Home Office

NON-TECHNICAL SUMMARY

Anti-cancer therapy validation

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 cells, Hypoxia, Tumour microenvironment, Therapy

Animal types	Life stages
Mice	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?

To generate robust proof-of-concept data that either drives forward or stops progression of 3-5 anti-cancer therapies. We are focussing on therapies that influence the local environment of the tumour (called the tumour microenvironment) and/or immune contexture, which is the number, location and type of immune cells in the tumour or in the bloodstream.

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?

According to Cancer Research UK statistics there are around 375,000 new cancer cases in the UK every year, and around 167,000 cancer deaths. Whilst significant progress has been made in the treatments for many types of cancer, some have seen little improvement over many years. In these latter cancers of current unmet clinical need, standard-of-care treatments are often poorly tolerated, with significant side effects and negative impact on patient quality of life. Using new therapies that are able to target more specific characteristics of cancer can have significant benefits to patients. Examples include the use of immunotherapies and molecular targeted agents. However even here, patients can show variable response and over time, the therapies can stop working. It is important to undertake further research and animal studies with new therapies for cancers of unmet clinical need, ensuring that we gain a fuller understanding of how, when and where to use them to maximise patient benefit.

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

This project will generate data, new knowledge in the area of cancer. We will learn more about the interaction between cancer cells, the tumour microenvironment and the immune system, which controls how well a tumour responds to treatment. We will generate proof of concept data i.e. information on the effectiveness and/or side-effects of new therapies. This proof-of-concept is needed to progress a specific therapy to clinical trials. Alternatively, the proof-of concept data may show that the therapy tested is not very effective and/or has unacceptable side effects, which is essential information to enable us to say that particular therapy is a “no-go” and should not proceed to trial in humans. Further, benefits will include publications and presentations, to both scientific and public groups.

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

Over the duration of the project the main benefactors from this research will be the research group, others at our establishment, other researchers and, potentially, pharmaceutical industry should they collaborate in the research programmes undertaken. Over longer timescales (5-10 years), progression of therapeutic approaches into clinical trial would yield benefit to patients and clinicians.

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

Work (with both positive and negative outcome) will be published in open-access journals, presented at scientific meetings, shared with public and patient groups and funding bodies. Resources will be made available to other researchers (e.g., data, animals, tissues) to enable collaborative work. Data, where not prohibited by patent law, will be deposited in publicly available databases such as MGI (mouse genome informatics), MGB (Mouse Genome Biology) and XNAT (imaging data). We have an internal system within our groups in the department and more broadly at the establishment to offer materials that are not being used for our experiments but may be useful for others e.g. we often donate bone marrow from our mice to groups working in that area. I have national and international networks with researchers in the same field and we routinely share our in vivo data outcomes in order to generate consensus guidelines and refine processes moving forward. I have personally been involved in creating these guidelines at national and international level.

Species and numbers of animals expected to be used

- Mice: 3100

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 because it is not yet possible to model fully the complexity of the interacting cell populations within a tumour, the dynamic state of the local environment of the tumour (called the tumour microenvironment) or complex normal tissue responses that may underpin toxicity in any lower species of animal or at any early life stage than adult mice.

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

Animals will be anaesthetised and implanted with identification chips and tumour cells under the skin or in specific sites in the body to match the human disease e.g. brain cancer cells into the brain. Tumour growth will be followed by direct measurements of tumours under the skin (eg using callipers or imaging or if the tumours are in another part of the body and can't be measured by callipers, via imaging) for which animals will be anesthetised and may receive a “tracer” that allows contrast to be seen between the growing tumour and surrounding normal tissue. If required to follow metastatic tumour growth and/or therapy response, surgical tumour removal will be performed. Therapies will be administered via standard injection routes, sometimes in combination with, for example radiotherapy (that is restricted to the tumour site) or standard therapy in the clinic currently e.g. chemotherapy or immunotherapy. Samples (blood, tears or tumour biopsy) may be taken to monitor changes over time. Prior to humane killing at the end of the experiment, animals may receive pathophysiological markers that allow clearer evaluation of tumour and/or normal tissue biology at post mortem. Animal weight, wellbeing and tumour condition will be monitored regularly throughout using refined approaches and appropriate interventions (e.g. dose reduction, decreased frequency of dosing, supportive measures such as mash or topical emollients for skin dryness) made where necessary.

