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NON-TECHNICAL SUMMARY

Regulation of immune responses 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, Immune responses, Lymphocytes, Immunotherapy, Metabolism

Animal types	Life stages
Mice	pregnant, adult, juvenile

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 will study immune responses to tumours with an overall aim of identifying new approaches for the treatment of cancer. We are particularly interested in understanding how responses of populations of white blood cells called T lymphocytes are turned on or switched off in response to tumours sited in distinct areas of the body and the role of metabolism in these processes.

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?

Despite advances in the treatment of cancer, the World Health Organisation reports that cancer remains the second leading cause of death worldwide. In recent years, novel approaches that harness the body's own immune defences against tumours, collectively termed immunotherapies, have proven highly effective in the treatment of otherwise incurable cancers such as malignant melanoma, a form of skin cancer. Nonetheless, for several cancer types these approaches have yet to bring benefit to patients, whilst even with "immunotherapy-sensitive" cancers, a proportion of patients do not respond. Therefore, there remains an urgent need to understand better how immune cells respond to tumours and the mechanisms that tumours exert to prevent effective immune responses. This work will aid our understanding of immune responses to tumours and assess novel targets with the aim of improving cancer immunotherapies.

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

Through studying the response of T lymphocytes and other white blood cell (immune cell) populations to cancer, our studies will enable us to:

1. Define the impact of cancers cells and their products on white blood cell function and metabolism

2. Determine how the availability of nutrients within the body affects the outcome of anti-tumour immune responses and tumour growth

3. Screen and target molecules that regulate immune activation in cancer

4. Test combinations of therapies to enhance clearance of tumours by the immune system

An immune response is frequently provoked during the early stages of cancer, yet one of the hallmarks of advanced cancer is the ability of tumours cells to evade these responses. Current immunotherapies seek to overcome the failure of immune responses and can broadly be placed in two main categories:

1) Drugs that enhance the responses and / or relieve suppression of immune cells already present within cancer patients.

2) Tumour-targeting immune cell populations that may be genetically modified to enhance their anti-cancer function, before injection into patients

Both pre-existing and transferred immune cell populations must overcome a characteristically hostile environment that is created within tumour masses. Our project will seek to gain an understanding of the responses of immune cells within these challenging environments, with a view to developing new approaches to improve immune function in cancer. Importantly, we suggest that this work will have implications for the development and future success of novel immunotherapies.

T lymphocytes and other immune cells need to maintain a supply of nutrients such as glucose and amino acids to provide energy to sustain their responses in cancer. Evidence suggests that fast-growing tumour cells can out-compete immune cells for these vital nutrient resources within tumours. Furthermore, inhibitory products present within tumours impact upon immune cell metabolism. How these conditions vary between different types of cancer / different locations within the body, and the role of individual nutrients in determining immune responses is not fully understood. Through our previous published work, we identified one particular nutrient as being important for T lymphocyte responses. In this project, we will define the role of nutrient availability in anti-cancer immune responses and test how manipulating T lymphocytes might improve their capacity to thrive in nutrient-deprived conditions. Furthermore, Identification of factors within tumours that impede immune cell metabolism may enable the development of ways to improve immunotherapies.

The ability of T lymphocytes to respond to cancer cells depends upon triggering through a cell-surface molecule termed the T cell receptor (TCR). Several current immunotherapies seek to improve the ability of T cells to respond to TCR triggering, whilst our previous published work has defined key mechanisms that regulate these processes. In the current project, we will seek to build on this previous work and determine the impact of deleting inhibitory factors within T lymphocytes with a view to improving anti-cancer immune responses. If successful, these approaches could be incorporated into the design of novel immunotherapies in the future.

The overall benefit of this work will be to increase our understanding of the regulation of immunity, identifying how and why immune responses fail and testing new approaches to revive anti-cancer responses. Therefore, this work has the potential for publication in cross-disciplinary scientific and medical journals. We envisage that this will also lead to new productive collaborations with clinical colleagues and industrial partners to enable us to translate our findings into real-world benefits.

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

A major benefit of this work will be to increase our knowledge of the mechanisms that regulate the induction and suppression of immune responses to cancer. Importantly, this knowledge will inform our future scientific aims and, through the refinement of our experimental protocols, will enable us to maintain the highest welfare standards for our experimental animal colonies.

Cancer represents a considerable health burden in society. A major goal of the proposed program of work is to test the role of pathways, nutrients and genes that are implicated in protective immunity to tumours. For example, using GA mice, we can test whether modification of target genes alters the ability of immune cells to respond to cancer and provide proof-of-principle for future studies to improve immunotherapies.

