Home Office

NON-TECHNICAL SUMMARY

Studying the biology of brain tumours

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, brain, treatment, inflammation, immunotherapy

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 improve our understanding of the pathways and processes that promote the development, growth and pathology of brain tumours.

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 important to undertake this work as different types of brain tumours cause significant illness and death in people throughout the world. Glioblastoma is the most common type of malignant brain tumour and is responsible for more deaths in people under forty years of age than any other cancer. Meningiomas are responsible for 1/3 of all brain tumours and cause significant morbidity, such as impaired coordination, altered behaviour and social problems in sufferers, and can be fatal. Vestibular schwannoma tumours are highly linked with the NF2-related Schwannomatosis syndrome, and are life-shortening and life-limiting tumours, with substantial morbidity including hearing loss. There are currently no effective treatments for these tumour types, principally due to the lack of understanding of the biology of the tumours. Animal models provide critical opportunities to identify and mechanistically test the processes and pathways responsible for promoting the development, growth and spread, and pathology, of brain tumours, which will directly inform the identification of new treatments for the conditions.

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

The work in this project will lead to significant new information on the pathways and processes that promote brain tumour development and related suffering and mortality. Direct outputs from the work will be peer-reviewed research articles, dataset resources that will be shared with the research community, and presentations, where we will disseminate our discoveries.

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

The outlined programme of work will provide essential insight into the most appropriate molecules and pathways to target as new treatments for the different types of brain tumour. In the short term, this will be of major benefit to researchers and clinical scientists working on human brain tumours, increasing our understanding of the diseases. In the mid-term, the work should provide the foundation for clinical trials of new therapies for brain tumours, which, in the long-term, will ultimately help improve the standard of care treatment for the different brain tumours, substantially reducing the morbidity and mortality of people with the diseases.

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

We will publish our results in peer-reviewed journals, in open-access format. We will also initiate new and build upon existing collaborations to enhance the impact of our results. We will disseminate

unsuccessful approaches or negative data through specific journals or online forums.

Species and numbers of animals expected to be used

- Mice: 2500

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 are the most appropriate species for this work as murine brain tumour models are the most well characterised of the various animal models (when using established tumour lines, as will be done in most experiments within this licence). There is a significant body of literature that results obtained in murine brain tumour studies are relevant for understanding human brain tumours. Murine models (using adult animals) are also highly informative for studying the efficacy of new treatments for brain tumours.

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

The general project plan will involve either utilising genetically modified animals that are predisposed towards development of brain tumours, or the implantation of brain tumour cells into targeted areas of the cranium or body by surgical routes. These approaches will be utilised to study different types of brain tumours with different locations, biology and severities. The models studied will involve benign vestibular schwannoma brain tumours, which in humans develop associated with the ear canal, meningioma tumours, which develop on the outer membranes of the brain, and malignant glioblastoma tumours, which form within the brain tissue. Experiments will utilise both male and female mice, to avoid experimental bias and to assess any sex-related effects on brain tumour biology.

Depending on the question addressed in each experiment, mice may receive injections to modulate the immune system or physiological processes, or may undergo surgery to modify tissue function (e.g. ligation of brain lymphatic vessels to change how cells and molecules drain out of the brain), or to enable visualisation of the brain tumour (e.g. removal of parts of the skull for microscopy analysis). They may be administered chemotherapeutic drugs, or undergo irradiation with bone marrow reconstitution to change the nature of immune cells. Injections can be by different routes depending on the research question and the nature of reagents administered (i.e. reagents may be injected directly into the brain or cerebrospinal fluid, or provided systemically into the blood). The vast majority of animals will receive less than 4 injections to manipulate the immune system or physiological processes, by a maximum of two different routes. Some experiments may be short duration of 7 -14 days (when assessing the biology of fast growing and malignant tumours or when studying the very early phases or brain tumour development); however some experiments may be > 60 days, when studying the development of slow growing or benign brain tumour models. Multiple manipulations in a single animal will be avoided, when possible. Cumulative effects (e.g. additive effects) of multiple

treatments will be minimised by allowing animals to fully recover from procedures such as surgery or irradiation and reconstitution before the animals undergo any subsequent treatments.

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

The different brain tumour models have the potential, related to their growth and effects on brain and nerve activities, to cause suffering. The models to be employed in the objectives are all well established and standard in the field. This ensures there is substantial information describing the trajectories of brain tumour development, supporting definition of the observable signs of suffering to appropriately determine time points of analyses in experiments, and to specify stop-go points and humane end points.

The suffering of animals with brain tumours will be minimised by closely monitoring all animals using a well-defined 7-point grading system assessing neurological activity (behaviour abnormalities such as tremors or circling), general signs of suffering (including hunching, piloerection, ataxia and lethargy) and monitoring weight loss (which is a very sensitive measure of animal health during brain tumour studies). Robust humane endpoints for weight loss (20%) and level of adverse effects (balance, behaviour) will prevent unnecessary levels of suffering. Most of the modulatory procedures performed, or the reagents administered, to manipulate brain tumour development or growth should not directly promote animal suffering. Analgesia will be given to reduce pain following specific protocols (such as following surgery to ligate lymphatic vessels or create a cranial window); however, the direct damage caused to the brain by the brain tumour lessens analgesia efficacy in that context. Moreover, brain tumours do not cause pain or suffering for substantial periods of time following development, and when the brain tumour begins to cause pain (identified using our clinical grading scale), the experiment will be terminated. All administrations will be performed via the most appropriate route through (when applicable) the careful control of injections.

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 total, on this licence, 37% of animals are expected to experience mild suffering, 23% of animals are expected to experience moderate suffering, and 40% (genetically altered animals bred within protocol 1 and control genetically altered animals within the brain tumour protocols) will experience sub-threshold suffering.

