Home Office

NON-TECHNICAL SUMMARY

Analysing mechanisms of cancer dissemination and therapy failure

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

cancer, metastasis, therapy, imaging

Animal types	Life stages
Mice	adult, pregnant, juvenile, neonate, embryo

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 over-arching aim of this work is to achieve a better understanding of the mechanisms governing the spread of cancer and its response to therapy. This knowledge will help to inform the development of better strategies for cancer prognosis and treatment. It will also enhance our understanding of mammalian tissue organisation.

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 a major cause of mortality in the UK, responsible for over a quarter of all deaths. Even modest improvements in treatment will benefit the lives of thousands.

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

The outputs from this work will include.

1. A better understanding of how the interplay between cancer cells and their microenvironment influences both how cancer spreads and its response to therapies

2. Improved treatment regimens for controlling or eliminating cancer in pre-clinical models

3. Improved understanding of how to mimic the complexities of the tumour microenvironment in reductionist systems

4. In practical terms, the new information generated will be shared via publications, research presentations at conferences (both national and international), and, if appropriate, the reporting of improved methods

5. Improved imaging methods and new prognostic strategies are also potential outputs, although not the immediate aim of this project

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

There are likely to be multiple beneficiaries from the outputs above. These include:

1. If any of the genes implicated are ‘druggable’ targets then we expect that biotechnology and pharmaceutical companies may utilise the information produced in their drug discovery programs. If possible we will try to develop some targets with the appropriate technology transfer teams.

2. Another possibility is that the identification of important regulators of cancer dissemination may lead to improved cancer prognosis. This would enable better clinical management of patients. Thus there is a small, but real, possibility of patient benefit arising from this work within 5-10 years. In support of this, work from the previous PPL has recently obtained funding to explore its importance in large human patient cohorts. This is evidence that the type of work here is being exploited to try to directly improve patient management.

3. This work will benefit the basic research community by increasing our knowledge of mammalian physiology and cell biology.

4. An improved understanding of the details of how the tumour microenvironment influences the metastatic process and how tumours respond to therapy should enable better in vitro models to be developed. Indeed, the development of improved ‘organotypic’ culture models is an active area of research in the Tumour Cell Biology lab . This may ultimate reduce the number of animals used in research.

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

A major mechanism to maximise the output of the work alongside the publication of primary research papers, will be presentation of the work at both big international meetings and smaller more methods oriented workshops. These latter formats have the advantage that details of approaches that were ultimately sub-optimal can be shared.

Our group already collaborates widely and this provides another avenue to share details of approaches that were ultimately sub-optimal and how methods were improved.

If sufficiently transformative changes are implemented to methods, then we will look to publish specific protocols papers and post on bioxriv.

Species and numbers of animals expected to be used

- Mice: 6000

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 aims to understand how cancer cells interact with other cells in the body, and how their ‘communication’ affects the spread of cancer and its response to therapy. To do this, it is necessary to work with animals that have similar organs to humans – for example, lungs and mammary glands. This leads to the choice of mice. The main cancer types that we study occur in adults, and occasionally, young adults. Therefore, we work with juvenile and adult mice.

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

Procedures will be performed that lead to the development of cancer in mice. In the majority of cases, this will be through injected cancer cells. Mice will then receive therapies similar to those being used or developed to treat patients. Advanced imaging methods will be used to monitor the spread of tumours and how they react to therapies. Surgical procedures, such as the implantation of devices to aid imaging or the removal of primary tumours, will also be performed on a subset of mice. Extensive post-mortem tissue analysis will be performed to maximise the information obtained from each animal.

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

Mice will develop tumours. Initially, these have little effect on the mice, but as they become larger they might affect mobility. Further, as tumours begin to spread they can affect weight, breathing, and behaviour. Depending on how much the animal is affected, the duration of the adverse effect may range from a small number of days to a small number of weeks.

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 severity is expected for about a third of the animals.

What will happen to animals at the end of this project?

- 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?

We are interested in understanding how cancer cells interact with other non-cancerous cells. Therefore, we need to work in systems where the non-cancerous cells are as similar to the human as possible, both in their intrinsic features and their tissue organisation. The latter point of accurate tissue organisation is particularly hard to recreate using reductionist in vitro systems. Large parts of our work involve either studying breast cancer, lung cancer, or metastasis to the lung and this requires the use of organisms that have lungs and mammary glands. The mouse is well suited to this as it is a small mammal with relatively simple husbandry requirements. Further, well-established methods exist for genetic alteration in mice, which facilitate analysis of how cancer cells interact with non-cancerous cells.

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

Yes, we have considered and use alternatives for much of the work in our group. Our group has made extensive use of complex co-culture models. Zebrafish models of cancer can be informative, but fish lack lungs and mammary glands, which are key tissues for the cancers that we study.

Why were they not suitable?

