Home Office

NON-TECHNICAL SUMMARY

Identification of drugs and drug targets with the capacity to modify skeletal homeostasis

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Bone, Cartilage, Joints, Homeostasis, Therapeutics

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult
Rats	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

We aim to advance our basic knowledge of skeletal system remodelling, repair and regeneration in order to translate understanding of mechanisms to new therapeutics for disease.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Failure of the skeletal system in the two most prevalent conditions, namely osteoporosis and osteoarthritis, produces massive socioeconomic and healthcare cost and their incidence is rising due to the ageing population. Paradoxically, inherited/genetic musculoskeletal diseases are also becoming more important in companion animals. With a view to finding new ways to restore function and lifelong health, our work aims to unravel how skeletal tissues achieve, retain and lose function. We will also assess therapeutics with the potential to reverse or halt disease processes.

What outputs do you think you will see at the end of this project?

New therapeutic entities that can modulate disease processes for clinical translation

An understanding of disease processes and tissue homeostasis

Publications relating to the identification of drugs and drug targets that control skeletal homeostasis

Presentations at national and international conferences

We aim to find approximately 10 targets, trial 5 drugs and predict most would show efficacy

Who or what will benefit from these outputs, and how?

Through this program of work, we expect to identify novel drugs and drug targets that can modulate human and animal skeletal homeostasis and disease. The investigation of rodent models of disease is expected to improve our understanding of the molecular and cellular processes that govern tissue homeostasis. This will give rise to unique disease intervention points that can be targeted with

biological and chemical entities. It is also expected that this study will inform us on novel clinical markers (biomarkers) that are representative of disease state, progression and response to treatment. These markers will be translated to clinical studies investigating novel therapeutics in skeletal disease. These results may be used to support clinical trials as well as to add to our understanding of disease pathogenesis. This will have benefit to both human and animal patients. Reagents/models/techniques developed in this project are likely to benefit other researchers working in the field.

How will you look to maximise the outputs of this work?

We will ensure dissemination of our work through collaboration with other groups working in the same and distinct areas of research, and through presentation at national and international meetings. We will endeavour to publish all data from the project. The PPL holder is a trustee on the Rare Bone Disease Foundation and Bone Research Society which will aid dissemination to non-scientists.

Species and numbers of animals expected to be used

- Mice: 5000
- Rats: 300

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The rodent (mouse and rat) animal model has been proposed herein to study skeletal homeostasis due to a number of reasons. Firstly, the relatively short lifespan of rodents allows the study of age-related disease over a period of time that is compatible with a research project such as this. Furthermore, genetic modification along with immunocompromised animals have allowed functionality of specific substances in the generation of the skeleton to be described. The use of rodents for the investigation of skeletal disease has also proved a successful strategy, further de-risking the choice of the proposed models. Indeed, rodents exhibits age-related degenerative skeletal diseases such as osteoporosis and osteoarthritis which are equivalent to that seen in the human population. We will use juvenile and adult rodents to ensure we have the capacity to assess both childhood and adult disease.

Typically, what will be done to an animal used in your project?

Rodents might receive substances (biologic e.g. protein or cells/tissue; chemical e.g. small molecule drug; or a tracer e.g. chemical that attaches to tissue or cells) with or without a surgical or mechanical intervention to modify tissue homeostasis. Some animals may be analysed for gait changes or hearing modification (not regulated procedures).

What are the expected impacts and/or adverse effects for the animals during your project?

The rodent models proposed have no/few clinical signs associated. Furthermore, it is very unlikely that rodents will reach a disease stage where humane endpoints are necessary. Examples of potential adverse effects include transient pain from ovariectomy in protocol 2 (up to 24hrs) and transient mild lameness from loading in protocol 2 (up to 48 hours) and joint surgery in protocol 3 (for approximately 7 days). Adverse effects associated with the administration of substances are likely to only be transient. Foxn1nu rats display an altered immune status, this is necessary to allow xenotransplantation of cells. The $Sost^{-/-}$ mouse may display pathological changes related to sclerosteosis disease, but are still largely unknown. Mice may suffer deafness, however this is currently under investigation. The STR/Ort mouse displays pathological changes related to osteoarthritis, which are observed by imaging (CT and histology). However, these do not result in any welfare concerns and no visual signs are present.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Mild (30%)

Moderate (70%)

What will happen to animals used in this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The processes involved in modifying skeletal architecture and mass and those involved in the degeneration of the joint that can only realistically be replicated in vivo due to the interaction of multiple cell types and the immune system.

Which non-animal alternatives did you consider for use in this project?

In vitro organ culture systems appear capable of at least partly replicating some of the events whereby these mechanical stimuli are applied and may therefore be useful in examining the immediate and short-term responses to such application, currently in only individual cell types. Individual cell types can be exposed to substances in vitro but these are unlikely currently to replicate the effects of such exposure in vivo due to effector cells also being mechanosensitive or in constant communication with neighbouring cells to control their biology. Attempts to bridge this in vitro divide are actively being explored, through organ-on-a-chip type approaches and the 3Rs Crack-it agenda but these are still in

their early developmental stages. Monolayer cell culture can sometimes be used to replicate selected aspects of both of these types of responses. 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. Examples include the exploration of the behaviour and response of primary osteoblasts and osteoclasts, osteocyte cell lines and organ cultures of embryonic metatarsals (mostly requiring derivation from animal limbs ex vivo). We have fully acknowledged their strengths, reviewed their use for others, but are aware and appreciate their limitations.

