Home Office

NON-TECHNICAL SUMMARY

Elucidating the neuroscience aspect of cancer biology to identify novel therapeutics.

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

Key words

cancer, nerve cells, therapy, metabolism, immunology

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

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?

To understand the interactions between tumours and the nervous system, and to develop more efficient cancer treatments by modulating this crosstalk.

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?

It is known that malignant cells of various cancers acquire nerve cell-like properties, becoming electrically excitable and expressing proteins typical for nerve cells. In the proposed project, we will explore if the appearance of nerve cell-like properties in cancer cells correlates with the extent of tumour growth in mouse models of lung and pancreatic cancers. Furthermore, tumours of various origin are often richly innervated by the nervous system, which may support cancer growth. We will also investigate if the nervous system sends signals to tumours to regulate their growth and determine whether tumours themselves “communicate” with the whole body by sending signals via the nervous system. Finally, we will check if stimulation or inhibition of such interactions between tumours and the nervous system affects tumour growth. We hope that answering these intriguing, relatively unexplored questions about the roles of nerves and neuronal features in cancer will lead to the development of novel therapeutic inroads for cancer patients.

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

In this project, we expect to learn a lot of new facts about how cancerous cells use nerve signalling to maintain tumour growth. The new information will be shared with other researchers and clinicians 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 therapeutics for cancer patients, including both drugs and devices.

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

In the short-term, research scientists and students of cancer biology would benefit from the knowledge we aim to generate in this project. In the long-term, novel therapeutics may be developed based on the new biological mechanisms that we will discover. 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?

I will seek to publish these results, attend conferences and give seminars to disseminate new knowledge obtained from the project. If any new therapeutics are successfully developed from the

project, I will also collaborate with clinicians and reach out to pharmacological companies for potential clinical trials in human patients.

Species and numbers of animals expected to be used

- Mice: 8,680

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 modelling the interactions between tumours and the nervous system *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 a nervous system closely resembling that of human. On the basis of such criteria, adult mice would be the most appropriate animal model for our project, because they have a shorter latency of tumour development compared to higher vertebrates, yet their physiology, anatomy and pathological manifestations better resemble those in humans compared to the features of other commonly used animal models, such as nematode (*Caenorhabditis 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, pancreas, and mammary gland. These primary tumours may metastasise to other parts of the body, such as the brain or liver, 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 viruses that will carry enzymes enabling such genetic alterations or by a transplantation of cancerous cells.

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, 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. Some animals will be irradiated and receive bone marrow transplantation to enable precise modulation of their immune system. 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. In some imaged animals, a transparent window may be implanted to aid visualisation of internal organs. Animals may also undergo surgical implantation of devices that would allow to stimulate the activity of certain cells by using light stimulation. Some complicated experiments, where the activity of nerve cells will be stimulated and recorded in vivo, may be performed on animals under terminal general anaesthesia. 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.

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 a 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). Mice with pancreatic cancer may have low blood sugar and because of the tumour burden, paralysis of hind paws may occur. 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 or 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. For example, mice injected with tumour cells into the spleen may develop mild bleeding from the spleen.

In all cases, tumour burden will be limited to the minimum required for a valid scientific outcome. Animals may display tumour ulceration, laboured respiration, or persistent diarrhoea, 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 or will undergo surgeries (60% in total), the severity will be moderate. For 20% of the animals, which would be, for example, genetically modified but without any obvious clinical signs, or receiving infrequent injections, the severity will be mild. The remaining 20% of animals will be used for tissue collection after being

killed by a Schedule 1 method or after a non-recovery procedure under general anaesthesia (e.g., perfusion/fixation).

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?

In order to understand the crosstalk between tumours and their environment, both locally and systemically, and to formulate novel approaches for cancer treatment based on the modulation of the interaction of cancer cells and nervous system, we need to use animals that develop tumours similar to those in humans.

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

Some information about the sensitivity of the interactions between cancer cells and nerve cells to different drugs can be gained from experiments in cultured cells. We will therefore use such *in vitro* experiments for more precise planning of the experiments in animals. In addition, data on the expression levels of different genes and abundance of proteins encoded by them, as well as on concentrations of various biologically active substances and metabolites obtained from cancer tissue samples may provide important clues regarding the role of the nervous system in regulating tumour growth. We will therefore employ computational modelling and bioinformatic analyses of such datasets to identify the most promising drug targets to be validated in experiments *in vivo*.

