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NON-TECHNICAL SUMMARY

Drivers of cancer drug resistance

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, Therapy, APOBEC, Drug resistance

Animal types	Life stages
Mice	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 cancers become resistant to treatment, to determine the role that a set of seven human genes (the APOBEC3 'A3' genes) plays in this process and to test whether blocking A3 function can improve therapeutic responses.

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?

If cancers are diagnosed at an early enough stage, they can be cured by surgery. Unfortunately however, many cancers have already spread beyond the tissue in which they first arose by the time they are diagnosed. These require chemotherapy and more recently, treatments referred to as targeted therapies and immunotherapies. These treatments often work well initially and patients become well again. However, it is sadly the case that often the cancer will recur after several years, months, or even weeks and at this point, the tumour cells can no longer be killed by the treatment that was previously effective. It is therefore an urgent priority that we understand how this phenomenon, often referred to as acquired drug resistance, occurs and how we can prevent it from happening.

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

The human A3 genes we study encode proteins that help to protect us from infection by many different viruses, including HIV and SARS-CoV2. They do this, at least in part, by attacking viral DNA or RNA and altering the genetic code that the virus needs to replicate. This protection appears to have come at a cost however, as there is strong evidence that many of the mutations found in the DNA of cancer cells are generated by off-target A3 activity. These mutations (‘mistakes’ in the genetic code) cause cancer and can enable cancer cells to become resistant to cancer drugs. Until now, it has not been possible to study this in mice, as they do not possess the human A3 genes that make mutations in cancer. By using a new mouse line that has all seven human A3 genes (generated and characterised under my current PPL), we will:

1) Demonstrate the effect of the human A3 genes on the ability of cancers to become resistant to a variety of cancer treatments that are of direct relevance to human patients.

2) Demonstrate the effect of the human A3 genes on the ability of the immune system to detect and respond to cancers.

3) Demonstrate whether inhibition of A3 gene activity can prevent or delay the development of drug resistance.

All these findings will be published in international peer-reviewed journals and presented at scientific conferences. They will also be shared with collaborators developing A3 inhibitors as cancer drugs. If successful, the studies conducted under this licence will directly contribute to the clinical testing of A3

inhibitors by the end of this project. If negative results are obtained, these will be published in peer-reviewed, open access journals wherever possible.

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

Researchers in academia and industry that are developing A3 inhibitors as cancer drugs will directly benefit from the information we generate, as our studies will provide important proof-of-principle that the strategy of A3 inhibition could be used to prevent drug resistance in cancer patients. These benefits will begin to be realised within the first 2 years of the project.

Due to the fact that we are collaborating closely with researchers developing A3 inhibitors, we anticipate that if these inhibitors are able to prevent drug resistance in one or more of our tumour models, clinical trials will commence by the end of the project and therefore it is possible that cancer patients will begin to benefit within 5 years.

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

We will publish all results from this project in peer-reviewed journals as soon as possible (i.e. once we are sure our findings are reproducible and interpretable, and allowing for the protection of any intellectual property arising by patenting - particularly important for the A3 inhibitor work). We also have a policy of posting pre-prints of our work upon initial submission for peer review, which means our manuscripts are freely available to all to read and use under a CC-BY open-access licence (the least restricted form of open-access publishing, in which content may be freely reproduced and used for teaching or other purposes, so long as the original source is cited) several months prior to final publication.

We are collaborating closely with both a small start-up company in the UK developing A3 inhibitors and large pharmaceutical companies that want to understand how tumours become resistant to their existing cancer drugs, and to drugs they are currently developing. This collaboration will greatly increase the impact of our work and will maximise the chances of patient benefit.

