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Non-technical summaries granted during 2013

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Project Titles and key words

- Hydrological disturbance to Little Ringed Plovers

  Flooding; Life history; Reproduction; Diet; Stable Isotopes

- Immunological studies in animal models of disease

  Immunology, T-lymphocytes, arthritis, vaccines

- Investigation and modulation of cell migration

  Inflammatory bowel disease, breast cancer, inflammation, therapeutics, immunology

- In-vivo behaviour of progenitor cells

- Genetics of Cardiovascular Development

  Heart development, congenital heart disease, genetics,

- Investigating the biology of musculoskeletal…

  cartilage, dwarfism, osteoarthritis, biomarkers, therapeutic targets

- Development and disease of the cardiovascular system

- Cortical and sub-cortical motor control

  Cortex; spinal cord; reticular formation

- Regulation of DNA replication and damage repair

  DNA replication, DNA damage, ubiquitin, SUMO, cancer

- Mechanisms of memory persistence

  Memory; reconsolidation; extinction; fear; addiction.

- Role of cellular and molecular therapies in liver repair

  Liver, stem cell, inflammation, liver fibrosis, trafficking

- Immunotherapeutic manipulation of human cancers

  Immunotherapy, Antibody Engineering, Tumour Antigen, Tumour Immunology

Hydrological disturbance to Little Ringed Plovers
Flooding; Life history; Reproduction; Diet; Stable Isotopes

- Summarise your project (1-2 sentences)

This project aims to investigate the impact of river flow variability on the behaviour and life history of the Little Ringed Plover. Using SIA (stable isotope analysis), it will reveal the impact of flooding on river birds, as well as behavioural adaptations to this environmental disturbance.

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

Objectives

1. To quantify the effects of flooding on the behaviour and life history of the Little Ringed Plover.
2. To measure the impact of river flow variability on nestling survival, through nest monitoring.
3. To measure the impact of river flow variability on diet and foraging ecology, through SIA.

Background

River flow disturbances are frequent and ubiquitous in riverine habitats. Despite this, few studies have explored the effects of disturbances on aquatic and riparian community dynamics. This research gap is of significance as an understanding of the interactions between river flow and ecological communities is increasingly recognised as important in the development of conservation and sustainable strategies.

The life history of the Little Ringed Plover is closely associated with river flow and floods can lead to nest inundation and loss of key foraging habitats. In addition, it feeds on riparian invertebrates, which vary in abundance with the extent of river flooding. We will determine if variations in river flow lead to costs in terms of decreased reproductive success and reductions in prey availability.

- Outline the general project plan.

Adult birds will be captured whilst brooding using metal funnel traps. Standard morphometrics will be taken from adults to assess body condition. Both adults and pulli will be ringed with uniquely numbered British Trust for Ornithology (BTO) metal rings and unique combinations of colour rings, allowing measurements of reproductive success to be calculated. SIA will be used to determine the invertebrate prey consumed by birds. SIA will be performed on toe nail clippings and feather down of nestlings and on blood samples of adults. This will be accompanied by invertebrate sampling in habitats surrounding the nest sites. Invertebrates carry distinctive isotopic signatures and the strength of the isotopic signal in the different bird tissues will inform of the prey species and their relative contributions to each bird's diet.

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

Procedures

1. *Removal of approximately 0.7 mg of toe nail clippings from adults and nestlings.*
2. *Removal of a single tail feather (rectrix) from adults so not to influence flight capability (Note: feathers from other parts of the bird's anatomy cannot be used as they do not reflect local isotopic signatures).*
3. *Clipping of feather down from nestlings.*
4. *Blood sample of approximately 30 µl taken only via brachial venipuncture.*

Adverse effects

1. Stress from restraint and transient discomfort from needle insertion and blood collection.
2. Nestlings may be stressed by removal from nest.
3. Stress due to capture and restraint.

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

The synthesis, analysis and subsequent publication of results from this study will further our understanding of the impact of flooding and variability in river flow on birds. It will provide insight into the impacts of these disturbances on avian ecology, as well as the behavioural adaptations that allow birds to breed successfully on a dynamic riparian system and answer some key questions regarding the interaction between resource availability and life-history trajectories of birds.

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

The Little Ringed Plover has been selected because of the close association of aspects of its life history with river flow. We will aim to capture and carry out the procedures described in protocol 1 on 60–300 over the lifetime of the PPL (based on equal fieldwork efforts to capture birds in each year of the study). Captured birds will be marked with metal rings with unique codes so that they can be individually identified. By keeping detailed records of the birds used to take tissue samples and their codes, we can ensure that no birds will be re-used throughout the duration of the project. We will also minimise the number of animals used in the project by taking blood samples, feather cuttings and toe nail clippings from each bird, thereby maximising the scientific value of data from each individual**.**

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

Animals have to be used in this research because we are examining the ecological, behavioural and demographic processes and outcomes of hydrological disturbances on a free-living bird species. These requirements mean that it would be impossible to study a non-animal or captive system.

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

SIA is the preferable method for such a study as it limits animal suffering whilst

minimising disturbance of nests. Other potential procedures are less preferable. For instance, visual monitoring of birds may provide some insight into foraging behaviour but would require repeated visits to nesting habitats during the breeding season, potentially risking nest desertion and nestling mortality. In addition, this species' behaviour could be recorded by using radio tracking technologies. However, this would involve the attachment of transmitter harnesses or gluing transmitters to the bird's body, which are highly intrusive procedures and would require a prolonged procedure period that will extend until the transmitters fall off the body (approx. 8 weeks).

