



Home Office

## NON-TECHNICAL SUMMARY

# Enabling Development of Therapeutic Drugs for Cancer

### Project duration

5 years 0 months

### Project purpose

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

### Key words

Cancer, Therapy, Drug Discovery, Pharmacology, Tumour Models

### Animal types

### Life stages

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Mice

Adult, Aged animal

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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 licence is to develop our understanding of how mechanisms involved in cancer can be modified for therapeutic benefit and to understand the properties of new test agents we generate by investigating their tolerability, the molecular, biochemical, and physiologic effects of the test agent (pharmacodynamics) and how the test agent moves into, through and out of the body (pharmacokinetics).

We will identify and/or test an estimate of 50-60 new candidate drugs, or their surrogates, in non-tumour and tumour-bearing animals to support the development of new medicines and benefit cancer patients.

**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?**

It is estimated that there were 18 million new cases of cancer worldwide in 2020. It is projected that by the year 2040 there will be 28 million new cases of cancer each year [<https://www.cancerresearchuk.org/>]. Cancer is a complex disease with more than 200 types described and the four most common cancers occurring worldwide are breast, lung, bowel, and prostate. These cancer types account for more than 4 in 10 of all cancers diagnosed worldwide. In the UK, someone is diagnosed with cancer every 2 minutes. Despite the recent advancements in treatment of cancer, there are still many types for which targeted therapies are not available. In other cases, therapies are available, but treatment is not effective for all patients and many patients who do respond to these targeted therapies will eventually develop resistance and their tumour grows back. Of those patients diagnosed in the UK, only 50% will survive for 10 years or more following their diagnosis (source <https://www.cancerresearchuk.org/>).

Our research project will use animals to support our efforts to discover and develop novel therapies for cancer to benefit these patients. The use of animals enables us to mimic complex aspects of the disease and drug properties that is currently not possible in a laboratory. Using animal models, we test the activity of our new drug candidates in a relevant way in a live animal. It is important to undertake our programs of work to support the development of new drug formats and technologies for cancer therapy. This program of work is expected to enable us to generate data that will be used by our team to progress new cancer medicines through the drug development pipeline.

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

The work undertaken in this project will provide important data to enable initial testing of our experimental cancer drugs in relevant animal models. This work will allow us to understand how our drugs distribute in the body after dosing and how quickly they are eliminated from it. It will develop our insight into how our drug candidates work, to establish safe doses to use in animals, and will help discover specific responses that we can measure as biomarkers. These can then be taken forward into larger, more complex animal models of disease to evaluate their activity. If successful, eventually human clinical trials would be conducted with the new experimental drug. Work undertaken will also help us to identify the most appropriate drugs to combine and will guide clinical teams on finding the right dose level and schedule.

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

As well as building datasets for pre-clinical work packages on new experimental cancer drugs, we expect to publish our results and present our findings externally to scientific peers.

### ***Amendment May 2025***

We have included now orthotopic tumour animal models to allow initial testing of our experimental cancer drugs. Orthotopic means grafting of tumour cells into a natural position and the ability to do this allows us to build more relevant animal models of human disease in which to test our novel experimental cancer drugs. 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.

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

This program of work will enable us to progress new cancer medicines through the drug discovery pipeline. 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. New experimental cancer drugs developed using this licence have the potential to benefit millions of cancer patients globally.

Some procedures performed under the authority of this licence are necessary 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 project licence will contribute knowledge to the wider scientific community through the publication, presentations at external conference 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 advance in drug discovery and animal modelling. We aim to publish both successful and unsuccessful experimental results in relevant scientific journals, and to share our data and learnings with collaborators to avoid other's repeating work. We present our research at international conferences for the benefit of the broader scientific

community. This public dissemination of our results has the potential to lead to new collaboration and opportunities to develop innovative new experimental cancer drugs and associated biomarkers.

### **Species and numbers of animals expected to be used**

- Mice: 14,500
- Rats: 400

## **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 an immune system that resembles that of humans in some respects. This means that we can grow tumours in these animals to investigate our new experimental cancer drugs and their effect on the immune response to tumours. We will also use immunocompromised animals: these animals bear defects in one or more immune components. These mice or rats allow us to transplant human material into them without rejection. We will create models, which we termed 'humanised'; by injecting both human immune cells as well as human tumours to model aspects of the disease that we cannot do in mouse only systems.

In most experiments, we will use mice, rather than rats. This is because many more tumour models are available for mice, and a wealth of published data exists to help guide our experimental plans and interpret our results. Occasionally we may use rats when a mouse tumour model is not available to us, e.g. when the experimental cancer drug is active only in rates or when we need to compare against other data generated in rats. An example of this is safety data, where rats are often the preferred species.