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

Most animals will be implanted with tumours. Tumour implantation and growth may cause some changes to skin condition. Animals will feel transient pain/discomfort upon administration of therapeutic agents. Weight-loss/reduced of weight gain compared with naïve animals may be observed over the time-course of therapy interventions. Late stage disease/pathology may induce change in well-being, condition and behaviour of very short duration as this would indicate termination of the experiment.

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)?

Moderate for >90% of animals, mild for <10%.

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?

We cannot yet model fully the complexity of the interacting cell populations within a tumour, the dynamic state of the tumour microenvironment or the systemic complexity that may underpin toxicity (e.g. cognitive decline following brain tumour treatment) in any non-animal system (e.g in cells grown in the lab). Ethically we cannot test these facets in the clinical setting and therefore must use species with physiology that best represents what we would expect to find in the human diseased state. Therefore, we need to use animals (mice) as they very closely model the complexity of whole organ system interactions in human disease. Animal studies will enable us to answer the key aims of this project, as they allow us to determine the holistic effects (ie effects on multiple systems concurrently such as the tumour, the immune system, blood flow, oxygen levels) of therapies, which is ultimately what determines their overall efficacy.

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

However, to replace the use of animals, where possible, we are working with materials scientists and engineers to develop complex systems outside the body/animal as better future mimics, which are informed by what we learn about cancer biology in our animal models.

Before initiating mouse studies we undertake comprehensive studies in tissue/cells grown out of the body, which include using 3D-cell systems and modified culturing conditions, such as manipulation of

oxygen availability, to mimic tumour conditions inside the body. Coupled with computerised models that tell us how the drug will interact with the chosen target in the body and predict how the drug will be absorbed into the body and processed so that it reaches the tumour (this is called Pharmacokinetics), these important pieces of data enable us to make informed go/no-go decisions as to whether a study should progress to using mice.

Why were they not suitable?

We routinely use these systems to do thorough testing of potential new treatments and the cancer biology associated with them to be sure we should progress to in vivo work. In some instances, non-animal systems are sufficient to answer basic questions (e.g. do the treatments make tumour cells grow slower or die) on potential new treatments and therefore can partially replace animals.

However, although these non-animal alternative systems are useful for making informed go/no-go decisions as to whether a study should progress to in vivo work, we are still unable to appropriately and reproducibly model the complex, dynamic interplay between the tumour, its local environment and the whole organism - and it is the complex interplay of these factors with the therapy that dictates if the therapy will work.

As a result of these limitations of non-animal models, to achieve the aims of this project studies need to be conducted in whole animals.

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 estimated to be used per annum is based on our previous experience of delivering analogous objectives to those here and the usage from other similar licences e.g. previous PPLs of collaborators and colleagues.

From this, we have a very good estimate of the number of mice it takes to deliver the package of data required by drug regulators to decide if a treatment should progress to clinic - the so called "go/no go decision making" for clinical trial.

For example, a series of experiments to get proof of concept in protocol 1 consists of approximately 60 animals and 25 studies are planned over the license period giving an expected requirement of 1,500 mice.

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

We will use the study design methodologies we have developed over previous licences that reduce the numbers of animals used by which aim to reducing variability, improving experimental consistency and confidence in outcomes. Inherent variability is minimised by using adult, same sex, age matched animals.

The data we generate for individual models is used as a cumulative resource for sample size calculations (ie we continually pool control data or single intervention data where we are interested in the impact of a novel combination approach). Having robust control/single intervention data to use in sample size calculations reduces the number of animals needed to demonstrate an effective outcome of a novel intervention .

To reduce the numbers of animals used in tumour studies, treatments will be initiated when tumours are of equivalent size which we have previously shown offers a substantial reduction compared to using time-matched treatments on tumours of variable size and underlying pathphysiological heterogeneity.

We will use the NC3Rs Experimental Design Assistant for new more complex studies- we have previously adopted this approach in our normal tissue work whereby we are integrating multiple assessments- behaviour, imaging, post-mortem tissue analyses - and which reduces the numbers of mice required to generate statistically meaningful data.

