Home Office

NON-TECHNICAL SUMMARY

# Preclinical models of cancer and metastases

**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, metastases, hematopoietic stem cells, gene therapy, immunotherapy

| Animal types | Life stages |
|---|---|
| Mice | adult, neonate, juvenile, pregnant |

## 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?**

Our overarching aim is to identify and validate new molecular and cellular mechanisms, therapeutic targets and therapies in cancer, especially for cancer that has spread from the site of its origin to other organs (metastases). This will provide a better understanding of the disease and enable us to combine this knowledge with our drug-delivery approach using a subpopulation of white blood cells to deliver therapies to cancer lesions.

**A retrospective assessment of these aims will be due by 03 December 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?**

While the treatment options for many primary tumours are improving, cancer becomes in most cases untreatable once it has spread to other organs (metastases). Cancer spread commonly occurs to several organs at the same time, requiring therapies that can target cancer lesions at multiple sites. Patients with brain cancer, being primary brain cancer (e.g. cancer that has developed in the brain) or metastatic brain cancer (e.g. cancer that has spread to the brain from other organs), have particularly poor prognosis. Our studies are therefore focusing on brain cancer and multi-organ metastases. It is important to undertake this work in order to improve our knowledge and enable the development of improved therapies for these patients.

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

We will generate new information that is expected to result in publications in scientific journals. This will include (i) information on how therapies that enhance the ability of our immune cells to kill cancer work in the brain and in other organs to which cancer typically spreads. We will use this information to develop new approaches that can further increase killing of cancer cells by the immune system. For example, we plan to modify a specific population of white blood cells, called T cells, to enhance their

ability to find cancer cells. (ii) information on what is needed by cancer cells to enable their growth in different organs, particularly in the brain. We plan to test whether manipulation of molecules that were shown to be important for cancer growth through our previous studies, can be used to inhibit/kill cancer cells.

Another expected output from our work is a patent under which we plan to further develop our technology using white blood cells to deliver therapies to metastases in the brain and in other organs, with an ultimate goal to develop a therapeutic product.

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

In short-term, the generated knowledge will provide novel understanding of cancer cell growth and its interactions with the immune cells in the brain and metastases in other organs, and thereby profit a wider scientific community by informing their work. We expect that in medium term (next 3-5 years) this knowledge will inform the development of improved therapies at the preclinical level by our group and others, such as approaches using modified T cells. Moreover, there is a potential for numerous existing drugs targeting components of the immune system to be repurposed as cancer therapeutics, based on the information generated through our studies. In the long-term, this is expected to benefit cancer patients by providing improved treatments and hopefully result in an improved survival and a better quality of life. This is further expected to benefit biotech industry owning such drugs, by expanding the market opportunities and generating additional profit. We plan to approach specific companies directly to discuss such opportunities (next 5-7 years).

We have provided a proof-of-principle for the delivery of therapy using white blood cells in our recent studies. Under this project license, we plan to further improve the ability of this therapy to kill cancer cells and to demonstrate its safety (next 2-3 years), and obtain a regulatory approval to move this approach into the clinic (next 5 years). We hope that this will ultimately result in improved clinical therapies for brain tumours and metastases in the long-term, and thereby benefit cancer patients. Development of this technology is also expected to benefit the private sector (life science company) engaging with our technology, by generating profit and opportunities to generate further products.

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

The outputs from this work will be maximized by effectively disseminating new information through talks and other forms of presentations at scientific meetings, internal and external seminars, educational talks and lectures, through collaborations with scientists and clinicians, and through open-access publications. We are part of an extensive collaborative network of clinicians, pathologists, and scientists sharing tissue resources and information.

Disease models developed and refined in our laboratory are being shared with other research groups through direct collaborations. We are part of an international consortium that has recently put in place and published a database of available brain metastases cell models for in vivo research, a resource that has been made available to a wider scientific community.

We have been previously including statements about negative results in our publications that contain positive results, and will continue to do so whenever appropriate.

The outputs are also being maximized through engagement with the private sector with interest to commercialize our approach, which is expected to enable the development of our technology into a product and its translation into the clinic.

