Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 23

Projects with a primary purpose of Basic Research into the Musculoskeletal system

Project Title and Key Words

1. **Use of tri-axial accelerometers to quantify energies during collision in laying hens**
    - Poultry, keel, welfare, production, accelerometer

2. **Redox pathways in osteoarthritis**
    - Redox pathways; osteoarthritis; DMM; mouse

3. **Musculoskeletal ageing and innervation**
    - Loading exercise, muscle, bone, innervations

4. **Skeletal mechanobiology, remodeling and repair**
    - Osteoporosis, osteoarthritis, remodelling, repair, loading

5. **Action of equine stem cells in tissue regeneration**
    - Horse, stem cells, regenerative medicine

6. **Destruction and regeneration of joints in arthritis**
    - Cartilage, bone, arthritis, regeneration, healing

7. **Animal Models for Muscular Dystrophy**
    - Dystroglycan, fukutin related protein, glycosylation, neuromuscular

8. **Using Zebrafish to Understand Gene Function and Develop New Therapies for Cancer**
    - Melanoma, Cancer, Development, Imaging, Drug-development

9. **Changing the soil: a new approach to treating arthritis**
    - Inflammation, fibroblasts, immune response, arthritis

10. **Cartilage repair, replacement and regeneration**
    - Cartilage repair, replacement and regeneration

11. **Identifying New Targets for Treating Muscular Dystrophy**
    - Muscular dystrophy, macrophages, dystroglycan, muscle

12. **Mechanism of statin induced myopathy**
    - Statin, myopathy, exercise

13. **Genetic basis of skeletal evolution and disease**
    - Bone, genetics, development, evolution, disease

14. Evolution of locomotion in amphibians

- Biomechanics, locomotion, amphibians

15. Gene Therapy for Neuromuscular and Cardiovascular Disease

- Gene therapy, muscle, heart, disease

16. Understanding Neuromechanical Systems Biology

- Locomotion, Motor neuron, mouse, optogenetics, proprioception

17. Genetic mechanisms of craniofacial malformation

- Craniosynostosis, skull, mutation, development, mouse

<table>
<tr><td>Project 1</td><td colspan="3">Use of tri-axial accelerometers to quantify energies during collision in laying hens</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Poultry, keel, welfare, production, accelerometer</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">3 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Keel fractures in commercial laying hens are a serious welfare and production issue that appears to be getting worse rather than better. Despite the gravity of the issue, the difficulty of visualizing fractures as they occur has prevented the research community and poultry industry from identifying the actual causes of fractures, and thus developing effective solutions. Our project seeks to provide this information through the placement of miniature accelerometers on the birds themselves that can track and record the frequency and energy of impacts that occur during normal bird activity within commercial poultry units.</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 colspan="3">Our project will be the first to provide objective information on the actual quantity of energy involved in causing keel fractures, while taking into account other influencing factors such as bone strength, bird age, and muscling. In doing so it will be possible to advise on improvements to the</td></tr>
</table>

	design of commercial poultry buildings in order to reduce the risk of fractures, thus bringing welfare benefits to very large numbers of birds.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use the Hyline breed of laying hen; a total of 120 animals will be used over a 16 week 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?	No harm is expected as a direct result of the procedures in this study. However, our house design is based on that of basic commercial housing systems, in which fractures develop, during the course of normal behaviour. Thus we do expect fractures to develop, as they would in a standard commercial environment, but we will not do anything to cause or increase the likelihood of fractures. Additionally, anesthetizing the birds has the potential for adverse side effects, but because the duration of anaesthesia will be short the duration of any adverse effects will also be limited. For example birds may experience a mild fall in body temperature when they are anaesthetised. Any adverse effects experienced by birds during the course of the study will be mild. The animals will be euthanized at the end of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Keel damage is a complex issue likely involving a variety of bird and environment factors, the interactions between which we are unable to fully model with current non-animal systems. In previous work, we were able to build a foundation of knowledge using recently euthanized animals, thus the current work seeks to build on that model and model those interactions in live animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals was arrived at using preliminary data where birds in a similar housing setup design developed approximately 8 fractures over an 8 wk period. Based on this expectation, a statistician within our research team calculated the approximate minimum number of animals needed

	to produce robust outcomes from the experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We will use the Hyline breed of laying hen, since this is a major genetic line used within commercial UK egg production. Birds will be monitored daily for signs of fatigue or injury and undergo a more thorough examination during a weekly radiograph procedure. With respect to anaesthesia, the PI is a qualified veterinary surgeon and a European Specialist in Veterinary Anaesthesia and Analgesia and will make sure appropriate precautions are taken to minimize the likelihood of adverse effects occurring, as well as to undertake the correct actions in the event that adverse effects do occur.

<table>
<tr><td>Project 2</td><td>Redox pathways in osteoarthritis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Redox pathways; osteoarthritis; DMM; mouse</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>2</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
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<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>
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<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Arthritis affects up to 8 million people in the UK and osteoarthritis (OA), the most common cause, involves about 60% of men and 70% of women over 65. At present there is no cure for OA with management involving long-term use of drugs for pain relief or joint replacement surgery. As well as financial costs to health services and the economy, there are incalculable costs due to the pain and suffering endured.

Osteoarthritis is a characterised by a progressive loss of cartilage, new bone formation, synovial proliferation leading to pain and loss of joint function. It is a multifactorial disease involving age, genetic and obesity-related factors but abnormal mechanical loading (for example, trauma) is a key part in the pathophysiology of the disease. In addition, it is recognised that damage from inappropriate levels of free radicals can result in cartilage damage and cell death.

This project will determine the role of free radicals</td></tr>
</table>

	in mechanically-induced osteoarthritis and highlight potential targets for early modulation of the disease. Specifically we will use a surgical model of osteoarthritis in mice genetically engineered with alterations to antioxidant systems to undertake this study.
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)?	Due to the high socioeconomic costs of osteoarthritis, the ability to identify targets to ultimately reduce or prevent the development of this condition will have great benefits on human welfare and society. Understanding the pathophysiological processes involved in the development of OA is important in identifying treatment targets to modify or ameliorate the progression of this debilitating condition.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult mice (including genetically modified mice) Approximately 120 mice over 2 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?	Osteoarthritis will be mechanically-induced in mice using a well-recognised surgical procedure involving destabilisation of the medial meniscus (DMM) of the knee. This results in a moderate severity of OA after 8 weeks, comparable to naturally occurring levels in humans. There are minimal adverse effects expected and despite the nature of the intervention, animals are able to ambulate well with no significant effects on behaviour and welfare. 8 weeks after surgery, the mice will be euthanised.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Osteoarthritis is a disease of the whole joint and therefore the use of mice is required to study the disease in full. Cell cultures and use of explants can provide some information but do not replicate the complex interactions between cartilage, bone, synovium and other soft tissues in vivo and the changes occurring due to abnormal mechanical forces experienced in disease. In addition, ageing is a whole organism phenomenon. However, we will

	take data generated from the whole mice model to study further aspects of the identified redox pathways into cellular based systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use appropriate statistical tests and measures to calculate the minimum, but statistically appropriate number of mice required for this study. We will review our project regularly in order to re-evaluate mice numbers. In addition, we will source and share any tissue where appropriate (e.g. non-operated controls) to reduce numbers further.
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 use of lower order species is not appropriate for this project. The use of mice is required due to the similarities in organ physiology, such as joints, to humans. Spontaneous models of mouse OA occur but require large numbers of mice and time and are therefore inappropriate. Models of OA utilising surgical instability are recognised in other mammalian species such as dog, guinea pig and goat but can results in severe disease and high morbidity. The DMM model developed for mice is a refined procedure resulting in a moderate form of OA by 8 weeks, unlike the anterior cruciate ligament transaction (ACLT) which results in severe OA and will not be used in this study. Mice following DMM surgery show excellent mobility and appropriate monitoring and analgesia are factored into the protocol design, including clear defined end points. Appropriate intervention will occur following recognition of any adverse or unexpected effects to minimise welfare costs to the animals throughout the project.

