Home Office

NON-TECHNICAL SUMMARY

What Drives Melanoma Spread and Testing New Treatments for Melanoma and Ovarian Cancer

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, immunity, immunotherapy, metastasis, melanoma

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

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 how gene expression and metabolic changes enable melanoma to adapt to new tissue environments and develop drug resistance, with the goal of developing and validating new therapeutic options. Where relevant, these treatments will also be evaluated in preclinical models of ovarian cancer.

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?

Each year in the UK, around 17,000 people are diagnosed with melanoma, leading to over 2,000 deaths annually. Additionally, over 7,000 new cases of ovarian cancer are identified, with more than 4,000 deaths each year. For most patients with advanced-stage disease—when the cancer has spread to other parts of the body—there is often little hope of a cure, and survival may be limited to a few years or even just months after diagnosis. Despite these challenges, advances in understanding the molecular, cellular, and metabolic processes that go wrong when cancer spreads have led to the development of drugs and cellular therapies that can significantly slow disease progression and, in some cases, even achieve remission. However, the number of these effective treatments remains limited, and treatment resistance continues to be a major obstacle. Expanding the treatment options and finding ways to overcome resistance are critical areas where further research is urgently needed.

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

By the end of the project, we aim to have validated several new potential therapeutic targets for treating advanced melanoma or ovarian cancer, which may also be relevant to other cancers. Additionally, we seek to understand how cancer cells develop resistance to immune checkpoint inhibitors—drugs that enhance T cell responses (T cells being a key part of the immune system). We will explore whether these resistance mechanisms can be reversed using other drugs. Further, we plan to design and evaluate the safety and effectiveness of a novel chimeric antigen receptor (CAR). CARs are built from multiple proteins and introduced into a patient's T cells, guiding these cells to recognize a specific protein on the cancer's surface and then activating the T cells to destroy the cancer cells. Our findings will be shared at scientific conferences and in research publications, and any newly developed reagents will be licensed for commercial development where appropriate."

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

Our research is driven by a commitment to improving outcomes for cancer patients. Over the next five years, we expect that scientists in academia and the pharmaceutical industry will build on the knowledge we generate and share, using it to gain deeper insights into tumour immune responses, including the development of immunological memory, and to create new treatments and diagnostic tools. However, we recognize that any new therapies arising from our discoveries may take several years to reach patients, as they must first be identified and undergo thorough testing before they can be safely prescribed.

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

We will maximize the impact of our work through active collaboration and by sharing our findings as early as possible, first via pre-print servers and then through Open Access journals. Aligning with our institution's policy, we commit to making negative findings publicly available when appropriate and feasible. Specifically, we will aim to publish negative results in cases where the findings hold scientific value, particularly when they may inform and guide the research community, reducing the likelihood of unnecessary repetition of animal-based studies. This commitment recognizes the importance of rigorous experimental controls and considers both practical challenges and the availability of publication avenues for such data. Additionally, we will present our research at international conferences attended by academics, clinicians, and industry professionals. Our current partnership with a biotech company allows us to directly transfer our knowledge to an organization equipped to develop cellular therapeutics and advance them into clinical testing.

Species and numbers of animals expected to be used

- Mice: 3800

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 will use adult mice for our studies because the mouse immune system has been extensively researched, making our understanding of it the most advanced among laboratory animals. The necessary reagents for working with mice are readily available and well-tested, unlike those for many other species. Mice accurately reflect the human immune system in immunotherapeutic studies, and correlative studies show that results can often be translated between the two species. Adult mice have a mature immune system, are large enough to host tumours, and are more practical for treatment, allowing them to tolerate side effects better than younger mice. Additionally, we will use mouse embryos to live-image the migration and invasion of fluorescent-reporter-tagged melanocytes and melanocyte precursors—cells that produce skin pigment responsible for tanning and hair colour. We hypothesize that important similarities exist between these cells and melanoma cells as they disseminate through solid tissues.

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

Some animals will be used solely for breeding purposes. In most animals, we will establish tumours, primarily melanoma, at both superficial and internal sites. We will track tumour development over several weeks to months, often using non-invasive whole-animal imaging techniques. Certain animals will receive therapies, including drugs and cellular treatments, by injection, by a feeding tube reaching the stomach or in their food or water, and we will closely monitor their responses over days to weeks to assess both the effectiveness of the therapy and any potential side effects. Monitoring may require non-invasive imaging that would require anaesthetising the animals. Strong evidence suggests that melanoma cells become more like embryonic cells during disease progression or in response to treatments. Although we do not yet fully understand the functional significance of this reversion, it is likely to influence how tumour cells interact with the tissue environments they invade and with immune cells. To gain deeper insights into this process, we will conduct live imaging studies of embryonic development.

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

Cancer growth within an animal, as in a human, can have negative effects such as weight loss and obstruction. However, we closely monitor weight and specific behaviours in the animals to ensure their well-being, implementing observation schedules, control measures including appropriate anaesthesia and analgesia, and humane endpoints to minimise any potential discomfort. Genetically altered mice developing tumours or serving as hosts for transplanted tumours exhibit a sub-threshold or only mild phenotype when bred to maintain a colony. Tissue sampling from the ear (a non-regulated procedure) may be necessary to evaluate the genotype of mice and will be undertaken by highly trained staff. The planned treatments will use the least invasive route possible to administer the agent at doses which avoid toxicity; more invasive administration such as by feeding tube will be undertaken by highly trained staff. In some cases, we may expose only a few mice to an unknown agent, escalating the dose with careful monitoring. If our monitoring indicates any adverse effects, we will promptly adjust the therapy dose or discontinue treatment, and we will humanely kill animals to avoid lasting harm. For imaging embryos, we will use mice wherein genetic alteration labels their melanocytes with a fluorescent protein. No adverse effects are expected from substances administered to pregnant mice to switch on the fluorescent protein, nor from the expression of the fluorescent protein itself. Prolonged imaging of embryos will require extended anaesthesia. Sufficient and terminal anaesthesia should mitigate any adverse effects experienced by the mice; additionally, signs of vitality (regular heartbeat and body temperature) and hydration will be monitored regularly and supported to ensure no mice undergo unnecessary stress. If signs of stress are observed, mice will be humanely killed to avoid lasting harm. At the end of our experiments, animals will be humanely killed, and tissues may be collected for further laboratory tests.