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immunological studies in animal models of disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>immunology T-lymphocytes arthritis vaccines</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td>Yes</td><td></td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td></td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objective of the project is to study immune mechanisms in two models of human disease: melioidosis caused by infection with the bacterium Burkholderia pseudomallei, and autoimmune inflammatory arthritis including Rheumatoid Arthritis. In the first model we will identify which proteins and immunogenic regions of these proteins (immune epitopes) are important in immunity to this infections, and in the second model we will determine how joint autoantigens induce immune mechanisms (pathogenic cytokine responses) that destroy joints.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>In the first disease model (melioidosis) we will be identifying proteins and their specific epitopes as candidates for future vaccine development. No vaccine is available at present despite melioidosis being an infection of huge economic and public heath importance. When we are clear what the important immune mechanisms are then we will use the arthritis disease model to try out treatments that target these immune mechanisms to prevent or cure arthritis which will inform future new treatments of Rheumatoid Arthritis in humans.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Mice - breed up to 3000 mice over 5 years.</td></tr>
<tr><td>In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will</td><td>For the majority of mice [>2000, protocol 19b 2] no adverse effects are expected as the procedures are of mild severity. A minority [>1000, protocol 19b 3] will be immunised to induce arthritis in up to 4 paws and are expected to show redness, swelling with</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

happen to the animals at the end?	mild deformity but no necrosis or systemic effects so the procedures are of moderate severity. Experiments will be terminated after 60 days or before by a schedule 1 method.
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 necessary to be able to induce and manipulate the immune response because pilot work of this nature is not ethical in humans and cannot be reproduced in artificial systems because of the complexity of cell and tissue interactions over a period of days or weeks in live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power calculations have determined the minimum group size for experiments to either provide sufficient tissues for ex vivo experiments or to achieve clear results when making comparisons between ways of inducing immunity or methods for treating arthritis.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use special strains of mice with defined genetics that have been developed to study diseases such as melioidosis and arthritis. The mice also need to be inbred so their responses are uniform to see clear effects. The mice we use have their immune responses tailored to study the immune responses in these disease. One type of mice are engineered to have an easily detectable number of lymphocytes that all recognise the joint antigens we study so they are easy too find and follow. Other types of mice express human immune response genes so that the proteins and epitopes we define are the ones that would also be recognised by humans if they are vaccinated.

Investigation and modulation of cell migration
Inflammatory bowel disease, breast cancer, inflammation, therapeutics, immunology

- Summarise your project (1-2 sentences)

We aim to identify the biological pathways responsible for cellular movement during inflammation and cancer, and to identify methods of preventing or reversing this process in animal models that are applicable to the human immune system, specifically the conditions of inflammatory bowel disease and breast cancer.

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

Inflammatory conditions and cancer are characterised by the abnormal movement of cells within the body. Recent scientific advances have led to the introduction of human-specific biological therapies for inflammatory and malignant diseases that have had some success in controlling this cellular movement. However as these therapies are specific to the human immune system, new human-relevant animal models are required to allow future discovery of targets for disease therapy, and also to test the safety of new medicines prior to safe human use.

- Outline the general project plan.

Our research project will utilise mice. We have recently developed a method of transplanting human white blood cells into mice that lack an immune system. We aim to use this model, and other specifically defined strains of mice to study and modify common immune pathways responsible for cell migration. We will also refine existing models of breast cancer and inflammatory bowel disease to study disease-specific biological pathways, in a bid to identify and test new targets for treating these debilitating conditions.

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

General mechanisms of cell movement and the human diseases of inflammatory bowel disease and breast cancer will be simulated in the mice. Medications may be used to limit or reverse these conditions. The applicant has substantial expertise in the models outlined in this application, many of which have been developed as a result of previous project licences. This experience has allowed us to minimise the risk of significant adverse events, which are not expected in the experiments outlined in this application. Furthermore this experience minimises the necessity for animal usage in pilot experiments, and maximises the translational value that can be obtained from this work.

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

We believe that the results of our research could lead to an improved understanding of the factors influencing cellular migration in human disease, and that the development of human specific models of disease could lead to a reduction or replacement of higher order animals required for experimentation in other laboratories around the world.

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

We expect to use an average of 120 mice per year over the course of this 5 year project. These will be a combination of typical form 'wildtype' mice, as well as some genetically modified mice, used specifically to study certain aspects of the immune system. This makes the information learned from these experiments of most relevance to human disease and the development of new treatments.

Statistical techniques have been used to ensure we use only the minimum number of mice for each stage of the experimental procedure.

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

Many experiments will utilise mice that receive a transplant with human white blood cells in order to maximise the clinical implications learned from these experiments. New models for inflammatory bowel disease and breast cancer will be derived, improving upon existing animal models, many of which have limited applicability to human disease and the development of new medications.

We aim to generate disease that is relatively mild and therefore suitable for longitudinal monitoring. In the inflammatory bowel disease models we will monitor animals sequentially using colonoscopy and non-lethal intestinal biopsy to observe the generation and treatment of gut inflammation. We believe this non-lethal monitoring will lead to a 3-4 fold reduction in the number of mice required for the model.

Whilst non animal methods of experimentation cannot be used to answer the questions posed in this project, as they lack the biological specificity needed to study such complex immune processes, cells in a dish will be used to help select compounds for investigation.

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

Our group has been carrying out research in this field for over 25 years. We have used this experience, and that of the worldwide scientific community to ensure the designed protocols consider animal welfare, and minimise suffering. Appropriate analgesia and anaesthesia is planned for each relevant model, and our experience with these models and techniques suggests that significant adverse events should not be anticipated.

In-vivo behaviour of progenitor cells

- Summarise your project (1-2 sentences)

We want to make blood progenitor cells in the laboratory because these can be used to replace faulty cells in the bone marrow of leukaemia sufferers. Also, we want to understand how ageing affects the properties of blood progenitor cells normally present in the bone marrow, however this means we need to test the laboratory made cells in whole animals to see where they go in the body and how well they work over prolonged time periods

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

  Bone marrow transplantation is used to treat leukaemia. This works because blood progenitor cells are present in the bone marrow and these can produce any type of cell normally found in the blood but it is difficult to get these cells from adult patients. We can make pluripotent stem cells (PSC) from the patients skin and subsequently turn those PSC into blood progenitor cells but we need to be sure that such “laboratory made” blood progenitor cells are able to do the same job as their natural counterparts before they can be applied to treating human diseases.

- Outline the general project plan.

We will inject the “laboratory made” blood progenitor cells into mice whose immune systems don't work so they won’t be able to recognise and reject the “foreign” cells. Moreover, we will knock out the existing bone marrow of the mice using radiation so most of the blood they make will be derived from the “laboratory made” blood progenitor cells.

