Home Office

NON-TECHNICAL SUMMARY

Enhancing the effectiveness of oncolytic virus-induced 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

cancer, cancer immunotherapy, oncolytic viruses, metastatic disease, relapsed disease

Animal types	Life stages
Mice	juvenile, adult

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 test the efficacy of oncolytic virus based anti-cancer therapies and gain a better understanding of the regulatory systems that exist to prevent cancer therapies from working. An improved understanding of the cancer environment will allow us to design novel treatments to eradicate cancer.

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?

We have developed novel oncolytic virus-based therapies for the treatment of cancer using human in vitro systems. However, before clinical translation can be considered it is important to validate the efficacy of these approaches using animal models that recapitulate the complexity of the tumour microenvironment and provide host cellular interactions that could influence anti-cancer responses.

Different types of cancer, or indeed cancers of the same tissue origin, have developed a range of strategies to prevent chemotherapy and/or immunotherapies from working. Moreover, the challenges that exist for the successful elimination of cancer at different stages of disease (e.g. primary cancers, metastatic cancer or relapsed cancers that have re-occurred after initially successful therapy) may also be different. Gaining a better understanding of the strategies used by cancer cells to survive will help us develop combination treatments that are effective against disease. This is important as many forms of cancer remain incurable and novel and effective therapies, that are safe and well-tolerated, are urgently required.

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

We expect multiple outputs to be obtained by the end of this project. For example, we will identify mechanisms that are used by different types of cancers to prevent therapies from working and develop novel treatments that can be translated into clinical trials to improve patient outcomes. Throughout this project, we will disseminate our findings by publishing data in peer-reviewed scientific journals and by presenting our work at scientific meetings, including local seminars and national and international conferences.

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

In the short term (within 3 years), researchers working in the field of cancer research or cancer immunology will benefit from these findings through knowledge gain. In the longer term (5-10years), we hope that the results obtained from animal models will be translated to cancer patients and that patients will benefit from the novel therapies identified (e.g., through increased patient survival and/or

the development of safer and better tolerated treatments). The clinical application of safe and well-tolerated treatments will ultimately increase the quality of life for patients undergoing treatment.

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

We will publish our research in high-quality peer-reviewed journals and work collaboratively with academic and clinical colleagues at a local, national and international level, to share our experience and expertise. We will also maximise outputs through the dissemination of results at relevant scientific meetings and by the delivery of seminars locally or at other universities.

Species and numbers of animals expected to be used

- Mice: 2500

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 will use established mouse models of a range of cancers (including ovarian cancer, colorectal cancer and multiple myeloma, amongst others) to characterise the cancer microenvironment and test the efficacy of novel treatments. Mouse models will be used because laboratory studies using cancer cell lines or patient samples do not (i) recapitulate the full repertoire of complex interactions that occur within the cancer environment or (ii) provide information about optimal drug scheduling and delivery routes.

We will use both juvenile (5-10 weeks) or adult mice (>12 weeks) to test anti-cancer agents. This will allow us to determine whether there are differential responses to treatment depending on the age of the animals used. The age of mice at the start of each study will be dependent on the specific cancer model and aim of the experiment.

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

Animals will be injected with tumour cells at the start of each study and then treated with therapeutic agents. Therapeutic agents could be delivered by injection into the bloodstream, the tumour or the peritoneum. Additionally, therapeutic agents may be delivered orally, either by oral gavage or in food and water. To assess the effectiveness of treatments, tumour burden will be monitored using imaging software, blood sampling, calliper measurements (tumour size) or at the end of the study, after the animals have been killed. The method of tumour monitoring will depend on the tumour model being used. During therapeutic interventions, it is possible that mice will be subjected to multiple procedures (up to 5 times/week). The duration of established cancer models is usually between 1-4 months; however, is also dependent on the cancer model. All animals will be killed humanely at the end of each

experiment and mice may be killed at specific time points depending on the research question being addressed.

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

General signs of ill health will be assessed throughout each study.

