Home Office

NON-TECHNICAL SUMMARY

Metabolism as a novel target for cancer therapy

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Cancer, Metabolism, Therapy

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

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 what factors determine how tumours metabolize different nutrients and how to manipulate these factors in order to develop more efficient anti-cancer therapies.

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?

The abnormal metabolism is one of the well-known hallmarks of cancer. Tumours have been demonstrated to consume more of various nutrients, including glucose and glutamine, and catabolise them differently from normal tissues. Different types of cancers can be stratified based on the expression of metabolic enzymes and genes encoding them, which can predict the aggressiveness of the disease and the therapeutic outcome. The catabolism of major nutrients through specific metabolic pathway in tumours fuels the synthesis of molecules vital for proliferation and survival of cancer cells as well as their ability to metastasize. Metabolic enzymes regulating these pathways in tumour cells have been proposed as plausible therapeutic targets. However, metabolism of tumours and their metabolic requirements and dependencies are determined by multiple factors including genes driving cancer, the tissue a tumour originates from and the interaction between tumour and non-tumour cells. We will evaluate how this interaction between genetic and non-genetic factors determines how tumours metabolise different nutrients and what nutrients and metabolic pathways tumours depend on. With this we hope to be able to dissect the complexity of tumour metabolism in order to understand how to target it for therapeutic benefit.

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

In this project, we expect to advance our knowledge of how cancer and normal cells metabolise different nutrients and how these processes are being regulated. We will share our results with other researchers in the form of peer-reviewed original research articles and reviews in specialised scientific journals as well as, potentially, in cancer biology book chapters. We also aim to develop novel cancer models, other technical resources such as protocols and analytical tools for cancer and metabolism analysis and, importantly, novel anti-cancer therapies.

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

Clinical and academic research scientists and students of cancer biology would benefit from the knowledge/resources generated in this project. The project will also facilitate the identification of key metabolic specializations between different organs in health and disease. Novel therapeutic targets

may be discovered for developing new drugs. Therefore, ultimately patients with various types of cancer may benefit from these novel treatments.

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

We aim to disseminate our findings to scientists in our own and other fields, as well as more widely in the public domain, by presentations at national and international meetings and publications in high-impact journals.

Species and numbers of animals expected to be used

- Mice: 26,000

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 is focused on contribution of major oncogenes disregulated in human cancers and tissue environment to metabolic changes observed during tumorigenesis in vivo to identify novel therapeutics for cancer patients. Therefore, we will need to use adult mice as a model for our project, because they have shorter latency of tumour development compared to higher vertebrates, yet their physiology, anatomy, metabolism and pathological manifestations better resemble those in humans compared to the features of other commonly used animal models, such as nematode (C. elegans), fruit fly (Drosophila melanogaster), or zebrafish (Danio rerio).

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

Most of the animals used in this project will develop primary tumours of the internal organs, such as the lung, liver, mammary gland and intestine. These primary tumours may metastasise to other parts of the body, such as the brain, liver or lung, causing formation of secondary tumours, as it happens in human cancer. The tumours will either appear spontaneously due to inherent genetic alterations or be induced by the administration of either viruses or plasmids that will carry either enzymes or genes enabling such genetic alterations or by a transplantation of cancerous cells or tissue pieces.

After tumour induction, animals will undergo different experimental procedures. Animals may be sampled for small portions of blood to monitor changes in various biochemical parameters related to cancer development. We may treat animals with chemical substances that switch on or off the function of some genes or proteins, that could be relevant to tumour growth, or with labelling agents that would facilitate visualisation of certain types of cancer cells or cells interacting with the tumour. Animals may be placed on modified diets lacking certain nutrients. The control animals in this case will be placed on artificial diets with controlled complete composition. Animals may undergo imaging to monitor tumour growth by using both modalities that are used in humans, e.g., ultrasound, MRI or PET, and by state-of-

the-art specific animal imaging approaches based on luminescence and fluorescence measurements. Most of the animals as well as control counterparts, which will not have tumours or will not undergo the abovementioned procedures, will be culled by approved humane methods and their tissues will be dissected and used in experiments in vitro to examine the molecular and cellular mechanisms of the processes driving cancer growth and metabolism.

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

Most of the experimental animals in our project will develop some forms of tumours. In general, tumours in mice either emerge spontaneously or are triggered by injection of viruses or plasmids that will carry either enzymes or genes enabling genetic alterations and or by transplantation of malignant cells. In mice genetically predisposed to develop cancer spontaneously, tumours typically develop in the period between 3 to 6 months; in some cases, up to 12 months. Depending on the site of the tumour development and its stage, animals will experience different adverse effects. Animals with liver tumours may develop anaemia, jaundice and ascites (abnormal build-up of fluid in the abdomen). Animals with lung cancer may be asymptomatic initially, but after several months may develop breathing difficulties and anaemia. In addition, a proportion of the lung tumours may be highly metastatic and in the end stage, lung cancer may be accompanied by disturbances in other organs affecting animal well-being. In animals with spontaneously emerging (autochthonous) tumours, these side effects typically occur at the end stage of tumour development, gradually developing over 1–2 months.