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

The most likely cause of adverse effects is the radiation treatment. From our own experience and over 30 years of research in this field by others, radiation doses are carefully controlled and mouse numbers kept to a minimum to achieve significant results whilst minimising adverse effects. In addition, irradiated mice will be carefully inspected at least twice a day for 4 weeks post-irradiation and will receive antibiotics to prevent infection whilst they regenerate a blood system.

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

This project will increase our understanding of laboratory-derived progenitor cells and how they behave following transplantation into a whole animal. Furthermore, we hope to develop new and improved ways to increase the number of progenitor cells that can be produced in the laboratory. Consequently, this project represents a vital step towards the use of progenitor cells therapeutically.

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

No more than 2,600 mice (including 1,000 immunodeficient mice) will be used in this project. The use of reprogrammable mice is essential for the study of progenitor cell ageing since we need to be able to convert aged progenitor cells back to pluripotent stem cells with high efficiency.
NSG mice are essential for functional engraftment of progenitor cells and NOD/SCID mice are needed for the assessment of stem cell pluripotency using the teratoma formation assay. We will ensure that the minimum numbers of animals are sued as follows; The use of anaesthesia to immobilise mice prior to irradiation will also minimise variation in dosimetry and thus reduce the size of groups required. The use also of genetically defined inbred strains of mice reduces the need for large-scale experiments designed to eliminate genetic variation.

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

Mice are needed for this project firstly because they are sufficiently similar to humans to generate meaningful data that can be translated to human development and disease and secondly because mouse genetics has advanced to such an extent that mice provide an extremely powerful way of analysing the role of key genes in haematopoietic development – a process that we cannot effectively mimic *in vitro*

That notwithstanding, large sections of our programme of investigation can be performed using in vitro techniques thereby reducing the requirement for animal experiments. For example, substantial amounts of data concerning the functional analysis of HSC can be obtained using in vitro colony assays. This will contribute to investigations of the functions of PSC derived HSC and HSC ageing. Similarly, we will perform preliminary screening for candidate genes that can influence PSC differentiation to HSC *in vitro*. Only when strong candidates emerge from this process, will transplantation of genetically modified HSC be performed.

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

I have designed the protocols to minimise distress of the experimental animals. Where invasive procedures will be carried out, we will use anaesthetics and analgesics as appropriate. Any loss of condition as shown in Appendix A will indicate removal from the procedure and killing by Schedule 1 method. The use of prophylactic antibiotics during myeloablative protocols will reduce adventitious infection. We will keep the immunocompromised recipients on isolators to avoid loss of viability due to infections. Where possible, the experiments will be carried out in collaboration with groups that have a large body of expertise with a particular model.

Genetics of Cardiovascular Development
Heart development, congenital heart disease, genetics,

- Summarise your project (1-2 sentences)

This project will use genetically modified transgenic mouse models to uncover genetic pathways that control cardiovascular development. We will focus on genes that are responsible for activating other genes (known as transcription factors), that when mutated result in congenital heart disease in the developing mouse. We will also investigate the molecular and cellular process involved in building the aberrantly developed heart and its associated blood vessels, as well as employing cell culture based assays.

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

Congenital heart disease is a major cause of morbidity and death in childhood in the West, with genes playing a major role. However, the genetic and developmental mechanisms underlying most congenital heart disease remain unknown. Some patients with congenital heart disease have syndromes where certain genes have become mutated or are missing but it is thought that the syndrome can be further affected by mutations in other as yet unknown genes. This research aims to identify genes that work together in a network to control normal cardiovascular development.

- Outline the general project plan.

In this project we will:

1. Investigate the genetic pathways that control normal cardiovascular development
2. Use transgenic mouse models to manipulate gene expression and examine the effect of gene loss or mutation on cardiovascular development
3. Investigate the cellular mechanisms that contribute to cardiovascular development
4. Investigate the molecular mechanisms that control gene expression in relation to cardiovascular development

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

The majority of mice used in the project licence will need to be identified by examining the mouse's DNA, and material for this will be obtained by taking a piece of tissue from the ear. This procedure is quick and should only cause mild and short-lived pain. As we will mainly be examining embryos or foetuses taken from pregnant females, the procedure of mating transgenic mice will be a normal and natural act that should have no adverse effects. In some cases we will need to administer substances by injection or oral routes, but we do not expect these to cause any adverse effects. All mice will be closely monitored following the administration of substances to ensure that no adverse effects occur. Very few adult transgenic mice will have clinically adverse effects as harmful effects will occur in the embryo before it is two-thirds of the way through gestation. These effects will result in some mice dying shortly after birth, mainly from heart defects.

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

Congenital heart disease affects approximately 1% of all births and is a major burden for

the patient as well as the health care system. By furthering our understanding of the genetic pathways that control cardiovascular development we may be able to devise screening strategies for prospective parents that can highlight any potential risk to the child.

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

We will breed up to 100 mice of each strain listed on the project licence per annum, of which 90% will be used for intercrossing with other strains. Similar numbers of the intercrosses will also be maintained. Embryos (which are less than two-thirds through the gestation period) or foetuses (which are greater than two-thirds through the gestation period) will be collected from timed matings for analysis. We will not exceed the 10,100 adult mice, 500 neonates and 9,000 foetuses over the 5 year duration requested on the project licence. 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. In the majority of cases we use embryos or foetuses for analysis. All experiments are designed to use the least numbers of animals. We regularly consult a statistician for advice so that experiments are properly designed and group sizes are the minimum number required to give an accurate answer. We also use a magnetic resonance imaging technique which allows for foetuses to be imaged without destruction, therefore the foetuses can be reused for analysis with other techniques. Moreover, the datasets generated of the foetuses can be shared with other researchers. Our use of *in vitro* culturing of embryos will also reduce the overall number of animals used since the unit of experimentation can be the single embryo, and not the entire litter.

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

Unfortunately, due to the complexity of the processes involved in cardiovascular development, there are no suitable cell culture systems that can be used to replace mouse models. 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. In the majority of cases we use embryos or foetuses for analysis or, if appropriate (for example when investigating processes that occur within individual cells), then cell culture experiments are used. Cells grown in culture are a useful tool to investigate how different proteins interact or how genes can be controlled (i.e. switched on and off) by other genes. These sorts of experiments will be conducted in parallel with the mouse studies to identify molecular and biochemical mechanisms that control the genes we are studying.

