



Home Office

NON-TECHNICAL SUMMARY

Discovery of cancer genes and assessment of their therapeutic potential

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 gene, Therapy, Cancer immunology

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?

Our goal is to identify genes which cause or contribute to the cancers and their spread.

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?

In the UK 350,000 new cases of cancer are diagnosed each year and there are around 165,000 cancer-related deaths. If we are to reduce cancer incidence and the associated suffering and deaths, we need to identify tumours earlier and develop new therapies. Cancer cells have alterations in their DNA, so called genetic changes. These genetic changes allow the body's immune system to detect and kill cancerous cells which can keep most of us cancer free for most of our lives. In many cases however, cancers can make them selves invisible to the immune system and these can then grow unchecked. To better control cancer we need to understand how they hide from our immune system.

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

New medicines, namely drugs that offer potential new treatments for cancer.

Basic Science benefits:

- Information on genes involved in cancer which may be useful for diagnosis
- New information that might enable new cancer drugs to be developed
- Publications and presentations at meetings
- Genetically engineered mice that can be used by others in the scientific community.

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

- Society will benefit from knowledge generated as this provides better understanding of diseases, disease mechanisms and methods to treat and/or prevent them. Scientific knowledge is gained over the shorter-term (2-5 years) while drug discovery and development will take 10-15 years.
- Research institutes, pharmaceutical companies and non-governmental organisations will be able to take information produced by our studies and use it to support basic research and the development of drugs that ultimately provide benefit to patients.

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

We will collaborate with other experts, combined efforts will increase output.

We will publish scientific papers in open access journals and deposit papers in appropriate archives thereby disseminating knowledge.

We will share both positive as well as negative results with the scientific community so that others are aware of both successful and unsuccessful approaches.

We will file patents, thereby placing in the public domain detailed knowledge of the discoveries we have made.

We will use the ARRIVE guidelines when publishing our work.

Species and numbers of animals expected to be used

- ♦ Mice: 14,500

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.

The mouse was selected for this project because of the availability of advanced technologies to genetically alter mice. This is done by adding or removing genetic information at very early embryonic stages, before an embryo has implanted in its mother's womb. The methods to do this are very efficient so fewer animals are needed compared to achieving the same goals in another species.

Adult mice develop many forms of cancer that are similar to cancers in humans, for instance lymphomas, brain, colon, ovarian and prostate cancers. Just like in humans cancers develop in older mice more often than younger ones. Genes have been identified which when damaged cause cancer in humans and mice. One example is a gene known as p53. Mice and humans that have just one copy of the p53 gene instead of the normal two copies, develop cancer much earlier than normal.

Many strains of mice are genetically identical. This enables tumours to be caused by injecting them with cancer cells. This technique has allowed experiments to be performed to discover anti-cancer drugs. Indeed almost all cancer treatments available today have been tested for their ability to cure tumours in mice. Mice, when used in this type of work, give valuable insight into basic disease mechanisms as well as providing a means to test new therapeutics.

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

To produce transgenic mice fertilised embryos are collected which are manipulated under the microscope. These are transferred to foster mothers who become pregnant, give birth and rear their pups.

The embryo transfer is done under general anaesthesia so the animal will be unconscious and unable to feel pain. A small cut is made in the skin to place the embryos in the reproductive organs (uterus or oviducts). Typically, the mice recover very quickly from surgery, they are actively running around, eating and drinking after 1 hour and are fully recovered within a few days.

A small number of male mice are vasectomised, which are used to mate with foster females. Vasectomy is conducted on anaesthetised males by making a small scrotal incision and cutting the thin tube carrying the sperm. The incision is closed and the mice recover from the anaesthetic within 60 minutes or so. A week or two later the wound will be healed and the mice can be used for mating with females.

Transgenic mice in this project will be bred and weaned. Where the line is not pure bred a tiny piece of tissue from the ear is taken to identify the mice and the bit of tissue that is removed used to identify the genetic alterations they carry.

Transgenic and normal mice are used to study cancer. Cancers may arise spontaneously in some strains but in others we induce cancer by injecting them with cancer cells or with viruses that carry genes which cause cancer. In some cases tumour cells are placed just under the skin of the mice. The growth of these cancers is monitored by feeling the small tumour lump under the skin and the mice are humanely killed before the cancer grows very large (less than 1.2cm²).

In other cases we need to precisely control where the cancer develops, for instance to induce prostate cancer we inject the prostate gland under general anaesthesia. In such a situation the mouse is unconscious and unable to feel pain from the small incision and injection. Typically the mice recover very quickly from these surgeries. Over the following weeks or months they are monitored closely for tumour development. As the prostate can not be seen, we monitor tumour growth by imaging - very much like a scan in a hospital. To do this we inject a dye into the body cavity and while the mouse is anaesthetised we perform a scan to see if a cancer is growing or has spread. We do this monthly to identify tumours before they become too large and impact the health of the mouse. Once a tumour reaches a certain size the mouse will be humanely killed and the tumour recovered for analysis.

