



Home Office

NON-TECHNICAL SUMMARY

ROLES FOR GENOMIC INSTABILITY IN CANCER AND AGEING

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
 - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
 - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Cancer, Ageing disease, Genomic instability, Phenotyping, Therapies

Animal types

Life stages

Mice

adult, embryo, neonate, juvenile, pregnant

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?

The aim of this project is to find genes that regulate genomic instability (GIN). The genes will be researched to find ways to prevent genomic instability beginning and building up.

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?

Genome instability (GIN) contributes to many diseases which, if prevented, would lead to disease improvement. Discovering ways to prevent GIN could lead to better treatment of cancer and ageing.

Our cells are exposed to factors that lead to DNA damage. The environment, such as UV light, radiation and chemicals, can cause the damage. Alternatively, damage can come from within the body during natural processes. For example, when the body uses water or converts food to energy and other life sustaining components. When these processes go wrong in the body, bad chemicals or molecules can be created that cause DNA damage. The natural processes in the body may go wrong because of faulty genes.

DNA can be damaged in a minor way but also in a major way. DNA can be damaged at different times when a cell is growing. How the body decides to fix damage to DNA depends on these circumstances and is called the DNA damage response (DDR).

DDR senses DNA damage and triggers DNA repair, cell cycle arrest or cell death. Failure of these responses results in GIN and the start of disease. This includes developmental disorders such as progeria, an illness where people show signs of ageing and die early. Also, some forms of ataxia, which can affect co-ordination, balance and speech. Such diseases may affect the brain, the nervous system, muscle function and the immune system. They can affect people at different ages, young and old. They may cause degeneration that results in loss of movement, the senses, premature ageing and ultimately death. DDR failure resulting in GIN also leads to cancer and ageing related disease including dementia. A better understanding of how GIN works provides potential routes for disease prevention and treatment.

There were 9.6 million deaths from cancer worldwide in 2018. Alzheimer's and other dementias led to 1.5 million deaths in 2017. As the world population ages the cases of cancer and age-related illness are set to increase. It is estimated that 47 million people live with dementia around the world. Finding suitable treatments for certain age-related disease has proven difficult. We believe that this area of research is crucial for discovering new ways to combat cancer and age-related illness.

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

Primary outputs:

- New genes of interest will be identified and described.
- Novel therapeutic strategies will be discovered.

New mouse strains will be created and mouse strains created elsewhere will be investigated further, providing new information about those strains. New data will be created on the effect of novel therapeutic agents on the process of genomic instability (GIN).

We will gain new knowledge of how certain genes work. We will also get information of how one or more genes work together. This information will allow us to make decisions on the best way to move forward in researching the many different genes involved with GIN. We will get new information on how certain genes and combinations of genes cause diseases to be worse. Importantly, we will discover gene combinations that will reduce illness and suffering. For example, we will find combinations of genes that will reduce the rate at which a cancer will grow. We will find combinations of genes that will stop or reduce the faulty development and ageing health issues associated with GIN. Biomarkers or gene targets will be identified. These secondary outputs will help us work toward our primary output to find particular drugs to combat the illnesses.

New cell lines will be derived from the embryos of the mouse strains studied. Newly created mouse strains and materials originating from these mice, including cells and tissues, will be made available to the scientific community.

Work will be published in peer reviewed journals.

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

Mice and tissues produced will be supplied to other researchers. This will include new mouse models and novel genetic crosses with details of their phenotype. This will enhance and speed up the research for these investigators.

Outputs such as biomarkers could be used in pre-clinical and selected clinical trials. A biomarker is something that can be measured to show what is happening in the body. Measuring biomarkers can indicate the presence of a disease. Cancers can produce biomarkers and so can the body's healthy cells in response to the cancer. Cancer varies between individual patients and using biomarkers can help show what is happening inside the body and identify the best approach for dealing with a particular case. Thus, finding new biomarkers improves our ability to both detect and treat cancer.