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

Most of our mice are used for maintaining genetic lines and have no abnormalities. As we are investigating the effect of genetic mutations on the developing embryo or foetus, the main procedure we carry out is by inter-breeding different transgenic strains so that embryos or foetuses can be collected from the pregnant dam. Because mating is considered a natural act, this does not result in any abnormal suffering for the mice.

Investigating the biology of musculoskeletal…
cartilage, dwarfism, osteoarthritis, biomarkers, therapeutic targets

- Summarise your project (1-2 sentences)

We propose a detailed study of disease mechanisms leading to abnormal cartilage growth (chondrodysplasias) and osteoarthritis (OA) using genetically tractable mouse models. This will lead to the identification of potential biomarkers and of novel treatment avenues which can be translated into the clinic.

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

Cells in our bodies exist embedded in a scaffold of various proteins, so called extracellular matrix (ECM) which they lay down during the course of their lives. ECM determines the biomechanical properties of tissues and contributes to the diffusion of nutrients and signalling molecules, and thus tissue homeostasis. Mutations in genes encoding ECM components lead to disease (for example increased matrix deposition leads to fibrosis and increased breakdown of cartilage matrix leads to osteoarthritis). The purpose of this project is to determine the mechanisms by which mutations in genes encoding the ECM components lead to chondrodysplasias and how they contribute to the more common complications such as osteoarthritis and lower-limb weakness. Numerous ECM mutations leading to musculoskeletal diseases have been previously described; however, the mechanisms leading to these conditions remain unknown. Our research to date highlighted the role of cell stress in the disease mechanism. However, we still don't know whether the stress responses we detected are protective or destructive in our models.

- Outline the general project plan.

We will use mouse genetic models of human disease to examine the role of cell stress in the disease progression. We will cross our mouse models with mouse lines in which stress pathways have been blocked (knockouts) and examine the effect on disease severity. We will also determine the impact of cell stress upon the onset and severity of mechanically-induced osteoarthritis. These studies will identify pathways which could be manipulated to reduce disease severity and potential therapies will be tested.

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

The mouse strains included in this application exhibit a mild short-limbed dwarfism and in our experience suffer no obvious pain or discomfort. Most our studies involve analysis of tissues after humane sacrifice of the animal. For the study of osteoarthritis, the animal will undergo a small operation (under general anaesthesia) on one knee to destabilise the joint and cause a reproducible osteoarthritis; as a result it will develop a slight limp. Potential treatments may be administered by injection which can cause momentary discomfort.

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

These studies will advance our knowledge of musculoskeletal biology and disease. They

will provide insight into how ECM contributes to tissue integrity and disease, and what role cell stress plays in chondrodysplasias and osteoarthritis. In addition, our experiments will identify potential therapeutic targets and test small molecule compounds which could be used in treatment of patients in the future.

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

Species: Mouse (*Mus musculus*). Number of animals: 10,750 - Breeding and phenotyping 10,000; Treatment 500; Osteoarthritis 250.

Chondrodysplasia and osteoarthritis can be studied in dogs and in Hartley guinea pigs. We rejected these models due to unknown genetic causes of canine chondrodysplasias and guinea pig OA, and the extended timescale of such study. Mice are the ideal model for our studies. Their skeletal system develops in a similar fashion to humans; they also develop OA similar to that in humans in response to joint destabilisation in a relatively short time frame; and they are genetically tractable. We have previously generated genetic models of specific human chondrodysplasias. Moreover, we can genetically dissect the importance of various pathways by crossing our mice with genetic knockouts of specified stress-related genes and determining the effects on disease onset and severity.

To generate statistically robust data, we performed calculations to predict the exact number of animals to be used. For quantitative aspects of the programme, age and sex-matched animals will be used. Where feasible, the animals will be littermates to reduce variation. Moreover, in order to reduce the numbers of animals needed for bone measurements, imaging under anaesthesia will be employed to collect data. The most reliable and reproducible mechanically-induced model of OA will be employed to minimise experimental variation in onset and severity of disease. For qualitative aspects, breedings where all the offspring have the desired genotype will be employed to reduce numbers of animals with unwanted genotypes. The number of observations will be the minimum necessary to provide an adequate description (single or multiple samples from 3 animals per group). Lines that are not required in next 6 months will be frozen down (sperm freezing if possible to reduce numbers of animals used) rather than maintained as minimal colony.

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

The use of animals is essential in our investigations. Although several aspects of musculoskeletal biology can be modelled in cell culture, the systemic process of bone growth involves integration of many tissues and cannot be modelled in cell culture; moreover, transgenic mouse models allow us to genetically dissect various disease pathways. We have applied advanced statistical analyses to ensure we extract maximum insight from the minimum number of animals used. We will use cell culture models where possible, to dissect the disease pathways in parallel with the animal studies and to biochemically test the likely efficacy of specific drugs prior to their use in mice.

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

Animal suffering should be minimised during the procedures for both ethical and scientific

reasons. Undue stress may affect the quality of data thus leading to larger sample numbers. Transgenic models of chondrodysplasias employed in our project all present with a mild short limbed dwarfism which does not impact on the quality of life of the animal. Potential treatments may be administered by injection which can cause momentary discomfort, however, the animals will be carefully monitored during treatment protocols by experienced technical and scientific staff, and appropriate anaesthetics and analgesics will be sued where necessary to ensure they do not experience undue suffering or distress.

Under the osteoarthritis induction protocol, most animals will experience some inflammation, (and possibly pain), and eventually arthritis, in their joint; as a result they may develop a slight limp. Post-operative pain will be controlled by the use of appropriate analgesics and the animals will be closely monitored immediately following the surgical procedure, focusing on the animal physical activity, grooming and well-being, in accordance with the LASA guidelines.

Development and disease of the cardiovascular system

- Summarise your project (1-2 sentences)

This project investigates how different stem cell types contribute to malformation and disease of the heart and its great arteries.