Species and numbers of animals expected to be used

- Mice: 600

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 mice as they are a long-established and very useful model for studying cancer development in humans. Importantly, we can engineer tumours with the same mutations that are seen in human cancers and that are treated with specific drugs that work only in tumours with these

mutations ('targeted therapies'). We also know that the immune system is a critical factor in determining how tumours respond to cancer treatment. Modelling this component necessitates the use of mice as opposed to alternatives such as frogs or fish. It is necessary to use adult mice, due to the way in which the tumours are induced at sites that are relevant to the human disease and due to the time it takes for tumours to develop, and for drug resistance to occur during treatment.

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

In a typical study, lung cancer development will be induced by the administration of viral particles directly to the upper airway / lungs, either by inhalation of a droplet through the nose (intranasal instillation), or through a tube into the windpipe (intratracheal administration) while the mice are under mild (isoflurane) anaesthesia (this procedure takes approximately 5 minutes / mouse). Tumour development will be monitored using non-invasive imaging (typically micro-CT scanning) under anaesthesiaat approximately 8 weeks post tumour induction and at regular intervals thereafter (typically once / month and no mouse will be CT-scanned more than 18 times). Mice will be treated with anti-cancer drugs known as targeted therapies, at doses that have been demonstrated to be safe, and these drugs will be administered either orally (oral gavage, maximum once daily) or by another suitable and approved route, most commonly intraperitoneal injection (maximum twice daily). These targeted therapies are much less toxic than conventional cancer chemotherapy drugs, as they specifically target proteins that become essential for the survival of cancer cells but not for healthy cells. The drugs that we will use primarily in this project have not displayed any significant toxicity in mice, even with daily dosing over periods of several months. Dosing may continue for up to one year and mice will be closely monitored for signs of drug toxicity and/or suffering due to lung cancer burden. In some cases, we will administer experimental drugs (A3 inhibitors) in combination with the anti-cancer drugs to test whether we can prevent tumours becoming drug-resistant. We will only use A3 inhibitors once we have received detailed information on their safe usage in mice and on any potential toxicities from our collaborators who are developing them. Animals reaching pre-defined humane endpoints, and any animal alive at 1 year after the initiation of treatment will be culled via an approved Schedule 1 method. Tumour induction will typically be conducted on mice between 8-12 weeks of age and tumours typically take between 8-20 weeks to grow to a sufficient size to commence treatment, depending on the model. Therefore, even those mice that receive treatment for the maximum of 12 months without experiencing tumour recurrence would typically be killed by the age of 20 months. We have included an optional step to keep mice alive to 2 years of age, in case of delayed tumour development, rather than wasting animals in which tumours develop more slowly than expected.

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

Mice injected with a drug are expected to experience mild and short-lived pain at the time of injection but we don't anticipate any longer-term discomfort. Similarly mice receiving drug by oral gavage will experience short-term discomfort but no lasting harm/discomfort.

Mice in which lung tumour development is induced may experience symptoms related to reduced lung function, most commonly breathing difficulties and/or weight loss. Mice displaying such symptoms will be closely monitored and culled as soon as a humane endpoint is reached.

Mice approaching two years of age may experience symptoms related to ageing, and all mice over 15 months of age will be closely monitored for signs of any such symptoms.

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)?

Approx 90% of mice in this project will be used for tumour induction studies, and they may experience discomfort of moderate severity. No animals are expected to experience anything more than moderate severity.

What will happen to animals used in this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

We need to understand how tumours develop and how our genes of interest contribute to this process in the context of a living organism. Factors such as inflammation, disturbed sleep cycles and obesity can all increase our risk of cancer development and it is impossible to model these in cells growing in culture. We also need to establish whether new drugs targeting the A3 genes are likely to be effective before they can be trialled in humans.

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

We have conducted extensive analysis of the A3 genes in human clinical samples using DNA sequencing and gene expression data from The Cancer Genome Atlas project and International Cancer Genome Consortium. We have also made extensive use of human cell culture models, which form the main focus of our current research and we will continue to use human cell cultures wherever possible. We have invested a lot of effort in generating A3 knockout and A3-tagged human lines. We have also been successful in generating cell lines from the lung tumours that we have generated in these mice under our existing licence. Wherever possible we will use cells derived from our mice rather than the mice themselves.

