



Home Office

NON-TECHNICAL SUMMARY

Developing therapeutic drugs for cancer including the establishment of tumour models

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
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

cancer, therapy, drug discovery, pharmacology, tumour models

Animal types

Life stages

Mice

adult, aged

Rats

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?

The aim of this project is to develop new and improved medicines for the treatment of cancer. As part of this aim, we also plan to develop new cancer models to try to improve the relevance of our animal studies to human disease, or to refine existing models.

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 2018, over 17 million new cases of cancer were diagnosed worldwide, while 9.6 million people died from the disease. It is estimated that by 2040 there will be 27.5 million new cases of cancer each year. In the UK, over a quarter of all deaths are cancer-related. Cancer is a complex disease with more than 200 types of cancer described and the most prevalent types are lung, colorectal, breast and prostate. If cancer is untreated, in the majority of cases it will lead to death. (Source: www.cancerresearchuk.org)

Despite recent advancements in the treatment of cancer, there are still many types of cancer for which therapies are not available. In other cases, therapies are available but they are effective for only a limited percentage of patients. In addition, many patients who do respond to existing therapies eventually develop resistance to these treatments.

Our research project aims to use animals to support our efforts to discover and develop novel therapies for cancer. The use of animals will enable us to mimic complex aspects of the disease in a way that is not possible using cells grown in a laboratory setting. By doing so, we can test the activity of our new drug candidates on tumour growth in a more relevant way in a live animal. The use of animals in our research enables us to develop new cancer drugs to treat patients who have terminal disease and few treatment options.

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

This work will provide important data to advance our experimental cancer drugs through the phases of research and development by evaluating their activity in animal models of disease. It will further help us to identify the most appropriate drugs to combine together and will guide clinical teams on dose levels and dosing schedules.

This work will also allow us to understand how our drugs distribute in the body after dosing, and how quickly they are eliminated from it.

This project will also increase our insight into how our drug candidates work and will help discover specific changes in the body that happen in response to the drug. These changes can then be measured in human clinical trials.

This work will further enable us to identify potential targets for new drugs in order to expand treatment options for cancer patients.

Work carried out under this licence will also support the development of new methods and technologies to treat cancer patients.

The development of new tumour models and/or improvement of existing models will allow us to test our experimental therapies in animals in a manner that more closely represents specific patient populations.

Importantly, results may also be used to determine which drugs should not be progressed further.

In addition to the outputs mentioned above, we expect to publish our results and to present our findings externally to scientific peers.

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

This programme of work is expected to enable us to progress new cancer medicines through progressive stages of development. Ultimately, if successful, these medicines will be approved for use in the clinic to treat patients with cancer who otherwise are likely to die from their disease. Drugs developed using this licence therefore have the potential to benefit millions of cancer patients across the globe.

The development of new or improved cancer models will help us to target the right patient populations with our drugs which will increase the likelihood that those patients will benefit from the treatment.

The work carried out under this licence is also expected to contribute knowledge to the broader scientific community through the publication of our findings, presentation at external scientific meetings, and through collaboration with experts in the field.

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

Our team has an excellent track record of publishing our advances in drug discovery and animal modelling. We aim to publish both successful and unsuccessful results in relevant journals and to share our data and learnings with collaborators to avoid others repeating work. In addition, we often present our research at international conferences for the benefit of the broader scientific community. These public discussions of our results have the potential to lead to new collaborations which provide additional opportunities to develop innovative new drugs and technologies.

Species and numbers of animals expected to be used

- Mice: We expect to use up to 51500 mice over the course of 5 years.
- Rats: We expect to use up to 800 rats over the course of 5 years.

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.

Mice and rats are the most common animals used for the type of work that we are doing. They have immune systems that resemble those of humans, which means we can grow tumours in these animals to investigate the effect of our experimental therapies on the immune response to tumours. A large selection of different tumour cell types are available for use in mice and rats such as from lung, skin, or breast cancers. We can therefore evaluate our drugs in many different types of tumours.

In the vast majority of experiments we plan to use mice rather than rats. This is because there are more tumour types available for mice, and there is a larger body of published data for mice to help guide our experimental plans and interpret our results. Occasionally rats may be preferred such as when specific tumour types are available for rats but not mice, when an experimental drug is active in rats but not mice, or when we need to compare our results with other data generated in rats. An example of this is safety data, where rats are often the preferred species.

We plan to use adult mice that are typically 6-12 weeks old at the start of the study, as the immune system is considered immature in mice younger than this age and therefore would not adequately represent the biology of the cancer patients we aim to treat. Occasionally we may need to use mice that are significantly older than this. This is especially relevant as cancer is generally a disease of middle to old age. For those occasions, we plan to conduct studies on animals up to 18 months of age as our previous experience has shown that this age is sufficient to see immune system changes as a result of ageing but before the mice begin to suffer serious effects of old age.