Heart, great arteries, mouse, transgenesis, progenitors

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

Malformations of the cardiovascular system (heart and blood vessels) are the commonest defects found in new-born babies and are a major cause of illness and death in infancy. Heart disease is the major cause of death in adults. Recently, data has accumulated linking subtle, sub-clinical, abnormalities in heart development with predisposition to some common forms of heart disease including diseases of the arterial wall such as atheroschlerosis and valve disease. We aim to establish the causes of these congenital defects and how they predispose to adult disease.

- Outline the general project plan.

We have established a number of mouse models that develop congenital heart defects and/or which develop adult pathologies that might be traced back to intra-uterine life. These mice, and others that become available to us, will be used to identify common pathways that are essential for normal cardiovascular development. Targeted disruption of key genes ("knockout" mice) and transgenic lines in which cardiac cell types are labelled fluorescently, will be bred to produce offspring that will be examined for cardiovascular defects and progression to adult disease. In some cases mice will be exposed to environmental stresses, for example a high fat diet, alcohol or other known cardiac teratogens, to identify interactions that cause cardiovascular malformations and predispose to cardiovascular disease in later life. In the first instance the offspring will be examined at a time point before birth to establish the severity of malformation. Where the anomalies are mild, some will be allowed to be born and develop to adulthood where analyses will be carried out in live animals to determine whether the cardiovascular system is functioning properly. Having identified cellular and molecular pathways that are essential for normal heart development, we will use our knowledge of these pathways to design potential preventative strategies to prevent the cardiovascular defects.

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

The majority of animals used in this project are used only for breeding and for the generation of embryos and fetuses for experimental analysis. The majority of these analyses will be carried out after humane killing. Some of these embryos and fetuses will be cultured in a test tube and treated with environmental teratogens and/or therapeutic agents to determine their effects on heart development. A few pregnant mice will be treated (either in their food, by injection or by mini-pump) with teratogens or therapeutic agents, again to determine their effects on heart development. Other adult animals will be given similar treatments to establish how this affects their progression to cardiovascular disease. These animals may develop mild signs of illness, but this will not be severe. Some adult mice will also be used for functional analyses to determine how disruption of

genetic pathways, environmental factors, or therapeutic agents, affect the function of the heart.

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

Determining the causes of congenital heart defects and establishing links with adult heart disease is likely to have a major impact on the management of these common diseases. Cardiovascular malformations are the commonest congenital defects and any strategies to reduce their incidence are likely to have a significant impact for healthcare provision. Heart disease is the biggest single cause of death in the Western world, and with the looming obesity epidemic this is only likely to get worse. Establishing links between some of these adult diseases and minor heart malformations will allow the design of targeted therapies, aimed specifically at those who will benefit most from them.

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

Mice are used throughout these studies. Most are transgenic animals that allow us to target specific cell types for analysis and mark them by their expression of a marker gene and targeted mutants for specific genes that are known to be important in cardiovascular malformation or disease. This project requires complex breeding programmes to be carried out and for this reason it is estimated that no more than 32,000 adult mice will be used through the 5 years of the license. The majority of these mice are used as adults, solely for breeding or to produce offspring for experiments. Some mice (approximately 1000 over the course of the 5 years) will be used as adults to investigate the pathogenesis of diseases of the great arteries. In the majority of cases, the form under analysis experimentally is the embryo or fetus. As approximately 8 embryos are present within each litter, this allows us to minimise the numbers of adults used to generate offspring for analysis. In all cases, power calculations will be carried out to ensure that enough animals are used to gather scientifically valid data, but that excessive animals are not used.

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

For the study of three dimensional form, as in the development of the functional vertebrate heart, animals have to be used as these processes cannot be mimicked by in vitro cell-based analyses. Similarly, in order to study how genes and environmental factors interact to cause congenital heart defects and adult heart disease, it is also necessary to use live animals as these interactions, and the effects they have on the architecture of a given tissue, cannot be mimicked in vitro. Many of our other studies utilise cultured embryonic stem cells and lower organisms (zebrafish). Mouse is only used as a model system when these other models are not suitable.

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

At every step we aim to minimise harm and suffering. Most animals are collected for analysis before birth. Those animals that are genetically predisposed to develop cardiovascular disease, or are given treatments which promote this, will be monitored

carefully for signs of distress. Treatments will be given by the least stressful method possible, for example by mini-pump when multiple doses are required. As we are aiming to understand the links between development and disease we do not need disease to progress far, and thus we will humanely kill the animals before they show overt signs of disease.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Cortical and sub-cortical motor control</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cortex; spinal cord; reticular formation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[3]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[4]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project has the overall aim of improving our understanding of the different brain and spinal cord centres involved in the control of movement, and translating the knowledge into improvements in therapy for patients recovering from injury, such as after stroke or spinal cord injury.

Specifically, we aim to understand the relative contributions of different parts of the nervous system to movement control in healthy animals, and how information is processed within each neural centre. We will then map how the surviving centres change after damage. We also aim to understand the processes which can change neural connections within these circuits, and to use this knowledge to devise stimulus protocols which can modify connections.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>Stroke is currently the leading cause of disability in the UK. There are around 150,000 new strokes annually, one quarter in individuals aged under 65. The UK has 1.2m stroke survivors, around half of whom live with a disability that affects their everyday life. Total care costs for stroke in the UK are estimated at £8.2 billion (all figures taken from The Stroke Association). Therapeutic options for improved rehabilitation are limited, especially for hand function – one reason for this is a poor understanding of the scientific basis for motor control, and the processes underlying its recovery after insult. The information gained by this project will allow us to devise principled new strategies for therapy to improve rehabilitation. If this leads to</td></tr>
</table>

[3] Delete Yes or No as appropriate.
[4] At least one additional purpose must be selected with this option.

