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NON-TECHNICAL SUMMARY

Studying host interactions in tumour and metastasis

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

cancer, microenvironment, metastasis, immune cells, inflammation

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

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 licence aims to support the work of the laboratory in performing mechanistic studies on the interaction between tumour cells and the healthy host tissue cells. We have a particular interest in the relationship between tumour and inflammation and its relation with the rest of the cellular environment of cancer cells.

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?

One out of two people will get cancer in their lifetime, making cancer one of the most common diseases. Over the last 40 years, cancer survival has doubled largely thanks to better treatments but despite this more than 1 in 4 deaths in the UK is still caused by cancer. The fundamental understanding of cancer complexity is instrumental for the development of new and refined therapies. The studies on the synergistic cooperation between tumour cells and the host tissue cells holds the potential for novel and more effective anti-cancer therapies. Indeed, the interaction of cancer cells with their microenvironment is implicated in every aspect of tumourigenesis, from onset to metastasis as well as in resistance to chemotherapy. Understanding the inflammatory component of the microenvironment is of particular interest for the enhancement of efficacy of host-dependent anti-cancer treatment like immunotherapy. However, this complex physiology needs to be addressed within an mammalian system. Mice have a comparable physiology and the tumour disease trigger the same cascade of events as in humans. In particular, the human immune-system's reactions to cancer are highly recapitulated in mice, which are fundamental part of any therapeutic intervention and an essential focus of our work.

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

The expected benefits of the work will be mechanistic understanding in the immediate future and therapeutic benefit in the longer period. They can be summarized as follows:

1. To assist in the design of future anti-cancer therapies. We aim to discover novel molecules and proteins involved in the tumour microenvironment that can be efficiently targeted to weaken tumours making them more sensitive to standard therapies and reduce the risks of recurrence.

2. We aim to understand the impact of environment and lifestyle (for example; diet, inflammatory reaction, microbiota) on the tumour microenvironment and the impact on spread (metastasis) and cancer cell reactivation (dormancy). This will help the management of cancer patients and potentially help identify patients at higher risk of recurrence.

3. By studying the involvement of certain inflammatory components and systemic signals in cancer, we aim to clarify the mechanism of disseminated dormant cancer cell re-activation.

4. We also aim to better understand novel relation between cells of the tumour microenvironment and particularly the relation between the nervous system, inflammation, and cancer initiation.

5. Finally, this work will benefit the basic research community by increasing our knowledge of tumour biology.

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

The output of this work will initially be mechanistic information on cancer progression, identifying new potential drug targets, which will be used by the pharmaceutical industry to guide future potential treatments. The work will also provide valuable information to guide doctors in the clinic for the management of cancer patients with disease that may have spread to other sites in the body.

The short-term benefit will include presenting at scientific conferences and supporting other in vitro findings followed up with the publication of novel discoveries in international scientific journals. In the mid and long term the benefit will reach the pharmaceutical industry and clinicians, resulting in improvement of care for patients.

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

We will look to maximise the outputs of any significant findings through interactions with the scientific community through presenting at seminars and conferences. We also aim, where possible, to present unsuccessful data to prevent repetition by other labs. We also work alongside the Translation team who support collaborations with industrial and clinical partners that would reduce the bench-to-bedside time and enable possible changes in clinical practice (use of new drugs/screening tools) to be trialled sooner.

Species and numbers of animals expected to be used

- Mice: 45000

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.

My area of research is cancer; therefore, mice are for me the species of choice.

The reasons why mice are the best choice as cancer experimental models, can be summarized as follows:

• the physiology of cancer in mice is consistent with the human disease,

• The genome of a mouse is easy to modify allowing the study of particular genes in the cancer process. Mice can also be modified to be genetically prone to cancer (spontaneous genetically engineered mouse models) and this can allow a less experimentally engineered approach to studying the development and spread of cancer.

