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NON-TECHNICAL SUMMARY

In vivo evaluation of new therapeutics, new targets, and new models for oncology drug discovery research

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 improve the relevance of our animal studies to human disease by refining existing models, and to evaluate the drug-like properties of our experimental therapies including the determination of the therapeutic window.

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 2022, nearly 20 million new cases of cancer were diagnosed worldwide, while 9.7 million people died from the disease. It is expected that by 2050 there will be 33 million new cases of cancer each year, and the number of cancer-related deaths will rise to 18.2 million. In the UK, it is estimated that someone is diagnosed with cancer every two minutes, and the rate of cancer cases per capita in the UK is higher than 90% of the world. Cancer is a complex disease with more than 200 types of cancer described. The most prevalent types are lung, colorectal, breast and prostate, with these four types of cancer accounting for more than half of all cancer cases in the UK. 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 ensure that our experimental therapies have appropriate drug-like properties to become successful drug products able to treat as many patients as possible. One example of a drug-like property is the pharmacokinetics of the drug, which is an assessment of 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. Other models mimic cancers of the colon, pancreas, or breast.

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. 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: 47000
- Rats: 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.

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 drug candidates in many different types of tumours. A great deal of effort in the field has also been put into developing so-called "humanised" mice, which can be either mice with specific human proteins expressed in place of their mouse counterparts, or are mice that have components of the human immune system. These "humanised" mouse models are important as many of our experimental therapies are only active against human cells or proteins and do not recognise mouse targets, so these models provide a way to conduct critical in vivo testing needed for progression of novel drug candidates to the clinic.

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.

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. 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 (subcutaneous) 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 (referred to collectively as orthotopic tumour models). For example, direct injection into breast tissue (into the mammary fat pad or milk duct (intraductal)) mimics breast cancer, while injection into the bloodstream (intravenous) 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 colon, the bone (known as intraosseous, or more specifically intra-tibial into the tibia 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 may not always be needed to determine 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 anesthetised throughout the imaging session.

In many cases, we will inject human immune cells into mice lacking their own immune system. This is how we create the "humanised mice" described in the previous section. The cells are typically injected either intravenously or into the peritoneal (abdominal) cavity.

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, small samples of tumour tissue, or urine/faeces 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. Other sampling routes may also be considered (such as from a vein in the leg). Small pieces of tumour material are collected from live animals under anaesthesia with a fine gauge needle. Collection of a urine or faeces sample from an individual animal can be done by handling the animal (the animal typically responds by naturally urinating or passing faeces), or the animal can be placed for a short period of time in a cage on its own until these samples are produced.

Experimental therapies are most commonly injected into the peritoneal cavity (abdominal cavity), intravenously, or subcutaneously (under the skin). Occasionally drugs may be administered orally (typically by gavage, which involves the insertion of a tube through the mouth and down the throat) or directly into the tumour. 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 to two months 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:

1. implantation of a microchip under the skin for identification (under anaesthesia)

2. injection/engraftment of human immune cells intravenously or into the peritoneal cavity

3. injection of tumour cells under the skin (under anaesthesia)

4. 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)

5. collection of a blood sample from the tail vein

6. humane killing of the animal.

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 PBMCs (peripheral blood mononuclear cells, which are the white blood cells found in the blood) or immune cells derived from human donors such as in 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 30-60 days of implantation, but tumours that grow more slowly could take 3-5 months before reaching the same limit. The slowest tumour type that we currently work with takes approximately 5-6 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 discolouration 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. Pain relief or anaesthetics will be provided when surgery is performed, and post-operative pain relief will also be given.

When GVHD develops in mice that have been injected with human PBMCs, it is progressive in nature, meaning that it is not expected to resolve on its own and will progress over a period of a few days to a few weeks if there is no intervention. Weight loss is the most consistent observation, but mice may also display reduced activity, hunching, decreased grooming, rough or ruffled coat, diarrhoea, reddening of the ears or paws, or altered breathing. We apply strict limits on the severity and duration of these clinical signs in mice to ensure suffering of the animals is kept to a minimum. Clinical signs that are moderate may be allowed to persist up to 5 days while animals with mild clinical signs can stay on study up to 14 days. In contrast, animals that progress to more significant clinical signs will not be allowed to continue on study and will be humanely killed.

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 (bone) 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).

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. Most are not expected to display any harmful phenotypes. However, 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 = 5%

Moderate = 95%

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 context of 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 in a spherical shape 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 also have limited use as they typically are formed from only one or two cell types, and so do not give adequate insight into the complex biology of an organ or a whole animal.