Rats used in this licence will also be adult stage, typically 6-12 weeks old, at the start of the study.

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

Animals are kept in high-quality facilities, free from pathogens (disease-causing organisms such as bacteria, viruses, and parasites) and with access to food, water, and environmental enrichment. In all the facilities used, the animal care staff are highly trained in rodent welfare and will ensure the animal suffering is minimised. Animals are housed in groups except in exceptional circumstances, for example when aggressive behaviour puts the welfare of the animal at risk or when cage-mates have been removed for experimental reasons.

Most of the animals will be used for studies that measure the impact of experimental drugs on tumour growth rates. The vast majority of tumours will result from injection of tumour cells under the skin which then grow into a tumour at the site of injection. However, in some instances we will introduce tumours into specific organs through specialised injection routes. For example, direct injection into breast tissue mimics breast cancer, while injection into the bloodstream leads to tumour growth in the lungs. Producing tumour growth in other internal organs requires surgery, such as to implant tumours into the pancreas or sometimes into the breast tissue. Tumours that grow just under the skin are easy to

observe and to measure their size using callipers. Tumours that grow internally are more difficult to observe therefore other methods are employed such as imaging techniques and/or clinical scoring systems to carefully monitor the well-being of each animal.

In some cases, it is not necessary to use animals bearing tumours. For example, tumours are often not needed to determine whether a drug is tolerated by the mice or to measure how much of a drug enters the bloodstream. In addition, we sometimes can use animals that do not carry tumours when we need to understand the impact of our drugs on specific aspects of the immune system.

On some studies, we will apply advanced imaging techniques (such as ultrasound or other imagers that can detect tumours or drugs labelled with special tracers) that allow us to track growth of tumour cells or to follow distribution of a drug throughout the body. To do so the animals will be anaesthetised throughout the imaging session.

Blood samples or small samples of tumour tissue may also be collected during some studies to measure levels of drug or other indicators of drug effect over time. Blood samples are usually of a small volume and are taken from a vein in the tail or at the end of the study if larger volumes are needed. Small pieces of tumour material are collected from live animals under anaesthesia with a fine-gauge needle.

Experimental therapies are most commonly injected into the peritoneal cavity (abdominal cavity), intravenously, or directly into the tumour. Occasionally drugs may be administered orally (typically by gavage, which involves the insertion of a tube through the mouth and down the throat) or rarely, subcutaneously (under the skin). At times these substances may only need to be administered once, but more often they are administered according to a schedule that requires multiple administrations. For example, drugs injected into the peritoneal cavity are typically given two to three times per week, whereas drugs given orally would typically be administered once or twice a day, often for the duration of the study. In most cases our studies last approximately one month but on occasion when tumours grow slowly could last for 5-6 months. Therapies are most typically administered early in the day so that there is plenty of time to watch for any unwanted or harmful effects.

To understand the impact of our work on the animals, here are examples of a typical study and a worst-case scenario for our most commonly-used method in which a tumour is injected under the skin:

A typical study involves:

- ◆ implantation of a microchip under the skin for identification
- ◆ injection of tumour cells under the skin
- ◆ administration of experimental therapies by one or more of the following routes:
 - ◊ into the peritoneal cavity (typically this will be done twice a week for three weeks)
 - ◊ intravenous (typically this will be done once or twice a week for 3-4 weeks)
 - ◊ orally by gavage (typically this is done once or twice a day for the duration of the study)
- ◆ collection of a blood sample

Worst-case scenario study:

- ◆ administration of experimental therapies by one or more of the following routes:
 - into the peritoneal cavity (maximum 30 injections over the animal's lifetime)
 - intravenous (maximum 14 injections over the animal's lifetime)
 - orally by gavage (maximum 120 administrations over the animal's lifetime)
- ◆ injection of immune cells by one or more of the following routes:
 - into the peritoneal cavity (contributes to lifetime limits for this route)
 - intravenous (contributes to lifetime limits for this route)
 - intra-tumoral (contributes to lifetime limits for this route)
- ◆ when animals are imaged (for reasons outlined above), this is done under anaesthesia. The maximum number of times an animal may undergo anaesthesia in order to be imaged is 5 times within a 7 day period, or 20 times in total over the animal's lifetime.

At the end of procedures, all animals will be humanely killed.

