Home Office

NON-TECHNICAL SUMMARY

Elucidating brain tumour cell plasticity and preclinical treatment options during malignant progression and tumour recurrence

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, Brain tumours, Tumour recurrence, Therapy, Malignant cellular plasticity - cancer stem cells

Animal types	Life stages
Mice	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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?

Better understand why brain tumours progress and recur despite treatment, and developing and testing treatment options.

A retrospective assessment of these aims will be due by 26 October 2026

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

Malignant brain tumours aggressively invade and grow within the healthy brain. Across the world, 250,000 people are diagnosed with brain cancer every year, and this devastating disease is responsible for 189,000 deaths per year, of which 3,500 deaths occur in the UK annually.

Brain cancer is devastating for patients and their loved ones. Even with the best treatment, patients with the most cancerous brain tumours live for only 12-15 months. On average less than 1 in 20 people live more than 5 years, which is a much worse outcome than expected for most other cancer types. Treatment fails because brain cancer cells are very adaptive and grow finger-like projections into the surrounding healthy brain. Unlike in other cancers (e.g., breast), brain structures infiltrated by the cancer cannot be cut out whole, so cancer cells always remain after surgery. These remaining cells resist being killed by radiation and chemotherapy so new tumours eventually regrow. Better anti-brain tumour treatment options are urgently needed, and the UK government and the world’s biggest cancer charity, Cancer Research UK, have both stated that brain cancer urgently needs more research to improve

outcomes for affected patients. Our main goal is to ultimately translate our most promising research findings into clinical applications.

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

The herein described combination of brain cancer development, growth and relapse animal models represents a rare resource for the UK; the absence of which has hampered the clinical use of results that were obtained in laboratory (preclinical) research studies. Clinical testing of potential brain cancer medicines often fails to show any overall survival benefit for patients. The reasons for these setbacks are complex, including potential failure to achieve sufficient concentration of the tested medicines in the brain tumour, underestimating the ability of tumour cells to adapt and survive the treatment, and failure to identify and target the optimal genes and molecules in brain cancer cells so that their growth and can be efficiently halted. This 5-year project seeks to model and compare key stages of a brain tumour patients' journey, which can include surgical removal of tumour mass and observation of the remaining disease that inevitably leads to brain cancer relapse. Biological investigation of the brain cancer at different stages is expected to identify targets that would be overlooked in conventional studies of just the tumour mass that can be largely removed by neurosurgery. Furthermore, potential medicines against promising brain cancer targets can be tested so that the results can inform the planning of how to safely use such candidate medicines in humans (a requirement before any clinical trial testing a potential medicine can start).

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

Short term, our project, objectives, and protocols are expected to offer several insights and possibilities for the wider research community, in particular holding key advantages over existing models that do not take brain cancer relapse into account. For example, target identification in tumour cells in the remaining disease after removing the 'bulk' of the tumour (by micro-surgery) will aid the development of new treatments, and validation of previously identified 'Achilles heels', for example using agents that inhibit genes and proteins that allow the tumour to grow uncontrollably.

In the longer term, a clinical patient pathway-mimicking brain cancer modelling strategy that tracks tumour growth and also recapitulates the 3-dimensional structure of a tumour cavity after neurosurgery enables cross-disciplinary collaborations and further research involving engineering (e.g. ultra-high precision image detection), biomaterials (e.g. localised drug delivery) and medicinal chemistry (e.g. repurposing of existing drugs that are effective in vitro but do not enter the brain due to the blood-brain-barrier that keeps many potential medicines out of the brain).

The goal is to develop our most promising laboratory findings to a stage where they can be tested in humans. Currently, we are in the process of building on published results working towards developing a (so called) lead compound (synthetic small molecule) from an experimental chemical that is not yet optimised for use in humans. Before use in the clinic, experimental chemicals showing promising preclinical efficacy in animal models need further improvement via the so-called drug development process. On average, it takes a new drug 12 years to get to market (including R&D, preclinical testing, human trials, and EMA/FDA approval). We hope to test improved (drug-like) analogues of the candidate medicines (synthetic small molecules) during this project, which may inform the planning of first-in-human clinical trials. However, even if all research progresses as planned, the impact for patients with regards to clinical trials and new treatment possibilities reaches beyond the lifetime of this project.