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)?

Minimal, if any, adverse effects are anticipated in the animals used purely for breeding. All procedures involving animals with tumours, or without tumours will be managed to ensure they do not exceed

moderate severity, and most will experience mild severity as we will limit the growth of tumours to avoid interfering with vital functions or causing pain and use safe doses of agents. Imaging of embryos is classified as a non-recovery procedure. Overall, we estimate that the lifetime experiences of mice on this licence will be distributed as follows: approximately 35% will experience sub-threshold severity, 50% will experience mild severity, and 15% will experience moderate severity.

What will happen to animals used in 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?

The multiple cell types implicated in cancer formation, progression, and treatment response are all present only in an animal host. Additionally, in the case of drugs or cell therapies, we intend to address whether the therapeutic will be absorbed, metabolised and distributed effectively to have their effect without causing significant toxicity. Ultimately this requires an intact, living organism (notwithstanding the caveat that mice and humans have important pharmacological differences).

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

Human tumours and tissue culture were considered as non-animal alternatives and will be used extensively before animal experiments.

Why were they not suitable?

Non-animal alternatives cannot be used to test all our hypotheses due either to our inability to manipulate the system (as with human tumours, outside the context of a clinical trial with its own attendant ethical issues) or as with tissue culture because we cannot yet replicate all the important features of the in vivo environment and certainly not holistically. Co-culture systems can begin to address interactions between different cell types a couple at a time but not the entire spectrum of interactions. In particular, immune responses require the orchestrated activities of multiple cell types located at distinct anatomical sites, with various cells needing to migrate from one anatomical site to another.

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?

For each experiment, pilot and historical data are used in power analysis to predict optimal group sizes. Numbers are then scaled to encompass the anticipated/desired number of experimental scenarios, incorporating controls, different doses and independent replication.

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

Experiments will be appropriately controlled and mice of the same age, genetic background and source used to reduce the variability and thereby produce highly consistent data. An on-site statistician has advised us on design and power analysis, alongside the use of the NC3Rs Experimental Design Assistant. Bias will be avoided by random allocation of animals to treatment groups. Where possible and practicable, endpoints will be assessed by a technician who is unaware of the treatment the animal has received. Experiments will be designed carefully to avoid other sources of bias such as week-to-week or operator-to-operator.

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

Efficient breeding is used to minimise the sizes of colonies of genetically altered mice and to generate the numbers required for experiments. Real-time monitoring of cells (e.g. luciferase mediated imaging of tumour cell development) has considerably reduced the group sizes required for significant experimental results.

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 provide an in vivo context (anatomy, physiology, metabolism) that is relevant to human cancer and can be manipulated to generate data applicable to human cancer treatment. Therefore, mice are the most suitable in vivo model for achieving the stated objectives.

Sampling blood and administering tumour cells or drugs via subcutaneous, intraperitoneal, or tail vein injections are procedures performed by highly trained staff, ensuring brevity and minimizing discomfort. Needles will only be used once for sampling or administering substances to the mice. By responsibly considering the potential adverse effects associated with these regulated procedures, mechanisms are in place to minimize discomfort, including appropriate anaesthesia and analgesic regimens for pain avoidance and relief.

To further reduce suffering in tumour-bearing animals and treated animals, they will be humanely killed as soon as tumour formation is sufficient to yield satisfactory data, and always before they exhibit severe pain, or lose significant weight, which will be closely monitored.

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

Cancers develop in vertebrates, and mice are more similar to humans than lower vertebrates, such as zebrafish. This similarity is critical for using reagents, like drugs developed for human targets, and for translating findings back to the clinic. Since cancers develop over many weeks to months, the use of terminally anaesthetized animals or immature mice is not practical. Additionally, immature mice lack a functional immune system, which is essential for our research.

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

Therapeutic agents may be associated with toxicity related to the underlying mechanisms of anti-tumour activity and off-target effects. Our experience indicates that this has most frequently manifested as weight loss in less than 20% of experimental animals. This issue has been effectively managed through close observation and by providing mashed food instead of hard pellets. Whenever possible, animals will receive analgesia as outlined in the relevant protocol to control adverse symptoms associated with the treatment. In all cases, a predetermined set of criteria will be used to assess whether the duration of these symptoms is excessive or if the animals appear stable and/or show improvement over time, suggesting that these effects are transient.

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

PREPARE guidelines will be consulted ahead of initiating animal experiments.

All tumour studies will be conducted in accordance with the guidelines for working in rodent models of cancer as described by Workman et al (2010). (Guidelines for the welfare and use of animals in cancer research. Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA. Br J Cancer. 2010 May 25;102(11):1555-77).

LASA guidelines will be followed regarding volume of blood samples, and drug dose volume limits.

ARRIVE guidelines will be adopted when reporting our findings.

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

By reading 3Rs literature and participating in 3Rs workshops locally and nationally. Through discussing refinements with my NACWO, NVS, colleagues and HO inspectors.