	even small improvements in function, it will translate into major social and economic impact.
What species and approximate numbers of animals do you expect to use over what period of time?	40 macaque monkeys over 5 years 250 rats 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?	Monkeys will be trained to accept some restraint (a neck collar), and to perform a behavioural task. They are motivated to perform the task by having restricted access to food, and occasionally fluid; food and fluid rewards are then given for correct task performance. After training is complete, they are surgically implanted with a headpiece to allow head stabilisation and electrodes to record muscle activity from the forelimb. Recordings will then be made from the central nervous system in the conscious state, whilst the animal performs the task. The most common adverse effects are associated with wound infections associated with the chronic implants. In a small proportion of animals, focal surgical lesions will be made on one side of the brain. In the days immediately following, these animals may need nursing help with feeding due to impaired movement ability. However, as in human stroke patients with small lesions they often show a rapid recovery. Rats may be prepared for recording by a surgery to inject neural tracers or novel genetic material, after which they are allowed to survive for a few weeks. Subsequent experiments involve terminal anaesthesia, and then making electrophysiological recordings or removing brain samples for analysis in vitro. Recovery from the initial surgery is unlikely to show adverse effects, and no adverse effects can occur in the final terminal procedure. The macaque experiments will have moderate severity, although the licence limit of ‘severe’ may be reached for short periods in some animals associated with the period immediately after a lesion. Rat experiments will be of moderate severity. At the end of experiments, all animals are humanely killed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project investigates the complex interplay of brain circuits in different regions, and as such must be carried out in intact organisms. The laboratory does run a substantial programme of experiments

	in healthy human volunteers and patients; however, these can only produce indirect data. Detailed understanding at the level of single neurons and their connections can only be achieved using the invasive approaches possible in animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use sophisticated multi-electrode recording methods, which ensure that the maximum of data is gathered from each animal. Experiments in awake monkeys often yield sufficient data for publication from just two animals. Experiments under terminal anaesthesia use advanced anaesthetic methods to maintain the animals in good condition for extended periods (around 70 hours for macaques); this again enables us to gather extensive datasets 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.	Some basic circuit properties can be investigated in rats. However, the neural centres and connections controlling movement differ in key aspects in primates compared with non-primate species. To ensure that our results are directly applicable to human patients, we must use old world primates such as macaques. Our techniques have been refined over many years, and we continually seek to improve them. All recovery surgeries are carried out under full aseptic conditions, with advanced anaesthetic regimes which produce rapid and uneventful recovery. Full programmes of post-operative pain management are in place.

Regulation of DNA replication and damage repair
DNA replication, DNA damage, ubiquitin, SUMO, cancer

- Summarise your project (1-2 sentences)

The aim of this project is to study the regulation of processes of eukaryotic DNA duplication and DNA damage responses. In particular the roles of small protein modifiers: ubiquitin and SUMO, in these processes will be investigated.

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

Problems during DNA duplication (DNA replication) are thought to be a major cause of the mutations that are observed in cancer and many other human diseases. Moreover, the process of genome duplication is of vital importance to cancer progression; it is exploited by the frequent treatment of cancer with radiotherapy and drugs that target DNA replication. These drugs can be very effective but do not only target cancer cells and therefore elicit significant side effects. It is therefore crucial that we fully understand this fundamental process to understand the development of these diseases and create novel therapies that target aspects of DNA replication and DNA damage repair mechanisms that are deregulated in cancer cells.

- Outline the general project plan.

Our aim is to understand regulation of the process of DNA replication and DNA damage repair by small protein modifiers – ubiquitin and SUMO. They can be both attached to substrate proteins in an enzymatic reaction and change the behaviour, function and fate of the modified substrate.

We will identify proteins modified during DNA replication and DNA damage responses by ubiquitin and SUMO and determine the function of the identified modifications. We will also identify substrates of specific ubiquitin or SUMO attaching enzymes (ubiquitin / SUMO ligases) acting during DNA replication and DNA damage repair processes.

All the research will be carried out using *Xenopus laevis* egg extract as an excellent cell-free system perfectly suited for biochemical studies of DNA replication and DNA damage response processes. Once the modified substrates are identified and basic mechanisms characterised using *Xenopus* egg extract, we will also investigate if analogous mechanism regulate DNA replication or DNA damage response in immortalized human cell lines.

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

Female frogs will be induced to ovulate by hormone injections and mature eggs then collected. Alternatively, ovulating females will be killed for the collection of immature eggs. The food will be withdrawn from animals injected with hormone for the duration of the procedure (2-9 days). As *Xenopus* frogs are fed twice a week it means missing one feeding.

To provide a physiological DNA template, male frogs are stimulated to produce sperm by hormone injection and are then killed for the collection of sperm.
In most cases, the material collected from several animals will be pooled, fractionated biochemically and extracts stored frozen until use in the laboratory.

Animals that are injected with hormones will experience transitory discomfort.

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

The areas of study proposed are highly relevant to understanding of human cell biology and of human disease. Understanding how DNA replication and DNA damage processes are regulated is key to our understanding of how mutations accumulate. This will have important implications for identifying factors underlying a range of human disorders, including cancer.

It is also possible that some of the identified factors may act as novel drug targets.

The proposed research is fundamental academic research and thus the short-term impact will be mainly academic.

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

We estimate using up to 3000 female frogs for egg production (protocol 1) and about 150 procedures of sperm/oocyte production (protocol 2) within the duration of the project. To reduce the number of animals used for these procedures we propose to re-use the females for egg laying purpose. The procedure itself is less stressful than buying and transferring new animals into the animal house. It also allows the females to settle in, increasing the yield and quality of eggs and hence reducing the need to use additional animals.

We have also developed protocols maximising yield and quality of the extract produced and we store much of the obtained material frozen – maximising the number of experiments that can be performed on obtained material and therefore minimising number of animals required for continuous supply of the extract.

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

Xenopus egg and oocyte extracts provide the only cell-free system capable of efficiently supporting cell cycle progression in the test tube. As such it is the only system allowing a robust biochemical analysis of this process. It is indispensable for specifically isolating and analysing replication factors acting on chromatin during DNA replication and DNA damage.
Once biochemical analysis will be performed using egg extract system we will follow with testing our hypotheses in mammalian immortalised cell lines.

