Home Office

NON-TECHNICAL SUMMARY

Development of Molecular Imaging and Theranostic Agents for Cancer and Inflammation

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Cancer, Inflammation, In vivo imaging, Theranostics

Animal types	Life stages
Mice	adult
Rats	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 overall aim of this project is to develop and evaluate new ways to image and identify cancer and inflammation. Some of these methods are aimed at providing information to develop new therapies for these diseases.

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?

Improving dedicated in vivo (performed in a living organism) imaging methods to better understand the biology of cancer and inflammation is desirable as there are many areas where improvement is needed in the management of these disorders. We aim to develop new imaging methods to improve our ability to detect disease and to determine if treatments are being effective. New therapeutic strategies are also expected to be derived from this work.

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

The end goal of this research is to develop and characterise imaging methods that may be of benefit to the treatment of human cancers and inflammatory diseases. The imaging tools that will be developed will increase our understanding of disease biology, provide means of predicting the efficacy of therapeutic interventions and help establish the basis for new therapeutic clinical trials.

This programme of work is expected to provide the following benefits:

- Development of imaging and theranostic (therapeutic approaches driven and tailored by diagnostic observations) agents as companion diagnostics to “fast-track” the process of drug discovery; and

- Identify the best imaging and theranostic agents for translation into clinical use.

- Publication in peer reviewed scientific journals, dissemination of findings, including unsuccessful approaches or non-significant data via open access and through platforms such as F1000 Research.

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

Short term increase in knowledge which will be public domain in the scientific community and will serve as reference for further research.

Useful imaging and theranostic tools generated from this programme of work may be used to fast-track the process of disease characterisation and drug discovery at the preclinical level.

This work is intended to provide the basis for clinical trials making use of the products of this research. These may benefit patients suffering from cancers and inflammatory disorders in the future.

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

The results generated from the research will be shared with the scientific community through internal meetings, conferences at both local and international level and peer reviewed scientific publications.

Collaborations with laboratories and institutions with expertise in different scientific areas to include chemistry, radiochemistry, chemical engineering, pharmacy, physics, basic oncology and immunology, clinical imaging and clinical and veterinary medicine have been established to carry out the proposed work.

Species and numbers of animals expected to be used

- Mice: 1500
- Rats: 60

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 are developing new ways to image cancer and inflammation and find novel ways of treating these diseases. While much of the preliminary work can be done in tissue culture and other in vitro systems testing in rodents is the mainstay of preclinical development of these imaging methods as it provides the most sensible way to demonstrate whether the new methodology has potential for further application in humans. Adult mice and rats, some harbouring specific tumour models will be utilised. These are the most commonly utilised and most accepted animal models in this type of research.

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

The procedures carried out on these animals are standardised, generally accepted and will be conducted abiding to humane treatment conditions of animals involved. Where possible healthy control animals will be utilised for imaging. Animals will be administered imaging agents by injection or ingestion and their distribution in the body will be measured with dedicated imaging equipment and modalities. These types of procedures are mostly commonly utilised in humans but we intend to

develop new and/or improved agents in this arena. Depending on the specific experiment we will be utilising different types of molecules or other agents. These will range from tiny amounts of radioactively labelled small molecules, peptides or proteins to larger structures such as synthetic nanoparticles or radiolabelled cells to be administered in the same way. Similar agents designed to alter tissue density for computed tomography, provide fluorescent light or generate signal to capture with magnetic resonance imaging will also be utilised. All these agents are designed with the intent to not alter physiology of the animal and will in most cases be utilised in quantities that will have no effect on the animals' wellbeing.

The imaging procedures are carried out under anaesthesia and can last from a few minutes to a few hours. In healthy control animals the imaging experiment is likely to only be carried out once. Imaging agents such as cells or other substances showing promising features for further development will be utilised in appropriate models of disease. The experimental work will involve generating tumour models (to start with, animals injected with tumour cells reproducing targets normally seen in specific human tumours that we plan to image). We will routinely measure tumour size either in awake animals with calipers or through imaging while under anaesthesia. We will on occasion administer drugs with food or water or more frequently use injection. Blood samples may be taken from the animals in order to measure levels of the imaging agents, drugs or for evaluating treatment effects. In some experiments tumours may be treated with radiation. In order to measure the effects of the imaging agent, some animals may need to be housed alone or food may be withdrawn temporarily on very rare occasions.

