Home Office

NON-TECHNICAL SUMMARY

Understanding epithelial tumour initiation

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Cancer initiation, Prevention, Epithelial cells, Development

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant, aged

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 identify the early cellular and molecular events that drive epithelial tumour initiation. This information will enable us to detect tumours early and prevent their progression.

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?

Cancer is a devastating disease. Every day in the UK, 1000 men and women are diagnosed with cancer and 450 die from the disease. Progress has been made in developing better drugs to treat cancer. However, the most impactful intervention one can make for cancer patients is to detect the tumours early. The evidence is clear, the earlier a tumour is detected the higher are the chances of surviving the disease. For example, data from Cancer Research UK shows that breast cancer patients for which the tumours were detected early they have 90% chance of responding to treatment and surviving cancer-free for more than 10 years. In contrast, patients for which cancers were detected at a late stage of the disease they have less than 10% chance of surviving cancer-free for more than 10 years.

In aim of this project is to increase our understanding of how tumours develop. In particular we want to identify the early changes that occur in the tissues before tumours grow. This information will allow us to develop accurate early detection methods and hopefully new methods of how to prevent tumours from developing all together.

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

1) Advancing our basic understanding of how cancers develop. It is only now that we have the technical ability to study the early steps of tumour initiation. We will share any large datasets we generate in this project with the scientific community so it can be used by others in their research.

2) Identification of new markers that can be used for the early detection of tumour initiation. These will be reported and shared through peer review publications.

3) Developing new genetically engineered mouse models that can be used to accurately study tumour development.

4) Patent and grant applications based on our findings to enable the development of early detection and therapies.

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

1) Scientists studying cancer biology. The large datasets and mouse models generated from this project will benefit their research. We deposit all our data in public repositories and also generate free user friendly websites that accompany the publications of these studies so everyone can access and benefit from the data.

2) Cancer Patients. The identification of new markers that can be used for the early detection of cancer will improve the clinical care provided for cancer patients. We aim to validate our findings in the mouse and pursue the translation of our findings as soon as possible.

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

Data generated from this project will be presented at national and international conferences as well as shared through publications and pre-print outlets such as BioRxiv. We will also continue to make user-friendly websites to access the single cell genomics data generated form the mouse studies.

It is important in science to also share unsuccessful approaches to minimize unnecessary animal use by others. We will aim to publish any unsuccessful and technically challenging approaches using BioRxiv so others can benefit from our experience.

Species and numbers of animals expected to be used

- Mice: 20,000

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.

Despite the significant improvement in cancer care over the last decades, we still have little understanding of the events surrounding tumour initiation. Most cancer studies to date, both in mouse and in human, have focused on characterising the tumour and how to shrink it. Now with the advances in sequencing technology we have the ability to study the very early steps of tumour development using genetically altered mouse models. This is important as it will reveal the earliest markers of cancer development, which can then be used for the development of: 1) early detection methods and 2) therapies that can prevent cancer development. These kind of studies are only feasible in the mouse, particularly when studying solid tumours (eg. Breast, Ovarian, Lung and Intestine). We will generate mice that are predisposed to developing cancer and investigate their tissues at the cellular level using novel sequencing technologies, prior to the detection of physical tumours. This analysis will reveal the very early cellular changes that occur in a tissue before tumours arise. It is impossible to perform these kind of pre-cancer studies in humans.

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

We will need to generate and maintain genetically altered animals.The majority of our work will be focus on tissue specific tumour initiation which will be achieved using tissue specific gene deletion/induction as this will minimise unwanted harm. The animals will be monitored for tumour development and in some cases treated with potential therapeutics. In addition, as most of our focus will be on the early steps of tumour initiation, we will have only a small proportion of our animals with fully developed tumours. In general mice in the project could be used for:

- the generation of new or re-derivation of genetically altered animals obtained from public repositories or research labs.
- the breeding and maintenance of genetically altered animals that can be used in the experimental protocols.
- the treatment with tumour inducing substances or cell labeling substances or contrast agents for Xray/CT scaning using one the following methods (Steps 3-5) :

1. oral gavage (a tube inserted via the mouth into the stomach to deliver a soultion).
2. subcutaneously (i.e under the skin).
3. intraperitoneally (i.e. into the body cavity).
4. intravenously (i.e into a vein)
5. implantation of a slow release pellet subcutaneously under anaesthesia.

It is not possible to fully predict the nature or severity of any potential defect from new genetic mutations. We expect if these new mutations predispose animals to tumour development, most of them (80-90%) will develop within 1year. In few animals (10- 20%), it may take up to 2 years of ageing for this new mutation to cause tumour development. Tumour inducing agents are highly mutagenic and hence increase the chances of tumour development. We expect 100% of mice treated with tumour inducing agents to develop tumours within 1 year. Transgene inducing/deleting reagents, in rare cases, may cause skin inflammation, thickening or flaking, hair loss or altered pigmentation, benign dermal cysts, skin erosions or tumours. General anaesthetic might be used if subcutaneous injections or implants are used (30% of animals). Animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Surgical procedures will be carried out aseptically. In the unlikely event of post-operative complications, animals will be killed unless, in the opinion of the NVS, such complications can be remedied promptly and successfully using no more than minor interventions. In the case of wound dehiscence, uninfected wounds may be re-closed on one occasion within 48 hours of the initial surgery.