<table>
<tr><td>Project 3</td><td colspan="3">Musculoskeletal ageing and innervation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Loading exercise, muscle, bone, innervation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
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<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Correct musculoskeletal innervation is crucial to maintain optimal function of this system and its ability to adapt to challenges. We hypothesise that mechanical loading of one tissue (e.g. muscle) will affect the innervation pattern of that tissue and that this may lead to compensatory changes in the innervation of the other tissue (e.g. bone). To verify this hypothesis, we intend to establish a model to:

a) Monitor changes in muscle innervation under mechanical loading of the muscle b) Assess to what extent changes in muscle innervation affect bone innervation and physiology under mechanical loading c) Assess to what extent changes in muscle innervation affect bone innervation and physiology under mechanical loading during ageing.</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 colspan="3">The goal of this work is to understand how innervation of the musculoskeletal system changes with age, and to what extent it can be modulated by a life-style based intervention, such as loading exercise, in order to preserve its function.
Elegant studies have shown an age-related decline in muscle innervation. However, to our best knowledge, little is known about bone innervation changes with age, the relationships between bone and muscle innervation, and the effect of loading exercise on the innervation of the musculoskeletal system.

With this project, we will be the first to use an integrated approach to monitor changes in bone and muscle innervation in response to mechanical loading.</td></tr>
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What species and approximate numbers of animals do you expect to use over what period of time?	Young and old mice (80 in total) 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 protocol described above is designed to cause hypertrophy of muscle tissue. Previous published work by workers in the UK using mice and rats indicates that this will not lead to any substantial disability and that following recovery from anaesthesia, animals were observed to use both legs within 1 hour. Published data also demonstrates that mice undergone this procedure do survive without problems, and can be kept alive for several days (up to 208 days) post-operation. Therefore we anticipate that this procedure will have no major effect on mobility of the mouse for feeding and drinking. In case of discomfort, suitable analgesia will be used for up to 3 days at which time point, mobility will be assessed. The assessment of muscle hypertrophy in these mice will be determined at the earliest time point possible, which we anticipate being between 14 and 28 days post operation. At the end of the protocol the mice will be killed by Schedule 1.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The need to study the interplay between nerve, muscle and bone *in vivo* means that it is not feasible to use biopsy material from humans. There is currently no suitable cell culture alternative to examining the effect of mechanical loading on muscle and bone innervation *in vitro.*
2. Reduction Explain how you will assure the use of minimum numbers of animals	The proposal has been designed with the help of statistical advice to minimise the number of mice used. Statistical power calculations were performed using Minitab Data Analysis Software based on previous published work and work from our laboratory. Calculations suggest that n=5 will be necessary to achieve statistical significance.
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	Mice are chosen for these experiments due to the similar physiology between mouse and human muscle. Mice are the lowest vertebrate group possible in this study and the availability of genetically altered mice will provide definitive data necessary to achieve the objectives for this study. Other species have been considered but deemed unsuitable for these experiments, such as

(harms) to the animals.	Zebrafish, drosophila or nematode. To minimise animal suffering, we will initially perform these experiments only in young mice and keep these animals for 28 days maximum post-operation. Analgesia will also be used when necessary. We will only proceed with these experiments in old mice once we are satisfied that the experiments performed in young mice do not cause any severe signs of discomfort or ill health.

Project 4	**Skeletal mechanobiology, remodeling and repair**	
Key Words (max. 5 words)	Osteoporosis, osteoarthritis, remodelling, repair, loading	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Disorders of the skeletal system may result from hereditary or acquired pathologic processes. Impairments may result from degenerative processes as well as traumatic events. Two of the most prevalent skeletal conditions are osteoporosis and osteoarthritis, both of which will increase even further as a consequence of increasing longevity and lifestyles. Those conditions result in fracture, chronic pain, impaired quality of life, higher levels of morbidity arid mortality and provide a challenge in terms of management and health economic coals. There is therefore a continuing need to advance our basic knowledge on the remodelling, repair and regeneration of the skeletal system in order to translate understanding of mechanisms to new clinical strategies in prevention and management of skeletal disease. Our project uses animal models of these disorders, genetically modified rodents and a range of specific protocols to further	

	understand the mechanisms and essential factors regulating skeletal tissues remodelling and repair from development to ageing with the ultimate goal of providing directions for drug developments to alleviate osteoarthritis and osteoporosis and the impacts of their consequences such as pain and fragility fractures. There is a continuing need to translate understanding of mechanisms to new clinical strategies to improve prevention diagnosis, control and treatment of skeletal disease. For example, despite considerable advances, the mechanisms controlling the response of cells to specific biological and defined mechanical stimuli have not been fully elucidated. This is true not only for bone tissue, which has been the initial focus of mechano-biological research, but also for other non-calcified musculoskeletal tissues where the lack of knowledge of these specific mechanisms is even greater. Our aim is to build upon current knowledge and extend understanding to variables such as genetics, mechanical environment, hormonal changes, diet and ageing. The fact that musculoskeletal disorders lead to significant human suffering, this work is focused on furthering our understanding of the mechanisms controlling normal physiological function of joints and bone in the pathogenesis of osteoarthritis and osteoporosis. In each of the protocols we are using, we aim to emulate some aspect of the human pathology in order to understand and interfere with the pathophysiological processes. In both osteoarthritis and osteoporosis, there is a multi-cell involvement with complex systems that cannot be addressed in tissue culture or non-animal model systems. In osteoarthritis, the main surgical model that we propose to use is akin to sports injury in people which go on to develop osteoarthritis, therefore these models are important paradigm for interventional studies to ameliorate the outcome. Our program of work will principally investigate:

	I) The identification of the molecular mechanisms and novel regulators involved in skeletal tissues repair and functions. 2) The response of specific skeletal tissues to defined mechanical and biological stimulation and how this response is compromised with age and disease. 3) The interactions between biological factors and mechanical loading for the maintenance of skeletal tissues during ageing. 4) The influence of age, disease, mechanical and biological factors on the repair processes of skeletal tissues and structures. 5) The effect of changes in diet to either induce or alleviate pathological skeletal conditions and to improve skeletal repair
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 of our project to gain better understanding of the factors and their mechanisms of actions regulating bone and joint physiology for diseases in which these tissues are affected. Overall, our research aims to improve the quality of life and mobility of people with bone and joint pathologies. Ultimately, we hope to help develop directions for treatment options for those pathologies.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 7000 rats and mice, mainly mice. The rodent species to be used are appropriate because their fundamental skeletal biology is very similar to humans in many regards and there is the advantage that genetic models, probes and antibodies are available. The most appropriate models of osteoporosis, joint disruption, fractures and type 2- diabetes arc in rodents. Sample sizes to be used are based on previous work and a calculation to estimate the minimum number of rodents required for establishing significant differences between groups
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	All procedures to be undertaken are performed in rodents arid do not exceed “moderate” in severity. We have developed over the years appropriate animal models of skeletal diseases and protocols that aimed at investigating the

the end?	remodelling responses of skeletal tissues to their mechanical and biological environment, All these experiments arc performed by appropriately trained experimenters and are essential for the success of this project. Animals will be sacrificed by Schedule I at the end of experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The processes involved in achieving adaptive changes in bone architecture and mass and those involved in the degeneration of joint that are likely promoted by mechanically-derived loads are incompletely understood and can only realistically be replicated using live animal models. in Wm organ culture systems appear capable of at least partly replicating the events whereby these mechanical stimuli are applied and may therefore be useful in examining the immediate and short term responses to such application, but they arc completely incapable of replicating the longer term osteogenic response in bone to create functionally appropriate changes in architecture and mass. These In vitro approaches also fail to produce the range of structural abnormalities in joint architecture that can he seen, sometime after, in response to abnormal loading in the intact joint. Monolayer cell culture can sometimes be used to replicate selected aspects of both of these types of responses but they fall short of providing integrated, organ.1evei, physiologically intact environment in which such responses are normally coordinated. These in vitro and cell culture based alternatives have been, and will be, used by us as replacements wherever possible to examine some selected aspects of the responses we aim to more fully decipher. We have fully acknowledged their strengths, reviewed their use for others, but appreciate their limitations.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We always aim to reduce the numbers of animals we use. Power analyses are always applied in order to identify the minimum number of animals that we need to use in order to answer the specific question being posed, For instance, have established that our tibial bone loading studies require group sizes of no more than eight to secure statistical