Why were they not suitable?

These alternatives are complementary to the animal studies, but cannot fully replace the work we are proposing here. In particular, it is not possible to achieve a similar structural and temporal complexity of interactions between tumours and nervous cells in experiments *in vitro*. In cell culture, nerve cells have limited viability and may not grow processes of the same length as *in vivo*.

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, each cohort we normally would need around 20 animals per group for preclinical trials, whereas transplantation models would normally need between 10–20 animals per group. We will take preclinical trials in small cell lung cancer (SCLC) model as an example: considering potential treatment combinations, each experiment would normally need at least four groups: control/vehicle treated; standard of care/chemotherapy; new drug; new drug in combination with standard of care/chemotherapy. If the new drug alone does not seem efficacious in pilot experiments, we will only test the combination using the minimal animal numbers possible, and see if we could observe any trend. If not, we will not pursue the full trial. Furthermore, for all novel therapies, the dosing frequencies and drug dosage needs to be optimised first. There are also at least three different trial designs: prevention, intervention and regression, which elucidate the role of a certain manipulation (whether pharmacological, genetic or surgical) at different stages of tumour progression. As such, for each new drug to be thoroughly examined, both in transplantation model and in autochthonous setting, in one trial, we would need at least (20 autochthonous + 10 transplant) × 4 experimental groups × 3 designs = 360 animals.

We plan to use mouse models of the following five major cancers: SCLC, lung adenocarcinoma, pancreatic ductal adenocarcinoma, pancreatic neuroendocrine tumour and mammary tumour (breast cancer). Therefore, we would need 360 mice × 5 cancer types = 1,800 animals if we want to run at least one trial per cancer type. We would like to run 10 trials in the course of the project, which will require 3,600 animals. In addition, we will run pilot exploratory experiments with smaller groups of animals, probing different approaches to study the relationship between nervous system and tumours. We will need approximately twice fewer animals in this initial phase (1,800 in total). Therefore, we will need 3,600 + 1,800 = 5,400 mice in experimental protocols.

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 may be suitable for experiments, we would need to produce 1.5×5,400 = 8,100 GA animals for the programme of experiments. These 8,100 animals will come partly from the breeding protocol on the current Project Licence (4,860 animals or 60%) and partly from other breeding colonies (3,240 animals or 40%). 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 580 animals. Therefore, we plan to use 8,100 + 580 = 8,680 mice over the period of this project.

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

We have consulted statisticians for the sample sizes used in preclinical trials. We will also take advantage of the online tools, including the NC3R Experimental Design Assistant to help us with experimental designs. In addition, we will perform smaller-scale pilot studies: if the preliminary results reveal an interesting trend, we will then perform a new set of properly powered experiments in a separate cohort.

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

We will first use *in vitro* systems to optimise the experimental conditions and to select for best gene and protein targets to be validated *in vivo*. We will also collaborate with bioinformaticians to mine human patient datasets and identify the most promising genes and pathways to be investigated before planning experiments *in vivo*. We will perform pilot studies for these candidates, and will take advantage of different imaging techniques and sampling methods to obtain multiple data points in one single experiment, thus maximising the output from each cohort of animals. We will work with engineers in our department to develop novel devices to be used in this project, aiming to increase the efficacy of such devices so that we could decrease signal variability and therefore use fewer animals for the experiments. We will also work closely with our histology experts and imaging specialists to obtain higher quality images and videos, so that we can use fewer animals for the imaging analyses. Finally, we will generate tissue banks to ensure that the materials could be utilised for different experiments or even shared with other labs.

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 mouse models in this project. Genetic alterations or transplantation methods will be used to induce tumour formation in these animals. 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. 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 modelling the interactions between tumours and the nervous system *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 a nervous system 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?

I will work with engineers in our department to develop novel devices to be used in this project, aiming to decrease the discomfort of the animals carrying such devices. I will also consult our NVS to improve the surgical methods.

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 Research.[2] In addition, I will also keep myself updated with 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).

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 and attend courses and seminars to learn any advances in the 3Rs.