to track cell fate in vivo and perform longitudinal studies without the need to kill the animals at each time point of the analyses.

Project 36	Innovative Treatments for Heart Failure
Key Words	Heart failure, Stem cell therapy, Gene therapy, Drug therapy
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

Heart failure occurs when your heart muscle doesn't pump blood as well as it should. This is a major cause of human death and disability. Currently, heart failure affects millions of people all over the world and produces a huge economic burden for treating these patients. Therefore development of new effective treatments is urgently needed. Previous research in our groups and others has shown that cell therapy (transplantation of stem cells), gene therapy (injection of gene to change expression of a molecule) as well as drug therapy are promising; however, outcome of clinical trials so far is not very satisfactory. Thus, for the future success of these new therapies, further laboratory studies to improve the protocol are required.

This project aims to develop safe and effective new protocols of cell, gene and drug therapy for the treatment of heart failure. In addition, our study aims to discover new biological information regarding the mechanism by which the heart becomes weakened and recovers from the damage, which is not fully understood currently.

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)?

Results obtained in this project will propose promising new treatments for heart failure. These treatments will be able to be applied to patients after some more pre-

clinical studies. We believe that these may improve survival and quality of life of a large number of patients. Treatment cost for these patients may also be reduced, thus this project has a great impact in patient care. In addition, collected data will add exciting new information in basic biology underlying development of and recovery from heart failure. This will advance our scientific knowledge and also suggest further novel approaches for the treatment of heart failure.

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

A total of 1,700 rats and 1,800 mice will be used to complete this project over 5 years. These include genetically altered animals, which are extremely useful to clarify the role of specific cells or molecules in the development of and recovery from heart failure. All possible efforts to reduce, refine, replace the animal use have been and will be made as stated in the 3Rs section below.

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?

Human heart failure includes several different types based on the cause, stage, and nature. Thus, the most effective treatment for each type of heart failure is different. We will therefore use four different models, each of which represent a major type of clinical heart failure. All these models are well validated and frequently used in relevant research. Unfortunately, to mimic clinical heart failure, which is the major cause of human death, these models needs to have substantial severity, causing about 10% death in animals. However, we have developed the least invasive models to minimise death and suffering of animals (see below 3R section). These heart failure animals will be treated with different protocols of treatments, including cell, gene and drug therapies, and outcome (safety and effects) will be evaluated to decide the most promising ones. At the end of the protocol, animals will be humanly killed and tissues and organs will be collected for further examinations, including investigations on the mechanisms by which heart failure recovers.

Application of the 3Rs

Replacement

Our project aims to develop new clinical treatments for heart failure. In order to evaluate safety and therapeutic effects of the treatment, monitoring of global function and structure of the heart as a whole organ for a long term after the treatment is needed. The process to develop and recover from heart failure is extremely complicated, involving multiple cell types (both locally resident muscle cells and non-muscle cells as well as recruited cells) as well as many molecules and signalling pathways. Although we have made all possible efforts, it is impossible to represent such complex processes by using computer-based systems, lower organisms and

embryo stages, cultured cells, tissue, and organs. Only living animals can be meaningful models for the purpose.

Reduction

A wide literature search has confirmed that our project is original, and that there is no duplication with previous reports. For quantitative experiments, animal numbers needed are statistically determined using power analysis. To assure reproducible outcome, which will maximises the information obtained from the minimum resource, experiments will be carefully designed and performed, including randomisation of treatment or control groups, allocation concealment, and blinded assessment of outcome, and explicit inclusion and exclusion criteria. In addition, tissues from the same animal will be used in as many analyses as possible to minimise the number of animals required.

Refinement

We will use rats and mice, which are the most suitable for this large-scale basic and translational study. General features on heart failure in rodent models are sufficiently equivalent to human patients (though not 100% same). In rodents, we will be able to use genetically altered animals, which is extremely useful to elucidate the mechanism underlying the development of and recovery from heart failure. There are a plenty of useful research materials (like antibodies) for rats and mice.