GIN is a hallmark of cancer and ageing and there are millions of people in UK and throughout the world that are affected by GIN related disease. The approach we will employ in this project and the outputs generated will help find new therapies to fight illness in those affected. Our work will provide benefit for those suffering from neuro-developmental disorders such as ataxia. We will be contributing to finding ways to combat dementia, including Alzheimer's and Huntington's. Other specific illnesses targeted include Hutchinson Gilford Progeria and Cockayne syndrome. Ultimately, cancer sufferers and patients of these diseases will benefit along with their carers and society as a whole.

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

All mouse lines we produce will be made freely available. Moreover, we will report all data that will be generated under this licence. Negative data is very informative but seldom published; following quality control and validation, all generated data, positive and negative will be published in open access databases or journals. We will collaborate with other researchers in the field and present findings through meetings and conferences.

Species and numbers of animals expected to be used

- Mice: It is expected that a maximum of 14,000 mice will be used over the course of 5 years, with 9,500 of these being used solely for breeding to generate mice for analysis.

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.

Using mice is the best test against data gained in the lab. Using ageing and cancer mouse models is the only approach available for full investigation of the processes involved with DNA damage response (DDR).

Using the mouse embryo allows us to make the mice we need for our work. The mouse embryo also allows us to do work without the need for live mice. Young mice are used because the genes we study may have an impact on development. Studying young mice is important when looking at illness relating to age because signs of illness can start earlier with age related disease. Much of our work will be done on adult mice since a lot of data can be gained at this stage.

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

The mice on this licence will be used to understand the role of genomic instability (GIN) in the promotion or dampening of tumour formation and ageing.

Some new mouse strains will be created. Different strains of mice will be bred together. Such strains will not have been bred together previously. We will study these new strains to see if they produce mice at the usual rate and whether they develop normally. We will look at the development at different life stages. Individual mice from these new strains may be examined over the first few weeks of their lives, have their weight, shape and size measured. Simple procedures may be carried out on these mice, such as a blood sample or x-ray image. Other mice from the new strains will be used to test behaviour.

In these cases mice will undergo non-invasive behaviour tests carried out on several days over a three week period to examine how the brain is working.

We will breed different strains of mice together to see if one strain stops the signs of disease in another.

Mice will undergo procedures where tumour development can occur or they may be allowed to age. These mice may carry an altered gene and are monitored to see if they develop a developmental defect, tumour or age related change.

Sometimes, mice will be injected with cells that produce tumours. All such mice are monitored daily for any signs of developing tumours. The mice will be checked to see if the tumours are making them ill and may be given drugs to reverse the tumour growth. Such mice will be studied for a few weeks.

Mice will have blood samples taken. Images including x-rays may be taken. Sampling and imaging procedures are quickly carried out as a single event.

If a mouse becomes sick unexpectedly it will be humanely killed and the mouse examined to see why it was displaying these symptoms. Samples will be collected for further analysis.

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

Many mice will suffer no adverse effect during the project.

Some mice will experience no more than a transient feeling of pain or suffering.

Few mice will develop at a slower rate and/or have developmental abnormalities, for example curvature of the spine. These effects may develop gradually over weeks and will not be allowed to prevent the mouse from feeding and drinking.

Some mice will form tumours. Tumours may last for a few weeks but will not be allowed to stop mice carrying on with normal behaviour. For short periods, the body weight of the mouse may increase or decrease in the presence of a tumour. Mice may move differently or have uncoordinated movement. Breathing may be more difficult. Skin could become more irritated in the presence of a tumour.

Some mice may show signs of ageing earlier. There may be gradual weight loss, over several days, and loss of fat and muscle mass.

Certain mice may experience loss of coordination or seizures. This is expected to be rare and for short periods.

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)?

A large proportion of the mice, possibly more than 5000 mice and particularly on the breeding licence, are not expected to suffer any adverse effect (sub-threshold).

About 30% of the mice we propose to use will experience pain or suffering that is classified as 'mild'. Particular mouse strains known to develop adverse effects or an illness because of their genetic alteration will not be allowed to develop more than short-term mild pain, suffering or distress. The genetic alteration in the mouse may result in a difference in growth rate, size or behaviour. However, these mice will have a normal lifespan and will be able to freely move and feed. These mice will have only one or a limited number of procedures carried out on them, which will result in no lasting adverse effect. Where these mice are used for more than one test, it will be ensured that the combined affect causes no more than slight or transitory pain or suffering.

