Home Office

NON-TECHNICAL SUMMARY

Development of humanised mouse models for study of cancer immunotherapy

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

Transplantation, Cancer, Treatment, Immunotherapy, Safety

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, 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 aim of this project is to study how the immune system recognises cancer cells, and test the safety and effectiveness of drugs that can kill tumour cells by interacting with the immune system.

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?

Cancer is one of the leading causes of death in the world. It is now recognised that the immune system has a critical role in how cancers develop, and that drugs that manipulate the immune system have great potential as better therapies. The proposed project will allow a better understanding of how the immune system recognises tumours, and to test the safety and effectiveness of drugs that can kill tumour cells by acting on the immune system. This could lead to development of new therapies that are more effective and cause fewer side effects to patients.

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

It is expected that the proposed project will ultimately enable novel models of human tumours to be used not only to study cancer biology in vitro, but also to enable drug development and screening. Moreover, it is anticipated that advanced immunotherapies can be developed and optimised that will enable highly effective tumour eradication without the devastating side-effect profile associated with classical chemotherapy agents. The insights generated by this project will be shared through publications to enable other researchers to incorporate the findings into their research programs.

More specifically, we expect to generate at least one new 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 novel therapy developed in this project.

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 immune-related therapies for treatment of cancers. 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 immunotherapies, who are essential for the ultimate production of these therapies, will also benefit from the findings of this study. In the long-term (5-7 years), this project will benefit patients with cancer. The benefit will initially be limited to those patients enrolled in clinical trials investigating the safety and efficacy of immunotherapies developed in this project. We hope that ultimately (7-10 years) large

numbers of patients will benefit from the findings of this study, once the immunotherapies 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, and 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: 3475

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.

This project will use adult mice. Mice are the lowest species with a comparable physiology that enables useful information to be gained relating to the safety and effectiveness of human immunotherapies. Importantly, genetic strains of mice are available that allow studies to be designed that can generate valuable information about specific cellular therapies. Moreover, mice can be used effectively to model the human immune system by injecting them with human immune cells, followed by transplantation with human cancer cells, thus allowing the study of the human immune response to cancer.

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

In the typical experiment, an adult mouse will receive sublethal irradiation, followed by adoptive transfer of human immune cells through injection into the abdominal cavity. After several (typically 4-12) weeks later, the animal will be surgically transplanted, on one occasion, with tumour (or control) cells or tissue into the abdomen under general anaesthesia. After 2-4 weeks, the animal will be given an immunotherapy agent by intraperitoneal injection on a number (typically 3-5) occasions over 1-2 weeks. The animal will then be killed by Schedule 1 methods (humane killing of protected animals under the Animal (Scientific Procedures) Act 1986), several (typically 4-6) weeks later. Some animals may undergo non-invasive imaging on a number (typically 3-5) of occasions. The typical animal will undergo one or two surgical procedures and be kept for approximately 16 weeks, when it will be killed electively while still well and without clinical signs. In some cases, the animals will be killed by removal of organs while under deep general anaesthetic.

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 tumour cell transplantation.

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

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.

When rapid adverse effects may be expected, animals will be monitored very frequently (up to one hourly) during the initial period (~6 hours) when adverse effects are most likely to occur (based on data from previous animal experiments and clinical studies).

Animals will also be culled if they experience clinical signs that approach the limits described in the project according to the Home Office guidelines.

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)?

Approximately 65% of animals will undergo a surgical procedure and will therefore experience moderate severity, or experience moderate severity as consequence of administration of immunotherapies. Approximately 25% of animals will only receive cells by intraperitoneal or intravenous injection 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 definitive examination of the efficacy and safety of anti-cancer drugs requires examination in intact animals, including those with a competent immune system. This is a necessary and pre-requisite ‘final’ step for the clinical translation of these cancer therapies and cannot be completed without animal experiments. Much of the proposed work is carried in the laboratory and using human tissue only, thus minimising the need for animal experimentation. Importantly, it is anticipated that this work will lead to

the refinement and optimisation of laboratory models of cancer which can be ultimately used to replace experimental use of animals.

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

We have 3 key strategies for the development and use of non-animal alternatives.

1. We intend to use humans cancer cells and tissue in the vast majority (>95%) of our studies. Animal cells will only be used if they are necessary control for the use of human cells, or where equivalent human cell alternatives is not available. We therefore anticipate using very few animals for the generation of tumour cells.

2. We will continue to make extensive use of sophisticated human cell culture systems, such as organoids and tumoroids technology, to study the safety, functions and immune response to cancer in vitro.

3. We have developed mechanism for perfusing human organs ex vivo, on a specialised machine, for prolonged periods in order to test the functions of transplanted human cells and tissue.

Why were they not suitable?

We are using all of these three alternative approaches to reduce the number of animals used in the proposed experiment. However, the definitive investigation of the immune response to tumours, and the safety and efficacy of immunotherapies requires an intact and functional immune system in an animal model.

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 and all experiments are conducted and/or data analysed in a blinded manner to reduce bias. When a new tumour cell or immunotherapy is under investigation, we will first perform pilot experiments with small animal groups (typically 2-3 animal per group) 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 by transplanting control and tumour cells in two separate sites (for example, into each kidney) in the same animal. 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 tumour, which 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 tumours and immunotherapies 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 tumour or control cells or tissue into animals to assess the tumour biology

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

3) Administration of immunotherapies to assess their safety and efficacy in reducing tumour growth

All experimental models have been refined to ensure they cause the least pain and suffering. 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 immunotherapies. As the immune response to tumours, and the action of immunotherapies takes days to weeks to manifest, experiments cannot be performed exclusively under terminal anaesthesia.

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 technician who ensures 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. These include enhanced environmental enrichment (such as extra card-board housing) and 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). Where adverse effects may be expected, we will perform particularly close and frequent monitoring of animals, including through the use of observation sheets and body weight records. particularly where. We will continue to strive to develop new refinements.

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), such as guidelines for 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.