	significance. Wherever it is possible we will also exploit contra-lateral limbs as controls in order to reduce the numbers of animals required still further; the possibility of exploiting such controls is another area in which future reduction in numbers may be achieved. This may not always be possible, however, but efforts will be made in all initial investigations to secure the validity of internal control samples. The principles of our experimental design have been already established in our on-going programme of study and so there is little need in performing studies to modify our bone loading programme. This is not necessarily the ease for joint loading but advances are being made all the time and it is our hope that during this particular programme of study that we will have identified an optirnised osteogenic loading protocol; an important step, as it will mean that we more fully understand the mechanical drivers of osteoarthritis — a vital advance in our understanding.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen to focus particularly on rodents. This decision has been made as it will provide us with the potential to explore the role of specific genes in the response we identify through the use of mutant and transgenic mouse models. Indeed, the choice to develop the tibial bone (de Souza et al., 2005) and joint loading model (Poulet et al., 2005) in the mouse was made with this purpose firmly in mind, These models are being replicated by other groups and represent the fore-front of this in viva approach to address questions in bone and joint mechanobiology. Animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. The alternative strategies which others have used to attain similar end-points frequently involve surgery and our use of surgical approaches will be kept to a minimum. Appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia.

<table>
<tr><td>Project 5</td><td>Action of equine stem cells in tissue regeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Horse, stem cells, regenerative medicine</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of this work is to define the mechanisms by which different equine stem cell populations function to aid tissue regeneration.

The specific objectives are to:

1) Understand if equine stem cells isolated from one horse can be used to treat another, un-related horse.

This would allow the production of a standardised “off the shelf” source of cells to allow horses to be treated immediately following an injury.

2) Can equine stem cells suppress an immune response?

This will determine if equine stem cells would be suitable for treating inflammatory conditions such as osteoarthritis.

3) What soluble factors are involved in stem cell-mediated immunosuppression?</td></tr>
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	Identification of these factors might allow their future targeting in the development of novel therapies. 4) Can equine stem cells promote the migration of beneficial white blood cells to injury sites? Some white blood cells can assist in tissue repair by degrading the damaged tissue and encouraging the formation of new blood vessels. Understanding if certain stem cells can promote this will help us to develop new treatments for poor tissue repair. 5) Are all equine stem cells the same in their ability to turn into musculoskeletal tissues? Some injuries, e.g. fracture, may benefit from the transplantation of new cells to the injury site. Understanding how good different sources of equine stem cells are at turning into different cell types will help us to develop new treatment strategies.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This work will provide new information about the properties of equine stem cells to help us to understand how they work to improve tissue regeneration. It will determine if equine stem cells taken from one horse are safe to use in other, un-related horses to allow the production of standardised “off the shelf” sources of stem cells which could be used immediately for the treatment of acute injuries. It will define the characteristics of different sources of stem cells with regard to their ability to turn into specific tissues and their ability to orchestrate repair by the body’s own cells (e.g. immune cells). This will enable us to tailor the use of different stem cells to treat specific injury types. It will advance our knowledge on stem cells and the signalling pathways that are involved in their functional properties. This will allow future optimisation of equine stem cell therapies in treating injuries and may assist in the development of human stem cell therapies. Horses suffer from a large range of musculo-skeletal and other tissue injuries which undergo poor natural regeneration. Developing new therapies for these conditions would have a significant impact on horse health and welfare and

	may also inform human medicine.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use horses and over its 5 year duration we expect to use a maximum of 10 animals.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The horses will be used to provide biological materials (stem cells and blood cells) to use in laboratory studies. These samples will be either whole blood or tissue biopsies. The expected level of severity is mild. At the end of the project the horses may be discharged from the act and re-homed if suitable. Alternatively, they may be kept for re-use on other project licences at the establishment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To successfully use stem cells for the treatment of injuries and diseases we need to understand more about how they work. Much of this work will be done in the laboratory, however, we require the use of animals for the isolation of tissue samples from which to derive stem cells and blood cells. Whenever possible we will use archived cells and/or harvest tissue from animals which have died for unrelated reasons.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To account for variability which occurs between animals we will need to isolate cells from multiple horses. Based on our previous experience up to 5 horses are likely be required to account for this variability and therefore over a period of 5 years we anticipate that up to 10 horses may be used. To minimise the number of animals used we will isolate multiple sources of stem cells from each horse.
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	Stem cells are already being used to treat tissue injuries in horses but little is still known about their function. In order to optimise the future use of stem cells we need to understand more about how they work. As stem cells may vary between species it is important that we learn more about the properties of equine stem cells in order to optimise future equine clinical applications. Therefore horses must

minimise welfare costs (harms) to the animals.	be used in this project. To minimise the harm to the animal any tissue sampling will be performed simultaneously to minimise the number of times it is performed. The handlers know the animals well and they will be closely monitored for adverse reactions.

<table>
<tr><td>Project 6</td><td>Destruction and regeneration of joints in arthritis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cartilage, bone, arthritis, regeneration, healing</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>yes</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 this project is to identify cells and molecules that can limit the destruction or induce the healing of joint tissues (bone, cartilage, menisci, ligaments) within the joints when they have been injured due to arthritis or following trauma.</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 surface of the bones, at a joint, are covered with an elastic, resistant and very lubricated tissue called cartilage, which guarantees the frictionless motion of our joints. Cartilage has a limited capacity for repair, and when damaged by arthritis or trauma, often fails to regenerate and this results in irreversible disability because of permanent pain, reduced mobility, and joint swelling. In fact, for this reason, arthritis is the most common cause of disability allowance in the UK and the most frequent cause of disability worldwide. Our long term research in this field has already yielded a potent cell-based product, now available worldwide, to repair relatively small, isolated cartilage defects,</td></tr>
</table>

	and we are now aiming to make these technologies more potent, safer, more affordable, and especially suitable to treat also large and diffuse defects such as those resulting from arthritis. Every success in this field leads to patients regaining their independence, ability to work, be mobile, and to look after themselves and their families.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mostly mice, but for specific experiments we may also use rats. We expect to use approximately 3,000 animals per year.
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?	Using methods in cells in culture we identify cells and molecules that, based on tests performed in the laboratory in test tube, appear to have properties that support the healing of joint tissues. The most promising of these cells and molecules are then tested in animals in models of arthritis or of tissue formation. Most protocols are mild to moderate in severity and the animals in general only experience from mild to moderate joint pain. In only one protocol it is expected to observe severe arthritis and the animals are expected to develop joint pain, swelling, and, in rare cases, even ulcerations. At the end of the protocols the animals are killed and their joints are analysed under the microscope and biochemically to confirm whether the treatment had any effects. Pain will also be measured.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have a wide array of tests that we can perform in the laboratory to test if cells and molecules can form cartilage. These tests efficiently replace most experiments in animals. These experiments drastically reduce the number of conditions and molecules that require animal experimentation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have carefully optimized our animal models so that the minimum number of animals is needed to measure differences and treatments. Therefore we will avoid situations in which the effects are going to be too small or too large to measure any

	differences effectively, and which therefore would otherwise require high numbers of experimental animals.
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.	Different strains of mice develop diseases with different degrees of severity. Therefore, models of disease that are perfect for a certain strain of mice may be too severe or ineffective in others. Since in our laboratory we have expertise with multiple different models that vary in severity, we will be able to match the appropriate model to each strain so to avoid the excessive suffering of a severe model in a susceptible strain.