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

The project team has been put together to maximise impact across brain cancer research by immediate collaboration and knowledge exchange with other UK-based groups. We have a wide-ranging international research network and have a track record of collaboration with industrial partners.

In addition to the earliest possible academic publication of sound research, for example via making our most timely manuscripts available via a widely used preprint server that features timely research before peer review, members of this project team regularly engage in laboratory tours and science activity days. We regularly give presentations for brain tumour patients and their relatives, fund-raisers, support groups, and charity ball/festival audiences. These events, away from the anxieties and pressures of the clinical environment, are key to discussing the personal effects of brain tumours on all aspects of the individual's life. It is obvious from our discussions that patients embrace novel and bold research approaches to finding better treatments, and that they are very engaged in contributing to research. A patient suffering from treatment side effects and morbidity who is offered some hope and retains some positivity is better prepared for all forms of treatment. The prospect of telling patients that we are going to be able to better understand their brain tumour, and test drugs against it, may help to facilitate a more 'positive mind set', while delivering the herein described objectives. For example, many patients experience contributing tumour tissue (including our established theatre-to-lab research tissue pathway) as a very empowering act in the face of a dismal prognosis.

Species and numbers of animals expected to be used

- Mice: 550

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.

Our ultimate goal is to translate our most promising research findings into clinical applications that ultimately benefit brain cancer patients.

The mouse models described in this project are required for mimicking the human disease and brain tumour patient experience by integrating research before and after the tumour is resected by neurosurgery.

Considering animals at a more immature life stage: our project revolves around models of adult brain tumours. Approximately 93% of primary brain and central nervous system tumours are diagnosed in people over 20 years old with people over 85 having the highest incidence. The average age at diagnosis is 57. Therefore, this project and its protocols are designed to increase our understanding of the cells driving tumour growth in the fully developed (adult) brain, including tumour recurrence.

Considering species that are less sentient: our project fills a gap in modelling brain tumour biology in the central nervous system environment, which we currently cannot replicate with cell culture approaches as they do not sufficiently mimic the anatomy of the brain. The human brain is unique; however, there is a body of evidence showing that many important aspects including tumour initiation, development, and invasive growth of tumour cells into brain structures (for example, the corpus callosum, the cortex, the striatum, and the ventricles) can be soundly studied in the mouse brain. Overall, in vivo brain tumour modelling is well-established in mice. As of November 10th, 2020 the search term 'brain tumours, mice' retrieved 25,205 entries in the pubmed database. In contrast, the search terms 'brain tumours, fish' and 'brain tumours, flies' retrieved 1065, and 456 pubmed database entries, respectively.

Considering animals that have been terminally anaesthetised: this can be considered whenever experimental endpoints are reached. It is however neither an option for the surgical procedure that is required to implant cancer cells into the mouse (with the purpose of initiating tumour development), nor for mimicking tumour relapse after surgical resection of the brain tumour. Moreover, non-invasive imaging procedures are required to monitor tumour growth, for example in presence/absence of a treatment strategy. All these procedures entail full recovery of the animals after they have been anaesthetised.

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

Typically, an experiment starts with the implantation of cancer (or control) cells (under anaesthesia) into the brain. Animals are anaesthetised by inhalation of anaesthetic agents and the head is fixed in a stereotactic apparatus. Analgesia +/- antibiotic are administered prior to making a scalp incision. All surgical procedures are performed using standard sterile aseptic techniques. A small hole is drilled into the skull. Cancer cells are injected into the brain using equipment that allows us to deliver precise cell numbers and fluid volumes into the desired location. The needle is subsequently removed, the skull opening is sealed with bone wax and the skin incision is closed with tissue glue. Animals are observed in a warming chamber until full recovery from anaesthesia. After full recovery from the surgical procedure, the typical animal may experience several (non-invasive) imaging procedures that enable us to measure and predict the tumour growth in the animal's brain.