Our project requires investigations of heart failure in clinically relevant settings. Human heart failure has several different types based on the cause and nature. The most effective treatment may be different among the heart failure types. We will use four different models that mimic major types of heart failure. All the models are well justified and have been widely used in the similar research.

These heart failure models have “severe severity”, but we have optimised the protocols to mininise the death and distress of the animals. Surgical and anaesthetic techniques have been refined. Post operatively, animals will receive intensive care for several hours in special recovery cages. Post-operative pain will be prevented using analgesics. Infection will be prevented by using antibiotics and aseptic procedures in a specifically-regulated recovery surgery room. Dehydration will be prevented by administration of fluids and limited blood collection. In an unlikely event in which animals do not recover from surgery well or develop unexpectedly severe heart failure, they will be humanely killed.

Project 37	Ischaemia-Reperfusion Injury and Shock
Key Words	Ischaemia, Shock, Kidney, Haemorrhage, Heart Research, Trauma
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;

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

Our project has two aims, firstly to increase our knowledge about the events that lead to organ injury and dysfunction in diseases associated with reduced blood supply (ischaemia) to organs. These include heart attacks, acute kidney injury and shock (which can lead to dysfunction and failure of multiple organs). Shock and organ dysfunction is the single most common cause of death in intensive care units and we would like to gain a better insight into the mechanisms that lead to organ injury and failure with the hope (see below) to develop new medicines to combat these conditions.

Our second aim is to work towards identifying new treatment approaches or medicines for these conditions. These include the use of known medicines (with a good safety profile) for new indications (diseases) either alone or in collaboration with the pharmaceutical industry. Much work is being done to develop new drugs for treating heart attacks, acute kidney injury and shock (caused by either blood loss or infections) but sadly these efforts have yet to translate into the development of new medicines which reduce the extent of organ injury and dysfunction in these conditions, which a major health and economic burden to society.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

We hope to gain a better insight into the mechanisms leading to organ injury and dysfunction in diseases where the blood supply to an organ is critically compromised (heart attack, acute kidney injury, shock). This will lead to publication and presentation of our discoveries. Ultimately, we wish to discover novel interventions that ultimately can be used in man to reduce the mortality and morbidity associated with ischaemia-reperfusion injury (hear/kidney) and shock. We have already demonstrated that it is possible to move our preclinical discoveries into clinical trials in man (translation). If successful this will benefit a large number of critically ill patients by reducing their illness (morbidity) and mortality.

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

We expect to use approximately 9000 mice and 12000 rats over the 5 year course of the licence.

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?

In our animal work, we induce kidney disease, heart attacks or shock by reducing the supply of blood (through surgery) to kidneys or heart, or (in the case of shock) by causing an infection or removing blood (to simulate blood loss after an accident). Whenever possible, the entire experiment will be conducted under anaesthesia. At the end of the experiment the animal is humanely killed and blood and organs are removed for analysis in the laboratory. In other cases the animals will recover from surgery to allow us to investigate the progression of the disease. Diseased animals may transiently display symptoms of kidney failure or heart dysfunction. Like patients, these animals are, where necessary, treated with pain killers, antibiotics and fluids to minimise any symptoms. At the end of each experiment, the most humane method of euthanasia is chosen.

Application of the 3Rs

Replacement

Whenever possible, our research is carried out using kidney and heart cells grown in our laboratory. These isolated cells from heart or kidney enable us to test the effects of new disease pathways or treatments in these cells, which provides us with useful information as to which therapies might work in the whole living organism. Once we have identified a new treatment strategy, it is essential that we test the benefit of this therapy in a whole living organism. We have carefully considered our procedures in order to minimise pain and distress experienced by an animal, and to enhance its well-being. The employment of animals of a lower phylogenetic scale would be of

little relevance to our studies as they are physiologically too different from humans. Likewise the use of computer modelling to predict intracellular mechanisms are still only predictions and need to be confirmed before moving on to humans; such modelling would complement data obtained from the above mentioned cell studies.