<table>
<tr><td>Project 7</td><td>Animal Models for Muscular Dystrophy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Dystroglycan, fukutin related protein, glycosylation, neuromuscular</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overall aim of the programme is to generate animal models for muscular dystrophy based on the genetic modification of specific genes. Neuromuscular diseases affect more than 60,000 people in the UK and an estimated 7 million people worldwide. Many of these conditions are lethal and involve long periods of substantial disability and a reduced quality of life. In the vast majority of conditions there are no effective therapies and certainly no cures. The main objective of this project is to generate and analyse mouse models for muscular dystrophy. Specifically we aim to concentrate on using and generating models for a severe form of congenital muscular dystrophy which is associated with defects in the brain eye and muscle and a milder form of disease in which only heart and skeletal muscle is affected.</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>We do not yet understand why some patients are born with defects in the brain and eyes as well as the muscles. Surprisingly mutations in the same gene also lead to a common form of limb girdle dystrophy in the UK which is frequently associated with a dilated cardiomyopathy. The reasons for this broad clinical spectrum remain unclear as does the precise mechanism by which the mutations lead to disease.

We have already generated mice with defects similar to patients at the severe and mild ends of the clinical spectrum. These mice are proving to be valuable models in which to understand the human</td></tr>
</table>

	disease which could not be obtained from patient biopsies. All our animal work has major clinical relevance to specific forms of muscular dystrophy and will enable us in the long run to understand disease progression and develop new forms of therapy.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use up to 15,890 mice over 5 years, this represents a mixture of normal animals and genetically modified mice with neuromuscular disease. In addition the mice with neuromuscular conditions will be bred with mutant and genetically modified mice that have other genetic alterations that might be expected to alter the course of the neuromuscular condition. Mice are used as the mammalian species because a wide variety of spontaneous mutants and genetically modified mice are available.
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 mice are expected to only feel mild discomfort associated with injections and none will undergo procedures of greater than moderate severity. The studies conducted under this project will contribute to a greater understanding of the mechanism underlying the disease process and the development of clinically applicable protocols for patients with neuromuscular disorders.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Muscular dystrophy cannot at the present time be modelled in tissue culture. Importantly we have recently shown using our animal models that a possible therapeutic strategy whilst working in tissue culture was actually detrimental in the diseased animal. We now know that the expression of this particular protein will need to be tightly controlled if it is to be used as a therapy.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the recently developed standard operating procedures (currently located on the treat-NMD website: http://www.treat-nmd.eu/research/preclinical/preclinical-efficacy-standards/). We have considerable experience with our models which provides knowledge of the variation in each measure and therefore accurate calculations of the required sample size to achieve a reliable result. Pilot trials using 3 animals per group are initially used to assess whether a larger scale experiment is required.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	All experiments will be conducted in mice as this species has been the most widely used for genetic manipulation and has the greatest number of spontaneous and induced mutants and genetically modified strains for this group of diseases

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	(dystroglycanopathies). The majority of the mouse models which do not die around the time of birth are relatively mild, those that are more severely affected are killed when they start to show clinical signs to reduce the level of harm. Most of the studies involve pathological examination of tissue at defined points during the disease process.

Project 8	**Using Zebrafish to Understand Gene Function and Develop New Therapies for Cancer**	
Key Words (max. 5 words)	Melanoma, Cancer, Development, Imaging, Drug- development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		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 objective is to understand the genetic scientific mutations that contribute to cancer development, scientific/clinical and how these mutations affect the development of addressed) cells in an animal. We are specifically interested in understanding skin cancer, and developing new drugs that might be useful to treat cancers.	
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 science is basic science and translational from this science. We expect to learn about the biology of science could be melanoma and other cancers, and then apply this humans or knowledge to the development of new therapeutics.	
What species and approximate numbers of animals do you expect to use over what period of time?	We use zebrafish (Danio rerio), and we plan to use numbers of about 13000 fish 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?	There are two aspects of our work that may cause moderate severity levels. First, we are developing zebrafish that have mutations, and these fish develop cancer. Often the fish appear healthy, in spite of the tumor growth (on the skin). Second, we will be treating fish with anti-cancer agents, and the fish may develop adverse effects to the drug. We will limit the potential of these effects by using the smallest concentration of drug we can. Any fish that is no longer able to swim well, or has evidence of suffering, or additional unexpected signs of disease or illness will be killed in a humane method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to use animals because we are unable to address our questions in any non-animal system and have it be directly relevant to human disease. We need to study the function of the genetic mutations and the potential drugs in the context of a living organism. Sometimes, we can address some of our scientific questions using cell cultures, or other systems such as yeast or in vitro, and we have the experimental set up in our lab to do these experiments when we can.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We ensure that the minimum numbers of animals are used by preparing our experiment ahead of time and using proper statistical methods, keeping healthy, fewer animals for our needs, and when possible using fish in the embryonic stages.
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 zebrafish because they develop cancers that are highly similar to human cancers, Like us, they are vertebrates, and they share many genetic and anatomical features with humans. Zebrafish are not mammals, so they are less similar to us than other animal systems, such as mice. However, we cannot use non-vertebrates (such as worms or flies) because these animals do not develop cancers that have the clinical features as humans. We have two

	dedicated staff looking after the zebrafish to maintain their health. Any fish that is no longer able to swim well, or has evidence of suffering, or additional unexpected signs of disease or illness will be killed in a humane method.

<table>
<tr><td>PROJECT 9</td><td>Changing the soil: a new approach to treating arthritis</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Inflammation, fibroblasts, immune response, arthritis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>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>Fibroblasts are tissue resident cells that act like the soil in your garden to help define and maintain the architecture of organs. Fibroblasts come in different varieties, or subsets, some of which become altered in disease. Our aim is to identify which subsets are most important in the development of arthritis and to explore whether changing them improves disease. Since different fibroblasts perform different functions in the joint, a key objective will be to determine which fibroblasts to target and which to ignore.</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>Patients with rheumatoid arthritis dream of having their disease cured. Although we have exciting new treatments that control disease activity, a formal cure of the disease remains elusive. We suggest that this is because current approaches to treatment do not take account of the role that fibroblasts play in the development of arthritis. Strategies which target fibroblasts have been shown to be highly effective in some forms of cancer and there is evidence to suggest that a similar approach could work in treating arthritis. A key strength of our work is that it combines both human and animal studies that run in parallel. An advantage of this approach is that we will minimize over reliance on mouse models of disease. This will place us in an ideal position to translate our findings into benefits for patients with inflammatory arthritis</td></tr>
</table>

What species and approximate numbers of animals do you expect to use over what period of time?	Mouse About 12,000 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 level of severity? What will happen to the animals at the end?	A question that many patients ask is why their inflammation begins and why it is confined to certain organs or tissues. In order to study how inflammation begins, resolves or persists at different sites we will induce inflammation in the skin, joints and peritoneum of mice to see whether by manipulating the fibroblast and white blood cells at these sites we can alter the course of inflammation and ultimately turn it off without inducing immunosuppression. Mice will get moderate disease in the tissues where we induce inflammation. The maximum time any one mouse is likely to have arthritis is 5 weeks as the arthritis is self limiting. The maximum time any one mouse will have peritonitis is 2 weeks. We will give mice treatments that lessen pain and discomfort that might occur as part of the inflammatory response. This will include pre emptive treatment with opiate analgesia. We have also adapted treatments used in patients with arthritis (e.g anti TNF biologic therapies) for use in mouse strains that have a genetic predisposition to develop arthritis. We have taken precautions to reduce any suffering from other procedures including, genetically altered animal phenotypes, gene inducing agents, anaesthesia, surgery, therapeutic agents, immunisations, blood withdrawal and irradiation by keeping the number of these procedures to a minimum. We have put in place mitigations as described below in the 3Rs to ensure that any suffering by an individual animal is minimized.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to study the inflammatory response three components need to be examined; time, place and cell type. While place and cell type can be examined in humans, it is unethical to perform multiple biopsies, and cell transfer experiments in humans with arthritis. Over the last decade we and our collaborators have pioneered a range of non animal *in vitro* models that have furthered our understanding of chronic inflammation by culturing human cells in the laboratory. However we have now reached a point where we cannot proceed to test our ideas without resorting to animal models as they are required before we can move to human