Once the tumour has grown, we may administer a substance (e.g., a chemical compound) to test whether this agent can reduce tumour size and spread and/or prevent further cancer growth in comparison to a control substance. This may require daily dosing of a substance (up to twice per day) depending on its half-life and other chemical properties.

Our project entails tumour excision surgery. Animals are anaesthetised and all surgical procedures will be performed using standard aseptic techniques. The previous incision from tumour implantation (see above) is reopened and a biopsy punch and fine suction tip used to take out a large part of the tumour, thereby mimicking the surgical technique utilised in human patients undergoing comparable brain cancer surgery. Animals are subsequently observed, and tumour relapse is measured by imaging (e.g., via small animal MRI) under general anaesthesia. At ~8 weeks post surgery, the animal is culled followed by whole brain retrieval for research analysis.

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

After tumour cell implantation, the animals are expected to recover quickly. Based on our experience, the animals show normal behaviour (no pain or distress) after full recovery from the procedure. The animals are monitored daily according to a scoring table that assesses the clinical signs that are associated with growth of the tumours as based on our pervious experience and observations. As a first sign of tumour burden, animals typically show slight under-grooming, which is often followed by an evolving hunched posture and slight reduction in activity. Whenever these moderate signs of animal distress appear, this will be reflected in the total 'score'. Reaching a pre-defined threshold means that the experiment will be ended as soon as possible. When reaching the first threshold, we will consider the the overall objective of obtaining sound results so that the animals are not wasted. In some cases, a prolonged period of tumour growth may be required, for example to determine how long a treatment can maximally keep the tumour under control. Under this scenario, the animals will be monitored up to three times per day to observe progression of under-grooming and hunched posture and appearance/accumulation of additional signs including reduced breathing activity, disorientation of movement, isolation, and increased periods of inactivity that all indicate an increase in animal distress. Any of observed signs will be noted, which counts for the overall score. We have pre-defined a threshold that, once it is reached, ensures immediate termination of the experiment regardless of any anticipated endpoint by human killing, before the animal experiences pain and suffering from the tumour burden.

Typically, animals show completely normal behaviour (score = 0) until the late stages of tumour growth. Once the critical tumour burden for causing symptoms is reached, the animal health score increases to greater 5 (e.g., reflecting slight under-grooming). A score greater 5 triggers twice per day monitoring. As soon as the animal reaches the next score bandwidth (>15), the animal will be monitored 3 times per day. Once the score reaches the final pre-defined threshold (>25), it is removed from the study by humane killing, independent of any other factors.

Like in human brain cancer patients, tumour-removing surgery can have adverse effects. For a small number of animals this could mean short term experience of severe (neurological) clinical signs such as strong disorientation leading to immobility. Hence, animals will be closely monitored during and after the procedure (hourly) to ensure that the animal's recovery period (several hours) stays within the expected scoring threshold (≤ 25). Any animals that are immobile after the recovery period (score = 26) will be removed from the study by humane killing.

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)?

This project entails to protocols.

- Protocol 1: moderate bandwidth (300 animals)

- Protocol 2: severe bandwidth (200 animals). Based on advice from collaborators (with expertise in such protocol) and input from neurosurgeons carrying out this procedure in patients, we expect that a maximum of 5% of the animals (10 animals total) may transiently (for several hours) fall into the severe category (likely during the immediate procedure recovery period) before removal by humane killing. We would like to point out that, if the procedure is successful, the tumour removal is substantially reducing

symptoms caused by the expanding tumour mimicking the situation in brain cancer patients after neurosurgery (the first line clinical treatment for brain tumours).

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

- Killed

A retrospective assessment of these predicted harms will be due by 26 October 2026

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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 ultimate goal is to translate our most promising research findings into clinical applications that ultimately benefit human patients. In this context we are pursuing three major (inter-related) research strands that aim to better understand as to why cancer cells in the brain are adaptive and not all equal, why, and how brain cancer cells survive and grow despite therapy. We aim to find solutions as to how we can therapeutically target the tumour cells more efficiently so that their growth can be substantially halted. It is important to note that all the underlying biology posing these questions is very dynamic happening in space and time. This biology cannot be meaningfully separated from its location and anatomy of the brain. We do not have tools that allow us to study brain tumour development and recurrence exclusively in a human brain (non-invasively) or in human cell culture, and therefore, we need animals to study brain tumour biology at the molecular level, which can provide answers to our research questions. The mouse models described in this project are required for mimicking the human disease and there is a body of evidence indicating that these in vivo approaches yield meaningful results in terms of studying the complex adaptive growth and invasion of tumours into the relevant brain environment. Here, we mimic the brain tumour patient experience integrating models enabling research before and after the tumour mass is removed by neurosurgery. There is currently no replacement for the herein described pre-clinical orthotopic tumour models using mice.