**Species and numbers of animals expected to be used**

- Mice: 3600

# 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.**

We are using mouse models of cancer and metastases because cancer in the brain and other organs mimics human cancer, and it has been repeatedly demonstrated that findings from these models and therapies initially developed in mouse models can be applied to the human situation. Thus, studies of cancer and therapies in these models is expected to enable clinical translation of our findings, which is our ultimate goal. We are using adult mice, because we are studying types of cancer that develop in adults. We are also using genetically modified animals of the following types:

(1) Mice that lack a specific cell type to enable us to study its function. This includes immunocompromised mice that allow for engraftment of human cancer and immune cells.

(2) Mice that lack or over-express a specific molecule to enable us to study its function.

(3) Mice expressing specific receptors on T cells (a subpopulation of immune cells) that allow for studies on immune responses.

(4) Reporter strains that express molecules that can be easily detected and therefore allow for tracing of specific cell types or molecules.

(5) Mice with modifications that result in spontaneous cancer development in order to mimic the disease.

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

Development of cancer in mice in order to mimic tumour growth in patients will be achieved by one of the following methods:

Protocol 1 and 2: development of metastases will be achieved by injecting cancer cells into the blood stream to mimic dissemination of cancer cells to target organs from the blood, as seen in patients. This will include injection of cancer cells into the internal carotid artery (to generate brain metastases), into the heart (to generate metastases in multiple organs) or into the tail vein (to generate lung metastases);

or a mouse will undergo a surgical procedure under anaesthesia to implant cancer cells into the brain to mimic large brain metastases.

Protocol 3: a mouse will undergo a simple surgical procedure under anaesthesia to implant cancer cells into the organ in which a tumour initially develops in humans (the mammary fat pad to mimic breast cancer) or cancer cells will be simply injected into or under the skin (to mimic skin cancer). Cancer cells then disseminate from this primary tumour to other organs, as seen in patients.

Protocol 4: we will use genetically modified animals that develop tumours spontaneously.

To further develop the therapeutic approach exploiting white blood cells for the delivery of therapy to tumours (~20% of the mice), the mice will undergo irradiation to ablate the existing white blood cells in the bone marrow and the modified progeny of white blood cells will be subsequently injected via the tail vein (Protocol 5). After this, the mice will be transferred onto one of the Protocols 1-4.

Following tumour development, mice will receive a therapy through injection under the skin or into the intraperitoneal space. To determine whether the therapy can inhibit tumour growth, tumour size will be measured 3-4 times during the experiment, either by a direct measurement of the tumour diameter if the tumour is visible (for example skin tumour) or by imaging that enables visualization of inaccessible tumours (for example in the brain). For the latter, the mice will receive an under-the-skin injection of an agent that enables tumour visualization and will be subsequently anaesthetized to perform imaging. Tumour sizes in mice receiving therapy will be compared to the control group without therapy to determine whether the therapy works. A blood sample may be taken from the tail vein. A typical duration of such experiment is 2 weeks. At the end of experiment the animals are culled under anaesthesia and organs are isolated for analysis.

Genetically modified animals required for the study will be bred under Protocol 6. To this end, mice will be grouped as required for mating.

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

Animals may experience slight discomfort from anaesthesia and surgical procedure for cancer cell implantation, with the vast majority of mice recovering rapidly, showing no symptoms or mild symptoms that do not persist longer than one hour. In a small proportion of mice (<3%) mild symptoms may continue for up to 48 hours. A small proportion of mice undergoing administration of cancer cells into the carotid artery may experience stroke after the surgery (~0.3% of all mice) and these mice are immediately culled humanely.

Administration of therapies, white blood cells and blood draw is associated with only a transient discomfort from needle sting. Irradiation has no effect on the animals.

The majority of mice display normal behaviour for the duration of the experiment. However, intracranial tumour growth and development of metastatic cancer lesions in other organs in mice under the Protocols 1-4 may lead to specific symptoms once the tumours become larger. At the experimental endpoint, the majority of mice experience no symptoms or mild symptoms characterized by slight under-grooming. A proportion of mice (<10%) is expected to display moderate symptoms including clearly detectable under-grooming, reduced activity and hunched posture, with the duration of symptoms

usually not exceeding 12 hours. Only a minor percentage of mice (<2.5%) may display severe symptoms, in which case a mouse is immediately culled humanely.

