Home Office

NON-TECHNICAL SUMMARY

Modulating the immune response in cancer

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

Cancer, Immunomodulation

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 identify novel mechanisms by which substances that modify immune responses (immunomodulatory substances) can initiate, enhance or maintain anti-tumour immune responses in vivo. These studies will not only provide fundamental understanding of the requirements for an efficient anti-tumour immune response, but should also provide proof of principle data for future therapeutic development.

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 the second leading cause of death worldwide, accounting for 9.6 million deaths every year. However, new therapies targeting pathways known to inhibit the immune system have revolutionised cancer therapy, and clearly demonstrated the importance of the immune system in tackling cancer. Unfortunately, a large number of cancer patients are non-responsive to such immunotherapies, highlighting the incomplete understanding of the mechanisms underlying the activation and suppression of the anti-tumour immune response. Therefore, it is critical to gain a greater understanding of the processes leading to a successful immune response during cancer and to identify novel targets and immunomodulatory substances that could be used alone, or in conjunction with current therapeutics, to improve cancer treatment.

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

A central goal of this project is to identify new understanding of the mechanisms by which immunomodulatory substances can initiate, enhance or maintain anti-tumour immunity.

One of the immunomodulatory substances that we will initially focus on (IMM-101) has already been tested in phase I and II clinical trials. As part of this project, we aim to generate vital preclinical data to help this promising therapy to advance to phase III trials and, ultimately, to the clinic.

This project aims to disseminate new knowledge by publication in peer reviewed journals and presentations at conferences, seminars and workshops. We hope that in the longer-term our work will contribute to new immunology-based therapies for cancer. In the 5 years of this project, we aim to continue our high standard of publication, averaging more than 5 research papers per year in highly-respected peer-reviewed journals.

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

This project aims to answer basic scientific questions. The new knowledge generated, and the unravelling of important fundamental immune mechanisms, will be relevant to a broad range of human and animal conditions, including cancer.

In the short term, we will gain novel mechanistic insights into how the immune system can be modulated to promote tumour clearance. This new information will offer the potential of enhancing

future treatment options for a broad range of disease, as the immune response is centrally involved in all cancers. Importantly, one of the immunomodulatory substances that we will initially focus on (IMM-101) has already been tested in phase I and II clinical trials, and has demonstrated safety and efficacy in advanced pancreatic cancer and melanoma, and is currently being assessed in a range of other cancers. Therefore, increased mechanistic understanding of this promising therapy is critical for its progress to phase III trials, and to patients.

In the longer term, we aim to discover novel therapeutic candidates through our fundamental research into anti-tumour immune responses. These novel candidates could be targeted individually, or combined with established therapeutics, to improve current cancer treatment strategies.

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

Communication of our findings will be primarily through publication in widely-read peer-reviewed journals, but also presentation at local, national and international congresses and institute seminars. To ensure maximum dissemination, only journals that allow open access without payment by the reader will be considered. To prevent unnecessary repetition of experiments by others, we will seek to publish all data generated under this project, including negative results.

To enable rapid translation of our findings to the clinic we will exploit new and existing collaborations, and with local clinicians as part of the translational environment within our institution. We have highly effective systems in place for technology transfer. Additionally, we aim to expand our current collaborations with pharmaceutical and biotech companies by presentation at national and international forums at which industrial representatives are present.

Species and numbers of animals expected to be used

- Mice: 9000

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 study adult mice because the immune system, tissue organisation and development of all mammals are similar, allowing mice to be a model for humans and other animals. We also use mice because scientists have created many genetically altered mouse lines that allow us to dissect in fine detail what happens during immune responses and in cancer. Genetically altered mice, and many of the tools designed to work with mice, allow us to define in precise detail how particular cells and molecules of the immune system work together to fight cancer. By manipulating these cells and molecules, we can identify the immune components that are most effective at preventing tumour growth, and use that information to help design future cancer therapies.

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

Typically, animals will receive a single or multiple injections containing an immunomodulatory substance (e.g. antibodies to neutralise a specific immune mediator or deplete a specific cell type, or cells to promote a particular response) and/or cancer cells.