About 35% will be classified as 'moderate' severity. These mice may show signs of disease but will not become severely ill or die as a result. Mice may lose more of their body weight, up to 15% weight loss. Some mice may have a more significant loss of normal movement or there may be more persistent evidence of an illness. Some mice will develop tumours. Growths will be monitored to ensure they do not go over a certain size and do not make the mouse severely ill or significantly impair movement. Surgical procedures, for establishing new mouse colonies, will result in pain for no more than a few hours and include administration of pain relief.

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

- Killed
- Used in other projects
- Kept alive

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 can test in the laboratory without using animals to provide useful data on how diseases work in humans or animals. We can also run tests without using animals to see how different genes work together to make a disease better or worse. Particular cells from animals can be used to test the effect of drugs that could stop a disease. With cancer and ageing as studied in this project, we can only confirm that lab tests have worked well by testing further with mice. When mice are used to find genes that affect cancer or ageing or to prove a drug works against a disease, it provides an avenue for finding medicines for humans.

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

Extensive analysis without using mice is integral to the project and is always the first option considered when new biological areas of interest are identified. Organoids from both human and animal origin are our first alternative to using mice.

Organoids are 3D organ-buds that can be grown in the lab. They can be made from the tumour and normal tissue of a patients cell. Organoids enable us to study how tumours develop and how they respond to treatments. Tests using organoids can reduce the number of mice needed for experiments and improve the chances of successful experiments using mice.

Why were they not suitable?

Accumulation of GIN is a multi-step, highly complex process involving cell and tissue interactions. GIN accumulation can act together with multiple genetic abnormalities. GIN associated disease including developmental disease, cancer and ageing affects all organ systems in a highly interconnected fashion. This means that GIN and the associated diseases cannot be fully modelled without a whole organism such as the mouse.

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?

This is based on previous experimental work for similar programmes of work done on licences held in other establishments whereby 15,000 mice were created and used over an 8 year period.

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

Mouse experiments are not carried out until there is sufficient evidence from testing in the lab. Animal sample size calculations are made to ensure as few mice as possible are used whilst still achieving good results. Online resources from trusted and experienced organisations provide software and templates that help with this.

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

Where possible we shall import existing mouse lines rather than generating new ones. We will utilise the archive at our previous establishment, where our current colonies are cryopreserved, and will use wildtype mice that are surplus wherever available. In general, we will order lines in as frozen sperm or

embryos for recovery. This will reduce the number of mice used for breeding between facilities as well as remove the harms related to the stress mice are under when in transit.

For certain studies, such as when cells are injected into mice for cancer experiments, we will use commercially available mice. This will reduce the need to produce and breed mice at the facility.

All mouse lines will be archived so that they may be distributed to other researchers worldwide. This will reduce the number of animals used globally, as fewer animals will be required to re-generate these archived lines.

In some circumstances, such as when certain mouse lines have been only recently created, less published data will be available thus we propose to perform small pilot experiments to determine the final experimental design.

Wherever possible, multiple experiments will be performed on the tissues collected from an individual mouse so as to maximise the use of the mouse.

All data generated from our research on the mice will be published in scientific journals available to the whole scientific community, reducing duplication of production resources and phenotyping procedures elsewhere. Wherever possible, the results of experiments that involve large datasets will be made publically available to serve as a resource for other scientists and clinicians.

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.

Mouse models of cancer and ageing will be used during this project.

Mice will only be bred if they are required for experimental analysis, distribution or archiving. Furthermore, the methods selected to analyse mice are all standard, robust, well validated tests that are commonly used in high throughput projects.

