Home Office

NON-TECHNICAL SUMMARY

Safety, efficacy and immunogenicity of regenerative cellular therapies

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

Key words

Regenerative medicine, Stem cells, Cellular therapy, Immune response, Tissue engineering

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

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?

The overall aim of this project is to study the biological behaviour of regenerative cellular therapies when transplanted into rodent models. The purpose is to confirm that the cellular therapies survive after transplantation, are safe, function, and to understand how they are recognised and affected by the immune system. Our priority is to work on development of cellular therapies for liver disease, diabetes, brain disorders (such as Parkinson's Disease), bowel disorders and blood disorders.

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?

Many patients with end-stage organ failure die of their disease or need organ transplantation to survive. In cases where treatment is available, such as insulin therapy for diabetes or dialysis for renal failure, current treatments do not cure the disease and the quality and duration of the patients' lives are severely affected. Cellular therapies, such as those derived from stem cells, represent a promising opportunity to develop methods to repair or replace diseased organs and tissues. The proposed work is essential to ensure that cellular therapies under development for human patients are safe and effective.

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

The proposed project is expected to lead to new discoveries about the function of regenerative cellular therapies, and how they are recognised and rejected by the immune system. More specifically, we expect to generate at least one cellular therapy and advance it to a stage that it can be investigated further in large animal models. In the long-term (5-7 years), we expect that the findings of this study will result in the design of at least 1 human clinical trial to test the safety and efficacy of a cellular therapy developed in this project. The findings of the studies will be disseminated widely through publications and presentations.

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

In the short-term (1-3 years), the primary beneficiaries of the proposed project will be other researchers who are also developing cellular therapies for treatment of medical conditions. We anticipate that the findings of this study will be of broad relevance to the research community in this field. In the medium term (3-5 years), we anticipate that industrial companies involved in the manufacture of cellular therapies will also benefit from the findings of this study. These companies and manufacturers are essential for the production of cellular therapies that are eventually used for treatment of patients. In the long-term (5-7 years), this project will benefit patients with conditions such as diabetes, liver disease, bowl disease, blood disorders or Parkinson's disease. The benefit will initially be limited to those patients enrolled in clinical trials investigating the cellular therapies developed in this project. We hope that ultimately (7-10 years) large numbers of patients will benefit from the findings of this study, after the

cellular therapies have been shown to be safe and effective in clinical trials and can be manufactured at large scale.

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

We will disseminate all findings of our studies, including unsuccessful approaches, through publication in peer-reviewed journals, presentation at scientific conferences, and through meetings with other researchers. All publications will be open access, including through platforms such as F1000Research. This project includes collaborations with a large number of researchers with expertise in complementary areas. This network will be utilised to maximise the dissemination of the new knowledge gained through this project.

Species and numbers of animals expected to be used

- Mice: 4675
- Rats: 200

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.

Mainly mice will be used in the proposed project. A small number of rats which may be used if the technical aspects of the surgery mean that it cannot be performed safely and effectively on mice due to their small size. It is anticipated that less than 5% of the animals used will be rats and that more than 90% of the mice used will be adults. Mice are the lowest species with a comparable physiology that enables useful information to be gained relating to the safety and effectiveness of human cellular therapies. Importantly, genetic strains of mice are available that allow studies to be designed that can generate valuable information about specific cellular therapies. In a small number of experiments (estimated at less than 5% of total animals used), new-born mice must be used in order to allow for studies investigating how the immune system rejects human cellular therapies. The new-born mice will be approximately 4 days old. Due to their young age, these mice will develop a more functional immune system, which will enable more informative data to be generated in a limited number of specific experiments.

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

In a typical experiment, the animal will undergo one surgical procedure during which it will be transplanted with a cellular therapy (for example, into the abdomen). The typical animal will then be kept alive for 4-12 weeks before being culled, so that the tissues can be harvested and examined. The animal may undergo blood sampling typically 2-4 times per month during this period. Approximately 50% of the animals will also receive an injection to give them human immune cells (such as white blood cells) to study rejection of cellular therapies. Approximately 25% of the animals may also undergo

a procedure such an injection of toxin or oral dosing of a drug into the stomach via a tube. The purpose here will be to induce a disease, such as liver failure or diabetes, to test the effectiveness of the cellular therapies. Approximately 10% of animals may undergo injection of a toxin or drug into the brain, followed by behavioural testing. The typical animal will undergo one surgical procedures and be kept for approximately 12 weeks. The typical animal will then be culled electively while still well and without clinical signs. In some cases, the animals will be culled by removal of organs while under deep general anaesthetic. Newborn mice will only receive one injection of cells to administer immune cells. All all procedures on these mice will be performed later when they are adults.