• In mice, there are many models that are commercially available, as well as well-defined techniques for production. There are also many scientific tools like genomic libraries, antibodies etc that are

largely available.•

In most cases where tumours will be induced, adult mice will be used, but there will be instances where juvenile animals may be used, for example; in order to pre-condition with dietary changes.

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

Typically, animals will develop tumours (either spontaneously through genetic modification or through transplantation of tumour cells by injection methods). In some cases, we will transplant tracer cancer cells (created by our lab) that can label the cells neighbouring the tumour, therefore tissue will be collected to allow further analysis.

Treatments (chemo/radiotherapy) could be employed in this setting. Drugs or antibodies will be used to study the roles of different host cell types on the tumour disease, generally by depleting or boosting them and looking at how the tumour develops, spreads and responds to treatments such as chemo or radiotherapy.

As we are interested in how environmental changes also impact cancer progression, we may use other interventions, such as; changes to the diet, infections with respiratory viruses, changes in gut bacteria (using antibiotic treatments). These interventions will have impacts on the host and we are interested as to how the changes caused by these will impact on the tumour and it's response to therapy.

The animals might have their disease monitored over time using non-invasive imaging techniques, particularly if they have internal disease. The duration of the experiments will vary depending on the model and will consider many features of the disease specific to the experiment, including the nature of the cancer cells, if transplanted (slow or fast growing, local disease or prone to spread) or the known timeframes in genetically engineered mice that form spontaneous tumours. We always aim to maintain duration of experiments to the minimum required to address the scientific need.

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

In most cases animals used in this license will form tumours. The size and impact of these tumours on the animal will differ due to model and method. For example, poorly aggressive tumour cells might grow well within the transplantation site but spreading to other areas of the body is poor and therefore the impact on the animal is low. However, with a more aggressive line tumours may spread early meaning that even though growth at the transplant site is not particularly high, the impact on the animal due to spreading to other areas of the body may produce more suffering for the animal.

We have much knowledge and experience of all the models that we use, both transplantation and spontaneous and are able to predict well the time frame over which the animals will not show adverse effects. The use of certain interventions can make this slightly less predictable, but the animals are monitored closely in this case to minimise any suffering and we aim to terminate experiments before the onset of undesirable adverse effects. Undesirable affects could be limited to the locality of the tumour (for example- ulceration) or be general signs of poor health, such as; weight loss, piloerection, hunching, increase breathing rate (especially when tumours have spread to the lungs).

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 expected severities predicted by this proposal are both mild and moderate.

For those protocols that involve tumour growth it is expected that a greater proportion (50%) will reach a moderate severity, due to repeated procedures, tumour burden or possible surgery.

When working with animal models it is essential to minimise any possible adverse effects of the experimental procedure. We closely monitor the animal's reaction to specific experimental procedures and pay attention to any sign of suffering that may occur. Cancer is a complex disease, however the knowledge of all the tumour models used will allow a good prediction of the time frame of the disease progression. Therefore, mice will be likely killed on the basis of a time period rather than based on their clinical signs. An experimental end point (time controlled) will most likely occur before a humane endpoint (as determined by deterioration of heath conditions) and result in only mild suffering for the animal.

When performing our metastatic studies, it will not be an experimental requirement for animals to reach a late stage of disease, but metastatic progression must occur. We will pay particular attention to mouse behaviour and monitoring specific to the organ targeted by the disease, and will in most cases, aim to terminate experiments before obvious signs of health deterioration appear. For example, in cases where tumours spread to the lungs, particular attention will be paid to the breathing behaviour of the mice both in resting condition and after a small physical challenge, however, some mice can drop in condition very quickly without many prior signs and may show more of a moderate suffering.

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

- Killed
- Used in other projects
- Kept alive

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?

In contrast to other tumour studies, here, a tumour is analysed in the context of its local tissue environment, as well as within the scope of the systemic changes induced in the host organism affected by the disease. Therefore, these studies must mainly be performed within the animal as the complexity of these changes and the number of players involved cannot be modelled in the laboratory.