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

Both proposed protocols are of mild severity and we refined the hormone injection procedure to ensure only minimal and temporary discomfort to the animals.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Mechanisms of memory persistence</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Memory; reconsolidation; extinction; fear; addiction.</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[5]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[6]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project concerns the mechanisms of memory persistence. In particular, the focus is on the processes of memory reconsolidation and extinction, which allow memory expression to be modulated. Memories can be retrieved over long periods of time, yet their expression is modulated to maintain their utility. The processes of memory reconsolidation and extinction are critical in maintaining memory expression and relevance. These processes operate in competition with each other; reconsolidation serves to maintain and update memories, whereas extinction suppresses the expression of memories that are no longer predictively useful. The mechanisms of reconsolidation and extinction are incompletely understood, as is the manner in which they compete. This is important, not only for the basic understanding of memory persistence, but also because targeting reconsolidation for disruption and extinction for enhancement are potential ways of diminishing problematic memories (e.g. in posttraumatic stress disorder and drug addiction). Moreover, the ways in which memories overlap in the brain is not well understood, and so the impact of targeting one memory upon other memories is important to determine.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td>The outcomes of this project will lead to a better understanding of the reconsolidation and extinction processes. This will help both a more complete understanding of memory persistence and will be informative for potential clinical applications of impairing reconsolidation or enhancing extinction to</td></tr>
</table>

[5] Delete Yes or No as appropriate.
[6] At least one additional purpose must be selected with this option.

	reduce the impact of problematic memories in conditions such as posttraumatic stress disorder and drug addiction.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 6500 rats will be used over the 5 years of the 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?	There are no long term predicted harms for the behavioural training and testing. The aversive stimuli used are mild and cause no lasting harm. A proportion of rats will undergo surgery to create lesions in the brain, to implant cannulae into the brain (for the subsequent delivery of substances directly into the brain), to implant electrodes into the brain (for the recording of electrical activity) or to implant an intravenous catheter (for the subsequent administration of addictive drugs into the bloodstream). These surgical procedures could be associated with adverse effects, such as weight loss, pain and potential infection of wounds, however in our experience these are rare and minimised through aseptic technique, use of peri-operative pain relief and good post-operative care. Overall, the expected level of severity is moderate. Rats will be killed at the end of the experiments, and for a proportion their brains will be extracted for further analysis.
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 required as the objectives of the project concern the behavioural expression of memories. This cannot be achieved without using awake behaving animals. We use rats as opposed to simpler organisms because they show a degree of similarity in behaviour and the brain mechanisms of memory to humans that will allow our results to inform normal and abnormal human behaviour.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The behavioural procedures that we use are well-defined and refined (e.g. by using computerised behavioural analyses), which allows us to use the minimum number of rats to show differences between groups. We have also refined our surgical procedures in order to minimise rat numbers and suffering.
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.	Some of the protocols involve surgical preparation of the rats (e.g. in order to target specific areas of the brain). All surgical procedures are carried out to the highest standards, and the welfare of the animals is of prime importance also to allow reproducible behavioural results. Other protocols involve behavioural training and administration of substances to the whole body. The substances that we use cause no lasting harm and we maximise the

	use of these mild severity protocols unless the specific objectives require surgical procedures. In this way, we minimise the suffering of the animals involved.

Role of cellular and molecular therapies in liver repair
Liver, stem cell, inflammation, liver fibrosis, trafficking

- Summarise your project (1-2 sentences)

This project will study the action and safety of cell therapy in models of liver damage as a way of informing and improving the use of cell therapies in clinical trials.

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

There is a large clinical unmet need in liver disease, for which cell therapy offers exciting new possibilities. Cell therapy has real potential to help reduce scarring in liver disease, reduce ongoing liver inflammation and also contribute to replacing liver cells. These studies will explore ways of enhancing our current knowledge by applying cell therapies to more clinically relevant models as a prelude to clinical studies. In addition these studies will look at ways of enhancing the action of cells by improving their action and delivery to the injured liver.

- Outline the general project plan.

Cell therapies will be studied that have the following actions:
1. Reducing liver scarring
Haematopoietic stem cells, mesenchymal stem cell and macrophages will be used as they have been shown to reduce scarring in mouse models and also in clinical studies.

2. Reducing liver inflammation (ongoing damage)
Mesenchymal stem cells and regulatory T cells will be used as they have been shown to reduce inflammation in mouse models.

3. Improving engraftment of new liver cells in the damaged liver
Stem cells that have been developed into liver-like cells will be used to replace missing liver cells in relevant models.

Mouse models will be used that closely mirror human liver diseases and thus allow these objectives to be addressed.

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

Potential harm results from the induction of liver damage, for which several models will be used. In the vast majority of cases when generating liver injury it is important to state that it does not cause overt symptoms of suffering in the mice. Weight loss (up to 40%) can be seen with some of the models and clear limits are placed on this before studies are terminated. There are clear guidelines in place in our facility to ensure that suffering in mice is minimised by either administration of pain-killers, stopping liver injury or termination of experiments.

Administration of cells has the potential to cause irritation at the injection site mice although in our extensive experience this has not been the case. As with the induction of injury we remain vigilant for any adverse effects and will follow unit guidelines in the event they occur. There is also likelihood of transient pain from administration by

superficial routes, and also from laparotomy when intra-organ methods of administration are used which will be controlled with appropriate pain-killers.

Non-schedule 1 methods of killing will be used, which will generally be carried out under terminal anaesthesia.

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

This project will provide important data that improve the action of cell therapy alongside our understanding of how cells exert their benefits. This will underpin new clinical trial submissions to the MHRA. Evidence of action and safety of cell therapy from appropriate mouse models will be a key part of submission to the MHRA.

As well as leading to the generation of new clinical therapies this will also provide insights into the mechanisms of liver damage. This in turn will drive additional new therapeutic options which may use drugs rather than cell therapies.

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

7100 mice will be used in total over a 5 year period, of which . Genetically altered mice will be used to more closely represent the clinical condition seen in patients. This will include models that recreate liver cirrhosis (Carbon tetrachloride), immune-mediated biliary damage (MDR2$^{-/-}$, Ova-Bil) and fatty liver disease (MCD). These models are widely regarded in the scientific community as being the closest models to the situation seen clinically.

The number of mice used will be based on statistical power calculations (80%) as used in clinical trials. Based on the anticipated benefit from a cell therapy and the variation in the parameter being measured, we will calculate how many mice are needed to provide a statistically robust answer. We have in-house access to statisticians as well as our own experience to ensure that these calculations are accurately performed.

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

The actions of cell therapies are complex and require interactions with a variety of different cells in the injured mouse. Moreover infused cells can have multiple actions in different locations after infusions into mice. This complexity cannot be captured in non-animal alternatives. This is reflected in the requirement by the MHRA for pre-clinical data before a clinical trial with new cell therapies can be considered.