Why were they not suitable?

Human cell cultures will continue to be our primary research tool and will inform our animal experiments. However, the key questions concerning the role of A3 genes in cancer development and progression are only addressable in an animal model. Furthermore, the development of A3 inhibitors as anti-cancer agents depends upon establishment of appropriate preclinical models for testing prior to trialling in human cancer patients.

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 planned a detailed series of mouse studies that we need to conduct over the entire period of the licence to achieve our objectives. For each experiment, we will use the minimum number of mice required to give us robust, meaningful data.

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

We've followed guidelines detailed in the 'The Designing of Animal Experiments: Reducing the use of animals in research through better experimental design' (Festing, Overend, Borja and Berdoy; 2nd Edition). We have also used NC3R's Experimental Design Assistant to help plan our 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 use pilot studies to help refine our estimates of expected effect sizes. We will collect multiple tissues from euthanised mice to learn as much information as possible per mouse. We will also derive and culture cells from mice and will use those for analysis and further experiments (e.g. drug treatments), rather than performing procedures on live mice wherever possible.

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.

This project is entirely based on the use of adult mice, in which we will model tumour development and treatment. We will use non-invasive imaging to monitor lung tumour burden, so that experimental mice that are not being used for experiments designed to measure time-to-reach human endpoint (a surrogate for terminal tumour burden) can be killed prior to the onset of any clinical signs of harm. We

have carefully chosen humane endpoints to ensure that mice experience no more than moderate severity.

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

We need to study tumour development and the genes in which we are interested act in humans over many years to generate the mutations in DNA that eventually cause cancer in adults. These genes are not relevant to childhood cancers, so we need to study tumour development in the adult mouse. While a number of cancer-relevant studies can be conducted in non-protected animals, such as nematodes or flies, modelling the complex interplay between A3 gene expression and inflammation and/or viral infection and cancer development requires a model as close to humans as possible, hence our use of mice. Furthermore, we anticipate using these mice as a preclinical model for A3-targeted drug development. Again it is essential to conduct such studies in an organism that best approximates the action of a potential drug in patients, while avoiding the use of (for example) non-human primates. Indeed, by generating the humanized A3 mouse model, we have enabled these studies to be conducted in mice, as opposed to in non-human primates, which are the only other species apart from humans to possess the 7 A3 genes.

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

All work will be conducted by trained personnel, who will be given further training in specific techniques where necessary. This licence contains only protocols categorised as mild or moderate and anaesthesia and/or analgesia are used wherever appropriate. Animals will be closely monitored during all procedures and where possible we will consider refining existing techniques or incorporating new methods to minimise any suffering to the animals.

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

Ullman-Cullere et al 1999 'A Rapid and Accurate Method for Assessing Health Status in Mice' Lab Animal Science; 49(3):319-323.

Wilkinson et al 2019 'Progressing the care, husbandry and management of ageing mice used in scientific studies' Laboratory Animals 54(3):1-14.

Turner et al 2011 'Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider' J Am Assoc for Laboratory Animal Sci 50;600-613.

Workman et al 2010 'Guidance for the welfare and use of animals in cancer research' Br J Cancer 102;1555-157.

Diehl et al, 2001 'A good practice guide to the administration of substances and removal of blood, including routes and volumes' J. Appl. Toxicol. 21, 15–23.

'The Design of Animal Experiments: Reducing the use of animals in research through better experimental design' (Festing et al 2nd Ed).

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

Our NTCO disseminates any 3Rs training or events at our quarterly Facility User meetings.

I will also continue to read the latest relevant scientific literature, attend conferences and consult policies on animal research from relevant funding bodies (Cancer Research UK, UKRI, NC3R's and the NC3R’s Oncology Network.