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

Our routine adoption of imaging allows continuous non-invasive monitoring of disease progression, reducing numbers and enabling us to intervene earlier with minimal detrimental effects.

Archived control data (non-treated animals) are used as a cumulative resource to allow recalculation of sample sizes and consequent reduction in group sizes. If several related agents are assessed, initial experiments use 2 animals per dose level. If both fall beyond the 95% confidence interval for the control data, the agent is taken forward.

Pilot studies will be used, for example if: 1) a model hasn’t been established in mice by the group previously, 2) the likelihood of a model spreading to other sites in the body (metastases) has not been established 3) the marker in the mice that we want to use to track the biology of the cancer and/or track the response to treatment has not been previously evaluated (these tracking methods could be imaging or tissue based: blood, tears, tumour biopsy). The numbers per group will not exceed 6 for (1), 12 for (2) [spread to other parts of the body (metastases) is less uniform i.e. more variable than tumour growth, so requires increased numbers] or 6 for (3). Data from these initial pilot experiments will be used to define group sizes using power analysis.

We run monthly "In Vivo User Group" meetings, which provides a forum for us to share tissue from upcoming experiments. Examples of this is providing colons from irradiated mice to a colon research group, harvesting bone marrow for the immune cells for immunology research and harvesting skin for use in skin research labs.

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.

Mice will be used as they are the species with the lowest neurophysiologic sensitivity in which a range of well-characterised models of cancer exist. Further correlative studies between mouse and man indicate the potential for results to translate between the species.

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

Mice of a more immature life stage or less sentient species lack the complex, dynamic interplay between the tumour and the micro and macro environment, particularly with respect to the effects on the immune system: a key readout of the efficacy of the anti-cancer therapies we are investigating. Therefore, we cannot achieve our aims by modelling cancer in any organism of lower order than adult mice.

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

We have refined and developed imaging approaches that inform tumour size, how their blood vessels are working, how much oxygen they have and, by extrapolation areas of the tumour that are still functioning (viable) and those that have been destroyed by the therapy (non-viable). Furthermore, data acquired from imaging studies is being used as a resource to develop data-derived mapping of tumours with a goal to stratify regions dependent on response. All of these approaches are fully translatable to the clinic.

We have moved away from using injectable anaesthetics by adapting equipment to allow gaseous anaesthesia use that allows more control of the depth and duration of sedation.

In our surgical techniques, we have moved away from using skin staples and reverted to stitching which enables more freer movement of muscle and skin layers whilst healing which reduces irritation to the animals. We also use a painkiller (eg buprenorphine) before a surgical procedure called a laparotomy, so that it is active by the time the mouse recovers from surgery.

We have found that for some tumour models, scabbing occurred, which can be reduced by implanting the cells at an increased distance away from the injection entry site.

We don’t cull immediately mice that haven’t grown tumours, but instead use them as company for mice remaining on study.

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

Adherence to LASA Guidelines, Workman et al, Joint Working group on refinement (superseding LASA for substance administration), PREPARE guidelines: readily available, fully researched guidelines that provide clear guidance on how to conduct animal experiments such that the harm:benefit ratio of the work is optimal.

We will also follow guidance developed by my group and collaborators groups, during the course of previous licences of which these are some examples:

CRUK Roadmap for developing imaging-based biomarkers

FOSTER guidelines for developing patient-derived models and preparing European wide guidance on the optimum use of osteosarcoma (bone cancer) models in animal research.

Collaborator - whose team will work under this PPL - has developed policy guidelines for the refinement of drug and radiotherapy studies at international level, which has been adopted by drug regulators around the world (FDA, EORTC and AACR).

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

Newsletters from the "NC3Rs" and Establishment "3Rs Managers". "NC3R" is an initiative that funds research into and shares information on the reduction, refinement and replacement of animal research.

"LASA Guidelines" and "Workman et al, Joint Working group on refinement" (superseding LASA for how to administer therapies) and "PREPARE guidelines" are all readily available documents that provide clear guidance on how to conduct animal experiments such that the harm:benefit ratio of the work is optimal.

We have presented data at and attended meetings in these areas and will continue to do so.

We have regular local meetings for all colleagues who work with animals under our PPL. We use this forum to share best practice and discuss ways we can refine our procedures. We work alongside several other groups locally, nationally and internationally.