



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 animal

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. For example, a new model has been included in this license which involves growth of tumours in the bones of the hind limbs. This provides us with a relevant model that mimics bone cancers such as osteosarcoma, or situations where primary tumours elsewhere in the body have spread to the bone.

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.

Amendment May 2025

We have included a new variation on the breast tumour animal model where tumours are implanted directly into the mammary duct (a thin tube carrying milk to the nipple) rather than the surrounding tissue. This will allow initial testing of our experimental cancer drugs in a different type of breast cancer. By using the right tumour models, we expect to produce higher-calibre results while minimising harms to animals. This leads to better scientific rigour, which is an important component of the 3Rs as it ultimately leads to more relevant results that are more reproducible. This in turn results in the need to use fewer animals in the long term. We have also increased the animal numbers to reflect the expected increase in use.

We have clarified some text around humane endpoints in the “Imaging” steps that was causing some confusion, and also corrected a section in the “Detection Agents” step that contained the wrong text.

We have also removed a sentence of text that prevented the use of two imaging methods.

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. Some procedures performed under the authority of this licence are necessary in order to allow tumours to grow, to better mimic the human disease, or to better understand how our experimental therapies are working. These are important aspects of our cancer models which help ensure we can test our experimental therapies in as relevant a model as possible.

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 53000 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 24 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.

Amendment May 2025

We have now included protocols in which to test our experimental cancer drugs in orthotopic models, this requires tumour cell implantation into an organ or area that is the natural microenvironment, e.g. breast tumour cells implanted into the central duct of the breast. Some of these tumour models may also spread to other tissues (metastasise), which mimics the clinical experience of some patients.

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, the bone, 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. On rare occasions animals may be shipped to us after tumours have been induced in another laboratory and begun to grow. These cases will be limited to superficial tumours only which can be easily monitored, and to those tumours that are expected to grow slowly. Animals may be shipped from within the UK or internationally. Shipment of mice bearing tumours is necessary so we can acquire tumour models that are slow growing and/or difficult to establish. This is because shipping tumour fragments or vials of cells would likely result in the loss of important characteristics of the tumour, and would also

significantly delay the development of new treatments for cancer by as much as a year or more, during which time patients would not have access to new treatment options.

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.

In contrast, in some cases tumours are necessary but they will not grow in the animal unless additional factors are administered. For example, some breast tumours require the presence of oestrogen hormone.

On some studies, we will apply advanced imaging techniques (such as ultrasound or other imagers that can detect tumour burden e.g. in the bone 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.

Several steps in the license permit the administration of various substances. These steps typically include a range of different options for administration routes such as oral, intravenous, subcutaneous, etc. It is important to have several different options to administer these substances since the best route of administration can be different for each substance depending on how they distribute in the body or are broken down in the blood and tissues.

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 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. A NACWO or NVS will undertake a physical check of the animals where it is necessary for animals to exceed 2 IP doses per day in a study, and also where PO dosing exceeds 30 doses in a study, to ensure they are fit and healthy to continue.

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 or into a specific site such as mammary tissue or bone
- 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 anesthesia. The maximum number of times an animal may undergo anesthesia 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, from the drug treatment, and from microchip insertion. Additionally, animals which receive human immune cells derived from human donors such as PBMCs (peripheral blood mononuclear cells) or cellular therapies may develop graft versus host disease (GvHD), which is a systemic disorder that occurs when the graft's immune cells recognize the host as foreign and attack the recipient's body cells. "Graft" here refers to transplanted human immune cells and "host" refers to the animal.

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. For some tumour models mice may develop a discoloration of the skin at the site of the tumour that does not have any welfare implications. This is considered to be a normal appearance of some tumour types, and we have not observed any signs of distress in the mice as a result. We do not know whether some tumours grown in rats will develop the same discoloration as we do not have any examples of this yet. Animals that have been implanted with tumours before shipping may experience mild stress due to the presence of the tumour in addition to the standard stresses associated with shipping. However, we only intend to ship animals that carry very slow-growing tumours which are not expected to reach the size limits mentioned above during transit, nor to interfere with the normal behaviour of the animal.

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.

Amendment May 2025

We have added additional adverse events (development of GVHD) for a specific type of experimental cancer drug called cellular therapies (such as engineered immune cells) as we now have a greater experience utilising these in our drug discovery pipeline. New expected adverse effects have also been added to some steps after gaining more experience with those models and procedures (bruising due to core needle biopsy, and development of lung metastases in some tumour models).

In certain instances, engineered (i.e. genetically modified) immune cells will be tested in murine models to demonstrate pre-clinical anti-tumour activity. Examples of engineered immune cells include chimeric antigen receptor (CAR) T cells and engineered T cell receptor (TCR) T cells. These T cells are specifically modified to express proteins that enable precise recognition and targeting of tumour cells. Additionally, modifications may be made to enhance their survival and persistence post-transplantation into the host. In addition to GvHD, unwanted effects may occur if the intended target antigen is also minimally expressed on healthy host tissues or if there is unintended reactivity against non-tumour tissues. In all cases, clinical observations will be observed on the acute and chronic scale (i.e in the hours and days following immune cell transplant). Animals will be classified 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.

