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NON-TECHNICAL SUMMARY

Cell therapy for muscular dystrophy with genetically corrected cells

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Muscular dystrophies, Stem cells, Ex vivo gene therapy, Muscle development and regeneration

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant, aged

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?

This project aims to develop a new therapy for muscular dystrophies, genetic diseases affecting muscles which lead patients to progressive paralysis and premature death.

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?

Genetic muscle diseases that appear in children affect a significant fraction of the population and essentially are treated with palliative, symptomatic therapies. No experimental therapy has shown to be clinically effective.

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

I expect to see scientific publications, at least one patent and a novel protocol to be tested in the clinics.

In addition, I will keep constant contact with patients' communities to update them on the progress of the work.

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

Humans and animals affected by muscular dystrophy will eventually benefit from these new protocols based on the results of this work.

Academic colleagues and collaborators will also benefit from the output of this work

By participating in the work to be carried out under this licence, students may benefit of the acquired knowledge on the disease and on possible therapies.

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

The applicant has been working for several decades in the field and has developed a vast network of world-wide collaborators, who will contribute and advise within their specific competence in various aspects of the work.

Communication to scientific meetings and importantly with patients' advocacy group meetings will help to disseminate the output of the work as soon as we obtain robust and reproducible results. Publication of negative results will be considered when it may help others to avoid repeating unsuccessful experiments.

Finally, we aim at publications on scientific journals and dissemination to a lay audience with media engagement when enough evidence has been accumulated.

Species and numbers of animals expected to be used

- Mice: 4,000

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.

We use mice because these animals represent a simple model of muscular dystrophy, which is milder than in patients; therefore the animals experience no suffering and only limping in the hind limbs around one year of age. Mouse and human anatomy and physiology are similar.

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

We will administer cells to dystrophic mice by intra-muscular injection. We may also administer drugs (e.g. anti-inflammatory drugs) alone or in combination with cells. Some drug (e.g. Tamoxifen) may be administered with an injection in the peritoneum.

In some case cells may be injected subcutaneously to test whether they may form tumours, in which case the animal would be humanely culled as soon as any tumour formation is detected. We will perform this protocol on 3 animals per subset and 4-5 subsets per year.

In some experiments biomaterials may be combined with cells and transplanted under the skin of the animals to test the formation in vivo of a novel bio-engineered muscle.

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

The procedures to be used have been carried out for many years and usually do not cause major adverse effects. For multiple injections or subcutaneous implantation animals are anesthetised and treated with analgesic drugs. Tumours are not expected, and we do not observe severe adverse events such as pronounced limping or infection of the wound.

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)?

All procedures described above are associated with moderate severity.

What will happen to animals at the end of 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?

To develop a new therapy, it is necessary to design an experimental strategy that must be tested on healthy and diseased cells in culture, having clear and reliable endpoints that may inform of the toxicity and efficacy of a given treatment.

As it is not possible to experiment directly on humans and cell culture of muscle cells can only provide preliminary information, it is necessary to use the whole animal.

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

We considered cell cultures, used for decades as first step before moving to animal work . Many groups, including our own, have tried to develop organ cultures of skeletal muscles, that could faithfully mimic a normal, functional muscle, but so far these provide inadequate information.

Why were they not suitable?

Unfortunately, these artificial muscles reproduce only some aspects of muscle anatomy and physiology and, most importantly, only last few days in culture, thus preventing the study of long term effects.

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?

We have two main projects: Firstly, developing and tests new, more effective, protocols of ex vivo gene therapy in different mouse models of muscle diseases. The animals are studied with appropriate controls and the numbers determined by statistical advice to obtain meaningful information

The second project focuses on the origin of the development of the muscle cell in these diseases and requires fewer animals.

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

Previous experiences indicate that 5 animals is the minimum required to produce robust and reproducible information.

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

Different animals will be used in pilot studies to determine the appropriate strain and genetic makeup, to give us the most meaningful results. We will consult with experienced statisticians.

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.

Mice are the most appropriate animal models for this project.

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

Mice are the most commonly used laboratory animal for their similarity to human anatomy and, to a lesser extent, physiology and their small size. The zebra fish model of muscular dystrophy is only useful for drug and genetic screening but is inappropriate for these studies.

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

We routinely administer anaesthesia and post-operative analgesia after any surgery or any potentially distressing interventions (such as multiple intra-muscular injection). In the many years that the anaesthesia/analgesia protocols have been in use, we have made refinements to the post-operative monitoring plan, including use of warming mats, more frequent observation until recovery, improved

awareness of welfare indicators and communication between the responsible researchers and facility personnel.

The procedures of cell implantation will only cause only a minor discomfort and may be alleviated by the use of analgesic drugs. When multiple muscles are injected simultaneously, the animal will be anesthetised. In some experiments, artificial muscles will be implanted subcutaneously, by creating a pocket in the skin, that will be sewed after insertion of the implant. In this case, general anaesthesia will be followed by analgesic drug administration

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

We will follow guidance from BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinements and LASA guiding principles.

As well as continually consulting with experienced colleagues, there are several constantly updated publications on the web that can be easily accessed in case of doubts and to remain updated of the best practice to follow. For example,:

Guidelines for planning and conducting high-quality research and testing on animals (https://labanimres.biomedcentral.com/articles/10.1186/s42826-020-00054-0)

The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research (https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000410).

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

The nature of the planned experiments does not pose major concerns on animal welfare. Nevertheless, we will consult regularly with the personnel with experience of the NC3Rs and in consultation with the scientific community as well as NVS and NIO. Any advances in the 3Rs procedures that can be utilised will be promptly implemented in our project.

We are also signed up to NC3R newsletters and updates, which we use to review our protocols and projects.