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NON-TECHNICAL SUMMARY

Understanding how metabolism contributes to cancer development, progression and treatment response

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Cancer, Metabolism, Metastasis, Microenvironment

Animal types	Life stages
Mice	juvenile, adult, pregnant, embryo, neonate, 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 aim of this project is to understand how metabolic changes in both tumour and normal cells can affect different stages of malignant progression, and how to target these changes for cancer therapy.

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 (in this protocol we are looking at pancreatic, lung, and intestinal, and possibly others in the future depending on how the project proceeds) is a major cause of premature death worldwide and treatments for late-stage metastatic disease are urgently needed. This project brings together established and novel systems of metabolic control with the recent appreciation of how host factors control cancer, and how these might be used for therapeutic effect.

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

The project will produce two broad types of output. Firstly, there will be peer reviewed scientific publications that will contribute to the general pool of knowledge about the role of metabolism in cancer (such as pancreatic, lung, liver and intestinal) . This will help to drive the understanding of this area and serve as a platform for further studies in our group and others. These publications will be supported by other means of dissemination of results, such as presentations at international research conferences. A second output will be the identification and validation of new targets for cancer therapy that focus on metabolic pathways. These may lead to the development of new drugs to affect metabolic enzymes or entirely new treatment approaches such as dietary modulation. Several pharmaceutical and biotech companies are already pursuing these avenues and will be well placed to take forward the information generated by the outputs from this project.

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

In the short term, the key beneficiaries of these outputs will be the general cancer research community, who will be able to build on the data generated through this project to increase our understanding of the mechanisms linking metabolism and cancer. In the mid and long term these outputs will be of benefit to cancer patients and clinicians, through an increased understanding of how to use existing treatments and the development of new therapies. This work is novel and does not seek to reproduce work of others but will be used to build on our previous and ongoing findings.

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

We will maximise the outputs of this work through peer reviewed publication in open access journals, seminars and presentations at international meetings and conferences. Where appropriate, we will work with the communications teams at our Institute to disseminate our results more broadly to the public, for example through press releases and lay talks. We will also maintain close links to industry partners and other external collaborators to ensure the most rapid translation of our work to patient benefit.

Species and numbers of animals expected to be used

- Mice: Mice: We expect to use up to 7,000 mice per year over 5 years. It should be noted that 50% of these will not undergo scientific procedures, but will be used solely for breeding and maintenance of colonies or if appropriate provision of tissues.

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 focuses mainly on the use of adult mice to study cancer development, with the occasional use of juvenile mice, for example in the study of dietary interventions.

Mice are the only suitable animals for these studies for a number of reasons.

1. The physiology of cancer in mice is consistent with the human disease. Our aim is to study the interaction of whole-body systems with cancers, and for this it is necessary to use a mammalian system that mimics these aspects of human physiology.

2. Mice are routinely used to model cancer in a whole animal. As a result, there is a vast body of information and large numbers of models that are used by the whole field. This means the design of our experiments will be informed by previous data, reducing the requirement for preliminary studies.

3. As mice are so widely used, the results from our project will feed into the growing understanding of metabolism in cancer. Our work will be relevant and complementary to other studies, so helping to take forward the entire field.

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

To study tumour (such as pancreatic, intestinal and lung cancer) development , mice will be exposed to genetic modification (which may be spontaneous or targeted to a specific organ or selected time point in life, such as after birth), known or potential carcinogens and transplantation of tumour cells. These procedures may also involve viral infection.

To study how host responses promote or retard cancer progression, we will use mice of different genetic backgrounds (e.g. to study metabolism we will use obese or diabetic mice) or mice exhibiting

co-morbidities , such as infection to induce inflammation, or we will use different diet to induce metabolic changes in the mice to see the effect on tumour development. The effect of aging will also be studied by maintaining the mice for two years.

The effect of different potential treatment options will be assessed using drugs, biological therapies such as antibodies or radiation.

Some mice will receive transplantation of certain immune cells to allow us to modulate and assess the role of the immune response in cancer.

Some mice will be exposed to different diets.

Tumour development will be followed by direct measurement of superficial tumours or non-invasive imaging of internal tumours, in some cases using genetic marking with a reporter protein such as iRFP (a fluorescence protein that will enable us to observe the tissues in-situ).

Tissues and fluids will be collected from these mice, either from live mice (e.g.blood, urine or faeces collection) or post-mortem (e.g. tumour and other organs).

The duration of these experiments will vary considerably depending on the model, ranging from a few days to years. In most cases we have experience and knowledge of the expected timeframes and experiments will only be maintained for the minimum duration to address the scientific need.

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

Mice will experience a range of impacts from extremely mild (for example some dietary changes) to moderate (in the case of aggressive tumour development or infection combined with interventions such as surgery). In the latter case we would expect weight loss and abnormal behaviour such as hunching. While we have significant experience and can predict the time frame and severity of each model, unanticipated events can occur and all mice will be monitored closely for signs of ill health or distress. Experiments will be terminated if they reach moderate severity limit.

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)?

Most of the animals in these studies will be expected to develop tumours. The impact and severity of each experiment will differ depending on which tumour model is used, and the aggressiveness of the tumour combined with the efficacy of any potential intervention. We anticipate that the tumour experiments will result in 50% mice reaching moderate severity as a result of repeated procedures. The other 50% will be a combination of mild and sub-threshold as a result of breeding maintenance of the different cohorts.

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?

Our project aims to understand cancer development in the context of the whole body, integrating the interplay between cancer cells and normal hosts systems to determine mechanism of cancer growth and spread, as well as the effects of systemic therapeutic interventions. The complexity of these interactions cannot be measured in tissue culture systems and the mouse provides an ideal mammalian model to carry out this work.