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

It is expected that most (more than 90%) of animals will recover rapidly and well from stem cell transplantation.

Injections with immune cells (such as white blood cells) is also generally well tolerated. Most (more than 90%) animals will not experience adverse effects from this procedure.

Animals that undergo a procedure to induce a disease, such as diabetes or liver disease, would be expected to display the effects of that disease (such as abnormal glucose levels in diabetes or abnormal liver function in liver disease). In most cases, this would only manifest in the form of abnormal blood results (for example, high glucose levels or abnormal liver tests) and the animals will not display any clinical signs. Injection of toxins into the brain followed by drug testing may results in transient abnormal behaviour such as circling. Circling behaviour is when the animal walks in a circle after administration of the drug. This behaviour is expected to last no more than a few minutes. The circling behaviour ends when the effects of the drug wear off and is not lead to lasting harm.

In some cases, the animals may experience weight loss, reduced food intake, reduce movement or an abnormal coat. In such cases, the animals will be culled if these clinical signs do not respond to treatment (such as high energy and easily digestible diet) and persist for up to 24 hours.

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 animals that undergo a surgical procedure will be expected to experience moderate severity. This is expected to be the case for the majority (65%) of experimental animals. A small proportion of animals (25%) will only undergo injections and will experience mild severity. The remainder (10%) may be culled for tissue and will experience subthreshold severity.

What will happen to animals at the end of this project?

- Killed
- Used in other projects

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 aim of the project is to study the safety and function of cellular therapies and how they are recognised by the immune system. Robust and reliable investigation of these characteristics of the cellular therapies requires the use of animal models that can mimic the biology and diseases encountered in humans. We routinely use extensive laboratory experiments to characterise the cellular therapies without the use of animals. However, complex responses such as their ability to reverse clinical signs of a disease, or rejection by the immune system, cannot be studied comprehensively without animal models. Importantly, data generated from animal models are essential for gaining regulatory permission for the ultimate use of these cellular therapies in human clinical trials.

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

The majority (more than 90%) of the cellular therapies used in this project will be derived from humans. Mouse cellular therapies may be used as controls in less than 10% of experiments. Importantly, this is itself an significant part of our Replacement strategy. Very few animals will be used to generate cellular therapies: human tissue will be used instead. Moreover, we have and will continue to make extensive use of specialised human cell culture systems, where human cells can be examined for their function in the laboratory. By growing cells in the presence of human immune cells, we are also generating important data about the therapeutic potential of, and the immune response to, our human cellular therapies. Similarly, we utilise human organs, supplied with human blood on specialised machines, as a site for transplantation of some cellular therapies. This approach further reduces the need for animal experimentation.

Why were they not suitable?

As outlined above, we make extensive use of human tissue and human cells, as well as sophisticated human cell culture systems and human organs examined on machines, to replace the use of animals in many studies. However, these alternatives cannot entirely replace the use of animals as they do not fully replicate the complete repertoire of the biology of the cellular therapies when administered to patients.

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?

The number of animals have been estimated based on the range of studies that are planned, as well as based on the previous similar studies we performed during the last five years. Based on our previous experience, we are able to predict, for each study, the number of animals that are required to generate reliable and reproducible data. Using our previous experience, we are also able to predict how many studies we can perform in a given time period.

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

We have generated a wealth of data from previous experiments that enable us to estimate the anticipated effect size and variation in the experimental data. This data will be used to ensure appropriate experimental group sizes. We routinely randomise animals to experimental and treatment groups. All experiments are conducted and/or data analysed in a blinded manner to reduce bias. When a new cellular therapy is under investigation, we will first perform pilot experiments with small animal groups (typically 2-3 animal per group). The pilot experiments will be used to confirm the appropriateness of the experimental design and to generate pilot data to enable group sizes to be formally calculated. We will also use tools such as the NC3Rs Experimental Design Assistant to ensure experiments are appropriately planned to generate reliable and reproducible data. We will also take into consideration any regulatory requirements relating to the reproducibility of the data, in order to ensure data generated from this study is suitable for informing design of future clinical trials.