Additionally, we have created separate scoring systems to manage the specific welfare associated issues for our orthotopic models such as altered gait from the intraosseous tumour model.

We have also learned that some tumour models can spread beyond the site of implantation to other tissues, and that taking small tumour samples through biopsy can cause bruising in some cases. These cases will be carefully monitored.

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.

Most animals will have a microchip subcutaneously inserted for identification purposes. We have found a small percentage of animals with delayed wound healing post-microchip insertion. This is more prevalent in Aged animals. We have put in place control measures to minimize these and those are found in protocols throughout.

Weight loss is a possible adverse effect from several steps in the license, including (but not limited to) the administration of substances such as hormones and gene induction/deletion agents. These mice are typically monitored more frequently as the amount of weight loss increases and can often be limited by providing dietary supplements.

Occasionally, the use of genetically modified mice is necessary for our work. We have recently begun using a new mouse strain that expresses human versions of two proteins important for controlling immune responses to tumours. This strain can display subtle jerky movements that appear as mild tremors or altered breathing similar to hiccups. These unusual movements are intermittent and occur for short durations (typically 10-15 seconds) most typically when mice are handled outside the cage, therefore they may be a response to stressful situations. Although the clinical signs may recur, they are very mild, do not worsen over time, and are not considered harmful. It is expected that the behaviour of the mice will return to normal once returned to the home cage. Advice from the Named Veterinary Surgeon or Named Animal Care and Welfare Officer would be sought if their behaviour doesn't return to normal or there are any welfare concerns.

An estimated 10-20% of mice of strain NSG (and their derivative strains, such as NSG-SGM3 and NSG-MHC I/II double knockout) may show swelling around the hocks. Any animals experiencing swelling around their hocks may be given an altered enrichment, pain relief and/or anti-inflammatories in consultation with the NACWO and/or NVS.

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 = 15%

Moderate = 85%

What will happen to animals used in this project?

- Killed

Replacement

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

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

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.

Amendment May 2025:

The total number of estimated animals has been increased by 1500. This is due to an increased demand for the more complex models of disease found in two protocols in this licence.

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>.

Amendment July, 2024:

This licence has been amended to include a broader range of administration routes and types of agents that can be used for some steps. This is necessary because our work has evolved, leading to the need to use different types of agents, some of which need to be administered via other routes to

have an effect. These amendments will lead to better scientific rigour, which is an important component of the 3Rs as it ultimately leads to more relevant results that are more reproducible. This in turn results in the need to use fewer animals in the long term.

Amendment May 2025

The new breast model involves implantation of tumours directly into the duct. It may be possible to implant more than one duct per mouse (up to 4) to reduce the number of mice needed overall.

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.

Another reason why we sometimes need to use more complex models is when our experimental therapies are not active in the mouse. This could be because they are specifically designed to target human cells or because the human and mouse biology are too different. In these cases we often need to use special mice in which the mouse immune system has been replaced by human immune cells. On some occasions we will introduce the human immune cells ourselves in our facility, but on other occasions we will import mice from a commercial supplier who has prepared the mice for us by performing a series of procedures that result in the growth and survival of a human immune system in mice. Once these mice have been prepared in this way, we can then inject human tumour cells under the skin as described above. This allows us to understand whether our experimental therapies can provoke human immune cells to attack and eliminate a human tumour.

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 inserting a microchip, we have instigated a refinement to use non-adverse handling (avoid pinch scruff), wherever possible, for a minimum of 3 days following microchip insertion in order to enable full wound healing in the nape of the neck of the animal.

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.

When shipping mice that have previously been implanted with a tumour, we will ensure the following conditions will be met prior to transport:

- Animals will have recovered from implantation procedure with healed wounds.
- Tumours will be confirmed as palpable and measurable before transport (around 200mm³ volume).
- Tumours will be expected to be slow growing and will not reach tumour size limits during transport and acclimatisation period. The standard acclimatisation period for national or international shipments set by the establishment will apply to these animals. Currently these are set at 7d for national shipments or 14d for international (overseas) shipments.
- Animals will have no other clinical signs and deemed fit to travel by responsible person.

Any animals experiencing swelling around their hocks may be given an altered enrichment, pain relief and/or anti-inflammatories in consultation with the NACWO and/or NVS.

When injecting tumour cells into more than one mammary duct, the maximum permitted diameter of each tumour will be decreased in order to limit the overall tumour burden.

When injecting tumour cells into hind limb bones, pain relief may be administered and a scoring sheet is available to assist with monitoring of pain, swelling, and mobility after the procedure.

Amendment July 2024:

This licence has been amended to include a broader range of administration routes and types of agents that can be used for some steps. This is necessary because our work has evolved, leading to the need to use different types of agents, some of which need to be administered via other routes to have an effect. Some agents will behave differently when administered by different routes, and this amendment will ensure we can explore these different routes in order to find the most effective route with the best welfare profile. Collectively, these amendments will help ensure that we can achieve the best possible outcomes from our studies. By using the right agents, administering them via the right routes, and using the right tumour models, we expect to produce higher-calibre results while minimising harms to animals.

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.