Injection of tumour cells, and the resulting tumour mass, may cause some pain and discomfort. However, in our experience tumour growth is extremely well tolerated. Hind limb paralysis/weakness is a common adverse event in some cancer models (e.g., multiple myeloma), however, with daily monitoring this symptom is observed early, and animals are humanely killed at the first sign of hind limb paralysis/weakness. Potential, but unlikely adverse effects for tumour growth and metastasis could include weight loss, hunching, tremors or convulsions or altered breathing; however, we have not experienced any of these adverse events to date. All animals will be humanely killed if they display any sign of discomfort or deterioration of health.

The administration of therapeutic agents by different routes may also cause pain and discomfort; however, pain associated with therapeutic intervention will be short lived. No adverse events are expected as a result of therapeutic intervention, except short-lived discomfort at injectable sites, during oral gavage, or following repeated dosing. It is possible that mice may develop a fever after treatment with some biological agents, although to date, we have not experienced this in any of our studies. If this was an anticipated possible side effect, mice would be monitored carefully for signs of discomfort and humanely killed if symptoms did not subside within a couple of 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 will be killed humanely at the end of the experiment and are expected to suffer no more than moderate suffering during the entire experiment. Moderate suffering is expected because of the cumulative effects of repeated procedures, although the treatment itself is unlikely to cause any adverse events except short-lived discomfort during treatment administration. Mice may also develop a fever after treatment with some biological agents, however, in our experience this is unlikely (<5% animals).

Moderate adverse events associated with injection of tumour cells, and the resulting tumour mass, may cause some pain and discomfort. However, in our experience tumour growth is extremely well tolerated. Hind limb paralysis/weakness is also a common adverse event in some cancer models (e.g., multiple myeloma). Potential, but unlikely (<5% animals) adverse effects for tumour growth and metastasis could include weight loss, hunching, tremors or convulsions or altered breathing; however, we have not experienced any of these adverse events to date.

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?

The aim of this project is to better understand the regulatory systems that exist within the tumour and identifying novel treatment strategies which make cancers more amenable to therapeutic intervention. Although we conduct many experiments in the laboratory to investigate the effectiveness of anti-cancer therapies, these models are not fully representative of what may happen in patients. This is because: (i) cancer cells do not exist in isolation but co-exist alongside other cell populations which support cancer growth and development; and (ii) the response to therapy involves a complex set of interactions between different cell types and at different locations within the body. Therefore, to identify treatments that have the best chance of success when translated to cancer patients, they need to be tested in animals that model the full complexity of cancer environment and that have a fully functioning immune system.

Unfortunately, it is only possible to model simple interactions between different cell populations in the laboratory. However, this will be done as much as possible to replace and reduce animal experimentation.

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

We use a range of non-animal alternatives to characterise cancer cells, the cancer microenvironment and the effectiveness of treatments. These include human cancer cell lines, human immune cells isolated from health donor blood, and cancer tissue and blood from cancer patients. We use these routinely in the laboratory, either cultured alone or in co-culture model systems to mimic cell-to-cell interactions that occur within the cancer environment. Animal work is only carried out when laboratory studies have been performed and data has confirmed that therapeutic strategies are effective against disease.

Why were they not suitable?

Cancer is a complex disease with multiple cell-to-cell interactions occurring within the tumour and unfortunately, these cannot be accurately recapitulated in the laboratory. Therefore, animal models are required to fully characterise the cancer environment and test the effectiveness of novel treatments. Moreover, laboratory-based studies do not allow us to: 1) determine whether therapeutic agents can reach tumour sites after different routes of delivery, 2) establish the best route of delivery, 3) identify off-target effects to non-tumour tissue, or 4) optimise treatment schedules in a complete living system capable of inactivating and/or clearing drugs from the body. Taking this into account, we rely on data obtained from well-designed animal experiments to determine the true efficacy of drugs before translation into 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?

This animal number has been estimated based on current and planned research activity. Protocols are based on previous work where statistical differences in tumour burden or survival have been demonstrated. Typically, group sizes require 6-10 animals (dependent on mouse model/therapy) to provide sufficient power to detect statistically significant differences. In vivo passaging of cells is required for some tumour models, where this is required animal numbers are low and determined depending on the downstream experiment; typically, tumour cells obtained from one animal is enough to seed tumours in 4 recipient animals.