Experiments might look at the immediate immune response in the first few days after administration of an immunomodulatory substance or tumour cells, or may last several months to allow full tumour development, or assessment of immune memory. These experiments will typically last between 1 and 28 days. For longer experiments, mice are likely to receive multiple doses of an immunomodulatory substance (e.g. once a week for 2-3 weeks). For some experiments, mice will receive an injection of tumour cells either prior to or after treatment with immunomodulatory substances. Depending on the substance, it may require multiple doses over a time period as tumours develop (typically up to 4 weeks). Experiments will end with animals being killed humanely, sometimes under terminal anaesthesia.

The cumulative experience of mice will typically be exposure to 2 or 3 procedures that may each cause short but usually separated periods of typically mild or potentially moderate degrees of suffering.

Separate from the above experiments, some genetically altered animals will be used only to breed and maintain animal lines.

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

The vast majority of animals will experience no adverse effects or only mild adverse effects.

Tumours grown on the flank can affect walking and normal behaviour and, in rare cases, can become ulcerated. However, the tumour models we will use are generally well tolerated and will rarely reach moderate severity. Although immune modulation can trigger systemic inflammation that can cause weight loss, piloerection, hunching and reduced movement, in most cases these effects should be mild or transient.

In all experiments, animals will carefully monitored and humanely killed before they exceed moderate severity limits.

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)?

We expect approximately 85% of mice to experience mild 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 mammalian immune system is highly complex, relying on the co-ordinated actions of multiple different cell types and molecules that collectively provide protection. As such, the insight in vivo experiments can provide on the mechanisms necessary for tumour protection is of significant clinical relevance. Importantly, we know the importance of the tumour microenvironment and its ability to suppress immune responses, thus limiting the efficacy of immunotherapies.

Unfortunately, in vitro systems are unable to reflect the cellular and molecular complexity of the immune system and tumour microenvironment. Therefore, the use of mammals is essential for gaining a better understanding of the mechanisms underlying immune protection that could be utilised for patient benefit.

Mice will be used in these studies because their immune system closely resembles the human immune system therefore giving a better chance for translating potential therapies. Additionally, a wide array of wild type and genetically altered strains of mice are available that will allow us to better decipher the role of immune cells and molecules in anti-tumour immunity. Finally, a vast range of reagents is available for analysing murine cellular and molecular interactions during immune responses.

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

1) Analysis of human biopsies.

2) Use of cell lines and in vitro systems.

Where possible, we will use in vitro assays to provide initial data on the effects of immunomodulatory substances on specific immune cells. This data will then be used to inform and complement our in vivo experiments.

Why were they not suitable?

The types of experiments required to track cell function in vivo are not possible with human tissue biopsies, nor can we experimentally manipulate humans.

Many location-specific features of cells are lost once they are removed from the tissue, which makes the use of cell lines impractical. In vitro systems typically allow for the study of one or two cell types in a highly controlled environment that is not reflective of the complex immune system in vivo. Therefore, to fully understand how different cell types and molecules co-ordinate an effective immune response we require in vivo experiments.

We will regularly review the literature regarding in vitro and zebrafish systems so that if new approaches are developed we can test them and potentially exploit them if they succeed.

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 mice has been estimated based on experience gained under my previous Home Office licenses, taking into account breeding strategies for genetically altered mice, and anticipated numbers of planned studies over the course of the license.

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

For all of our experiments, in-bred mice are used to reduce experimental variation, which makes it possible to use fewer animals to achieve statistical significance. For the majority of our studies, mice from the same litters are used for control and experimental mice, reducing variation that can occur due to differences in the microbiota. Overall, our experiments are designed to reduce the number of variables (for example age) to as few as possible and thereby reduce the number of control groups required.

We work with the NC3Rs Regional Programme Manger to ensure all lab members are introduced to the NC3Rs experimental design assistant and encouraged to use it. Everyone in the lab is trained in statistical methods and these are regularly discussed at lab meeting, to ensure all agree the best methods are being used. This includes randomisation and blinding, whenever practically possible. Tissue-sharing is a major tool we use to reduce animal usage.