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

The majority of the animals in this project should not experience any adverse impact as they are used for mild procedures like breeding. Mice are typically breeding for 4-9 months.

Some of the animals will experience transient pain from the minor surgery required for embryo transfer, vasectomy and inducing tumours in specific organs. These procedures will be conducted under anaesthesia so the animal will remain in a state of sleep/unconsciousness during the surgery. Once they have recovered from anaesthesia any pain will be managed with drugs for a few days.

The procedures used for tumour cell inoculation under the skin will involve needle pricks. In procedures which involve anaesthesia, the mice will be expected to be subdued and lethargic for an hour or two but then recover fully as the effects of the injection and/or anaesthesia wear off. Some animals may have an altered immune system that may make them more susceptible to infection. To keep mice with immune deficiencies healthy, they are maintained in a very clean facility where they are not exposed to harmful viruses or bacteria.

The tumours will be allowed to grow to a size where they may cause some discomfort and may cause other clinical signs. We will carefully monitor mice with tumours to make sure that the tumour is not having a major impact on the health of the mice. We monitor weight, breathing, mobility and general condition of the mice. We also routinely measure tumour size, which may involve feeling it through the skin or using an imaging technique like ultrasound.

Tumour growth will occur over a period of between two months and one and a half years and can be unpredictable, so monitoring will be continuous. Tumour bearing mice will be humanely killed at the point that we judge that the tumour burden is having a significantly health impact or if tumours don't grow within 18 months.

All animals will be killed humanely at the end of the procedure.

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)?

Mouse : Mild severity : Greater than 96%

Mouse : Moderate severity: less than 4%

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

- Killed
- Kept alive
- 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?

Cancers are usually composed of tumour cells mixed with and normal cells. As they grow new blood vessels form to provide the tumours with oxygen and nutrients. Very often tumours are attacked by cells from the immune system which recognize the cancer cells as "foreign" and in some cases the tumour is controlled or even eliminated. In many other cases the tumour is able to "hide" from the immune system.

When we detect cancers they have usually been growing over a very long period of time, years in humans and many months in mice. During this time the cancers change so they are composed of a mixture of similar but non-identical cells. Cancers that arise in different people have some similarities, but each one is unique.

To control cancer we need to identify the genes which are able to support the growth of cancer and the mechanisms cancers use to hide from the immune system. Studies of cancers isolated from people and mice over the last 10 to 15 years have given us long lists of "cancer genes". To identify which combinations of genes are contributing to the cancer's initiation and growth requires studies in animals because cancer cells grown in the laboratory are in a very simple environment compared to when they arise naturally in humans. It is not possible to replicate the complexity of how a cancer grows, interacts with normal cells and manages to evade the immune system in a laboratory setting.

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

Cells isolated from some cancers can be grown in the laboratory. These culture systems have improved in recent years allowing cancer cells to grow into tiny but more complex structures that better resemble some aspects of normal tumour growth. These so called "organoid cultures" are three-dimensional cultures usually composed of a small number of related cell types. While their three-dimensional nature can more closely resemble some aspects of simple tissues they are very simple compared to the highly complex environment one finds in a mouse or human tumour. Organoid cultures are useful for conducting screens for drugs or mutations that can alter a cancer cell's growth but these systems do not have an immune system component, thus drugs which prevent a tumour hiding from the immune system can not be found using such simple systems.

Why were they not suitable?

To study how a cancer evades the immune system and to find rare immune cells which can kill tumour cells it is necessary to study cancers in the presence of a functional immune system. This can't be replicated in the laboratory. Similarly, the process whereby a cancer spreads from its primary site can only be studied in the whole animal system.

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 numbers of animals estimated to be used for this project are based on many years of experience in generating mice with altered genes. They are also consistent with the usage in the existing project license.

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

Genetic manipulation: More than 90% of the animals used in this project will be used for establishing and breeding combinations genetic alterations together. The numbers of mice can be estimated from

prior experience using genetically modified ES cells to make mice as well as the probability of generating mice with the desired combination of alterations based on patterns of Mendelian segregation.

- Where possible we establish mice which breed true.
- We test the performance of each genetic alteration before breeding them together.

Cancer studies: Around 5-10% mice in this project will be used to deliver the core scientific objectives, which involve inducing cancers and collecting the resultant tumours and analysing them.