Typically, we plan to use adult mice (6-12 weeks old) at the start of our studies as the immune system is considered immature in younger mice and would not represent the biology of the cancer patients we aim to treat. Occasionally we may use significantly older animals. This is especially relevant as cancer is generally a disease of middle to old age in human patients. For those studies we plan to use animals up to 24 months old, as our previous experience has shown that this age sufficiently demonstrates the changes in the immune system associated with age but before mice begin to suffer serious effects of aging.

Rats used will also be adult stage (approximately 6-12 weeks old) at the start of study. We do not intend at present to undertake studies with aged rats.

### ***Amendment May 2025***

We have now included protocols in which to test our experimental cancer drugs in orthotopic models, this requires a surgical procedure of tumour cell implantation into an organ or area that is the natural

microenvironment, e.g. pancreatic tumour cells implanted into the pancreas.

### **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. Animal care staff are highly trained in rodent welfare and ensure 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.

We will use both tumour-bearing and non-tumour bearing animals for our projects. It is not necessary to use animals bearing tumours, for example, in some cases to determine whether a drug is tolerated by the mice or to measure how much of a drug enters the bloodstream, or to understand the impact of our drugs on specific aspects of the immune system.

For those animals bearing tumours, these will be used to test the tolerability of drugs where the tumour microenvironment plays an essential role in the activity of the new experimental drug, or where the tumour is needed to determine the kinetics and/or mechanism of action of the new drug. We use cell lines derived from mouse and human sources, and patient-derived xenograft (PDX) models of many different tumour types to model human disease. PDX models are generated by transplanting human patient tumour material into host immunocompromised animals and are maintained by passaging tumour fragments from host to host. Both cell line and PDX derived tumours will result from the injection of tumour cells or tumour fragment implantation under the skin where they grow into a tumour at the site of injection. They are easy to monitor and measure their size with callipers, and additionally we also use a tumour condition scoring system to assess tumour condition. In some cases, tumours will not grow in the animal unless additional factors are administered. For example, some breast tumours require the presence of oestrogen hormone. 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 PDX 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.

### ***Amendment May 2025***

Our studies will utilize orthotopic tumour models that better recapitulate (mirror and predict) the clinical circumstances and/or patient disease patterns. These tumours will result from either surgical implantation or specialised injection of tumour cells or tumour or self organizing 3D tissue cultures (so called organoid) fragments into a specific organ (e.g. pancreas).

In some studies, we will use advanced imaging techniques (such as bioluminescence imaging) that allow us to track tumour growth or to follow distribution of a drug throughout the body. To undertake

these imaging sessions the animal will be anaesthetised.

Blood samples may also be collected during some studies to measure levels of drug, or human immune cell components in humanised models. Blood samples are of a small volume and are taken from a vein while the study is ongoing, whereas larger blood samples are taken at the end of study if a greater volume is needed.

Our experimental cancer drugs are commonly injected into the peritoneal cavity (i.p.; into the abdomen), intravenously (i.v.; into the tail vein) or directly into the tumour. Occasionally drugs may be administered orally (p.o) or under the skin (s.c.; subcutaneously).

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, they could last for 6 months.

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.

To understand the impact of our work on the animals, here is an example of a typical study with an immunocompromised host animal:

1. implantation of a microchip under the skin (s.c.) for identification
2. injection of human immune cells into the tail vein (i.v.)
3. injection of tumour cells under the skin (s.c.)
4. administration of experimental therapies by one or more of the following routes: i.p. into the peritoneal cavity (typically this will be done twice a week for three weeks); intravenous i.v. into the tail vein (typically this will be done once or twice a week for 3-4 weeks); orally (by gavage force-feeding ; typically this is done once or twice a day for the duration of the study)
5. collection of a blood sample
6. 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 or in humanised models from graft versus host disease (GvHD). GvHD 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.

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 or deletion agents. These mice are typically monitored more frequently as the amount of weight loss increases and weight loss can often be alleviated by providing dietary supplements.

We will humanely kill any animals that have developed large tumours to minimise unnecessary suffering using well-defined tumour burden limits. Generally, studies with subcutaneous tumours will reach these limits within 30 days of implantation if they are not treated with a drug, 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 6 months to reach the same size without any drug treatments that might inhibit 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. 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 bear 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 anaesthetic and pain relief (analgesia) will be provided when surgery is performed.

