Home Office

NON-TECHNICAL SUMMARY

Genetic analysis of metastatic cancer

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

cancer, metastasis, therapy, genetics, imaging

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

The main objective of this project is to reproduce genetic alterations observed in human cancer in a mouse system in order to better understand the basic biology of cancer and the process through which the cancer spreads from the initial site to various other organs. Specifically, we will utilise a highly refined set of genetically engineered mice to test the importance of specific pathways in driving disease progression in cancer. Ultimately, success in this work will represent a significant advancement in the field of cancer research through the analysis of tumours and new therapies in a realistic environment.

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?

More than 1 in 3 people in the UK will develop some form of cancer during their lifetime. Fortunately, the overall survival of most cancer patients increased remarkably in the past decades through early detection, but also improved treatment protocols that include the surgical removal of tumour tissue and conventional chemotherapy and radiotherapy. However, the majority of these approaches are associated with severe side effects. Moreover, the prognosis for patients with relapsed and/or aggressive tumours remains extremely poor. Notably, the vast majority (i.e. about 90%) of metastatic cancers that have spread to secondary sites are refractory to treatment and are therefore incurable. Thus, there is a pressing clinical need for further research, like ours, to identify the basic mechanisms that drive cancer progression. This knowledge is essential to assist the design of future anti-cancer agents and improve the outcome of patients exhibiting aggressive clinical behaviour.

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

We anticipate that our research will advance scientific knowledge about the formation and progression of tumours. This will be achieved through observational studies of tumour development in murine models that satisfactorily reproduce the human disease. This information will be of significant interest to other scientists in academia and in industry, as the results could open up new possible avenues for the development of anticancer drugs for patients with malignant disease. To ensure the dissemination of our work, we will publish our results in scientific journals and present our findings at seminars and conferences.

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

The primary potential benefit of this proposed work relates to new knowledge about tumour growth and malignancy. This information will have a major impact in the biomedical research as the data can serve as a basis for the development of rational therapeutic strategies to combat metastatic cancer. Thus, the potential secondary benefit of this work will go beyond basic cancer research, possibly leading to the future development of novel pharmacological interventions.

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

A number of avenues will be taken to explore the medical application of our results within the lifetime of this project. Specifically, we will interact with research business managers employed by the

establishment to facilitate interactions with industry and translate the potential value of this project to benefit society as a whole. In addition, we will share best practice with the local and national research community using similar models. In particular, the use of imaging will allow us to define more humane refinement of end points and study parameters and we can share these protocols with other teams working in the same area to enable them to do the same.

Species and numbers of animals expected to be used

- Mice: 19500

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.

Mice constitute important models for the characterisation of complex cellular processes. One advantage relies on the ability to dissect biochemical reactions *in vivo* by the use of targeted genetic deletions. This type of molecular manipulations is particularly successful in mice and has contributed substantially to an increased understanding of biological processes involved in normal development and pathogenesis. In addition, the genetic conservation with humans and comparable disease aetiology make mice highly suited for cancer research studies.

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

This project will create, breed and maintain mice with appropriate genetic changes. For the majority of animals, we anticipate that these genetic alterations will have negligible adverse effects and be of mild severity. Some mice will be utilised for tumour studies. Substances which are intended to alter tumour growth may be given by a number of routes, e.g. in the diet or drinking water, by direct application onto the skin or by experienced licensees via oral gavage or by needle injection, on one or more occasions. We may also make use of special imaging techniques to view the primary tumour as well as their spread in the living animals. Careful monitoring of tumour bearing mice is crucial for our studies and we have strict criteria when animals are to be killed. In particular, tumours will only be allowed to grow to a particular size and if the animal’s normal behaviour is altered they will be killed. At the end of the experiment the mice will be killed painlessly according to an appropriate procedure. Autopsies may be carried out to expose adverse effects undetected by external examination and the information used to refine future studies.

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

We will do everything possible to minimise adverse effects relating to tumour growth. In particular, we will not allow mice to carry on with fully developed tumours, but we will kill them in a humane way before any signs of suffering will develop. For this reason, the most likely level of severity in most cases will be mild. However, in some cases, some animals will be moderate for their welfare impact. Any

suffering will be minimised by careful monitoring to allow for the early signs detection of general lack of wellbeing. Moreover, if there are indications of ill health from gavage or skin ulceration after substances are applied directly to the skin, then the animals will be removed from the study and humanely killed.

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)?

In the majority (about 70% of mice) the severity will be mild, but never more than moderate.

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

- Killed
- 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 need to utilise mice because an important component of tumour growth is based on the interactions of tumour cells with the host organism (so called tumour microenvironment).