Reduction

All work involving animals in our laboratory is limited to highly experienced staff, thus, ensuring that the welfare of experimental animals is protected. We always carefully considered our experimental design and have used both our previous experience of *in vivo* studies and performed Power calculations to ensure that the least number of animals are used in order to generate precise data with the highest chance of demonstrating a significant response. We also consider performing small pilot studies in order to elucidate whether or not it is appropriate to proceed to a major experiment. Whenever possible, we will use cultured cells (e.g. from the heart or kidney) to obtain preliminary data which will allow us to modify the main study (e.g. to help decide appropriate doses of drugs) in order to use fewer animals, earlier endpoints and/or less-invasive procedures. Whenever possible, we will use imaging procedures (MRI or CT) for longitudinal studies aimed at evaluating the progression of disease; and this reduces the overall number of animals needed. Where possible we will use genetically modified mice, in which key proteins are deleted that we believe play a key role in the disease process. This helps us to target our experiments more efficiently and to reducing the number of animals required.

Refinement

We have carefully considered our procedures in order to minimise pain and distress experienced by the animal, and to enhance its well-being. Over the last 5 years, our previous licence has also undergone several amendments resulting is many refinements, which have led to fewer animals being used. Anaesthesia and analgesia are used whenever appropriate. Whenever possible and appropriate, we also use supportive therapy (including antibiotics and fluids) to minimise the effects of a disease on the experimental animal. Whenever possible, we aim to use murine models in vivo models of research. To further this end we are increasingly using genetically modified mouse strains allowing us to target our experiments better, using smaller and fewer experiments and consequently resulting in fewer animals used overall. Careful consideration has been given to the endpoints and measurable parameters obtained from each procedure and as much information as possible is gained from each animal ranging from haemodynamic and biochemical data through to immunohistochemical and histological analysis of tissues. At the end of each experiment, the most humane method of euthanasia is chosen.

Project 38	The long-term effects of prenatal hypoxia on cardiomyocyte function
Key Words	Programming, pre-natal hypoxia, cardiac, mitochondria, myocyte
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

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

The overall objective is to assess the long-term effects of low oxygen levels (hypoxia) during development on mouse heart function. This goal will be realized by addressing the following specific objectives in fetal, juvenile and adult mice previously exposed to hypoxia during development. In addition, we will run control experiments on snapping turtles which are naturally hypoxia tolerant, providing us with a model to identify adaptive vs. pathological responses

1. To measure heart cell contractile force and calcium regulation
2. To assess mitochondrial function in heart cells
3. To characterize gene expression and modification of key proteins involved in heart cell function

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 main benefit of the study is the advancement of current understanding of the cellular and molecular mechanisms underlying the developmental origin of cardiovascular disease. We hope to identify cellular targets for drug intervention to protect people from developing cardiovascular diseases later in life. All of the findings will be published in peer reviewed leading scientific and clinical journals as appropriate to ensure wide dissemination of the research findings. The information is

of direct benefit to basic scientists, physiologists and clinical cardiologists and will provide key information enabling better management of cardiovascular disease.

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

Wildtype mice, approximately 850 animals over 5 years Juvenile snapping turtles, approximately 240 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 levels of severity? What will happen to the animals at the end?