**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)?**

Protocols 1, 3, and 4: moderate category; all mice are expected to experience moderate severity

Protocol 2: severe category; <2.5% of the mice are expected to fall into this category and the remaining mice (>97.5%) are expected to experience moderate severity

Protocols 5 and 6: moderate category; <10% of animals will fall into this category and the remaining mice (>90%) are expected to experience mild severity

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

- Killed

**A retrospective assessment of these predicted harms will be due by 03 December 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?**

Use of animals is required to achieve the aims of this project because a whole organism is needed to study systemic processes that involve multiple organs, such as trafficking of immune cells between the lymph nodes, the blood and the tumour. Immunotherapies work only when all these components are present, and can therefore only be studied in vivo. The development of approaches for improved delivery of drugs to brain tumours requires a model in which the injected modified white blood cells travel to the bone marrow, where they mature, and from there via blood vessels to the brain. This complex process requires a whole organism and can therefore only be recapitulated in animal models.

Cancer cells growing within their natural organ environment strongly differ from cancer cells growing in the cell culture. Sole analysis of cells grown in cell culture is therefore unlikely to identify good therapeutic targets. Complex compositions of different organs cannot currently be recapitulated ex vivo, and therefore these studies at least in part require use of animal models.

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

We are periodically searching the literature for replacement possibilities for our in vivo models using all available resources. Up to date there are no alternatives that could completely replace in vivo models needed for our studies in order to achieve our objectives. However, certain components can be replaced by non-animal alternatives. Non-animal alternatives that we are currently using include an in vitro migration assay, which mimics certain steps of immune cell migration observed in vivo and can be partially used to replace in vivo studies. We also established a protocol for culturing slices of human brain metastases ex vivo, which can replace the initial studies looking at whether drugs can inhibit cancer growth, which would be otherwise performed in animal models. Notably, brain metastases-derived cancer cells don't readily grow in culture and robust protocols for their growth could so far not be established. We use in vitro assays to demonstrate that therapeutic molecules used in the context of hematopoietic stem cell gene therapy can inhibit cancer growth, and to validate a role of specific molecules in cancer. We plan to use a co-culture of cancer cells and blood vessel cells in the future to mimic interactions between cancer cells and blood vessels. Analysis of publicly available data extracted from comparison of human tissue samples under different conditions can also identify differences between conditions of interest and can omit a need for an in vivo experiment, whenever such data are available. We are also considering a use of organ-on-chip models that simultaneously mimic multiple organs.

**Why were they not suitable?**

There are several non-animal alternatives that are suitable to address our aims and we are including these in our studies, as described above. However, current lack of understanding of the in vivo interactions and systemic processes hamper the development of organ-on-chip models that would sufficiently resemble the in vivo situation to allow meaningful studies of systemic interactions involving multiple organs. It is expected that generation of further knowledge using in vivo models is required to enable the development of such complex in vitro models.

**A retrospective assessment of replacement will be due by 03 December 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?**

The overall number of animals predicted to be used in this project was estimated based on the use in our previous projects. For each experiment, we use statistical approaches to calculate the minimal number of animals that will allow us to obtain significant results.

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

In order to ensure that a minimal number of required animals is used in each experiment, we perform power calculations using NC3R's Experimental Design Assistant. Whenever feasible, one control group will be used as a control for multiple treatment groups, omitting a need for multiple control groups. We will use male and female mice in experiments whenever possible. Multiple readouts will be used within one experiment, reducing the number of required experiments. Tumour growth will be monitored by non-invasive longitudinal imaging in live mice, omitting a need for multiple time points at different stages of the experiment, thereby reducing the number of required animals. Our techniques for tissue analysis are being refined to allow for detection of multiple parameters in one tissue sample, reducing the number of mice required. Tissues are harvested for multiple projects whenever possible. Harvested tissue from each experiment that is left over is stored and inventoried, so it can be used in the future when new questions arise, omitting a need for additional experiments.