- Mice might undergo direct injections of viral particles that can induce breast cancer.

More than 99% of animals are expected to make a rapid recovery. Analgesic agents will be used to minimise pain wherever possible. In rare cases (<1%) it is possible that adverse effects from anaesthesia could occur. The procedure is not expected to result in any frequent or chronic adverse effects. By removing the dead skin from the nipple non-surgically, we expect that the already low risk of infection will be further minimised. Mastitis is a possible adverse effect of injecting into the mammary duct. However, the risk of mastitis is greatly reduced by using a blunt needle which is as short as

possible and is not inserted into the mammary gland. The fluid pressure is sufficient to carry the viral particles into the gland with the needle positioned at the tip. Mice that show signs of redness/inflammation (mastitis) following completion of an intraductal injection will be given a mild anti-inflammatory and monitored closely. Mice that display persistent mastitis (not resolved 72 hours after injection) will be killed by schedule 1 method. Viral constructs (for example constructs inducing oncogene expression) might lead to the development of tumours.

- Viral reagents might be delivered via inhalation to induce gene expression, deletion or editing directly in the lung.

Delivering the viral agent (eg. Adenovirus or Lentivirus) for tumour induction is done under general anaesthetics. This is a non-invasive procedure where a small drop of viral agent is applied at both nostrils. Tumours - Tumour incidence varies with method of induction and genetic background. The majority of studies do not require advanced tumour development therefore 75% of tumour bearing animals will be killed by a Schedule 1 method before moderate adverse effects are exceeded. 25% of animals will harbour advanced tumours. General anaesthetic will be used but animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Any animals that fail to do so or exhibit signs of pain, distress or of significant ill health will be killed by a Schedule 1 method.

- Mice might be injected with cancer cell lines to perform xenograft tumour growth assays. Cells mignt be injected subcutaneously or through the tail vein.

Post-surgical infection is unlikely (1%) as aseptic techniques are used. However, in case of an infection developing mice will be killed by a Schedule 1 method. General anaesthetic will be used but animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Any animals that fail to do so or exhibit signs of pain, distress or of significant ill health will be killed by a Schedule 1 method. Wild-type cells injected subcutaneously are not expected to develop tumours. However, tumour cells or cells carrying novel cancer mutation may develop tumours.

At the end of all experiments mice will either be humanely killed, or tissues and organs collected under deep, terminal anaesthetic unconsciousness‘ or similar.

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

To be able to study the early steps of tumour development we will need to breed mice that will develop solid tumours. We will focus on breast, ovarian, lung and digestive tract tumours. For some mouse lines they will be administered with non-toxic gene inducing agent via injection, water or food. In some cases mice will be treated with carcinogenic agents to induce tumour initiation. In all cases tumours will develop within several months. As most of our focus will be on the early steps of tumour initiation, we will have only a small proportion of our animals with fully developed tumours. In some cases, a potential therapeutic agent or preventative agent (eg. Vaccine) will be administered in cancer prone mice to determine their effect on tumour initiation.

General anaesthetic will be used for surgical procedures. Animals are expected to make a rapid and unremarkable recovery from the anaesthetic within two hours. Surgical procedures will be carried out aseptically. In the unlikely event of post-operative complications, animals will be killed unless, in the opinion of the Named Veterinary Surgeon, such complications can be remedied promptly and

successfully using no more than minor interventions. In the case of wound dehiscence, uninfected wounds may be re-closed on one occasion within 48 hours of the initial surgery.

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)?

Mice:

- Mild 40%
- Moderate 60%

What will happen to animals at the end of 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?

Cancer is a complex disease that develops in intact tissues. Studying primary human tumours at the molecular levels have been very informative as demonstrated by the international cancer genome consortium (ICGC) and the cancer genome atlas (TCGA) and ICGC studies. However, it is necessary to have a realistic model, which is amenable to genetic, and biochemical studies whilst maintaining tissue architecture. The mouse allows us to perform such detailed genetic and biochemical studies whilst maintaining the 3D tissue organisation and normal physiological environment. We will aim to use laboratory based cell lines whenever possible to perform validation and biochemical studies. However, cell lines have their limitations as they are a) self-selecting populations of cells that can survive on plastic and b) grow in a 2D environment. We will switch to animal experiments when the data at hand clearly supports the need for it.

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

Cell lines and primary organoid cultures. Organoids are small, three-dimensional tissue cultures that can be grown in a dish in a lab into such an ordered pattern that they mimic a lot of the complexity of an organ and remove the need to repeatedly collect animal tissue.

Why were they not suitable?

The mouse allows us to perform such detailed genetic and biochemical studies whilst maintaining the 3D tissue organisation and normal physiological environment. We will aim to use cell lines whenever possible to perform validation and biochemical studies. However, cell lines and organoids have their limitations as they are a) self-selecting populations of cells that can survive on plastic/matrigel and b) grow in a 2D environment.