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?

The mouse numbers are estimated based on our recent historic use of each model, with an adjustment to account for recent changes in the group size and strategic priorities. For example, our workhorse protocol, where tumours are implanted subcutaneously into the flank, has been decreased by approximately 12% compared with our current PPL. This is based on our retrospective use. On the other hand, we expect to use more complex orthotopic models of disease, therefore more mice have been allocated to those protocols. For example the breast orthotopic model has been increased 4-fold versus our current PPL.

We anticipate we will infrequently need to use rats, therefore this number has been set relatively low to allow for approximately 100 animals per year.

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/experimentaldesign-assistant-eda.

We also strive to design studies using the most appropriate animal models for each experimental question, and the best route of administration for each experimental agent, since this may impact their effect. By doing so, we aim to achieve more relevant results that are more reproducible. This in turn results in the need to use fewer animals in the long term.

For some studies we use mice that are injected with human immune cells from blood donors. The use of multiple donors in a single study can reduce the total number of animals required by approximately 50% compared with running several studies with a single donor, and also improves the relevance of the results.

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

For some studies we use mice that are injected with human immune cells from blood donors. We routinely pre-screen these donors for specific characteristics both in vitro and in vivo to ensure they are suitable for further testing. By doing so, we prevent failed studies which would need to be repeated and thereby reduce the overall number of mice needed.

We also carefully track the welfare concerns of our studies in a database that allows us to better estimate how many mice are expected to reach a humane endpoint before the scientific results are achieved. By doing so we can adjust the size of the study to ensure we reach the planned scientific objectives and avoid unnecessary repeats.

Pilot studies are also routinely conducted when new situations arise (for example new therapies, new analytic methodologies, new models). These are done with small numbers of mice and then when the approach is optimised we can progress to larger studies with confidence that the study will accomplish the scientific objectives.

In addition, we strive to combine multiple requests for animal tissues so that as few mice as possible are used for this purpose.

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 where possible especially when tumours are not superficial and cannot be measured by callipers. 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 delay any procedures that require the animal to be restrained via the scruff (such as to measure tumours) for a minimum of 3 days following

microchipping wherever possible, in order to enable full wound healing in the nape of the neck of the animal.

When dosing animals with unfamiliar test substances, we dose as early in the day as is practical 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 recurrence. 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 to minimize the number of injections administered to the animals. We aim to select the most appropriate route of administration for our test agents and the most relevant tumour models in order to produce the highest calibre results possible which ultimately minimises harms to animals.

In addition, a non-surgical method of tumour implantation in the mammary fat pad has been developed, and for subcutaneous implantation of tumour fragments under the skin. These methods are 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.

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 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.

Mice harbouring a human immune system (called humanised mice) present particular challenges and we refine their use in the following ways:

- Use of a scoring system to monitor a condition called graft-versus-host disease (GVHD) that occurs when the human immune cells injected into the mouse (graft) attack the organs of the mouse (host).
- Preliminary assessment in mice of the human cells from each new blood donor for specific characteristics to test their suitability for further testing.
- Implementation of an in vitro assay to determine whether each donor's immune cells can respond to different tumour types before use in mice.
- Use of several different donors in a single study to reduce the overall number of mice and to improve the relevance of the results.
- In many studies we are able to utilise a particular strain of mouse which is much less likely to develop GVHD.

We also plan to implement some changes and explore new methodologies regarding husbandry practices. For example:

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- In order to better manage weight loss on our studies, we plan to implement the practice of alternating types of dietgel (regular or bacon-flavoured) in weekly rotations. This has been successfully piloted in 2025 and will be rolled out across all studies in 2026.
- We are currently testing whether the provision of Rainbow Foraging Bits (Bio-Serv) can help with hyperactivity in mice, through the encouragement of natural foraging behaviour. If successful, this would likely also be implemented more widely across our studies when hyperactivity is a concern.
- We also intend to continue to explore the use of Digital Ventilated Cages (DVCs) for applications such as monitoring of hyperactivity.

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), and the OBSERVE guidelines published in 2024 (De Vleeschauwer et al. can be found at https://doi.org/10.1038/s41596-024-00998-w). 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, to a team working on an ex vivo (taken directly from a living organism) tissue slice culture system in 2017, and this year (2025) our team received a special commendation for our efforts to refine the way we manage humanised mouse models, and for our innovative approaches to reducing & replacing the use of thousands of mice.

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.