#### ***Amendment May 2025***

In certain instances, engineered (i.e. genetically modified) human or mouse immune cells will be tested in mouse models in which we will test their anti-tumour activity. Examples of engineered immune cells include chimeric antigen receptor (CAR) T cells and engineered T cell receptor (TCR) T cells. These are a special kind of treatment that modifies a patient's own immune cells (T cells) to specifically express proteins that enable precise recognition of tumour cells. Additionally, modifications may be made to enhance their survival and persistence after transfer into the host. In addition to GvHD, off-target effects may occur if the intended target antigen is also expressed at low levels 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 tumour models where specific adverse events may occur such as diarrhoea in a colorectal tumour model.

Treatment of animals with cancer therapies may also lead to unwanted effects like 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 of these effects will be mild and of short duration; however, some animals may experience moderate effects. We expect that approximately 65% of animals will have moderate adverse reactions to drug substances whereas the remaining animals will not experience any adverse reactions.

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 (NVS) or Named Animal Care and Welfare Officer (NACWO) would be sought if the movements/breathing patterns began to appear while in the home cage, if they became constant while out of the cage, if they significantly worsened after performing procedures, or additional clinical signs or weight loss were present that could not be explained by other factors.

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 project licence (PPL):

Mild = 25%

Moderate = 75%

**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 in the laboratory 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.

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

Our organization regularly uses a range of in vitro methods. These experiments take place in a test tube or laboratory dish, outside a living animal. 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 3D experimental systems, such as the tumour slice culture system and patient-derived organoids. These methods preserve the 3D structure of a patient tumour and are expected to be more representative of the biology of the whole tumour compared with the 2D experiments mentioned above.

Most commonly, new experimental cancer drugs will undergo analytical tests such as stability and to detect impurities and will include where possible potency testing in a relevant assay. However, due to the wide range of different types of drug substances and modalities that we test, it is not possible to provide specific go/no-go criteria that have broad applicability across our portfolio. Animal studies will only commence once lead candidate drugs have been validated extensively in the laboratory to identify a small panel of lead candidates (typically 3-6 drugs).

### **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 or organoid cultures are a valuable addition to our experimental toolbox, however they can have a short lifespan and can show modification of cellular function but do not demonstrate that this effect can shrink a tumour.

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 usage over the last several years, and projected usage of the new orthotopic models in our current drug discovery programs of work..

**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>.

**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.

Using data analyses from past projects, we have analysed the tumour tissues of the most frequent types of tumours that we grow in mice 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.

Additionally, we are developing tumour models that better recapitulate the human disease by using hormone administration, gene expression and depletion agents to create the specific aspects of tumour biology we are targeting. We hypothesise that this also will lead to an overall reduction in animal number because they will ultimately lead to more relevant results that are more reproducible. This in turn results in the need to use fewer animals in the long term as our new candidate drugs will be tested in human disease relevant models.

## 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 (subcutaneous tumour). 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. We use subcutaneous tumours as described here around 90% of the time to test tolerability of our novel experimental cancer drugs.

In around 10% of cases for our novel experimental cancer drug testing, we may require different types of cancer models in which to test them which is more representative of human disease. 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. We continuously seek refinement in our surgical models, implementing recent advancements, and strive to always utilise methods and procedures to refine the techniques

**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. 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. To this end for our humanised models, we have developed a GvHD (graft versus host disease) Scoring System that we are constantly refining to ensure welfare and business need are balanced. 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. 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. Additionally we are constantly refining our surgical implantation techniques including that for patient-derived xenograft (PDX) tumour fragment passage. When unexpected events occur, these are thoroughly investigated to find out what happened so action can be taken to prevent a reoccurrence.

We carefully monitor tumour burden including the use of whole-body imaging techniques when possible. When utilising imaging to monitor tumour burden, before animals undergo repeated anaesthesia, they are assessed and weighed before undergoing the procedure. The imaging substances such as luciferin or contrast agents and radiotracers are not expected to cause any harm.

Depending upon study design, animals may receive whole body radiation or focussed radiotherapy. Generally, no adverse effects are expected at the intended doses that will be used under this project. Occasionally though, mild weight loss and/or piloerection may be expected, especially if more susceptible mouse strains are used.

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 200mm<sup>3</sup> 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 7 days for national shipments or 14 days 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.

Aged mice up to 24 months old will be used only when necessary for studies requiring certain aspects of the biology of aging. We note that animal younger than 15 months old are considered middle aged, rather than old, so when studying immune responses in old age studies may require aging animals up to 24 months old.

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

Our practices 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/>).

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/](https://www.lasa.co.uk/current_publications/)

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

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

Our organisation is committed to the 3Rs and continues to follow advances in the community. We routinely hold Symposia and invite external speakers to talk on these topics and we have a dedicated 3Rs contact who regularly disseminates information related to 3Rs.

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 e.g. 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.