	studies
2. Reduction Explain how you will assure the use of minimum numbers of animals	A key strength of our work is that it combines both human and animal models so that each can be used to inform the other and therefore minimize an over reliance on mouse models of disease. This will give us the option to stop the line of research at any stage where our findings fail to show any significant increase in our understanding of chronic synovial inflammation. We are running these studies in parallel with studies that explore the same markers in immune mediated inflammatory diseases in humans. Using non-invasive strategies such as imaging of joints in the same animal over time will also reduce the number of animals required allowing statistically significant differences to be obtained from less mice We have also put in place strategies to reduce the clinical symptoms of arthritis and inflammation in such mice by treating them with anti-TNF antibodies in the same way that therapy is given to humans with rheumatoid arthritis. Not only does this help reduce unnecessary suffering for the mice but it will reduce the number of mice needed in our breeding programme Experiments will be designed to ensure that minimal numbers of mice are used to obtain biologically significant results. The following general principles apply. i) Pilot experiments, where therapeutic interventions are planned will use small numbers of animals with appropriate controls; e.g. 2-3 mice. ii) Full experiments will incorporate the principles of randomisation, replication and local control. For designed experiments, the level of significance will be set at 5%, and the power will be set at 80%. The least practicable difference between groups will be set at no less than 25%. Generally, the expected coefficient of variation (i.e. standard deviation/mean) will be about 15%. For a 4-group experiment, this means a group size of about 7 animals/group. Blocking will be used routinely. Factorial designs will be used where applicable. The assumptions of the model used to fit the data will be tested before any formal statistical analysis is done (whether parametric or non-parametric). iii) Full experiments will not normally be

	repeated. Where there are doubts about the reproducibility of data, an experiment may be re-run, but using a different batch of the critical reagents or a different strain of mouse.
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 best model for the study of persistent disease because: (i) The main components of their immune system is shared by humans; this is essential where immune responses as opposed to the function of individual genes is being studied and thus will produce satisfactory results (ii) A wide range of wild type and genetically manipulated strains of defined genetic makeup are available; (iii) An extensive range of reagents is available for analysis of immune responses (iv) They are the most acceptable animal model that shows the least degree of neurophysiological sensitivity and will suffer the least pain, suffering, distress or lasting harm. (v) There are no other alternatives to this work. We have refined and streamlined as much as possible the models of arthritis that we use. Importantly we will use a research strategy of moving from lower degrees and duration of arthritis towards protocols with more chronic and persistent arthritis Where necessary, male mice transgenic for the gene of interest will be mated with WT females in order to exclude indirect effects on the progeny derived from gene overexpression in the pregnant female. When using genetic mouse models we will as far as possible use inducible models where genes are switched on conditionally (i.e. at a specific time and in a specific place). This will limit the number of mice used per experiment and the duration of any potential harm or suffering to the mice affected by a change in expression of that particular gene. We will also ensure that appropriate pre emptive pain relief, husbandry and housing measures are adopted to minimize harm to the mice

<table>
<tr><td>Project 10</td><td>Cartilage repair, replacement and regeneration</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Cartilage repair, replacement and regeneration</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>To determine the best attachment method for, and efficacy and durability of, novel articular and meniscal cartilage replacement therapies.</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>Articular and meniscal cartilage replacements could allow patients to prolong use of the knee, increase mobility, reduce pain and significantly reduce the need for total knee replacements (TKR) or at the very least postpone the need for such radical treatment as well as offering a treatment option for those patients deemed too young for TKR.

The same technology could also be applied to other joints for example the hip, ankle, shoulder and foot to alleviate similar problems in those areas.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Maximum of 300 Sheep over 5 years</td></tr>
</table>

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?	Due to the moderate nature of the procedure and the previous experience we have had with sheep knee surgery, there should be minimal discomfort to the animals after the initial surgery phase. In previous studies the majority of animals have returned to normal behaviour patterns within 3-4 weeks of the surgery. The animals are usually kept for 6 months after the surgery date at which point they are euthanized and the treatment site and surrounding tissue taken for further examination.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cartilage is a complex tissue which interacts with the surrounding bone and joint tissue and whose growth and maintenance is affected by the mechanical stimulation of joint motion and weight bearing. These conditions are almost impossible to replicate in the laboratory or in dead tissue when looking at healing / remodelling especially over longer time frames (i.e. months).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Both hind legs will be taken at termination to allow comparison of the treated knee with an untreated one that has experienced similar conditions. For significant product changes, a 1 or 2 animal study will be performed first, followed, if successful, larger studies - groups of no more than 10 animals for each test plus the use of non- invasive imaging (e.g. X-ray , ultrasound, etc) where possible to allow data collection at various times without causing undue stress/ trauma or the need for euthanasia.
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 sheep model is considered the closest mechanical representation of the human knee joint and the animal weight is also reasonably similar. Due to the limited pain reception within the joint there should be little discomfort for the animal from the surgical site any discomfort would more likely arise from the surgical access which should be limited to a small cut of less than 10cm which should be easily controlled by standard pain relief. Also, there should be no need to splint or otherwise restrict the animal.

	It is not possible to use smaller species as the size difference (and therefore the size of implant used and the load applied to it) compared to humans makes direct comparison or even extrapolation far less accurate.

<table>
<tr><td>Project 11</td><td>Identifying New Targets for Treating Muscular Dystrophy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Muscular dystrophy, macrophages, dystroglycan, muscle</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>Yes</td><td>~~No~~</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Muscular dystrophy is a progressive muscle wasting disease that is life shortening and has no cure or long lasting, effective treatment. The aim of this project is to identify and test targets for the development of new therapies for muscular dystrophy.</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 potential benefits that we are likely to derive from this project are the identification of new treatments or drug targets that could improve the quality and quantity of life for muscular dystrophy sufferers. Identifying new treatments will ultimately be of benefit to the patients and their carers, and furthermore will reduce the economic burden on the healthcare system and to society as a whole.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use</td><td>We expect to use on average approximately 1000 mice per year for a five year period.</td></tr>
</table>

over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	These mice will go through one or more of the tests we have outlined in this project licence. The tests chosen result in no lasting harm to the mice and any adverse effects would be mild when they do occur. Minor discomfort/stress is the main effect of any of the tests e.g. discomfort due to manual handling, withdrawal of blood, injection with reagents, removal of a small amount of tissue from the ear or tail for isolation of DNA to enable genetic identification, some stress through placing the animals in a new environment (e.g. a maze, or a different type of cage) while the test is being carried out.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	*In vitro* assays cannot accurately mimic the complexities of neuromuscular disease which often involves both nerves and muscles, but can also involve connective and higher neuronal tissues and the immune system. However, we will continue to use muscle cells in culture, and zebrafish embryos below the age of protection (5dpf). Through our use of both these approaches we have gained considerable insight into the aetiology of DMD at the cellular level, valuable information that could then be applied to higher animal models such as the *mdx* mouse. As our knowledge and understanding of the disease mechanisms obtained *in vitro* continues to increase, we will use this information to further inform and refine our experiments carried out on mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to reduce the numbers of animal used in testing potential therapeutic agents for the treatment of muscular dystrophy and to further our understanding of the molecular mechanisms of the disease process we will continue to use muscle cells in culture, and zebrafish embryos below the age of protection (5dpf). In addition, numbers are kept to a minimum by: the use of highly skilled and

	well-trained workers; using tests that we know from a great deal of experience give reliable results (and therefore do not need to be repeated); using statistical analysis to enable us to use the minimum number of animals required to detect a difference; using techniques that ensure the maximum possible data is gained from each animal e.g. testing muscle strength, analysing muscle morphology and blood analysis; and making use of sperm and embryo freezing to archive all animal lines for future use.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have chosen the lowest organism that is able to reasonably mimic the human diseases we are studying. The mouse is a suitable organism for studying conditions such as neuromuscular disease. Anatomically, the musculature is similar to the human, and the physiological responses to muscle use and wasting are very similar to that in humans. Additionally a very large body of published literature exists on the use of mouse models of neuromuscular diseases against which the findings from our studies can be compared. We aim to keep any distress, discomfort or pain felt by the animals to a minimum and have carefully considered the humane end points in the proposed experiments to ensure that mice do not suffer unnecessarily. The tests chosen result in no lasting harm to the animals. To help reduce any stress or suffering that animals may be subjected to, the mice will be observed daily and any animal showing lethargy, hunched appearance and subdued behaviour will require alerting the NACWO and the appropriate PPL holder or a designated deputy the same day.