A significant proportion of our animal use is related to breeding programmes for genetically altered lines. We follow the advice of our animal facility staff to optimise breeding, and regularly discuss numbers at lab meeting to ensure we do not overbreed. Where possible and appropriate, we use substances that can target or block immune processes in wild type mice, to reduce use of genetically altered mice.

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

We routinely perform pilot experiments to determine the optimal number of mice to achieve statistical power. Experiments are then performed on a minimum of two separate occasions to ensure reproducibility, following which data pooled from experiments are statistically analysed to reveal less pronounced effects without increasing overall animal use.

We have many years of experience in planning animal experiments and we plan our research to ensure that all animals are used most effectively. We often combine experiments to ensure that multiple organs are used to address multiple objectives at once. Careful discussion between multiple researchers is required to avoid compromises. Due to high variability in immunological and tumour models in vivo, especially in genetically altered models that we have yet to assess, we will adjust groups sizes as required, should subsequent power calculations indicate that this is necessary.

The increased use of genetically altered animals has led to more complicated breeding strategies and, as a result, larger colonies. We reduce the numbers of these animals in our experiments by using littermates as controls wherever possible. Additionally, when a particular strain is not being used experimentally we work closely with the animal technicians to develop a breeding strategy that maintains low numbers of stock animals.

In many experiments, we will use bone marrow to carry out pilot studies in vitro, therefore only a few animals are used to initially test new hypotheses. Importantly, we continue to work closely with collaborators who can supply bone marrow samples, therefore reducing the number of genetically altered animals that have to bred to facilitate experiments.

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 will use mouse models to study the immune response in cancer. Mice represent the most appropriate species for in vivo study of cancer immunity, because of the extensive knowledge of their physiology as it relates to humans, the genetic and biological tools available and the ability to be easily bred and handled.

The cancer models we will use do not cause significant pathology, and doses and timing are carefully managed such that the animals will experience minimal suffering. As our experience of these tumour models develops, we will look to refine our approaches to ensure robust experimental results whilst minimising pain, suffering or distress.

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

To our knowledge, no other species of lesser sentience can fulfil the requirements of this project to the same extent as the laboratory mouse. We are studying long and complex immune processes, and trying to understand how the adult immune system handles tumours, and how different cells communicate to orchestrate an appropriate response. Only adult animals would give meaningful results. To define the impact of immunomodulation on tumour growth requires mice to be monitored for several weeks, so procedures cannot be carried out on terminally anaesthetised mice.

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

All procedures will be performed by trained and skilled personal licence holders, who will handle animals with care. Animals will be monitored for adverse effects using score sheets previously developed in conjunction with the NVS and NACWO. These score sheets have proven to allow for objective measurements of clinical signs associated with adverse effects to determine when humane endpoints have been reached.

In line with the establishment's policy, we will adopt the latest techniques in animal handling (e.g. cupping) to significantly reduce the stress associated with procedures. Furthermore, where possible, the least invasive methods for dosing and sampling will be applied.

Anaesthesia and analgesia will be provided where suitable (e.g. for humane restraint, during or in recovery from surgery). The best aseptic technique will be used during surgery.

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

We follow LASA guidelines, and consult the recommended https://www.nc3rs.org.uk/3rs-resources on a regular basis, including watching videos of best practice techniques. For specific models, we read papers from other groups doing similar experiments, as well as consulting directly with other researchers to discuss the most refined procedures.

We will continue to use the NC3Rs Experimental Design Assistant to ensure we design experiments that will allow us to achieve statistical significance whilst minimising the number of animals we need to use.

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

Our animal facility includes a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. We consult closely with them and take full advantage of the extensive resources provided to ensure we are following current best practices. We will continue to work closely with our local NC3Rs representative to ensure we stay informed about the advances in the 3Rs. For example, we have recently attended an NC3Rs Experimental Design Assistant workshop to ensure we are using the EDA to the fullest extent in order to achieve our scientific aims with the minimal number of mice.