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 aim to identify the earliest cellular and molecular events that occur prior to epithelial tumour development. To achieve this we will use Genetically Modified (GM) animals which we have extensive experience in using. The experimental design overlaps considerably between the various epithelial tissues. Calculations were also informed by reference to the National Center for the replacement, refinement and reduction (NC3Rs) experimental design assistant. Mice will be randomly assigned to experimental or control groups whilst maintaining a comparable age, size and sex if possible (this is not feasible for breast cancer studies). The team of researchers will each be assigned a role to ensure that experimental and control groups remain blinded to the researcher designing the experiment. We have also performed this type of analysis previously and based on that published analysis we now have an informed estimate of the number of animals needed. In some instances pilot experiments might be needed to determine the number of mice necessary to achieve robust statistical results when experiments are ready to be conducted in full.

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

For new and pilot studies we will consult the NC3Rs experimental design assistant following the PREPARE guidelines. In addition, we consult with our bioinformatics collaborators ensure experiments are powered appropriately for downstream computational analysis.

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

Most of our GM animals have complex genotypes we have carefully planned breeding strategies to maximize the number of suitable experimental animals and control littermates. All animals will be humanely killed at the end of experiments and tissue samples taken for further experiments. Where possible mouse tissues will be shared amongst the research group. Where appropriate pilot studies will be conducted to determine feasibility and efficacy. Depending on the tissue and scientific question, mice will be randomly assigned to experimental or control groups. In addition, we try and run most experiments with at least two team members to enable blinding of the groups thus minimising bias.

Finally, when ever possible we always try and store and share tissues with the extended research community to maximise the amount of data and information collected from each mouse used.

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 the mouse to model and study the early steps of cancer development. We will generate genetically altered mouse models that represent the types of cancer seen in the clinic. We will breed these mice and monitor them for tumour development and tissues will be collected from these animals and analysed to identify the earliest signs of tumour development. Importantly, these models generate tumours with predictable latency and growth thus allowing termination of the experiment at an earlier stage when tumours are still relatively small. Thus, our tumour models mean that most mice are likely to exhibit only moderate adverse effects. The small number that exhibit severe effects will be killed immediately. We will use tissue specific genetic alterations which will minimise unnecessary harm for the animal and model the disease more accurately.

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

Cancer is a complicated disease that starts well before a tumour is detected. It is also a fact that cancer cells do not grow in isolation but do so by interacting with and manipulating the healthy cells in their surroundings, which is called the cancer micro-environment. By understanding, how cancer cells manipulate the micro-environment during the early stages of cancer hold the key to the development of new methods of early cancer detection and therapeutics. Therefore, it is not possible to use more immature stages or terminally anesthetised animals to answer this important and complex biological question. Mouse physiology is sufficiently similar to that of humans thus, enabling us to model human disease with a high degree of accuracy. This is facilitated by detailed knowledge and comparison of mouse physiology and genetics.

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

We will aim to house the mice in social groups of up to 5 to enhance their social interactions and experience. If mice undergo surgical procedures we will administer pain medication and use post-operative bedding, heat mats if anesthesia was prolonged and provide mash for easy access to food. Animals are expected to make a rapid and unremarkable recovery from the anesthetic within two hours. In the uncommon event that animals fail to do so or exhibit signs of pain, distress or of significant ill health they will be killed by a Schedule 1 method unless a programme of enhanced monitoring and care is instituted until the animal fully recovers. Any animal not fully recovered from the surgical

procedure within 24 hours (eating, drinking and return to normal behavior) will be killed by a Schedule 1 method.

Tumours - Tumour incidence varies with method of induction and genetic background. The majority of studies do not require advanced tumour development therefore 75% of tumour bearing animals will be killed by a Schedule 1 method before moderate adverse effects are exceeded. 25% of animals will harbor advanced tumours. Tumour burden will be measured using non-invasive methods such as Calipers. We will also explore the use of more advanced and quantitative non-invasive devices such as the Biovolume 2000 (https://www.biovolume.com/).

Pain management - After consultation with the veterinarian and if needed, pain management treatment could be provided in flavored jelly, paste or milk shake formats.

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

We adhere to the guidelines published in Workman et al (2010) Guidelines for the welfare and use of animals in cancer research. BJC 102, 1555 - 1577. We also consult the PREPARE guidelines when designing and planning animal experiments. It is also important that we report how animal experiments were conducted so that others can learn from our experiences and minimise potential animal suffering. For this we adhere to the NC3Rs ARRIVE guidelines on reporting of In Vivo Experiments. For surgical procedures we adhere to guidance from LASA such as the 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery (https://www.lasa.co.uk/current_publications/).

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

We are advised of advances in the 3Rs via regular email correspondence with our dedicated technician who regularly attend relevant 3Rs meetings. We also keep up to date through the use of the NC3Rs website pages (https://nc3rs.org.uk/resource-hubs) and 3Rs tools in-house and external resources such as Norecopa https://norecopa.no/databases-guidelines. In addition, we also regularly consult the Jackson Laboratories website which is a valuable online resource that include the latest updates in animal husbandry and care. (https://resources.jax.org/)