Important and immediate outputs for the work will include scientific publications and talks at national and international scientific meetings. The work will be of value and of great interest to fellow

researchers in the fields of immunology, immunometabolism and cancer immunotherapy. The project will also help identify novel approaches to manipulate immune cell responses in disease. Thus, it is expected that the knowledge gained from our animal experiments will be translated to pre-clinical human studies with our colleagues within our establishment's hospital campus, that could generate intellectual property rights and inform the generation of new human therapeutics in the future.

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

Our research involves collaboration with groups within our institute as well as national and international colleagues and the current project will seek to maximise the benefit to all partners through:

- transfer of knowledge and expertise. For example, international collaborators will provide access to protocols and reagents for investigating metabolic processes whilst our group will provide in vivo samples

- provision of training and new skillsets to postgraduate student and postdoctoral researchers, thereby facilitating and enhancing their career development

- enhanced quality of scientific publications through complementary experimental approaches and technologies. In this regard, our in vivo approaches will complement studies using clinical samples and / or lab-based molecular techniques.

We will publish the results of our studies in leading scientific journals and present the work at relevant national and international conferences. We have previously accelerated the dissemination of our research by posting our results as "pre-print" publications to the bioRxiv server (bioRxiv.org). This enables us to share our findings months before acceptance in peer-reviewed journals as well as to receive feedback from the scientific community. We will continue to use social-media platforms (e.g. Twitter) as well as traditional media outlets to inform and engage with both the scientific community and non-specialist public on our own and related scientific research.

Species and numbers of animals expected to be used

- Mice: We anticipate using up to 4750 over the 5 years that this project will run. Note that that up to 500 mice will be transferred from protocol 1, under continuous use, to protocols 2 and 3.

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 species of choice is the mouse as it can be genetically modified in a manner that is not currently possible with other mammalian species. Furthermore, the use of well-characterized inbred mouse strains maximises the reproducibility of in vivo research. Importantly, a wealth of commercial reagents

are available for the study of the mouse immune system and for intervention in and treatment of mouse models of cancer. We will investigate immune responses of young adult mice to ensure the reproducibility and cost-effectiveness of our studies.

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

The purpose of this project is to study immune responses to cancer. Therefore, in typical experiments mice will be injected with cancer cells either into the skin, a vein or the peritoneal cavity with or without substances or immune cells that we hypothesize will modulate the immune response and growth of tumours. We will study the growth of tumours in mice using advanced imaging techniques and may take blood samples to monitor circulating immune cells or nutrient levels. Experiments will vary in duration depending on the growth rate of tumours. This can vary substantially in different models from 7-70days. Wherever possible we will use techniques that have the least possible impact on animal welfare and undertake experiments of the shortest possible duration.

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

Injection of cells or substances with a needle causes mild and transient pain at the injection site. In some circumstances we may use temporary anaesthesia for a few minutes to minimise discomfort for animals resulting from injections or for imaging procedures. Injection of cancer cells leads to the formation of tumours that in a minority of cases (<20% in protocols 2 and 3) may cause moderate suffering such as difficulty with breathing (lung tumours resulting from intravenous injection) or the accumulation of fluid in the peritoneal cavity. Such suffering will be short in duration as we undertake regular monitoring of mice and humanely cull any animals to prevent unnecessary suffering. Through the experiments, we hope to define fundamental aspects of the processes underpinning immune responses in cancer and assist in developing novel medicines to improve the treatment of these diseases.

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)?

The majority of mice will experience mild or subthreshold suffering. For example, much of the analysis of experimental mice is initially carried out in the laboratory using fresh cells and organs from the mice generated by breeding. Therefore, ~80% of our mouse colonies will be humanely culled with no regulated procedures performed beyond being bred. For mice that receive tumour cell injections, <20% of mice will experience moderate suffering.

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?

Our aim is to understand complex biological processes in immune cells, as this information will be most relevant to the design of future therapeutic approaches. Whilst some basic questions can be addressed using immortalized cell lines, such approaches have numerous limitations, most importantly that these cells are by definition quite distinct from primary cells. Immortalized cell lines frequently have mutations in key signalling and metabolic proteins which enable their survival and growth out with the body. As such, these genetic alterations render the analysis of signalling and metabolism in cell lines as quite distinct.

Wherever possible, we study immune cell function in the laboratory; e.g. tissue co-culture of lymphocyte populations with tumour cells. However, to understand the complex interplay between immune cells, cancer cells and environmental factors such as nutrients, requires the use of in vivo animal models. This is particularly prescient when the aim of the programme is to study the role that individual cell types and molecules play in these highly complex immune responses, as proposed here. These diseases involve the interplay of multiple tissues and factors that cannot be modelled using currently available tissue culture systems.

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

We will study immune cells from healthy blood donor and cancer patient samples to complement our animal studies.

Why were they not suitable?

Study of human cells in the laboratory will enable us to begin to assess the translational implications of our animal work. Nonetheless, if we are to define mechanisms of immune regulation and metabolism in cancer and assess whether molecules can be targeted therapeutically, the use of some animal experiments is essential.