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

We have considered cell cultures including three dimensional structures mimicking brain tissue - so called organoids. Our group has helped to pioneer a system that enables the real-time investigation of brain tumour cell infiltration into (early-stage) cerebral organoids (sometimes referred to as 'mini brains'). This is a powerful tool, for example in the early stages of investigating a potential anti-brain cancer treatment, and for observing brain tumour cell behaviour and adaptive phenotypes during the

highly dynamic brain infiltration process. We have shown that brain tumour cells and cerebral organoids self-assemble to form 'assembloids' within 48 hours. Hence, this technology is scalable and can help to reduce the number of animal experiments, but it is not yet a replacement for the more complex procedures that mimic the brain cancer patient pathway required for this project.

Why were they not suitable?

Brain tumour organoid structures still lack the concerted organisation and anatomy of an animal brain. They could be an alternative for animal brain studies in the future; however, it has to be pointed out that the use of miniature organs (organoids) that would closely resemble an animal (or human) brain in organisation and function would also raise ethical concerns, especially if these structures would gain consciousness. Currently, this level of brain organoid development is not possible (far from reality).

A mouse brain is a suitable structure for studying brain tumour growth providing conclusions that can inform the human disease (for example, tumour migration from one brain hemisphere to the other). We are also able to mimic the first line treatment of the human patient pathway, which is surgical resection of the tumour. No current in vitro model can faithfully capture the complexity of the brain tumour microenvironment found in vivo providing the 3-dimensionality of the tumour and residual cancer-harbouring zones post-surgery.

A retrospective assessment of replacement will be due by 26 October 2026

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 based on our published previous work and our calculations need to consider that even control tumours derived from the same cell model source can show growth differences. Our project aims are to establish and demonstrate the utility of two complementary in vivo models and then investigate a significant difference in effect between these groups. We have considered that several substances (e.g., newly made analogues of the small molecule inhibitor KHS101) may be tested and/or validated (using different regimes) within the lifetime of this project. We estimated animal numbers needed to establish the tumour resection models (~50) and we need to allow for a general engraftment failure rate of ~20%, and failure of tumour recurrence of up to 50%.

The given total numbers are also based on currently funded work as well as an estimate of project requirements that may become relevant within the next 5 years due to future grant applications (some currently pending). For imminent work, we have calculated the average cell number expected for each tumour for establishing a recurrent tumour cell library, and the number of investigated tumour regions of interest and serial sections required to investigate biological replicates for each of the models used.

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

We used the Design Assistant from NC3Rs (and the EDA report) to calculate the numbers for the imminent project work (to be carried out within the first three years of the project).

We use up-to-date ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) to compare compatibility of our research design with the ARRIVE checklist of recommendations. Our study design also typically incorporates the use of both male and female animals in order to eliminate any potential sex bias in the research outcomes. Moreover, we base our statistical considerations and group size determination and randomization on the literature and previously-published work.

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

We have established (and are using) a comprehensive *in vitro* (cell culture-based) investigation system that enables the determination of a specific cellular behaviour in a controlled (simplified) environment before increasing the complexity by utilizing *in vivo* models. Whenever assumptions required for the calculations of animal numbers can not be faithfully made due to insufficient pervious experience (data) and/or published information, we carry out pilot experiments with the goal of filling the gaps leading to assumptions that can predict the statistical power/scientific soundness of the planned experiment.

Our project entails the generation of a tumour recurrence (cell and tissue) model resource that will be shared with the wider research community.