For transplantation models, the time frame between tumour transplantation to the humane endpoint is shorter, typically between 1 and 2 months. Some of the adverse signs mentioned above for spontaneous cancer models will therefore develop over a shorter period of 1–2 weeks. In addition, mice undergoing transplantations may be impacted by the effects of injections, which could cause local haemorrhage and damage to the organs being injected.

In all cases, tumour burden will be limited to the minimum required for a valid scientific outcome. Animals may display tumour ulceration, labored respiration, or persistent diarrhea, but they will be immediately culled after any of these symptoms is observed. The animals will be also monitored for weight loss and body condition score, and mice dropping below the set criteria (e.g., 15% weight drop) will be immediately culled to prevent excessive suffering.

Animals that will undergo surgical procedures may experience mild to moderate pain immediately after the surgery and they will be given analgesics to minimise post-surgical pain.

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)?

For the majority of mice that will be developing tumours in the course of the project and/or will undergo surgeries (80% in total) the severity will be moderate. Approximatly 20% of these animals will be used for tumour and other tissue collection after being killed by a Schedule 1 method or after a non-recovery procedure under general anaesthesia (approximately 20%). The rest will be either administered different gene/protein modifying or therapeutic agents, and/or placed on modified diets or subjected to food restrictions. In all of the cases the overall severity will not exceed moderate level. For the rest of

the animals, which can be either genetically modified but without any obvious clinical signs or wild type and/or receiving infrequent injections, the severity will be mild.

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?

To identify mechanisms of tumour growth and metabolism in physiological condition, which are meaningful for human clinical studies, we need to use animal models because they develop tumours similar to human ones. In addition, the metabolic interactions between tumour and a host as well as effect of local and systemic effect of dietary manipulations can only be meaningfully studied in vivo.

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

Evidence will be collected from initial studies using in vitro cell culture /organoids/tumourspheres model systems, where appropriate, as well as by surveying the mammalian and other literature. In addition, we will use non-regulated procedures to collect expression data from fixed non-GM mammalian tissues and functional/expression data from genetically and/or environmentally manipulated in vitro system. Those generated data sets will be analysed by bioinformatics, and thus to identify the most promising candidates to be validated in experiments in vivo.

Why were they not suitable?

Organ- and cancer-specific metabolic pathways are highly influenced by nutrients, oxygen, circulating hormones and other aspects of the complex physiological environment inside the body. It is not yet possible to recapitulate all of these parameters in vitro, nor to mimic the metabolic crosstalk between different organs and tumours –a key aspect of this research project.

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 numbers were estimated based on our previous experiences, statistical modelling, and predictions for exploratory experiments. For spontaneously occurring tumours (autochthonous models), due to the high variability in tumour progression, for each cohort we normally would need around 20 animals per group for study, whereas transplantation models would normally need between 10–20 animals per group.

We will take as an example an experiment where the requirement of a metabolite both produced by a biosynthetic pathway and available through a diet is being evaluated for the progression of tumours. In this case animals are either administered an inhibitor of the biosynthetic pathway producing the metabolite or place of a diet lacking this metabolite or a combination of both treatments is being used. Considering these potential treatment combinations, each experiment would normally need at least four groups: control/vehicle treated/control diet; metabolic inhibitor/control diet; vehicle treated/experimental diet; metabolic inhibitor in combination with an experimental diet. Furthermore, for all novel therapies, the dosing frequencies and drug dosage needs to be optimised first. There are also at least two different regimes: prevention and intervention. In the liver model we will test these combinations in the inducible liver models and in the model induced by hydrodynamic transfection (3 oncotypes per each model): (20GEM + 10 hydrodynamics) x 3 oncotypes x 4 experimental groups x 2 designs = 720 animals. For 3 treatment combinations we will need 2160 animals (Metabolism of Liver Tumours).

Additionally, to evaluate how our findings would translate in human cancers we will use xenograft models. For 5 xenogfrats we will perform treatment combination experiments: 5 x 10 mice per group x 4 experimental groups x 2 designs = 400 mice. For 3 treatment combinations we will need 1200 mice. 1200 = 1200 mice with xenografts.

For these experiments we mostly plan to use mouse models of the two major cancers: mammary gland and liver, comparing at least 3 oncotypes per each type.

As mammary gland tumour models, we will also propagate 15 breast cancer PDXs and will perform initial multiomics analysis (10 animals for propagation + 10 for analysis): 20 x15 = 300. For 5 PDXs we will perform treatment combination experiment: 5 x 10 mice per group x 4 experimental groups x 2 designs = 400 mice. For 3 treatment combinations we will need 1200 mice. 1200+300 = 1500 PDX mice. Some of these PDXs will be propagated and used in the experiments with fat pad clearance.

Altogether, we will use 2160 GEMM animals and 1200 animals with xenografts for the metabolism of liver and mammary gland tumours each, In addition, 1500 animals with breast cancer PDXs. 8220 animals altogether.