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

Our studies include the use of tissue culture protocols such as the standard 2D cultures that uses established cell lines, also 3D organoids (from tumour and normal organs, such as liver, pancreas, intestine and lung) and co-culture systems (i.e. culturing two or many different types of cells together to mimic as close as possible to in vivo situations). These will be used extensively to establish hypotheses, understand mechanisms and examine direct interventions in cleaner, simpler systems. For example, we will be culturing adipose tissues and cancer cells together, and see the effect of adipose tissue on cancer growth or other parameters such as migration of cancer cells. The results from these studies will guide the mouse work and reduce the number of in vivo experiments required, and finetune the subsequent in-vivo hypotheses.. We will also regularly search in the literature, and attend meetings for unpublished new data, to see if there are any new methods that offer better non-animal alternatives, should there be any that is applicable to our aims.

Why were they not suitable?

The value of organoid and co-culture systems is constantly improving, and we will continue to modify and upgrade this work over time. However, even the most sophisticated cell culture models cannot reproduce the complexity of the tumour microenvironment, tumour dissemination to distant organs or the effect of systemic whole-body changes on cancer progression. These questions can only be accurately modeled within the context of the whole live 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 used in each of the studies is based on extensive (over 10 years) previous experience using the same models, published literature, advice from colleagues using the same models and the advice of our in-house statistical experts . In most cases power calculations will be used to estimate the number if the parameters are known.

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

1. All experiments will be designed to use the minimum number of mice required to achieve statistically meaningful results. This will be developed with the advice of in-house statisticians, where required.

2. In all cases, experimental designs will ensure the extraction of maximum data, for example by measuring primary tumour and metastasis in the same animal or harvesting multiple organs from the same mouse for analysis.

3. Tumours in genetically engineered mice tend to develop over broad time scales, making it difficult to know exactly when to harvest mice for the most informative results. Published data on the models and online tools such as NC3Rs Experimental Design Assistant will provide guidance for the optimal number of animals needed to address the questions. Non-invasive imaging techniques or endoscopy will be used where possible to accurately follow tumour growth over time, limiting the number of mice required to hit the appropriate point of tumour development for the study.

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

1. We constantly optimize our breeding strategies to minimize the number of animals needed to achieve the desired genotypes for our genetically engineered models. Where possible, orthotopic tumour transplant models will be used, which do not require the breeding of genetically altered animals and use fewer animals per study.

2. Studies from cell culture models will be used to ensure that only the strongest hypotheses are tested in the mouse

3. Animals will be shared between experimental groups, where possible. For example, normal control animals can be obtained from our breeding colonies where they would not normally be used for a study.

4. Where possible we will share tissue from experiments to enable multiple ex-vivo studies

5. Most experiments will be based on previous studies, allowing us to predict the required numbers of animals. .

6. Using advice from HO efficient breeding of GA animals (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773

553/GAA_Framework_Oct_18.pdf) we constantly monitor animal use and archive lines by cryopreservation when not required over a period of time.

7. Where possible, unwanted genotypes from genetical breeding experiments will be utilized for other experimental studies – this is coordinated across the research group prior to starting the mouse study.

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 project uses genetically engineered mice, models of cancer induction by carcinogens and tumour cell transplantation. Infection models and other models of metabolic syndromes such as obesity and diabetes are also used. In all cases protocols to limit pain and suffering (e.g. ultrasound guided injection to limit surgery) are used. The conditional and inducible nature of many models ensures lesions are targeted to the cell type of interest (if the tissue specific cre allele is available for the tissue of interest) in adults, reducing non-specific off target effects.

None of the protocols exceed moderate severity levels. All mice on procedures are constantly monitored and humanely culled when exhibiting signs of altered health status and /or tumour burden or other specified end point is reached.

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

Less sentient animals do not possess the physiology (e.g organs, hormones, metabolism and immune system) that will allow us to understand the interaction of tumours with these complex host systems which is the focus of our studies. Only mammals can provide the level of complexity required to make these studies relevant to humans. For example, to study effect of insulin signaling that is a key factor in metabolic disease, it is known that cold blooded animals is not a good model as the pathway is not well conserved and their energy metabolism is much different compared to warm blooded animals such as mice and human. Many genes are also not conserved in lower animals as well.

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

We take advice from the experts within various fields of medical research and share our own experiments of refinements and improvements. We are highly motivated to minimize harms and stress to our mice as this also allows for more accurate and reproducible experimentation. For example, we have moved to imaging guided transplantation of cancer cells – a technique that avoids surgery. We also follow local NVS policy on post-operative care and pain management. We also increase

monitoring of the tumour models to determine the earliest end-point possible, and will use orthotopic models if applicable.

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

For all studies we will refer to the Guidelines for the welfare and use of animals in cancer research (Workman et al, 2010) and ensure best working practice. We consult the NC3Rs guidelines and monitor refinement where such practices are published (NC3Rs/LASA websites). We also consult the Guiding principles aseptic surgery:https://www.lasa.co.uk/PDF/LASA, and Guiding_Principles_Aseptic_Surgery_2010.2.pdf Refining procedures for the Administration of substances: https://doi.org/10.1258/0023677011911345

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

Training programmes in monitoring tumour development are mandatory for all users for each of our models. The trainers are specialists and users in specific tumour models and are shadowed by the trainee during the routine checking of the animals for symptoms. The trainees will be considered trained once the trainers are satisfied with their ability to recognize the signs of tumour development, humane end-points, and what samples to be collected and processed. We received frequent updates and recommendations from our NIO and we have an in-house NC3Rs regional programme manager who will provide guidance to the lab to ensure the latest 3Rs recommendations will be implemented whenever possible. Also, we keep ourselves up to date (e.g. from reading literature and attending conferences) to recent developments regarding better mouse models of various human diseases that are relevant to our study in cancer and metabolism