Where possible, we will use imaging and blood sampling to monitor tumour burden longitudinally in the same animal to avoid unnecessary killing. This will reduce the number of animals used.

As part of good laboratory practice, we will write standardised protocols for each experiment including the study objective(s), experimental plan (animal numbers/groups and schedules) and methods that will be used for downstream analyses of results. While the overall numbers of animals may appear large, 2500 animals over 5 years equates to 500 animals/year to cover a range of different research projects, carried out by multiple staff. The estimated use for protocol 2 and 3 (therapeutic intervention protocols) is 1000 (protocol 2) and 1300 (protocol 3) animals over 5 years. An additional 200 animals are expected to be required for protocol 1 (in vivo passage of cells). In total, this is the equivalent of 500 animals/year for the project.

A typical study would have a minimum of 6-10 animals/treatment group, simply testing one agent vs controls would be 12-20 animals for one small experiment, and 3 biological repeats of the same experiment would require 36-60 animals. Moreover, more complex testing of combination therapies, which requires testing of single agents as well as the drug combination, means that these numbers rise rapidly. The maximum estimated number of animals required for this project were therefore calculated are based on the last 5 years' experience of working with different cancer models, alongside current and predicted research activity.

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

Protocols are based on previous studies where efficient statistical differences have been demonstrated. In therapeutic studies (protocol 2 and 3) typically 6-10 animals are required per treatment group (dependent on mouse model) to provide at least 80% power to detect differences using analysis of variance. Routine passaging of cells (protocol 1) will be decided based upon the number of animals required to generate sufficient cells for subsequent downstream studies. Normally

tumour cells from one animal will be passaged into 4 recipients, with some cells remaining for the generation of frozen stocks to be used at a later date.

We will continue to re-evaluate animal numbers in line with new developments and technology. In particular, we would like to improve in vivo tumour imaging which will enable us to carry out longitudinal experiments (with repeated measurements) in the same animal, rather than having to kill cohorts to obtain the same endpoints (i.e. tumour burden) ex vivo. Advances in these areas will improve experimental design and group randomisation prior to therapeutic intervention

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 continually review animal numbers throughout the course of this project license and try to establish more sensitive methods for monitoring tumour burden (e.g. imaging technology). For each experiment, we will ensure that tissue from each animal is fully utilised, this will allow us to thoroughly assess biological changes and maximise the output from each study. For example, we will endeavour to share tissue that is not needed for our study with other researchers, so all tissue is fully utilised.

For new studies, where data on therapeutic efficacy is not available, pilot studies and power calculations will be carried out to estimate the size of the likely therapeutic benefit before group sizes are finalised. Statistical advice will also be sought throughout the programme to take into account any new developments and reduce animal numbers.

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.

Mouse tumour models

Mouse models will be used because these are the lowest form of mammal recognised as relevant for human cancer. Mice do not develop spontaneous cancer at a rate compatible with experimentation, therefore tumour cells will be implanted into the mice. In general, we will use two types of mouse models to study cancer and the effects of new or existing cancer therapies. The first model uses mouse tumours implanted into mice with a fully functioning immune system. This is important as the therapeutic agents we test are act on immune cells (e.g. termed immunotherapies) and harness the immune system for their anti-cancer effects. Tumour models will depend on the study but will encompass models of both haematological malignancies and solid malignancies. A second model uses human tumour cells implanted into mice that lack part of their immune system or mice that have been engineered to develop parts of the human immune system. These models will be used less frequently but are important when mouse models of cancer do not closely resemble the characteristics of human

disease. As tumours grow in each of these models, the health of all animals will be closely monitored to avoid pain and discomfort associated with tumour growth. If necessary, animals will be humanely killed.

Methods

Throughout this project, we will use practices that minimise stress, harm and pain to animals. For example, we will only use single-use needles for injections to avoid pain from dulled needles. For tumour cell injection we will do this via the tail vein (for models of haematological malignancies), intraperitoneally to model ovarian cancer or subcutaneously for other cancer types (e.g. colorectal cancer). After tumour cell implantation, we will monitor tumour burden using various methods depending on the tumour model, these include bioluminescence (IVIS) imaging, blood sampling, assessment of animal grith/weight, calliper measurements, and/or monitoring of disease-related symptoms. These studies will be done with and without therapeutic intervention.