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 continue to reduce the number of animals we use through a number of simultaneous strategies:

1) We will share excess breeding animals with other researchers and use any animals culled as tissue donors for use by our group or by other researchers.

2) Where possible, we will use each animal as its own control. For example, we will transplant two cellular therapies in two separate sites (for example, into each kidney) in the same animal in the same operation. This will reduce inter-animal variation and reduce the number of animals used.

3) By generating large quantities of human cells, where appropriate, we can continue to perform new studies using the same human cells. This reduces the variation associated with different human donors and reduce the number of animals used.

4) By monitoring animals for prolonged durations, and through the use of non-invasive monitoring techniques (such as imaging), we can generate longitudinal data about cellular therapies without the need to cull animals at numerous timepoints.

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.

The experimental models and techniques used in this project can be broadly divided into three groups:

1) Transplantation of cellular therapies into animals to assess the survival and safety of the cellular therapies

2) Reconstitution of the animals with a human immune system to assess immune response to the cellular therapies

3) Generation of a disease state in animals to assess ability (function) of cellular therapies in reversing disease

All experimental models have been refined to ensure they cause the least pain and suffering. The disease models have been designed so that biochemical abnormalities (e.g., high blood glucose levels or abnormal liver function tests) can be used as experimental endpoints in the vast majority (more than 95%) of experiments, without animals displaying harmful clinical signs. Importantly, none of the procedures are expected to result in severe clinical signs (such as persistent abnormal behaviour or persistent weight loss). Animals will be culled if they display clinical signs that do not respond to treatment (such as easily digestible food or pain relief medication). Animals therefore will not be permitted to experience lasting harm.

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

Mice are the least sentient animals that can be used to generate valuable data to investigate the therapeutic potential of human cellular therapies. As the response to, and the function of, cellular therapies takes hours to days to manifest, experiments cannot be performed exclusively under terminal anaesthesia. Rats will be used in a very small number of experiments (approximately 5%), because their larger size will enable some surgical procedures to be performed more successfully and without adverse effects.

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

In close collaboration with the staff at our animal facility, we have a robust mechanism for the post-operative monitoring of our experimental animals. Animals are also given routine post-operative pain relief medication, which has been proven to be effective in previous similar studies. When adverse effects may be expected, we readily increase the frequency of monitoring to identify animals that may be experiencing adverse effects. We also have our own dedicated animal technicians who ensure that our animals receive close attention if there are any concerns. We have achieved a number of significant refinements during the previous series of studies, including: enhanced environmental enrichment (such as extra card-board housing); use of high-energy or tasty diets to prevent weight loss; improved techniques for transplantation of cells in the kidney or abdomen (such as using special needles to

shorten the duration of the procedure) and administration of toxins into a specific organ to prevent spread of the toxin in the whole body. Where adverse effects may be expected, we will perform particularly close and frequent monitoring of animals. These will include the use of observation sheets and body weight records. We will continue to strive to develop new refinements.

A important aim of many of the proposed experiments is to determine how long cellular therapies survive after transplantation. Where possible, we will use imaging methods to monitor the survival of the transplanted cells. Such imaging can be performed using a short anasethetic (lasting less than 5 minutes) and without any harm or surgical procedure on the animal. Importantly, this means that we can obtain important data from the same animals over several weeks, without having to cull animals at multiple time points.

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

All experiments will be conducted and reported in adherence to best practice guidelines including those published by the Laboratory Animal Science Association (LASA). We will follow guidelines on record keeping, performing surgery, education and training, and reporting of experimental results. We will also follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines on experimental conduct including study design, randomisation, avoiding bias and statistical analysis of results.

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

As a current project license holder, I am closely involved with the activities of the 3Rs committee at my institution, including the development of recommendations and dissemination of information relating to advances in 3Rs. I intend to continue with my activities, including through review of relevant publications, guidelines and best-practice information.