In addition, we will run pilot exploratory experiments with smaller groups of animals in lung and intestinal tumours, probing different approaches to study the relationship between metabolism and tumours. We will need approximately twice fewer GEMM animals in this initial phase (2,200 in total). Therefore, we will need 8220 +2200 = 10420 mice in experimental protocols, out of which 6520 are GA animals.

Considering that some animals may not develop tumours or may be excluded from the subsequent experiments due to various reasons before overt tumour development, as well as taking into account that some animals will have to be initially generated from breeding of GA mice, where not all progeny will be suitable for experiments (some genotypes can be 30-25%), we would need to produce on average 3×6,520 = 19,560 GA animals for the programme of experiments. For those we will need 1222

breeders (8 mice per litter, 4 litters per breeding pair). Altogether – 20,782. Some of the breeders will be on doxycycline inducible diet (200). Finally, we envisage that we may need to generate new genetically altered lines of mice or import some novel genetically altered animals from other laboratories. For this purpose, we will need 1500 animals. Therefore, we plan to use 3,900 (PDXs/xenografts) +19,560 (GA) + 1,222 (breeders) + 1,500 (new strains) = 26,182 mice.

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

The efficiency of animal usage is maximised in consultation with animal technicians by careful control of breeding to match research needs with respect to numbers, phenotypic uniformity and health. In addition, we will also take advantage of the online tools, including the NC3Rs Experimental Design Assistant to help us with experimental designs.

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

This programme of work will make optimal use of several tissues, fluids and cell types per individual mouse. We will aim to collect organ samples from multiple body sites and to provide other affected tissues to appropriate scientists, so that they do not have to breed mice specifically for their experiments. This highly integrative approach will maximise the information obtained from the minimum resources. Cryopreservation of gametes, embryos, tissues and cells is a routine procedure and will ensure that the minimum number of mice is bred.

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.

Mice have been selected for the majority of this work as it is an appropriate model for providing insights into human diseases and it is the species in which reliable transgenic and knockout technologies are most advanced. Wherever appropriate, we will minimise the adverse effects associated with genetic alterations by using inducible or conditional alleles to delete gene activity from specific tissues rather than from the entire mouse.

Genetic alterations or transplantation methods will be used to induce tumour formation. These models possess relevant pathological features similar to those in human patients, allowing us to investigate basic tumour biology and to perform preclinical trials. This will obviate the need to use higher vertebrates. Furthermore, whenever possible, we will perform experiments in transplant models, which usually exhibit a faster disease course, thus minimising the duration of distress and lasting harm to the

animals. We will only perform key experiments in the autochthonous settings, particularly when we will need to elucidate how tumours develop, evolve, and interact with their native microenvironment.

In all surgery, analgesia will be provided according to contemporary best practice and advice from the NVS/NACWO. Good aseptic surgical techniques, heat & fluid therapy will be provided as necessary. In the case of cancer models for example, we will follow the guidelines in Workman et al, British Journal of Cancer (2010) 102, 1555 – 1577.

Whenever possible, we will use terminal anaesthesia to perform surgical experiments in animals to decrease their pain and suffering.

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

This project is focused on understanding the role of metabolism in tumourigenesis in vivo to identify novel therapeutics for cancer patients. Therefore, we will need to use a model system that is able to establish tumours and has metabolism closely resembling that of human. Therefore, mice would be the most appropriate animal model for our project. Less sentient animal models, such as nematodes or flies, will not be suitable for our purpose. Whenever appropriate, we will carry out experiments in terminally anaesthetised animals, but for some of the proposed experiments, we will investigate various methods to intervene with the tumour progression. In such cases, we will need to observe the animals over a prolonged period of time until the assigned endpoints are reached.

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

For all manipulations we will adhere to local or national guidelines that aim to minimize suffering. Many of the genetic and dietary manipulations as well as the administrations of gene inducers/repressors or other agents are standard and previous refinements from the literature will be used. If, however, there is insufficient information available, new manipulations will be pre-screened in small-scale pilot studies to obtain indications of the minimum dose and exposure time that is likely to be effective, thereby minimising any potential suffering.

We will also consult our NVS to improve the surgical methods and procedures we are using.

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

Our experiments will be planned in accordance with the recently formulated PREPARE guidelines.1 For surgical procedures, we will follow the guidelines set out in LASA Guiding Principles on Preparing for and Undertaking Aseptic Surgery. With regards to the experiments in cancer models, we will adhere to the Guidelines for the Welfare and Use of Animals in Cancer Research2 and will monitor body condition according to system developed by Ullman-Culleré and Foltz3. In addition, I will also follow the latest advancements in relevant fields, by attending conferences, reading journal articles, and collaborating with experts in these areas, to ensure that the experiments will be conducted in the most refined way.

1. Smith, A. J. et al. PREPARE: guidelines for planning animal research and testing. Lab Animals 52, 134–141 (2018).

2. Workman, P. et al. Guidelines for the welfare and use of animals in cancer research. Br J Cancer 102, 1555–1577 (2010).

3. Ullman-Culleré M.H. and Foltz C.J., Lab Anim Sci. 1999;49(3):319-23

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

I will keep following up the latest publications in the fields as well attend courses and seminars and follow NC3Rs and NORECOPA websites to learn any advances in the 3Rs.