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

In our studies, the likeliest sources of adverse effects are from the size and condition of the tumour, from surgical procedures, and from the drug treatment. Animals will be classified as mild or moderate severity according to a scoring system that is based on the degree and duration of clinical observations such as body weight, activity level, posture, and body condition. This scoring system is currently under development, but will take into account the cumulative experience of the animal. For example, two clinical signs of short duration and low intensity might be classified as mild whereas the appearance of three clinical signs for longer periods of time and greater intensity would likely be classified as moderate.

We will humanely kill any animals that have developed large tumours to minimise unnecessary suffering through the use of well-defined tumour burden limits. Generally, studies with subcutaneous tumours will reach these limits within 30 days of implantation, but tumours that grow more slowly could take 2-3 months before reaching the same limit. The slowest tumour type that we currently work with takes approximately 5 months to reach the same size without any drug treatments that might inhibit the tumour growth.

Animals undergoing surgical procedures are assumed to experience pain due to the surgery and so will be classified as moderate severity. Pain relief or anaesthetics will be provided when surgery is performed.

Treatment of animals with cancer therapies may also lead to unwanted effects similar to those experienced by human patients. While humans may experience fatigue or fever soon after receiving the therapy, we observe similar responses in rodents such as reduced mobility, hunched posture, and

piloerection (bristling of fur). Animals that have been treated with vaccinia virus may also develop lesions that are usually found on the tail. Most of these effects will be of short duration and mild but some animals may experience moderate effects. We expect that approximately 30% of animals will be classified as moderate as a result of adverse reactions to drug substances, and the remaining 70% will be mild or lower severity.

At the end of procedures, all animals will be humanely killed.

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)?

For both mice and rats, we expect the following proportion of severities under this PPL:

Mild = 36%

Moderate = 64%

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?

Although we do many experiments using cells, molecular biology, and computer modelling, it is still necessary to use some animals for research so that we can more accurately assess the interaction of cancer cells with other cells and organs within the body. Isolated cells and organs do not reproduce the complex nature of *in vivo* (in a live animal) biology. The use of animals also allows us to understand cancer in the organ of origin or as it spreads throughout the body. An important aspect of our work is to understand how the immune system can be harnessed to attack tumours, and it is not possible to fully recreate these complex interactions outside of a living animal. In addition, regulatory agencies often require animal studies prior to approval of a drug for use in human clinical trials.

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

Our organization regularly uses a range of *in vitro* (taking place in a test tube or laboratory dish, outside a living animal) methods. Such methods may include experiments containing a single cell type or multiple cell types grown together, which allows both direct interaction and indirect communication

between cells. These types of studies are well-established, 2-dimensional experiments that are useful to understand the specific ways that our experimental drugs affect cellular function.

More recently, we also have developed a more complex experimental system called 'tumour slice culture system' which involves the maintenance of thin slices of human tumour tissue in a dish. This method preserves the 3D structure of a patient tumour and is expected to be more representative of the biology of the whole tumour compared with the 2D experiments mentioned above. We are also investigating other 3D systems such as spheroids which allow cells *in vitro* to grow in all directions similarly to how they would grow *in vivo*, compared with traditional 2D methods in which cells are grown on a flat surface in a petri dish.

Why were they not suitable?

Cell-based methods are useful to gain an understanding of the way that our experimental drugs impact the function of different cell types outside the body, but do not adequately test whether the drugs remain stable after they enter the body, can reach the site of the tumour, or whether they are capable of inhibiting tumour growth in a live animal.

The 3D assay systems such as tumour slice cultures are a valuable addition to our experimental toolbox, however the slice cultures have a short lifespan. In addition, they can show modification of cellular function but do not demonstrate that this effect can shrink a tumour. The spheroid cultures are still being developed.

None of the alternatives investigated can demonstrate how our experimental therapies are broken down by the body, nor can they provide any information about how to schedule dose regimens. Unlike experiments using animals, they also do not permit identification of specific signals produced in the body which can be linked to tumour growth inhibition.

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?

These numbers were calculated based on our use over the last several years, and an expectation that we will increase the number of our studies by approximately 30% beginning this year. This is due to a planned expansion of our team and the number of projects under investigation.

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

Our organization includes a team of statisticians who have implemented a Good Statistical Practice program where animal experiments are formally reviewed before they start. This review is conducted by a researcher and a supporting statistician team to ensure the goal, experimental design, and data analysis align. This process ensures the design is robust and the minimum animals are used that are needed to answer the scientific question.

In addition, the following guidelines and online tools are also used to influence the design of our animal studies:

- The PREPARE Guidelines, found at <https://norecopa.no/prepare>
- The NC3Rs Experimental Design Assistant, found at <https://www.nc3rs.org.uk/experimental-design-assistant-eda>.