To reduce mouse numbers we will:

- Conduct careful quality control on all samples used, such as cell lines and viruses, to make sure that when they are injected into mice there is a very high chance that useful data will be collected.
- Use standard operating procedures to ensure that we get the maximum amount of useful biological information from each mouse.
- Consult widely to identify and where appropriate introduce improvements in methods to maximise the recovery of data from every mouse
- Apply technologies which enables us to gain the maximum amount of information from every sample collected.
- Use the NC3Rs Experimental Design Assistant

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

There are many measures we take to reduce mouse numbers:

- We use colony management software to keep track of mice in the animal room, set up just the right number of breeding pairs to produce the mice we need.
- We conduct pilot experiments using small numbers of mice to assess the performance of a cell line or virus before initiating larger experiments, or not conducting them at all in cases where the pilot does not provide a good basis for continuing.
- We stop breeding and preserve mouse lines in cryogenic storage if they are no longer required
- We use the most advanced and efficient methods to make genetic mutants which avoids cycles of mouse breeding.
- We share post-mortem tissues

- We limit "noise" by controlling as many variables as possible including genetics (inbred lines), age, sex, and environment.
- In induced tumour models we use randomisation and blinding to control for operator bias.

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 with cancer pre-disposition mutations

We use mice with genetic alterations (mutations) in cancer causing genes. If these mice are available from other investigators or public repositories we will import these. If they are not available we'll generate these ourselves. We use refined models so that cancer is limited to the organ we are studying - for instance the prostate.

Some genetically altered mice develop cancers spontaneously. To limit their suffering, we conduct regular screens for signs of cancer and if detected more frequent screens are then implemented.

Mice with immune deficiencies

We use mice with immune-deficiencies to explore the role of aspects of the immune system in controlling cancer. Although, some parts of the immune system are missing in these mice, they are very healthy in the environment in which they are kept.

Transplantable cancers

To conduct controlled experiments, we use cancer cell lines which we implant into mice, either normal mice or ones with immune defects. The tumours are usually injected under the skin which allows us to monitor the size of the tumour to limit any suffering.

Organ specific cancers

These are induced by combining a specific genetic background which may pre-dispose the mouse to develop a specific cancer type with local induction of the tumour by administering a cancer causing agent like a virus. In such cases we will endeavour to design the experiment so that the growth of the cancer can be detected by an imaging technique which will allow us to intervene to prevent suffering.

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

Cancer is a late onset disease in adults. It develops from a single cell that arises in mature organs over many months, thus an immature life stage is not suitable. The immune system is also not mature at an earlier stage (immature life stage).

Some aspects of cancer can be modelled in zebrafish, for example they develop a type of skin cancer known as melanoma, but zebrafish have a very different immune systems to humans and mice, their organ systems are very different to ours and lower organisms can't be genetically engineered to the extent possible with mice.

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

I don't anticipate many refinements to transgenic methods as these are already very standardised and efficient, though where these are reported we will test and implement them.

Where possible we will employ highly experienced animal care staff who are familiar with the specific strains, experimental activities and careful handling of the mice. Familiarity with the balance between welfare and experimental needs, observing recording and reporting expected and unexpected outcomes at the cage-side with score sheets is another essential aspect of minimizing welfare costs. An important balance needs to be struck between to much disturbance of the mice while allowing time pre- and post-procedures for acclimatisation and recovery. To improve post-operative recovery heat mats are used to warm mice as well as changes to the environment such as deeper bedding and nests can be provided.

Where possible the mice will not be disturbed unless required for routine husbandry, daily checks or experimental purposes. Environmental enrichments like tubes and nests will be provided. We will however monitor harms via observation and body weight if appropriate and in situations where this does not induce more stress to the mice.

Where possible mice will be acclimatised, for instance when moved between facilities or animal rooms before any experimental procedures are conducted.

We consult existing literature to ensure we use the latest refinements in experiments. Work will be carried out in state-of-the art facilities by highly trained technicians and scientists, all of whom are dedicated to the highest standards of animal welfare. We use imaging and/or calipers to accurately assess the size of a tumour and these are monitored against previous data to enable prediction of the possible disease course.

The scientists and technicians work closely with the trained and highly experienced personnel in the facility and the veterinary surgeon to ensure that animals experience minimal adverse effects.

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

I use guidance from the following sources:

- The National Centre for the Replacement Refinement and Reduction of animals in Research (NC3Rs) <https://www.nc3rs.org.uk>
- The International Mouse Phenotyping Consortium (IMPC) <https://www.mousephenotype.org>.
- The International Society of Transgenic Technologies (ISTT).
- Some of my team members are members of The Laboratory Animal Science Association (LASA) and attend an annual conference where information on best practice is often exchanged.
- The PREPARE guidelines have been consulted
- The Workman et al., publication, "Guidelines for welfare and use of animals in cancer research" (2010) has been consulted.

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

I periodically check website for The National Centre for the Replacement Refinement and Reduction of animals in Research (NC3Rs) <https://www.nc3rs.org.uk> which has many excellent standard operating procedures and videos and consult Norecopa, <https://norecopa.no>.

I check the website for the International Society of Transgenic Technologies (ISTT) for advances in the field and observations and experience in implementing these.

Implementation of a technical variation will usually be conducted with a pilot experiment to gain confidence in the actual method and its reported advantages, ideally with suitable controls. Once this has been assessed and shown to be an improvement then this will be introduced in the standard operating procedure and then implemented as a routine.