All procedures are mild, and it is not anticipated these will have adverse effects. However, mice will be monitored daily for symptoms of disease or adverse events associated with treatment. Our work is based on the humane treatment of animals and all work will be carried out by fully trained staff to ensure the highest standards. Discomfort will be limited to unavoidable procedures that are required to conduct valid research. If at any time any animal is showing signs of ill health or continued distress they will be killed humanely.

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

A whole organism is required to establish the effectiveness of novel anti-cancer treatments. As such, mouse models are extensively used in the study of cancer, cancer immunology and to test cancer immunotherapies. There is a wealth of information available on mouse models and they are reliable and reproducible models of the disease. Mice are also very similar to humans genetically, and the process of cancer development is conserved between mouse and humans. Mice that lack a fully competent immune system also allow the growth of human tumours and also allow us to study human disease in a living organism.

Less-sentient species (e.g., zebrafish) can be used to study tumour development; however, restricted tumour size means that are unlikely to develop the full repertoire of immune evasion strategies that prevent the immune system from eradicating the tumour. Thus, this makes it difficult to establish the true effectiveness of novel immunotherapy strategies. For our studies, which are aimed at examining the efficacy of treatments against primary tumours and/or metastatic disease, mice are the most reliable to model tumour progression and treatment delivery.

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

General signs of ill health and discomfort will be assessed using the principles of the grimace scale for mice. Moreover, mice will be monitored regularly for other signs of discomfort, including any changes in behaviour, weight loss and movement. We will also attempt to identify earlier endpoints for our studies, to minimise harm and potential discomfort. To minimise harm to animals, we will strive to use refined mouse models of cancer which identify earlier endpoints that do not rely on the animals showing signs

of being unwell, suffering and pain to mice. Where possible, we will use specialised non-invasive imaging techniques (carried out under anaesthesia) that can detect the disease at much earlier stages. We will make sure that each cancer model is reliable and reproducible, this will also allow earlier endpoints to be used. For example, increased monitoring around the expected study endpoints (e.g., via imaging, blood sampling, calliper measurements or detailed visual inspection for early disease-related symptoms) will allow mice to be humanely killed before significant disease-related symptoms develop. Some of these refinements will also allow reduce the number of mice required and tumour burden and biological readouts can be observed and monitored in living animals.

Only animals that are in good health will be use and animals which lack a fully functioning immune system will be housed in ventilated cages to minimise their risk of infection. Any procedures that could lead to pain or discomfort will be performed with anaesthesia or appropriate pain relief. All animals will be regularly monitored for possible adverse effects that may occur in response to the tumour growth, procedures or treatments. If required, we will seek advice from the Named Veterinary Surgeon (NVS) and Named Animal Care & Welfare Officer (NACWO) (e.g. for administration of antibiotics if an infection is suspected). Small pilot studies will be used to identify optimal treatment regimens including drug dosing, timing of therapeutic intervention and route of administration before proceeding to larger scale studies.

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

Procedures for establishment and monitoring of tumour growth, metastasis and animal welfare will follow best practice guidelines (e.g. NCRI Guidelines for the welfare and use of animals in cancer research, Workman et al. 2010 Br. J. Cancer). We will also consult the NC3Rs website for information on the 3Rs. Published guidelines (Guidance on the Operation of the Animals (Scientific Procedures) Act 1886) which highlights recommended volumes for blood sampling and number of procedures will be followed.

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

I regularly receive NC3Rs online resources and updates and am a member of our local HO licence holder committee which meets regularly to discuss animal welfare and important developments. I also attend local, national and international conferences and read peer-reviewed literature to remain up to date with new advances. Importantly, animal experimentation will not be carried out until we have generated a significant amount of in vitro data demonstrating the effectiveness of novel therapies against human disease, and the appropriateness of selected mouse models.