It is currently not possible to replicate the complexity of mammalian tissue structures in culture models. For example, the grape-like architecture of alveoli and their associated blood vessels cannot be mimicked even in state of the art 3D cultures. A second issue, is that the immune system only functions effectively in an organismal context with appropriate white blood cell movement and function within lymph nodes. Finally, metastasis is the transit of cancer from one organ to another. To study this requires not just recreating the environment of a single tissue, but to have multiple tissues linked together via the blood and lymphatic circulation. This is not achievable in simplistic non-animal models.

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 estimate is based on several factors. First, we have taken into account the numbers of mice used in the previous five years' experience. Second, we have taken into account the current number of researchers within the group. Third, we continually re-evaluate the numbers of mice required for each experiment using power calculations. Indeed, our laboratory employs a highly trained statistician who assists with experimental planning. This will allow us to determine the number of animals required per experiment. By combining this with the group size and availability or resources for data analysis, we are able to estimate how many experiments we will run per year (roughly 20) and therefore the numbers of mice required.

Numbers of mice used for breeding are based on best practice and we maintain between 5 - 10 strains.

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

Experiments will be designed to not falsely detect effects and not miss effects ($\alpha = 0.05$ and $\beta = 0.1$ in technical terms). Based on our previous experience of how variable our measurements are and how big the effect we are looking is, then most experiments involve 5 – 10 mice per group and 4 – 10 groups. If the necessary in vivo data does not exist, then experimental design will be informed by a combination of prior in vitro data generated in the laboratory, existing publications, and the cumulative experience of >15 years mouse tumour work.

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

Production, Breeding, Maintenance and Phenotyping

Mouse lines are routinely maintained by keeping 2-3 breeding pairs, with around 3-4 litters/year total 75-100 animals per strain/year. For crosses to enable characterisation specific phenotypes it is likely that 5-6 breeding pairs will be kept with 6-8 litters/year total 350-400 animals per strain. We anticipate maintaining up to a maximum of 10 lines at any one time.

To minimise breeding, lines under sporadic use are maintained at lower levels, and frozen whenever practicable. Lines will be maintained in collaboration with other licences wherever possible to minimise redundant breeding.

Defining mechanisms of cancer dissemination and therapy failure

We always aim to maximise the amount of data we get from each mouse. Whenever possible we try to use the same mouse for both intravital imaging and analysis of spontaneous metastasis. We take care to divide tumours into multiple pieces so that we can perform histological, transcriptomic, and flow cytometry analysis on tissue for the same mouse.

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We also utilise a new designs of imaging windows to obtain longitudinal data about changes in tumours and their response to therapy in the same mouse. This enables more data to be obtained for each mouse. Further, we are now using repeated imaging in the ear, which can be done with no surgical intervention. These experiments with repeated tracking of tumours are more powerful than simple endpoint assays as they reveal the ‘full history’ of the tumour. Therefore, we need fewer mice to draw robust conclusions. We are committed to implement further longitudinal imaging modalities in the coming years.

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 mice in this project. As the focus is cancer biology, this necessitates the generation of tumours. For the generation of primary skin and breast tumours, we will most often employ intra-dermal

and sub-cutaneous injections. For the study of lung tumours and lung metastases, intra-venous injections will be the route of choice. On rarer occasion, we will study metastasis to other organs and therefore use other injection routes. When using genetic models, we will endeavour to use external agents to deliver a special gene editing enzyme that initiates tumorigenesis. This reduces the complexity of the mouse crosses and avoids generating tumour prone mice outside of experimental requirement.

We take care to ensure that the extent of tumour burden is the minimum required to observe the cancer behaviour that is being studied in the specific experiment. In particular, the use of microscopic resolution imaging methods means that we are able to evaluate tumour spread with greater sensitivity and, therefore, a lower overall tumour burden in the mouse.

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

We have considered zebrafish and fruit fly cancer models, but these do not replicate key structures that we are interested in, such as the mammary gland and lungs. We do use terminal anaesthesia to obtain very detailed information about tumours. However, to understand properly responses to therapy it is necessary to analyse the same tumour before and after giving treatment, hence the use on non-invasive longitudinal imaging.

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

We try to minimise any possible adverse effects. In particular, the use of fluorescently labelled cells coupled with microscopic analysis of tissues enables us to detect small metastases. This reduces the overall tumour burden needed to be able to detect metastasis from primary sites. Similar benefits are expected from the use of non-invasive fluorescent or bio-luminescent imaging. If the purpose of the experiment is simply to observe cellular behaviours in the primary tumour, then we would not grow the tumours to the larger size that some of the metastasis experiments require. Regular monitoring by BRF staff is in place and we strive to keep updated with the latest environment improvements, such as enhanced environmental stimulation.

Surgical procedures will be performed with suitable anaesthesia and animals monitored post-surgery to ensure that they recover well. Where appropriate we will also use suitable analgesia. An example of our commitment to refinement is that we were the first group in the UK to implement an improved design of imaging window.

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

We are aware of NC3Rs, and ARRIVE guidelines. We also discuss with colleagues in other research groups new improvements that lead to refinement.

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

We will stay up to date via regularly communication with BRF staff, other sientists in the field and regular visits to the following website https://www.nc3rs.org.uk/3rs-resources .