Adverse effects in mice: 1. Maternal reduced food intake, activity and weight: • Severity band, mild • Dams exposed to hypoxic environments are known to exhibit a decrease in food intake (up to 40%) and a substantial decrease in physical activity, leading to a decrease (~20%) in maternal body weight. 2. Maternal preeclampsia-like symptoms: • Severity band, moderate Hypoxia during pregnancy can cause maternal preeclampsia-like symptoms such as hypertension, pro-teinuria and kidney pathology. 3. Intrauterine growth retardation (IUGR) and physiological and morphological defects associated with prenatal hypoxia • Severity band: Moderate. • Prenatal hypoxia causes IUGR and a host of physiological and morphological defects, some of which persist into adulthood. 4. Disease susceptibility in offspring. • Severity band: Moderate. • Although we are not specifically inducing this, it is possible that offspring exposed to prenatal hypoxia will ex-perience disease susceptibility in association with aging (i.e. cardiovascular diseases, such as heart failure) later in life. Adverse effects in turtles: 1. Retained "conciousness" following decapitation Severity band: moderate We acknowledge that the CNS of reptiles is tolerant to hypoxia and hypotensive conditions so it cannot be assumed that decapitation causes rapid loss of consciousness All animals will be sacrificed according to Schedule 1 at the end of the protocols

Application of the 3Rs

Replacement

Cell lines and culture:

Adult cardiac myocytes are terminally differentiated and cannot be maintained in tissue culture conditions. There are no suitable cell lines that can be used to fill these purposes. Moreover, we will be studying the long-term effects of prenatal hypoxia on cardiomyocyte function, which cannot be reproduced using cell culture techniques nor can they be suitably modelled using computer simulations given the lack of understanding of the fundamental processes.

Human volunteers

Human tissue is; i) of limited availability, ii) rarely not already diseased and iii) nearly always subject to pharmacological interventions.

Alternative species

Since we wish our findings to be clinically relevant and translational to human diseases of the heart, the use of other less sentient species, such as lower vertebrates (reptiles, fish and amphibians), is not appropriate for the main study animal as the structure and function of lower vertebrate hearts differ significantly from mammalian hearts and mammalian hearts are known to be significantly more sensitive to hypoxia than lower vertebrates. Nevertheless, we have utilised a lower vertebrate, the snapping turtle, as a control species that naturally experiences developmental hypoxia to compare to the mouse findings and seperate adaptive vs. pathological responses.

Reduction

Experimental design has been discussed with, and approved by, our statistical advisor. In order to minimise the number of animals required, sample size has been estimated for each experiment based on existing published data and the use of power analysis (desired power of 0.8, $\alpha = 0.05$). These estimates will be updated and recalculated throughout the project as we generate new data.

Refinement

Experiments concerning mice:

We are committed to using the most translationally relevant model. We have chosen the mouse as our main experimental species for several important reasons:

1. Mice have a short generation time and an accelerated lifespan (2 years) which allows the long-term effects of prenatal hypoxia to be studied within a reasonable timeframe.
2. Our ability to directly manipulate the mouse genome provides an incredibly powerful tool to identify and confirm molecular targets for drug intervention.
3. Due to their small size and short generation time, maintaining mice requires less resources and space, and the time required to perform research is manageable.
4. The mouse has large litter sizes which allows the generation of multiple, identically reared progeny.

Steps to minimise welfare costs to animals:

1. Basic requirements for good rodent housing and husbandry will used at all times.
2. It is not possible to house pregnant mice in groups, but once pups have been weaned, mice will be housed in stable, compatible groups.

3. The following parameters will be measured during the protocol to ensure animals remain within the outlined severity limits: Body weight, body condition scoring (BCS), food and water intake and cardiovascular status. Control animals not subjected to any procedures will be used as a benchmark for normal changes in these parameters.
4. Oxygen levels will never be reduced lower than 9%
5. When animals are first put into the chamber, oxygen levels will be normal (21%) for 24 hours and then reduced slowly (over another 24 hour period) to avoid shock.

Experiments concerning turtles;

1. Basic requirements for good reptile housing will be used throughout the procedure
2. The head of chelonians can be exposed and extended by turning the turtle upside down allowing placement in guillotine with minimal handling
3. The head will be immediately immersed in liquid nitrogen following decapitation which instantaneously freezes the head with no risk of on-going brain activity