Where possible we will use T cell suppression assays to test function of cells such as mesenchymal stromal cells. This helps reduce and refine the number of animal studies we need to perform.

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

Models have been selected that closely mimic the varied clinical scenarios seen in patients. These include models of inflammatory biliary disease (Ova-bil and MDR2 ko),

fatty liver (MCD) and liver fibrosis (repeated CCl_4). The chemical and dietary models selected are commonly used and are deemed representative in the scientific and regulatory community. These models develop liver injury but do not demonstrate any sign of suffering.

Suffering will be reduced/stopped by the continued adoption of our unit guidelines. These ensure that side-effects are looked for and when found injury is either discontinued or the experiments are discontinued.

Routes of cell administration will for the vast majority of studies be by ways which do not cause any discernible harm in our experience. Only rarely will surgical routes of administration such as intra-splenic or intra-hepatic routes be used, and this will largely be reserved for infusion of liver-like cells in keeping with the literature and clinical practice.

Repeated general anaesthesia will only be used when non-invasively imaging the distribution of infused cells in mice. This reduces the number of mice that might otherwise be used. Modern anaesthetic agents will be used which shorten recovery times for mice.

Moderate severity is based on the clinical outcomes seen with the models outlined in this licence. This is in keeping with our previous experience over the preceding two licences.

<table>
<tr><td>Project Title (max. 50 characters)</td><td>Immunotherapeutic manipulation of human cancers</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Immunotherapy, Antibody Engineering, Tumour Antigen, Tumour Immunology</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[7]</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[8]</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Our studies focus on how the immune system engages with cancer and when it fails to engage with cancerous cells. The exact nature of the targets recognised by the immune system in detecting and controlling cancer remains poorly understood. We have identified some altered molecules that, in healthy individuals, the immune system recognises. In patients these responses seem to be lost and may underpin why we develop cancer in later life. We need to establish that these immune responses are able to target cancerous growths in a whole animal system, which have not previously been assessed. Furthermore, we would like to assess why these immune responses fail and if they can be supported in an animal.

Additionally, we have developed a new technology that allows the working parts of the immune system that target viruses to engage with cancer. Even patients with cancer have strong immune responses to these viruses and the ability to harness these to instead target cancer is of considerable interest. We use antibodies to carry fragments of viruses to the tumour and release these onto the cancerous cell mimicking a viral infection. We have established extensive laboratory research demonstrating that this approach is indeed very effective. We need to assess whether this</td></tr>
</table>

[7] Delete Yes or No as appropriate.
[8] At least one additional purpose must be selected with this option.

	approach works in an animal before clinical trials can commence.
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 these studies are substantial. Immune-based therapies are amongst the most promising in development. If we are able to demonstrate that immune cells recognising the altered-antigens we have identified are able to kill tumours then this will provide strong evidence to move our studies into the clinic. Furthermore, if we are able to demonstrate that modifications to antibodies are able allow immune cells to control tumours this will allow the planning and development of first-in-man studies. Additionally these studies will allow us to optimise and improve on the current therapies we have.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate we will be using no more than 8800 mice over 5 years. The similarity between the mouse and human immune system, the availability of genetically manipulated mice and the extensive range of reagents available make the mouse the model of choice for in vivo studies. Typically, the experimental group size is 4 to 6 mice depending on the type of experiment, to allow for statistical significance. Consultation with a statistician and weekly data discussions within the group will be maintained to ensure the minimum number of animals is always used.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The majority of the expected adverse effects are of moderate severity (e.g. transient lower food and water uptake, intermittent vocalisation, lack of grooming) . Potential general adverse effects include minor stress levels due to the repetitive handling and injection of substances and weight loss that won't be higher than 20%. Mice with tumours may develop enlarged lymphoid tissue and other clinical signs as sign of tumour spread and in the case of sub-cutaneous tumours these may very occasionally ulcerate when the animal will be killed.. Mice infected with the viruses as described within this license are not expected to develop more than mild symptoms such as subdued and/or hunched posture but only transiently. Substances will be administered in a controlled manner and in concentrations and/or frequency that do not impact dramatically on the general wellbeing of the mice but have an effect on the tumour growth. No

	severe adverse side effects are expected from the small surgical procedures included in this license. The well trained staff will carry out these minor surgical procedures (such as implantation of a mini-pump device for constant controlled administration of novel agents under the skin) under aseptic conditions and therefore infections are expected to be only minimal. All animals will be closely monitored for adverse effects and will be humanely culled if these are apparent. Multiple analysis will be carried out from a single mouse so we can obtain the most data possible.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The in vivo work builds on data obtained from extensive validation and optimisation *in vitro* studies carried out within the group. In addition, we will use *in vitro* assays to analyse the functional properties ex-vivo when possible. However, it is not possible to re-create the complex re-circulation of immune cells between the tumour and the lymphoid tissue and the interactions between tumour cells and the local microenvironment in vitro. Therefore, in vivo studies need to be carried out if the novel immunomodulatory agents are to be taken into clinical trials. By conducting both such studies in parallel we will be able to identify correlations between the in vivo and the in vitro data which is important for clinical translation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	By following the response of individual tumours rather than average of groups, by using the latest technologies such as IVIS imaging (to monitor tumour burden and aid implementation of humane endpoints) and genetically manipulated mice or cells and regular review of the data collected
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.	Good laboratory practise, regular review of techniques and constant contact with other fellow researchers will ensure the best model with the least welfare costs to the animals will be employed for the study in question. We will use inbred mice as their immune system share many similarities with the human immune system and the extensive range of reagents allow us to perform clear, reproducible experiments so the data obtained can be translated onto a clinical scenario. All mice undergoing procedures will be closely monitored and the models used are standard and well established in immunology and cancer research. Tumour burden will be maintained to the minimum required for a valid scientific outcome and

	closely monitored by in vivo imaging so tumour spread is controlled and within the limits of severity of this project license. Infection with virus will be only carried out with already published minimal amount of virus with only mild welfare costs to the mice. Only the minimum amount of substances shown to have an effect on tumour growth in preliminary experiments with the lowest welfare cost to the mice will be used for further experiments