Particular attention will be given to limiting the number of disease model animals utilised to that strictly necessary and ensuring an adequate time window for the experiments is used to avoid exceeding severity estimates. Imaging with the new methods will be used at different stages of this process and will be the main objective of this research. In select cases the agents developed may be tested for their ability to inhibit cancer growth in time course experiments. All these procedures will be carried out using standardised and most refined methods available

At the end of the experiments animals will be killed by a humane method such as overdose of anaesthetic and the organs and tissues collected for further experiments. Tissues may be frozen and stored for use in future studies.

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

Most imaging studies will be carried out in unconscious, anaesthetised animals and with careful monitoring there should be no side effects.

As cancer and inflammation are main focuses of the research, disease specific models will need to be utilised for some of the experiments. This will involve implanting tumours or inducing inflammation which can affect animal wellbeing and careful consideration will be given to minimise the impact of this. The tumours may take 1 week to 3 months to grow to a stage required for imaging and treatment studies. For most of this duration the animals will be unaffected by the tumour growth. When and if they do experience signs of distress these may include weight loss, ulceration of the tumour or impaired movement, whether due to the tumour or the treatment. If 10% weight loss is maintained over 3 days, then additional support will be provided such as easy access to soft and palatable feed and pain relief. In addition, animals showing signs of ill health e.g. hunched posture, piloerection or

subdued behaviour which persists for 24 h and cannot be ameliorated by minor veterinary interventions, will be humanely killed before the severity limits are reached.

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)?

Mice

Non-recovery: 16.6%

Mild: 50%

Moderate: 33.3%

Severe: 0%

Rats

Non-recovery: 50%

Mild: 50%

Moderate: 0%

Severe: 0%

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?

Cancer and inflammatory disorders are dynamic processes that require assessment in living organisms. Use of non animal alternatives to these types of studies are very limited but will be utilised to all extent possible. It is necessary to conduct studies on animals as the in vivo behaviour of imaging agents, cancer cells and the immune system cannot be replicated in vitro or by computational methods.

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

Before considering experiments in living animals, the imaging agent candidates will be tested on computer models, cells or animal or human tissues (in vitro studies).

Imaging methods will initially be tested using objects called imaging phantoms that stand in for animals. Use of imaging phantoms will be applied where possible in order to minimise the number of animals required for imaging experiments for the purpose of optimisation of acquisition parameters and setup of the imaging procedures.

Tissue culture experiments assessing the biological properties of imaging and therapeutic agents being tested will also be carried out preliminarily to obtain information on the suitability of the procedures being developed.

Why were they not suitable?

In vitro and computational methods are not fully representative of the biological characteristics of cancer cells and the immune system within living organisms. These non-animal alternative methods can only provide preliminary information on the properties and target selectivity of the imaging and theranostic agents to allow preliminary screening and selection of the best agent for further evaluation in animals. Only animal models can reproduce the complex interactions between organ systems that may affect the behaviour of the imaging agents.

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 estimated number of animals for each experiment are based on statistical consultation, references from published scientific papers and our previous research experience and published track record in conducting studies on small animal imaging and therapeutics. A minimum of 10 animals per group is often used in imaging studies, and each imaging study may involve 3 or more imaging and treatment groups.

In most experiments the same animal is imaged at different time points which will reduce the overall animals necessary for the research (e.g., scan one group of animals from 0 min to 2 hours in order to obtain the time dependent distribution of imaging agents into various tissues rather than different groups of animals that are killed at various time points within that 2 hours).

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

Computer modelling and in vitro studies on human/rodent cell lines and tissues will be used to decide whether the imaging agents can proceed on to animal testing.