The following databases were searched for alternatives:

The John Hopkins Centre for Alternatives to Animal Testing (http://altweb.jhsph.edu)

Animal Welfare Information Centre (https://www.nal.usda.gov/programs/awic)

European Centre for Validation of Alternative Methods (http://ecvam.jrc.it/index.htm)

Fund for the Replacement of Animals in Medical Experiments, FRAME (http://www.frame.org.uk/)

NC3Rs (https://www.nc3rs.org.uk/3rs-resources/search?topic%5B0%5D=504)

Why were they not suitable?

In vitro cell and organ culture systems cannot replicate the discrete interaction of cells and tissues to elicit changes in skeletal architecture and mass, or the integrated response of the joint as an organ towards degeneration. These in vitro approaches also fail to produce the range of structural abnormalities in skeletal tissues that can be seen, sometime after, in response to abnormal homeostasis. Monolayer culture can fall short of providing the integrated, organ-level, physiologically intact environment in which such responses are normally coordinated.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

Numbers of rodents will be used to generate a robust, statistically significant result that is of biological/disease importance. However, we will also ensure that we do not use any more animals than are necessary to demonstrate this effect. These numbers can be determined, by prior understanding of the effect that is being assessed and the variation within the animal population and experimental intervention being examined.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

We review previously published research to gain insights into the range of variation within the intended study model and the significance of the effect size we aim to achieve. This effect size must hold biological or disease relevance for us to proceed. The NC3Rs Experimental Design Assistant will provide algorithm-generated feedback on potential adjustments, such as identifying sources of bias or nuisance variables, and visually representing experimental designs for group or external discussion when appropriate. We leverage additional online resources, such as GLIMMPSE, to ensure optimal sample sizes for maximizing the likelihood of a positive outcome, particularly in studies involving repeated measurements, thereby enhancing the statistical power of our comparisons. Whenever feasible, we utilize stored tissues from prior studies to minimise the need for animal use.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will only breed animals for specific experiments or to maintain a colony. In novel experiments, we will conduct pilot studies, where appropriate, to help inform our future experimental design. Wherever possible, surplus tissue from animals will be shared with other researchers.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We have chosen to focus primarily on rodents. This decision has been made as it will provide us with the potential to explore the role of specific drugs and drug targets in the skeletal response along with the use of mutant and transgenic mouse models for further investigation. Mice are well respected as the animal model with the lowest neurophysiological sensitivity for the study of skeletal development that is equivalent to humans, which have been shown to be relevant for the study of drug targets (including RANKL and Sclerostin) for the development of osteoporosis therapeutics.

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 endpoints 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 use of tailored levels of analgesia. We have also made more routine, the monitoring of changes in animal gait through the use of short-term video recordings for locomotor analysis. These allow us to determine the relationship, for instance, between the emergence of gait asymmetries and the onset and advance of osteoarthritis.

For administration of substances we take great care to consider fully the routes of administration to limit the number of occasions on which an animal is handled and dosed. A prior example of our refinement in dosing strategy is the switching from oral gavage to supplementation of the drinking water for a specific compound. This refinement was made possible by our keeping abreast of the most recent advances in the field and by seeking out collaboration from the manufacturer, the industry partners and others academic researchers using the same compound. We will continue to seek out the most up to date information that will facilitate similar refinement in our approach.

Why can’t you use animals that are less sentient?

Rodent models allow us to examine responses in skeletal homeostasis, which are crucial when evaluating treatments and disease mechanisms in humans or other mammals. Mammalian models are needed to accurately assess the effects and efficacy of treatments. We may administer treatments at a juvenile stage, so efficacy can be assessed towards childhood disease.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

Rodents will be closely monitored for signs of suffering or welfare problems through regular observational assessment and in-study weighing, however, we do not anticipate specific harms as the models we are studying do not elicit chronic pain symptoms or are associated with welfare issues. Any animal showing signs of an adverse effect will be assessed daily using our study specific score sheets, which has humane endpoints defined. We will not perform procedures that are associated with expected pain, other than transient discomfort or that which can be easily controlled with post-surgical analgesia.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow guidance generated by the NC3Rs in terms of husbandry.

We follow LASA guidelines (wherever appropriate) for administration routes and blood sampling protocols.

Work conducted within the BSU, and supported by local staff, is performed with attention to the Culture of Care, promoted by the PREPARE guidelines, which is fully supported by this research team.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will conduct regular 3Rs assessments for our work and utilise online tools and information and advice from the NC3Rs including their Resource Library and Resource topics. We will communicate with our NC3RS liaison officer and institute changes whenever we can to improve animal welfare, reduce numbers of animals required and refine the methods. Additionally, the licence holder sits on the scientific advisory board of FRAME and as such is constantly kept up to date with advances in the 3Rs.