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

The mice will be bred only specifically for planned experiments rather than continuously and the numbers of required breeding pairs will be calculated based on the average litter size for a particular strain. Between the breeding cycles we will only maintain two cages of males and females each. We will share harvested tissues between the project, and maintain an inventory of all left-over stored tissues to enable us to use them for additional analysis whenever new questions arise, thereby reducing a need for additional experiments. We will use in vitro assays to replace in vivo studies whenever possible (see "Replacement").

**A retrospective assessment of reduction will be due by 03 December 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.**

We will use mouse models of cancer and metastases. Depending on the scientific question, we will use different methods to model primary tumours and metastases, as described in detail under the "Project harms". Each chosen method results in the development of tumours in organs in which cancer that we are primarily focusing on (breast, melanoma, brain cancer) initially develops in patients, or in organs to which the cancer typically spreads. This is important, because the tumours grow in their typical environment, which influences their characteristics.

Discomfort and distress of animals will be limited to unavoidable procedures required for the conduct of sound research. A priority will be given to a method that causes least distress for the animal while allowing for modelling of a specific cancer type. For example, whenever possible, brain metastases will be generated by injection of cancer cells into the heart rather than into the carotid artery, as the latter requires an invasive surgical procedure as opposed to a non-surgical injection. Surgical procedures will be performed under anaesthesia and pain relief medication will be given prior and after the surgery. Animals will be monitored daily and will be culled humanly if showing adverse effects.

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

Modelling of cancer in adult mice is required because this is the life stage at which the types of cancer that we are studying occur in patients. Characteristics of the immune system and characteristics of organs in which cancer develops, such as for example the tightness of the blood vessels in the brain, differ between life stages, and it is therefore important to use the life stage that adequately mimics the situation in patients.

We are using mice because the immune system and the tumour microenvironment in mice better recapitulate human situation than in less sentient animals such as Zebrafish, and therefore information obtained from studies in mice is expected to be better translatable into human situation. Mouse model is an established host model for studies on cancer progression and immune system, and therefore well characterized. A body of literature supports the correlation of cancer biology, biology of brain disorders, and therapeutic responses to immunotherapies between mouse and human.

Studies over a longer period of time are required to allow for the development of different stages of cancer and thereby adequately mimic cancer progression in patients, and such prolonged studies are not possible in terminally anaesthetized mice.

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

All surgeries are performed under general anaesthesia. Analgesics are administered to minimize pain. Injection of cancer cells into the carotid artery may cause stroke immediately after surgery in a small proportion of mice (<0.3%). These animals are immediately killed humanely in order to prevent further suffering. Over the years we were able to refine this surgical procedure and minimize the occurrence of stroke, through measures such as efficient disruption of cell clumps prior to the cell injection, optimization of injected cell numbers, and use of mice strains with a low stroke susceptibility. Whenever possible, we are replacing this surgical procedure with a less invasive non-surgical injection of cells

into the heart. This procedure was also refined through ultrasound guidance to increase the precision of cell delivery.

Tumours growing under the skin or in the mammary fat pad are removed or the animals are culled before the tumour exceeds diameter of 1.5 cm. At this size the tumours have minimal effect on the animals. In case of tumours that are not accessible on the surface, monitoring of tumour size by imaging enables us to terminate experiments prior to the occurrence of symptoms caused by cancer growth.

Survival data are often critical to demonstrate clinical translatability of novel therapeutic targets and agents. To this end, we optimized a monitoring protocol for mice undergoing survival experiments and towards the end of experiment the mice are monitored up to 5 times a day.

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

We are following the PREPARE and ARRIVE guidelines.

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

Our group stays informed though the NC3R website and we are aware of the available 3R online resources. We attend and participate in local events organized under the umbrella of the Animal Welfare and Ethical Review Committee. We also participate in interdisciplinary workshops and seminars that bring together different disciplines, including engineers focusing on the development of non-animal alternatives such as organ-on-chip models. We periodically review and discuss the literature with a focus on advances in modelling of in vivo systems.

**A retrospective assessment of refinement will be due by 03 December 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?