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

Mice and humans have the same basic organ systems, skeleton and reproductive cycles. These similarities, coupled with the technology available to manipulate the mouse genome, make the mouse the best to copy human diseases. There are no, less sentient animal models for tumour growth that can be used. Using non-mammalian species of lower neurophysiological sensitivity is not possible since they lack appropriate tissue physiology. Using mice at an immature life stage would not be possible as we need animals to be mature since we are often looking in to the effect of ageing.

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

Whenever guidance materials are updated with recommended refinements, we will strive to include them into our procedures. We will regularly review literature and discuss possible refinements with peers and experts.

At all times, mice are handled calmly and following advice for best practice, for example tunnel handling.

Where mice are imported in to the facility, they will always be given a minimum of one week for acclimatisation prior to use in procedures or further movement.

Non-surgical methods of transferring embryos will be considered for mouse production. This will be assessed based on the ability to recover suitable numbers of live mice. All surgery will be undertaken in full compliance with Laboratory Animal Science Association aseptic technique guidance to minimise infection risk. Mice will always be provided with pain relief for surgical procedures, prior to commencement of surgery and, if required, following surgery. The group has over 10 years experience in refining surgical procedures, including the development of improved aseptic technique and rigorous analysis of surgery competency. We will continue to refine skills and techniques to improve surgical outcomes.

When creating new lines we will use optimal methods for design and production. For example, it is our intention to create new GA mice using CRISPR technology, which significantly reduces the number of animals required for both surgery procedures and later during breeding.

Mice which have had anaesthesia will have frequent monitoring until fully recovered and additional post recovery checks in animal holding rooms. When general anaesthetics are necessary, practicable combinations with the least adverse effects will be used. For example, gas anaesthetics will be used wherever possible.

It is our intention to incorporate image recording technology for monitoring of some behaviour tests to improve our ability to successfully blind the experiments.

Tumour growth experiments are carried out with extra monitoring of the mice involved. Mice will be checked using advanced welfare checks. These checks will be done more regularly than standard welfare checks and by staff trained to spot particular problems. Monitoring protocols can be changed or updated based on the particular mouse strains being looked at. For example, mice expected to experience adverse effects at an earlier age will have monitoring more regularly at an earlier stage of their life. Similarly, mice expected to develop tumours quickly will be monitored more regularly and at an earlier stage of the study.

When dosing mice for treatment drugs via oral gavage, we will be using a refined dosing technique using plastic/silicone gavage tubes that reduce trauma and make visualising delivery easier.

If there is a requirement to dose mice with treatment drugs beyond 30 days, usually administered via the intraperitoneal route, we will consider the option of mini-pumps for continuous dosing as an alternative.

Prior to recovering strains for breeding we will examine health observations previously recorded on a database that will allow us to accurately predict the onset of certain clinical signs of illness. We will then make notes on the database used by animal care staff so that they can quickly respond to welfare concerns. Furthermore, we will create a simple reference document listing all strains that will be used on this licence, including description and time of onset for particular health concerns.

For all tests it is important that the animal has no additional stress, therefore mice are handled calmly and habituated to testing rooms as well as arenas if possible.

Where mice are housed in modified cages for testing it will be for the minimum time needed to gather data. Mice will be removed from test scenarios if suffering from an adverse stress reaction, or other unexpected adverse effects.

Technical refinements will be developed throughout the project and disseminated to other researchers and collaborating institutes.

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

The work in this project will be undertaken in accordance with the principles set out in the Guidelines for the Welfare and Use of Animals in Cancer Research: British Journal of Cancer (2010) 102, 1555-1577 (referred to subsequently as the 'NCRI Guidelines') and in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017).

We will be following ARRIVE and PREPARE guidelines.

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

I, and delegates from the research team, will work together to adopt new developments in the 3Rs at the establishments the work will be carried out in. We will work closely with named persons and managers involved with promoting welfare, where possible, attending events promoting the 3Rs hosted locally and through institutions such as NC3Rs, LASA and the IAT. The International Society for Transgenic Technologies (ISTT) provides additional contacts and materials for keeping up to date with production technologies emphasising the 3Rs.

Any new methodology, techniques or practical refinements will be tested against established techniques and where results are not compromised and animal welfare improved will be implemented accordingly.