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

Targeted lab-based assays using 3D scaffolds and organoid cultures will be designed accordingly with the gained information used to reduce mouse workload. For instance, in order to assess specific interactions between tumour cells and certain components of the microenvironment or of the immune system. This approach will also have the advantage of identifying the impact of these components on each other in a “clean” system where all the players are known and better controlled.

Why were they not suitable?

Even the most sophisticated lab-based model systems cannot convey the complexity of the tumour microenvironment or the impact that tumour disease can have systemically on an animal. In order to investigate the impact of infiltrating cell types alongside both the local and systemic response of tumour we can only do this within the complexity of the animal.

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 animals to be used have been estimated on the basis of the previous 10 years of work of my lab and the current landscape of projects of the lab going forward.

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

We employ several strategies to try to limit the number of mice in the study:

- Firstly, we will always aim to maximise the amount of data we get from each mouse and when possible, we will use it for the study of both primary tumour and metastasis.
- Also, we will limit the use of genetic models (that often require many generations breeding) using orthotopic transplants of labelled cells and treating the mice with chemical agents either to block immune-system components or to generate tumours.
- We also use the minimal number of mice needed for statistical significance when testing the experimental hypothesis based upon pilot experiments to inform on numbers required. We can always relay on our in house statistician for any additional advise whenever we need.

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

Whenever possible, we will share animal tissue from experiments to enable multiple studies-ex vivo.

We are committed to improving education and training for those working under this project license.

We breed many of the genetically altered animals ourselves in order to promptly and, often transitory, adapt colony sizes to respond to the experimental need and reduce wastage from overbreeding.

We also take lead from HO efficient breeding of GA animals (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773553/GAA_Framework_Oct_18.pdf) and take decisions to archive lines by cryopreservation when not required over a period of time.

Obtaining wildtype mice from in house facility-shared breeding allows better efficiency for larger colonies.

We we also always run pilot study to estimate the effect size and the directions for future experimental settings.

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 spontaneous genetically engineered models of cancer alongside more controlled experimental models of inducing cancer, including transplantation techniques. Our work at present is mainly focussed on breast cancer and pancreatic cancer but it is not limited to this. We minimise the use of surgical methods, wherever possible, by employing ultrasound guided injection techniques which drastically reduces the suffering experiences by the animals.

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

Other animals that are suitable for these types of studies lack the organ and physiological complexity needed for translation to humans. This complexity needs to be recapitulated to make the investigation of tumour microenvironment possible. The requirement here is to have a physiology which is as close as possible to humans, and only mammalian organism have the same complex immune system, hormonal infrastructure and basic metabolism as humans.

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

We have access to cutting edge techniques and experts within various fields of medical research. We actively share refinement and improvements in techniques and seek to constantly improve our models

to ensure that we are minimising any harms to the animals, as this also helps to improve the accuracy of our study and reduce artefacts caused by stress. For example; the use of imaging guided techniques to reduce surgery as a means of transplanting cancer cells to internal sites. We also follow local NVS policy on post-operative care and pain management to minimise any harms to the animal.

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

Unless otherwise specified, the work in this project will be designed using the principle outlined in PREPARE guidelines for planning animal research and testing (2017) and in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery.

With regards to cancer models we take advice from the "Guidelines for the welfare and use of animals in cancer research".(Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA; Committee of the National Cancer Research Institute. Guidelines for the welfare and use of animals in cancer research. Br J Cancer. 2010 May 25;102(11):1555-77. doi: 10.1038/sj.bjc.6605642. PMID: 20502460)

LASA Guidelines will be followed for Administration of substances.

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

We regularly receive updates on advances in the 3Rs from within our establishment from NC3Rs and NORECOPA. Where we are placed to refine techniques without impacting the scientific validity of our work we aim to implement advances.