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

We ensure small scale pilot studies are carried out for new tumour types or experimental methods which enables us to design our studies using the minimum number of animals needed to achieve our scientific objectives. We also have implemented innovative study designs to reduce animal numbers where possible. We have also developed a new technique to take small samples of tumour tissue from live animals with a small needle, similar to a biopsy. This reduces the number of mice used for some studies because it avoids the need for separate groups of mice to both analyse the tissue and to monitor the growth of the tumour.

During our previous projects we have analysed the tumour tissues of the most frequent types of tumours that we grow in mice in order to define their cellular, molecular, and genetic characteristics. This characterisation has been important for reducing animal use since it ensures we can select the most appropriate tumour type for each experimental question, thereby reducing the overall number of experiments and allowing us to maximise the benefit gained from each mouse. A large amount of data has been generated from our characterisation work and is archived for use by all our scientists to guide the design of future animal studies.

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.

The most common type of tumour that we use involves injection of tumour cells under the skin resulting in tumour growth at the site of injection. This is the simplest method available to grow tumours in rodents and carries the least welfare risks since the tumours are not located in vital organs. They are

also the easiest to monitor since they can be easily observed and measured under the skin. This method is preferred except in specific cases when we need to understand more complex questions. For example, we may need to understand the spread of cancer from one site to another, the influence that specific cell types and organs have on tumour growth, and the responses of tumours to our therapies in these varied settings. In these cases, we will use more complex experimental methods in which tumours often develop inside the body cavity. These more complex studies also may involve surgical implantation of tumours. Where possible, non-surgical methods will be used for implantation and imaging methods used to monitor tumour burden.

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

Rodents are the lowest species of mammal (meaning they are the least sentient, or least aware of feelings and sensations) that allow us to adequately study the complexity of human cancer and immune system biology. Because many of our experimental therapies are designed to impact the immune system, it is essential that we use adult animals with mature immune systems in our research. Our studies also monitor the growth of a tumour over a period of weeks to months therefore it is essential that the animals are conscious as the use of anaesthesia (an agent that induces a state of unconsciousness) would not be possible for such an extended period of time. In addition, the behaviour of conscious animals also often alerts us to adverse reactions to our therapies.

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

We are committed to refining our procedures to minimise harm to the animals and have a track record of doing so. We ensure small-scale pilot or tolerability studies are carried out for new tumour types, experimental methods, or therapies. We carefully monitor tumour burden including the use of whole-body imaging techniques when possible. We also use tumour-free mice in some cases when tumours are not essential. We have implemented innovative study designs to reduce animal numbers and enhanced health checks to minimize suffering. General welfare of the animals is assessed by checking body weight and watching for the development of clinical signs such as activity levels, appearance of the coat, posture, and body condition. When dosing animals with test substances, we dose as early in the day as is practical in order to maximise the length of time that animals are monitored during the day. When unexpected events occur, these will be thoroughly investigated to find out what happened so action can be taken to prevent a reoccurrence. When two or more drugs are scheduled to be injected at the same time, we will also combine them into the same syringe where possible in order to minimize the number of injections administered to the animals. In addition, a non-surgical method of tumour implantation in the mammary fat pad has been developed and is used in preference to surgical methods wherever possible. We have also introduced the use of an improved method for judging the size of a tumour growing inside the body cavity in our pancreatic tumour studies. This involves the use of a standard set of clay balls of different sizes. The feel of the tumour under the abdomen can be compared against the clay balls to determine the approximate size of the tumour.

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

Our practices are principally guided by the Workman et al guidelines published in 2010 (Workman, P et al. Guidelines for the welfare and use of animals in cancer research. 2010. British Journal of Cancer. 102 p1555). In addition, we follow the ARRIVE guidelines developed by NC3Rs for publication of our work in peer-reviewed journals (ARRIVE Guidelines version 2.0 released in July, 2020 can be found at <https://arriveguidelines.org/>) and our practices incorporate many of the guiding principles of the PREPARE guidelines (Smith et al., PREPARE: guidelines for planning animal research and testing. 2017. Laboratory Animals.) LASA (Laboratory Animal Science Association) also has a range of published guidance documents with principles that can be applied to our animal studies which are found at 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?

Our team is actively involved in promoting the 3Rs and participates in global 3Rs activities including an annual poster competition. This award was given to individuals working on the development of alternative models under a previous project license in 2009, and also a team working on an *ex vivo* (taken directly from a living organism) tissue slice culture system in 2017. Our team also is aware of advances in 3Rs through the NC3Rs and establishment websites and via participation in conferences and events sponsored by organizations such as LASA (Laboratory Animal Science Association, IAT (Institute of Animal Technology) or NC3Rs.