<table>
<tr><td>Project 12</td><td colspan="3">Mechanism of statin induced myopathy</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Statin, myopathy, exercise</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">To determine the mechanism by which statins cause skeletal muscle pain and weakness</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 colspan="3">Skeletal muscle pain and weakness (myopathy) is seen in up to one quarter of the 6 million people in the UK who take statins. These effects can be exacerbated by exercise. Myopathy is the main reason that people stop taking statins. Because statins reduce cardiovascular disease and deaths from cardiovascular disease it is very important that we understand why they have these effects on skeletal muscle. Once the mechanism of myopathy is known, new treatments can be developed which can be taken along with statins to prevent their deleterious effects on skeletal muscle. This will improve statin use and so improve cardiovascular health.</td></tr>
<tr><td>What species and approximate numbers of</td><td colspan="3">Rats, around 300 over 5 years</td></tr>
</table>

animals do you expect to use over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our previous work has shown that the highest statin dose that we will use is well tolerated over a 2 week period with no adverse effects on the animals' wellbeing. Despite this, we do see evidence at the cellular level that there are changes in skeletal muscle. Here we intend to increase the period of statin treatment to up to 4 weeks. On the basis of what we already know for the 2 week treatment, it is unlikely that this will cause more than mild discomfort in skeletal muscle. If there is any evidence of severe discomfort, animals will be humanely killed immediately. At the end of the experiment, rats will be humanely killed and samples of skeletal (and cardiac muscle) taken for many different types of analysis of muscle function, structure and biochemistry.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Treatment of living animals with statins is required to allow us to model the situation of people taking statins. We do have a limited supply of skeletal muscle from statin-treated patients which we can use for some experiments, however it is difficult to properly test the mechanism of myopathy with these samples because people who take statins may have diseases or will be taking other medication which complicates the interpretation of results. In addition, not all the drugs we wish to use to test our proposed mechanism are licensed for use in man. For some types of experiment it is possible to use human cultured skeletal muscle cell lines, but this is not appropriate for the current project because statins must be chronically administered in living animals in order to mimic the clinical situation. When statins are given to a living person or animal, changes in cholesterol synthesis lead in turn to other tissue (e.g. liver) specific changes. This effect cannot be mimicked using a culture of skeletal

	muscle cells alone.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have performed calculations that tell us the minimum number of animals required in order to be able to test our hypothesis. For every animal we will derive the maximum amount of data that we can from it. We will also share tissue from these animals with other scientists who are interested in statin effects on e.g. the brain/nervous system.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mammalian species are required to properly replicate the effects of statin treatment seen in man. Rats have similar genes to man. Much work has been previously performed in these species to support the hypothesis for our research. Suffering of the animals will be minimised. Animals will be monitored very closely and our local veterinary officer will advise about acceptable levels of symptoms. If any animals show symptoms beyond this level they will be humanely killed.

<table>
<tr><td>Project 13</td><td>Genetic basis of skeletal evolution and disease</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bone, genetics, development, evolution, disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td></td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>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>Skeletal development is a fundamental biological process with a major impact on human health. Yet, the genetic mechanisms that instruct each and every one of the 206 human bones to develop into a unique shape at a unique anatomic location are largely unknown. This project aims to identify which genes regulate skeletal development, and how are they turned on and off in spatial and temporal manner to precisely control when and where each bone develops. How has the activity of these genes changed in the human lineage to lead to human-specific skeletal traits will be addressed.</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>Defects in skeletal development lead to many human congenital syndromes with debilitating effects to patient’s quality of life. Bone diseases are common among the population, especially among the elderly. Half the adults of the world are going to develop one of the many known skeletal conditions, which include osteoarthritis and osteoporosis. Many of the bone conditions affect bone density, bone</td></tr>
</table>

	turnover or bone structure. These affect bone strength, and the biggest problem associated with bone disease is bone fractures, which in many cases are the first sign of skeletal disease. They can be quite debilitating and are a chronic burden on both individuals and society. While for some of the patients the genetic cause of bone disease is known, mainly due to our ability to screen for mutations in the protein coding regions of candidate genes, many clinical cases remain unsolved. In these cases, the genetic cause has been difficult to pinpoint because the mutations are either in novel skeletal development genes, or they are in the vast intergenic regions in the human genome that contain hundreds of thousands of gene regulatory switches that turn genes on and off in temporal and spatial manner. My studies aim to uncover the genes and the regulatory switches that play a key role in skeletal development, and they can be prime candidates to screen in patients with skeletal disorders. Understanding the genetic basis of skeletal development and disease can help us identify novel mechanisms to strengthen bones and may uncover new targets for therapeutic intervention in skeletal disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of 5 years, I expect to use 50,000 threespine stickleback embryos and larvae, 10,000 stickleback adults, and 12,500 mouse adults.
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?	Threespine sticklebacks with diverse skeletal morphologies will be used for genetic mapping studies to identify genes and gene regulatory switches that control skeletal development. The stickleback embryos and larvae will then be used in experiments to investigate the function of the identified genetic factors. Mice will be used to investigate the function of surprising sequence changes in the human lineage compared to chimpanzee that may explain the evolution of human-specific skeletal traits. We do not anticipate adverse effects, and all procedures will have mild level of severity. The end point of all animals will be

	humane euthanasia by an approved Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are examining the development of individual bones at specific anatomic locations. The different bones that form the skeleton are specified at different stages during development, and bones continue to grow and develop until adulthood. The traits being studied in these experiments are whole animal characteristics. There are no possible alternatives to using live animal model, because we cannot recreate skeletal system development *in vitro*, and we do not yet know enough about skeletal development to use computer models. Our findings will help the research community to obtain a comprehensive list of all the genetic factors that regulate skeletal development. This will allow us to move away from live animal models in the future and use computer and 3D tissue culture models to perturb and experiment with the system for the purposes of developing novel therapeutics to treat skeletal disease.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Successful genetic mapping of traits to causative genes and sequence changes depends on the analysis of sufficient number of animals. To minimise the number of animals, we always first do a pilot study to identify the rough location of the trait locus, and we then gradually add more animals until we have a candidate interval with reasonably small number of genes whose function can be individually examined. In the follow up studies on the candidate genes, all our experiments are done using defined animals with homogeneous genetic backgrounds (inbred) that are tested under identical environmental conditions. This helps reduce the variability of results within the experimental and control groups and decreases the number of animals needed for detecting statistically significant differences in bone size. We will work with statisticians at our Integrative Biology section and at the Statistics section of our host institute

	(Imperial College London) to help us predict the minimum number of animals we will need to use to detect statistically significant results in our assays.
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 using the simplest possible vertebrate, a small fish, to do the genetic crosses for mapping skeletal traits. There are many stickleback populations around the world that differ dramatically in the shape and size of their bones, making them one of the only systems available for detailed genetic analysis of new skeletal traits that have been selected in natural populations of vertebrates. We are also using the simplest possible mammal, the mouse, whose skeletal development processes resemble human, husbandry is well established, and has all the necessary genetic and experimental tools available to study the developmental pathways that may have changed during the course of human evolution. The strength of the mouse model is the relative ease with which we can manipulate its genome to recreate the human and chimpanzee alleles and study the function of the changes that have occurred in the human lineage. The particular bones that have changed in the human lineage have homologs in the mouse. Mice are also known to be a good model of human skeletal disease. No adverse effects are expected on the animals, only procedures with “mild” severity levels will be used, and animal health will be monitored daily. Animals showing abnormal behaviour will be humanely euthanized using an approved Schedule 1 method.