All experiments will be designed in consultation with radiochemists, biologists, imaging scientists and animal facility staff via study plans. Imaging data analysis plans will be developed under the guidance of trained imaging scientists and statisticians consulted where required.

Pilot studies will first be conducted on small groups of terminally anaesthetised animals to select the best imaging agent for further evaluation as well as to determine the number of animals required to obtain robust data.

Reduction in animal use is further achieved in longitudinal experiments where the same animal can be imaged over a refined timeframe and acts as its own control, without the need for a separate control group. In addition, tissues will be harvested from the animals after the last imaging timepoint for archival and further in vitro evaluation. This will reduce the number of animals required.

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

Where possible tissues obtained after the animals are killed will be utilised to generate pilot data for future experiments. Being a part of a large biomedical research campus with many research groups working on different aspects of cancer and inflammation, such tissue may be obtained from other groups within the university via internal mailing lists. Such expertise will also be consulted and used to refine our techniques.

Resources such as the below are consulted.

https://norecopa.no/PREPARE

The Experimental Design Assistant - EDA | NC3Rs

https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction

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.

This project will utilise normal mice and rats as well as mouse models of tumour and inflammation. Rodents are the most appropriate model for the preclinical development of imaging and theranostic agents. The biological characteristics of cancer and the immune system in rodents are similar to those

of humans. Several well-defined genetically modified strains and protocols are available, and resources exist for the development of additional models. Most of the imaging methods used are either non-invasive or minimally invasive. Animal suffering will be minimised by careful observation of the animals undergoing procedures. Guidelines for the assessment of clinical signs will be strictly followed, and experiment on the particular animal or cohort will be immediately terminated and the animal will be humanely killed upon observation of clinical signs classified as above moderate to severe. Dedicated imaging equipment for use in mice and rats will be utilised in this study. These scanners are designed to closely mimic state of the art clinical scanners and have been developed with imaging of rodents in mind as these models are well recognised to be the ideal preclinical model for this type of setting.

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

Rodents are widely recognised as the least sentient species to carry out research of this type. Cancer and inflammation are complex diseases that many aspects of pathogenesis and response to therapy are widely approached utilising rodent models. Rodents are the best model to use instead of higher organisms such as higher mammals or non-human primates. There are well-defined protocols and regulated procedures for studying cancer and inflammation in rodents.

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

We will have regular discussions and reviews with our in house animal welfare team, including the NVS and NACWO, who have a wealth of knowledge on best practice for implementing refinements to optimise animal welfare, (such as non-aversive handling techniques).

We will use scoring systems to monitor and record the clinical signs in animals. Monitoring will be increased in later stages of disease models. Trained animal technicians will support in evaluating welfare and pain monitoring using animal grimace scales.

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

Refining procedures for the administration of substances.
https://journals.sagepub.com/doi/pdf/10.1258/0023677011911345

Research Animal Training: https://researchanimaltraining.com/

PREPARE guidelines and checklists will be consulted. https://norecopa.no/about-norecopa

NC3Rs website https://www.nc3rs.org.uk/.

Focus will be given to the updated ARRIVE guidelines https://doi.org/10.1371/journal.pbio.3000410 updated in 2020 to design experiments that can be properly executed and reported.

Other resources from these websites will be consulted.

(LASA) Laboratory Animal Science Association

(AALAS) American Association for Laboratory Animals Science

(FELASA) Federation of European Laboratory Animal Science Associations

(ICLAS) International Council for Laboratory Animal Sciences

(InterNICHE) International Network for Humane Education

Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/laban.1217

Workman et al (2010) Guidelines for the welfare and use of animals in cancer research | British Journal of Cancer (nature.com)

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

The local University Biomedical Services has extensive documentation regarding 3Rs including links to publications, portals and websites with information pertinent to all commonly used laboratory animals. This information is continuously updated. Staff involved in animal handling and experiments undergo rigorous and continued training on these issues and have all these resources available.