A retrospective assessment of reduction will be due by 26 October 2026

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

Orthotopic tumour models are currently the most refined method to address tumour development in a location-specific manner as the tumours can grow in their typical environment. These cancer models are well established in mice and are required for biomedical research. To address tumour complexity, it is important that tumour migration and invasion patterns in the mouse model mimic the situation in patient tumours. Importantly, our patient-derived xenograft models reflect hallmark features of aggressive brain cancer including extensive migration of tumour cells along typical migration routes that are also found in the brains of human patients. Moreover, these models utilize cells derived from patient tumours and as such reflect well the heterogeneity of tumour profiles in different patients.

Discomfort and distress of animals are limited by providing a pain relief agent (analgesic; METACAM; effects typically lasting 24 hours) directly after the mice have been anesthetized so that they do not experience significant pain from the procedure once they awake from the narcosis. We will consider relevant refinement(s) of the surgical procedures and imaging procedures described in this protocol. Intracranial cell transplantations in mice will be performed under anaesthesia and pain relief medication will be given after surgery. Animal will be monitored daily and will be culled humanly when showing adverse effects or other signs indicative of toxicity.

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

Considering animals at a more immature life stage: our project revolves around models of adult brain tumours. Approximately 93% of primary brain and central nervous system tumours are diagnosed in people over 20 years old with people over 85 having the highest incidence. The average age at diagnosis is 57. Therefore, this project and its protocols are designed to increase our understanding of the cells driving tumour growth in the fully developed (adult) brain, including tumour recurrence. We aim to identify new treatment targets by ensuring that findings from the herein described animal models are relevant to patients.

Considering species that are less sentient: our project fills a gap in modelling brain tumour biology in the central nervous system environment, which we currently cannot replicate with cell culture approaches as they do not sufficiently mimic the anatomy of the brain. The human brain is unique; however, there is a body of evidence showing that many important aspects including tumour initiation, development, and invasive growth of tumour cells into brain structures (for example, the corpus callosum, the cortex, the striatum, and the ventricles) can be soundly studied in the mouse brain. Due to its anatomy, the mouse brain also serves as a suitable model organ for other brain diseases such as neurodegenerative disorders. It has the advantage that neurosurgical procedures that mimic the tumour resection therapy in human patients can be performed. As an in vivo model, mice also have the advantage of being bred in many strains, some of which maintain an immunodeficiency. This allows us to implant human cells -derived from patient tumours- into the mouse brain (forming the herein described xenograft tumours). In addition, there are mouse cell tumour models that can grow invasively in wild-type mice with an immune system, Hence, an investigation system can be established that complements the absence of immune cells in xenograft tumours and genetic and phenotypic differences in mouse compared to human tumours.

Overall, in vivo brain tumour modelling is well-established in mice. As of November 10th, 2020 the search term 'brain tumours, mice' retrieved 25,205 entries in the pubmed database. In contrast, the search terms 'brain tumours, fish' and 'brain tumours, flies' retrieved 1065, and 456 pubmed database entries, respectively.

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

Animals are monitored daily and their health status is recorded using an established animal welfare scoring system. During and after procedures, animals are closely observed to ensure full recovery as indicated by normal animal behaviour and posture. Pain caused by surgical procedures is managed by administration of analgesics. Non-invasive disease monitoring (via imaging) that requires fixation of the animals for a short period of time is carried out under transient anaesthesia.

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

We follow the latest version of ARRIVE (Animal Research: Reporting of In Vivo Experiments). Currently: The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, PLOS Biology; https://doi.org/10.1371/journal.pbio.3000410.

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

We stay informed though the NC3R website and are aware of the available 3R online resources. We also discuss advances relevant to our protocols with other groups locally and collaborate nationally.

We carefully assess the *in vivo* methodology in newly-published research papers to identify potential advances in our field. We are passionate about further developing animal-free investigation systems for brain tumour research and consider these approaches highly relevant for reduction of animal numbers. Our procedures are informed by clinical neurosurgery and we aim to mimic procedures as closely as possible in mice (including aseptic techniques, disease monitoring, and pain relief).

We attend and participate in local events organized under the umbrella of the Animal Welfare Ethical Review Body (AWERB).

A retrospective assessment of refinement will be due by 26 October 2026

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?