<table>
<tr><td>Project 14</td><td colspan="3">Evolution of locomotion in amphibians</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Biomechanics, locomotion, amphibians</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">1. To discover at what point in frog evolutionary history frogs gained the ability to perform many (as opposed to few) locomotor behaviours.
2. To determine whether the evolved versatility of frog limbs (i.e. the ability for multiple behaviours) is due to the evolution of unique muscle properties (high speed & strength) and/or the evolution of unique bone shape.

3. To generalize our understanding of frog limb versatility towards discovery of ‘design’ principles that enable animals and humans to perform many different tasks with their limbs. Then to propose how to apply these principles to the design of improved human prosthetics.</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</td><td colspan="3">The successful completion of this project will shed light on how animals control the motion of their limbs to perform varied tasks. Specifically, we are likely to discover how the internal properties of the muscles and bones work together with the brain to help control limb motion. These principles will be</td></tr>
</table>

project)?	directly useful to biomedical engineers because the properties we discover in animals can be built into the design of prosthetic limbs to enable human victims of amputation to regain the ability to perform multiple tasks more efficiently.
What species and approximate numbers of animals do you expect to use over what period of time?	**Frog (F)** and **Salamander (S)**: *Salamandra salamandra OR Ambystoma tigrinum* (S) *Xenopus laevis* (F) *Kassina maculata* OR *Kassina senegalensis* (F) *Epidalea calamita* (F) *Pelophylax ridibundus* (F) *Trachycephalus resinifictrix*(F) *Hyla cinerea* (F) The maximum number of individuals required for each species is 50 per workflow over 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 level of severity? What will happen to the animals at the end?	Roughly 1 in 5 animals will experience mild discomfort such as mild exercise fatigue or stress from being introduced into a new laboratory environment. Additionally, animals may experience moderate discomfort following surgical procedures in which case they will be administered drugs to alleviate the pain. After the experiments conclude, animals will be euthanized by humane methods. The remaining animal cadavers can later be beneficial because they will be used to develop and further refine experimental procedures to minimize animal suffering for future experiments.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The central aim of this project is to understand the evolution of locomotor animals. The biological processes such as nerve impulses, muscle flexion and limb movement are extremely complex. Thus, they are only poorly understood compared to other branches of biological and biomedical study.

	Consequently, there is currently insufficient data available to draw conclusions in the absence of further data recorded from living animals. To greatly reduce the number of animals required, this project employs extensive use of computer simulations and robotic models to further our understanding. However, a relatively small number of animal experiments must be performed in order to confirm the accuracy of the computer and robotic models.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals required will be reduced with the aid of accurate computer simulations that will eliminate unnecessary experimental tests. Additionally, we will use sophisticated experimental design and statistical methods which have been previously developed by mathematicians to obtain similar results with the fewest number of animal subjects.
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 species were chosen on the basis of their ease of care in captivity and for the great wealth of information available regarding their welfare. Specifically, we will use this information to provide each animal with the optimal living conditions (light, moisture, temperature) and terrain. For example, some species will require water with artificial plants whereas others prefer loose material for making a burrow. Additionally, each of the species selected (or their close relatives) have been shown to behave naturally in experimental settings, suggesting that these species are robust and suffer relatively minor adverse effects due to being held in experimental settings.

Project 15	**Gene Therapy for Neuromuscular and Cardiovascular Disease**	
Key Words (max. 5 words)	Gene therapy, muscle, heart, disease	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall objective of this project is to test pre-clinical gene therapy protocols for cardiovascular disease and inherited muscle-wasting diseases prior to clinical trials.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will study different gene delivery systems for likely to derive from this use in gene therapies for neuromuscular and project (how science could be cardiovascular diseases to restore or compensate for advanced or humans or damaged gene function. This is important because, as animals could benefit from the yet, there are no restorative therapies for disabling and lethal conditions like Duchenne muscular dystrophy projec1. (DMD), which affects 1:3500 boys, and there are no non-invasive ways of reversing severe coronary heart disease (CHD) which causes 82,000 deaths/year in the UK. The major potential benefits will be the development of (i) efficient gene delivery systems applicable to a number of lethal diseases, (ii) gene replacement and repair strategies for DMD, (iii) non-surgical treatments for coronary heart diseases. We believe that this project will progress into clinical	

	trials and lead to the development of therapies for DMD and cardiovascular diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 4,500 mice will be used over the course of this 5-year programme of work.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Animals will only be used in our experiments if they are in excellent health. These will be housed in conditions of very high supervision and welfare. The mice under study here will undergo treatment regimes to correct genetic defects they carry, and occasional surgical procedures. Regular checks will ensure that any rarely expected adverse effects are rapidly discovered and managed. Effective pain relief and anaesthetics will be used rigorously to minimise the severity of the procedures to moderate. The animals will be used under Schedule 1.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The primary purpose of the research programme is to develop human gene therapies for muscular dystrophy, muscular atrophy and cardiovascular disease. Towards this goal a range of gene therapy reagents will be designed and routes of administration evaluated for effectiveness and safety. Extensive development studies will be performed in vitro on cells. Having examined the appropriate websites There is as yet no alternative available for evaluating physiological responses to gene therapies in the context of cardiovascular and neuromuscular diseases (www.frameuk.demon.co.uk, www.nc3rs.org.uk). These animal experiments are essential steps in translating new gene therapies into human clinical trials. We have considered the use of animals ethically, but as we are not aware of an alternative way of direct gene expression in vitro that would enable this proposed study to be carried out successfully and in a practical manner, we believe their use to be justified in this instance.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before embarking on regulated procedures, all gene therapy reagents will be extensively analysed and developed in vitro in cells to ensure that only the most effective test therapies are taken forward to animal experimentation. Group sizes will be predicted from prior knowledge of the scale and variability of measured parameters to ensure adequate statistical

	power. Expert advice on experimental design, group sizes, statistical power calculations and relevant statistical tests has been sought.
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 primary purpose of the research programme is to develop human gene therapies for muscular dystrophy, muscular atrophy and cardiovascular disease. A range of animal models exists for some of these conditions (mouse, rat, rabbit and dog), however, mice are a widely accepted logistically favoured experimental model for pre-clinical and therapeutic studies. The following clinically-relevant mouse models of human disease will be used in these studies: MDX Mouse: The mdx mouse is a naturally occurring animal model of human DMD. This mouse model is the least severe, most cost-effective and most extensively studied mammalian model of DMD. Mdx mice exhibit no profound behavioural phenotypes, but do exhibit biochemical, histological, mild activity, and muscle electrophysiological changes. The *mdx* mouse serves as a very appropriate animal model for testing a range of gene transfer strategies. OPMD Mouse: Oculopharyngeal muscular disease is caused by a mutation a protein involved in RNA metabolism. The OPMD mouse is a model of this disease. Up to the age of 6 months, OPMD mice exhibit no profound behavioural phenotype, but do exhibit some muscle atrophy, reduced body weight and biochemical changes. Beyond 6 months of age the OPMD mouse exhibits progressive muscle atrophy and weakness leading to severe motor dysfunction by 12 months. This is the only animal model available of OPMD. ApoE-/- Mouse: The ApoE-/- mouse lacks apolipoprotein-E and hence has elevated serum low density lipoprotein (LDL) and cholesterol levels which leads to cholesterol-rich deposits and atherosclerosis. However, the ApoE-/- mouse does not exhibit any obvious pathophysiology and reduced life-span and provides an excellent model for studying gene therapies for CVD. Myostatin (MSTN)-/- **mouse:** The MSTN-/- mouse is a null GAA which expresses no myostatin hormone from skeletal muscle, leading to increased muscle bulk and decreased fat deposition. However, the

	MSTN-/- mouse does not exhibit any obvious pathophysiology and reduced life-span and provides an excellent model for studying regulation of muscle growth. The measures we will take to minimise welfare costs to the animals include implementation of NC3Rs ensuring numbers used are minimized by careful project design and the procedures are carefully refined to ensure severity is as mild as possible. Animals will only be used in our experiments if they are in excellent health. They will be housed in conditions of very high supervision and welfare. They will be supervised by skilled and caring technicians on a daily basis and supervised regularly by an independent veterinary surgeon. The animals will be housed communally and in enriched environments to maximise social / welfare / rehabilitation considerations.