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?

Depending on the consistency of the results obtained in an experiment, more or fewer animals are required to support or reject our hypotheses. We can use online mathematical tools or get advice from statisticians in order to perform statistical tests to assess the robustness and reproducibility of our

experimental findings. We have used the results of our previous projects and data from similar experimental systems to predict how variable our experimental data are likely to be and therefore estimate how many animals will be required. In all cases, we use the minimum number required to generate meaningful data.

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

We have substantial experience in using and setting up the experimental protocols detailed in this application. In this regard, in many cases estimates of animal numbers have already been established either through analysis of previous experiments or small-scale pilot studies to establish variability.

We also take advantage of online experimental design tools, particularly the Experimental Design Assistant from the NC3Rs. This tool enables researchers to input the key components from each stage of an experiment; from numbers of animals and numbers of experimental groups through to data measurement protocols and statistical analysis. This provides assistance in the design of robust and reproducible experiments that minimise the possibility for subjective bias in the measurement and analysis of data and reduce animal numbers to the minimum required to achieve the scientific objectives.

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

In all cases, we ensure that mice are bred in the most efficient manner. Where practical, established genetically-altered mouse breeding colonies are bred in a manner that all offspring will have the required genetic modifications and can be used for experimental purposes. The size, frequency, numbers and health of litters from breeding mice are closely monitored by animal facility staff and researchers so as to maintain efficient colonies of adequate but not excessive animal numbers.

In many cases, we can minimise animal numbers by using multiple tissues from one animal to assess several experimental parameters; e.g. tumour tissues, blood and lymphoid organs such as the spleen can all be harvested and assessed in the laboratory.

When setting up new experimental protocols, pilot studies using small numbers of animals will be performed to enable us to determine the optimal strategy required to answer the experimental objectives.

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.

For protocol 1, the majority of mouse lines have no defects beyond alterations in or loss of immune cell populations and suffer no ill effects when they are maintained in filtered cages in our specific-pathogen free animal facility. In addition, we will use mouse models of cancer to model immune responses and therapeutic approaches to the treatment of cancer (protocols 2 and 3). In these experiments, cancer cells grown in the laboratory are injected into animals with a needle and a tumour will form at the injection site or in the case of intravenous injection, in the lungs. It is therefore unavoidable that some animals will experience suffering. The severity of these approaches will be kept to a minimum with ~80% of mice in protocols 2 and 3 experiencing a mild form of suffering. For some mice, growth of tumours may result in moderate levels of suffering. However, regular monitoring of animal symptoms as well as measurement of tumour growth by imaging techniques and/or visual inspection ensures that mice do not experience unnecessary suffering and are humanely culled. Through this work, we will be able to address fundamental questions about the immune response to cancer and help in the development of improved medicines to treat these diseases.

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

We wish to model cancer and immune responses in adults. Importantly, studies of adult mice are widely recognised to be relevant for the study of immune processes and cancer whilst many therapeutic breakthroughs have been made following initial mouse studies e.g. the use of several immunotherapies for the treatment of cancer. Furthermore, the ability to genetically manipulate mice is essential to test our specific hypotheses whilst the availability of key reagents and therapies to assess mouse immune function is central to our experimental design. With this in mind and to best of our knowledge, there are currently no non-sentient or lower sentient alternatives to the use of adult mice for our work.

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

Animals undergoing experimental protocols are monitored routinely by researchers and animal facility staff using specific checklists to assess wellbeing, discomfort and suffering. The frequency of monitoring is increased upon the development of specific symptoms. Anaesthetic is used whenever appropriate to minimise pain/discomfort and advice from veterinary surgeons sought whenever unexpected or unexplained signs of distress, discomfort or pain develop.

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

All animal suffering will be limited to unavoidable procedures required for the performance of our experimental protocols. For general guidance on the conduct and refinement of animal experiments, the NC3Rs provide a wealth of resources and information online (https://nc3rs.org.uk/refinement-refining-animal-experiments-minimise-pain-suffering-and-distress). Relevant academic literature on the care and use of mice in research will be referred to, including NC3Rs published guidelines (1). Furthermore, specific guidelines on the conduct of cancer research in experimental animals will be adhered to at all times (2).

1. Prescott MJ, Lidster K (2017). Improving quality of science through better animal welfare: the NC3Rs strategy. Laboratory Animal 46:152-156.

2. Workman P et al (2010). Guidelines for the welfare and use of animals in cancer research. British Journal of Cancer 102:1555-1577.

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

We will continue to subscribe to regular e-mails from NC3Rs, that contains updates on best practice in animal husbandry and experimental design. All researchers undertaking experimental protocols under this project will review relevant scientific literature, particularly in specialized peer-reviewed journals such as Laboratory Animal, that pertain to the conduct and refinement of mouse cancer models and will implement any advances in the design of subsequent experiments.