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 can only address the majority of our questions when a complete immune system and central nervous system, as well as auditory system, are present in their normal anatomical and physiological configurations (for example where the brain tumour can connect with draining lymph nodes to establish anti-tumour responses, when peripheral cells can migrate to and enter tumours, and when brain tumours can influence nerve function and hearing), or when the brain tumour is in a clinically relevant environment (for example where the brain tumour is acted upon by the various cellular, circulating and structural components within the cranium): the use of animals is, to a significant extent, unavoidable in our experiments.

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

When we have simple and reductionist questions, such as how brain tumour cells directly interact with specific immune cells or brain cells (i.e. nerve cells), or how they respond to specific molecules, we will establish *in vitro* co-culture systems (culturing more than one type of cell together, such as brain tumour cells with immune cells) to study these defined interactions. These *in vitro* systems will also be utilised as a strategy to test and prioritise treatment and modulatory approaches for subsequent employment *in vivo* within animals, to examine the pathways that influence brain tumour growth and pathology.

Why were they not suitable?

Such *in vitro* co-culture approaches are suitable for only very specific questions as during the course of brain tumour development, growth and spread *in vivo* within animals, the interaction between cancer cells, immune cells and brain cells is often shaped by a myriad of factors, including circulating immune cells and immunological mediators (produced in the brain but also recruited from other parts of the body), and the multifaceted communication with other brain resident cells. Thus, for the majority of our investigations to obtain accurate and clinically relevant results, we need to study our objectives within intact physiological tissues, *in vivo* within animals or *ex 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?

We have estimated the numbers of animals based upon our previous experience of running similar project licences in the last 15 years, as well as from consulting relevant literature and engaging with collaborators employing similar models. Thus we have accounted for the nature and requirement of the projects we have immediate plans to perform, including the numbers of times experiments must be repeated, the numbers of different experimental groups in experiments, and the numbers of mice required in different groups. We have also estimated the number of animals to be used based upon future plans and collaborations.

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

We calculate the required group size using data from previous experience, and published work. This ensures that we have sufficient ability to detect a biologically relevant effect using as few animals as possible. We also perform sample size calculations based upon pilot and preliminary experiments to ensure we perform subsequent experiments with the correct number of mice to detect statistically significant results. We also adhere to ARRIVE and PREPARE guidelines for reporting of research involving animals, which outlines appropriate study design (e.g. control groups and sample sizes), how to avoid experimental bias, and the analytical framework for simple and complex experiments.

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 perform pilot experiments when undertaking new experimental approaches so we can discontinue uninformative or inappropriate methodologies and so we can also evaluate the variability and magnitude of experimental effects. This will allow us to accurately assess the numbers of mice to use in future studies. We also consult the literature when we are performing similar approaches as others have previously performed, in brain tumours or in other models. This allows us to predict the strength of expected effects within our experiments, and therefore, the numbers of mice that need to be used to detect statistically and biologically relevant results. We will carefully manage maintained colonies (i.e. by employing short-term harem breeding) to ensure we have sufficient numbers of mice for planned experiments but ensuring we do not have surplus mice. Any unneeded mice will be shared with researchers, who have authority to receive 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.

Mice are the most appropriate species for this work as murine brain tumour models are the most well characterised of the various animal models (when using established brain tumour lines or genetically

engineered models, as will be done in most experiments within this licence), giving us essential background information that is lacking in other systems.

Mice are also the animals of choice for immunological investigations as so much is known about their immune systems, different well-characterised inbred strains of mice exist with differing responses to disease, there are a large number of genetically modified murine strains available for use, and all the reagents that we require (such as for modulation of the immune system) are available.

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

To obtain informative results in this project we need to utilise an organism with a complete immune system and physiologically equivalent brain and nervous system to that in humans, in which the different types of brain tumours can be established. This precludes the use of less sentient drosophila models. Whilst zebrafish models for Glioblastoma exist, these are less established for Meningioma and Vestibular Schwannoma. Challenges still exist for accurate and reproducible establishment of orthotopic xenograft Glioblastoma models (which is the injection of human-derived brain tumour cell lines into the Zebrafish brain). All technologies and methodologies required for addressing the project objectives are established in mice, and are not easily transferrable to Zebrafish.

We must also use adult mice with a fully formed and functional immune system. Otherwise, our results would be difficult to translate to the study of human brain tumours. We will perform certain protocols under terminal anaesthesia but due to the length and course of experimental brain tumour models, it is not possible to perform all work under anaesthesia.

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

Due to the overall purpose of this work - to study the factors responsible for development of and treatment response of brain tumours - we do need to allow experiments to proceed to the point where animals will experience some suffering, recapitulating the development of symptomatic brain tumours in humans. However, in our experiments we do not require animals to progress to experience severe suffering (i.e. experiments will be terminated before brain tumours cause severe suffering). Moreover, animal suffering will be minimised by providing analgesia, when possible and when required. For example, whilst we can provide analgesia following surgery, we are unable to provide analgesia to mitigate the effects of the brain tumour, as the analgesia itself will modify animal behaviour and the course of the experiment. Multiple treatments to manipulate the immune system or physiological process within a single animal will be avoided, when possible, with a maximum of two separate approaches applied in any animal.

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

We will follow NC3Rs guidance and we will continually assess our experimental designs in relation to advances within the relevant brain tumour and immunology literature.

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

We are committed to identifying and implementing new advances relevant for our work. We survey the literature for new refinements and experimental approaches. We actively engage with our institution (as well as receiving updates by email) to improve best practice in experiments. We are also on the NC3Rs mailing list to obtain newsletters with new advice, guidelines and information. Standard operating procedures for users working with animals are in place within our institution, the adherence to which is compulsory, which incorporates advances in animal handling and ensures animal welfare.