<table>
<tr><td>Project 16</td><td colspan="3">Understanding Neuromechanical Systems Biology</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Locomotion, Motor neuron, mouse, optogenetics, proprioception</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>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 colspan="3">The overall aim of the project is to build a greater level understanding of the role and activity of all of the components of locomotion: the brain, spinal cord, musculoskeletal system, and external world. For this we need to integrate existing results with a new class of results from precisely perturbed, intact, freely behaving animals in order to build better predictive computer models.</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 colspan="3">This project will yield a greater knowledge of the control of locomotion which will in turn allow a better understanding of neuromuscular disease, better design of artificial limbs and bio-inspired robots.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect</td><td colspan="3">All work will be done in cell culture or in mice. Many of the assays in mice will be carried out</td></tr>
</table>

to use over what period of time?	under terminal anaesthesia or post-mortem. We expect to use less than 900 mice over the 5 project period including mice used for post-mortem and non-recovery studies.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Breeding of transgenic mice is not expected to cause any adverse effects. Some of the experiments with transgenic mice will be carried out under anaesthesia without recovery and so are non-recovery. The minority of experiments, up to 80 mice will involve surgery with recovery which will be up to moderate severity. At the end of all experiments the mice will be killed by a Schedule 1 method and the tissues examined post-mortem.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	While we can model locomotion based on previous studies, this does not allow accurate modelling of the interaction of signals from the nervous system. To study these we need to use conscious whole animals. The data from these experiments will be used to refine the model of locomotion allowing more accurate computer simulations in the future.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will only breed as many transgenic mice as are required for the experiments and colony maintenance. We will use the minimum number required to optimise the implants and to compare the effects of optogenetic and electrical stimulation of nerves. Finally we will use the minimum number for the treadmill based experiments. While it is difficult to be precise at this stage we expect to use only 8 mice per experiment using the treadmill and we anticipate being able to run a number of experiments with the same mice.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having	All experiments will be conducted in mice as this species has been the most widely used for genetic manipulation. The use of optogenetics to temporarily block parts of the nervous system is a refinement on previous experiments

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	that required cutting of the nerves. All mice undergoing any surgery will be routinely given pain relief post-surgery with additional doses as required with the assumption that responses will be similar to man unless otherwise shown.

<table>
<tr><td>Project 17</td><td>Genetic mechanisms of craniofacial malformation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>craniosynostosis, skull, mutation, development, mouse</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>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>“Craniofacial malformation” describes an abnormality of development of the face or skull. This has many serious consequences, affecting functions such as vision, breathing, hearing and eating. These malformations frequently also affect normal mental functions and cosmetic appearance.

The primary focus of this project is craniosynostosis, the premature fusion of one or more of the cranial sutures, narrow gaps between the bones of the face and skull that allow normal growth during fetal life and childhood. Fusion of a suture prevents further growth at right angles to the suture line, causing growth distortion. Craniosynostosis may be caused either by alterations in genes (mutations) that interfere with cranial suture function, or by environmental factors such as external pressure on the developing fetal skull. Treatment of craniosynostosis requires major</td></tr>
</table>

	surgery, involving the removal and repositioning of the skull bones. Advances in knowledge might in the future enable the prevention of craniosynostosis, either through better genetic counselling and predictive testing or through new medical treatments. To understand why craniosynostosis occurs, we need to know how the cranial sutures develop where they do; and how their continued function (maintenance of suture patency, and ongoing addition of bone at the suture margins) occurs during growth. These questions can only be studied in living tissues, and it is ethically impossible to study these processes in humans. The laboratory mouse is the species chosen to undertake this work, for reasons described in the section below on 3Rs. Based on this background, this project has two major objectives: First, to improve understanding of the timing and variety of mechanisms by which the sutures develop and remain patent, and how mutations of particular genes disturb these processes to cause craniosynostosis. Second, to use this information to develop new, scientifically rational treatments for craniosynostosis, by inhibiting the abnormal developmental processes that have caused it to occur. To achieve these aims we will study particular genetically modified strains of mice that reproducibly develop craniosynostosis. We will monitor how the skull develops in embryos and after birth, and study how this relates to changes in the expression of genes, proteins or cellular processes. Having established these baseline observations, we will then treat the mice with substances that might delay or prevent the onset of the craniosynostosis, and monitor these mice compared to an untreated group.

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)?	Based on this work we hope to obtain clues to identify new disease-causing genes and improve the genetic counselling provided to families in which these conditions have occurred. The demonstration that treatment with a specific substance prevented the onset of craniosynostosis, a detailed description of the natural history and biological mechanism of this effect, and monitoring for any adverse side-effects, would be essential before exploring the use of such treatments in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 4348 mice over five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The genetically modified mice with craniosynostosis that we study are usually able to feed, drink and reproduce normally. In some strains, overgrowth of incisors sometimes occurs; this can be treated by trimming the teeth. All animals are monitored and any showing significant distress or weight loss would be humanely killed by a Schedule 1 method. In mice requiring treatment with a substance, administration in drinking water will be preferred, but in some cases gavage or injection might be necessary. Treatments with novel substances might have unanticipated side-effects, therefore mice undergoing such treatments will be monitored daily and any mouse exhibiting moderate distress will be killed by a Schedule 1 method. In cases where genetically modified strains of particular interest are not already available, it may be necessary to generate these as part of the project. This involves standardised protocols (superovulation and oviduct or uterine transfer) of no more than moderate severity. All mice will be killed by a Schedule 1 method once their experimental or reproductive purpose has been completed.
Application of the 3Rs	
1. Replacement State why you need to use	The cranial sutures are complex 3-dimensional structures containing multiple cell types. These structures need to stay open for several weeks for

animals and why you cannot use non-animal alternatives	bone growth to occur. At present there is no artificial system able to recapitulate these complex developmental processes.
2. Reduction Explain how you will assure the use of minimum numbers of animals	When analysing new mutant strains, wherever possible we will make use of animals available from repositories. In a minority of cases (e.g. twice in the previous 10 years) we may need to derive the mutant strain as part of this Project. Use of best practice at our institution will ensure that the minimum number of mice are used to derive the required genetically modified strain. Useful strains will be preserved so that they do not need to be made independently by other researchers in the future. For experiments in which we wish to compare the effects of a treatment with a control group, we will use 8-10 mice in each group. This is a minimum number that will give an effect that is statistically significant, where difference in effect of the treatment between the two groups is relatively large.
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.	Amongst model organisms (including chicks, amphibians, and fish), only mammals have a pattern of cranial sutures similar to these structures in humans. Amongst mammals, the mouse is the most suitable for intensive study because of its small size, short reproductive cycle, and the availability of well-developed genetic technologies to manipulate the mouse genome, and associated resources of genetic strains. The models chosen are those that recapitulate the phenotype of the equivalent human disorder (for example, craniosynostosis) most reproducibly. Experimental methods chosen are widely used and yield the most precise data. We have ten years of experience in the husbandry of mice with disorders of skull development. The health and nutrition of these mice will be monitored regularly with a simple scoring system, with particular attention to the occurrence of incisor

	overgrowth (see section on adverse effects). A soft diet will be provided. During this licence we will explore the use of additional refinements including the use of a spinning disk for teeth trimming and non-surgical embryo transfer device for embryo rederivation. This will be guided by best-practice experience within our animal unit. Where it is necessary to give treatments by gavage or injection, the minimum volumes will be used and the route chosen to minimise distress to the animal. These procedures will be undertaken by competent staff using best practice aseptic techniques. No Protocols involve substantial severity.