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

We will continue to employ non-animal alternatives to complement our *in vivo* studies in mice. For example, we will confirm the therapeutic applicability of our discoveries using cell culture systems. Moreover, we will test the clinical significance of our results by using credible computational modelling built using publicly available data from cancer patient materials.

Why were they not suitable?

While cell cultures and computer modelling constitute alternative approaches, conclusions drawn using such systems need to be tested in murine models that can simulate the cellular complexity of tumours. Additionally, metastasis (the process of cancer spreading to other organs) happens *in vivo* in the whole organism and there are no experimental alternatives.

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 utilised in this project will be kept to a minimum by using optimum crossing designs. The demand will be assessed before breeding and crossing. Colonies will only be maintained while there is an experimental plan and funding allocated. Moreover, the group sizes and the replications will be kept to the minimum possible to achieve statistically robust data. Importantly, we will keep consulting with statisticians as new studies begin to ensure that the optimal number of animals is used to obtain meaningful results.

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

Every opportunity will be taken to decrease the number of animals used for each experiment whilst still maintaining the statistical relevance of the subsequent data. In particular, experiments will be designed with repetitive measurements of the tumour volumes in the same animal. This has greater statistical power and animals need only be killed at the end of experiments rather than at each time-point, thereby drastically reducing the numbers of animals being used. The use of non-invasive imaging techniques that allow tumours to be studied in the same animal will also improve our study design and reduce number of animals required to answer specific questions. Moreover, we will always aim at maximising the amount of data we get from each mouse. We may also employ national on-line design resources, e.g. the Experimental Design Assistant (EDA) tool created by the NC3R, and consult with the NC3R team who provide periodic onsite help, to improve our approach.

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

An effective means of achieving reduction will be to avoid producing unwanted mice by carefully regulating our breeding colonies. Breeding will be optimised by replacing breeders before their reproductive performance declines. Wherever possible, colonies will be organised to produce only the genotype required by our studies. Cryopreservation will avoid wastage from the need to maintain colonies by continuous breeding. We are very familiar with the tumour models included in this application. Together with considerable experience and expertise available in animal husbandry, this will ensure careful management of colonies, matching supply to demand and thus avoiding the production of surplus animals.

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.

The mouse is one of the best model systems in cancer research that has greatly contributed to advance knowledge and improve treatment. This is largely due to the availability of different models that exhibit distinct cancer phenotypes consistent with the human disease. This advantage will allow us to utilise the most appropriate and refined systems for discovering clinically relevant mechanisms that drive or suppress the development of tumours.

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

An invertebrate model would not be appropriate since cancers do not arise in invertebrates and other differences in metabolism would greatly reduce the significance of findings for humans. The mouse is the lowest vertebrate in the evolutionary tree that can be genetically manipulated to produce the required genetically modified model.

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

We operate within a very tightly regulated, clean and well administered facility that has an excellent track record for animal care and safety. In all cases, we will avoid pain, suffering or lasting harm to the animals by the use appropriate anaesthetics and procedures.

Frequent monitoring will be used to assess progression of tumour burden, animal health and behaviour, so humane end-points are reached well before onset of clinical adverse effects. Moreover, we will be kept inform of any potential harmful events through our online system, so that we are always aware of issues with our animals and can respond to them quickly. Overall, adverse effects associated with tumour studies will always be limited to the minimum required for a valid scientific outcome.

Administration of compounds will be carried out by appropriately trained and skilled personal licence holders who will use specifically designed gavage tubes, fine needles and aseptic techniques to minimised stress due to restraint and momentary discomfort from oral gavage or needle insertion. Re-gavaged and repeated injection will be permitted only if the animal has fully recovered from previous procedures. Importantly, cancer therapy will be given at dose levels known to be tolerated. Any evidence of unanticipated toxicity would indicate reduction in dose and/or frequency of dosing.

Additionally, we will refine our approach by incorporating non-invasive tumour imaging where possible. This will provide opportunities to shorten our experiments, prevent potentially malignant tumours from escaping detection, and ensure that humane endpoints are adhered to, as more accurate detection and analysis of tumour growth informs decision making. Animals will have full recovery between periods of anaesthesia, rehydration and maintenance of body temperature during imaging sessions.

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

We will be guided and directed by the most up to date documents on the welfare and use of animals in cancer research. Additionally, we will follow relevant ARRIVE guidelines to ensure that our studies are reported in enough detail to add to the knowledge base.

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

Main sources of information include:

i) The NC3R team who provides very helpful advice;

ii) The NC3Rs and the BSF newsletters sent by email to all users of the animal facility;

iii) The National Centre for the Replacement Refinement and Reduction of Animals in Research (https://www.nc3rs.org.uk/resource-hubs); and

iv) Peer-review scientific papers, oral communications/posters in conferences and discussion amongst scientific colleagues, reporting alternative *in vitro* models to the use of live animals.