Home Office

NON-TECHNICAL SUMMARY

Provision of an outsourced drug discovery platform for the 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, tumour models, therapy

Animal types	Life stages
Mice	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 ultimately to develop new and improved treatments for cancer. We plan to develop new, or refine existing cancer models to improve the translatability of our work from animals to human medicine, resulting in the selection of the best drug candidates to take forward for further development.

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?

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, with the number of new cases rising year on year (Ferlay J, et al. Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; 2020). In the UK, over a quarter of all deaths involve cancer with the most common types being breast, lung, colorectal, prostate, skin and stomach cancer. In most cases, if cancer is left untreated it is highly likely that it will lead to death.

One cancer we hope to target is the particularly difficult to treat triple negative breast cancer (TNBC), which accounts for 15% of all breast cancers. There are currently fewer treatments available to treat this rare type of breast cancer, meaning that mortality rates are higher. Specific data on average age at death from triple negative breast cancer is not available, but with non-triple negative breast cancer mortality increases with age. In the UK in 2016-2018, almost half of deaths from breast cancer (48%) were in people aged 75 and over (Cancer Research UK website). However, younger women (<40 years old) are 1.53 times more likely to develop TNBC than older women (>60 years old), so age at death is likely to be lower in people with TNBC.

Whilst there have been significant advances in treatments for many cancers, deaths as a result of cancer still remain high. This is partly due to the type and number of cancers that exist and the fact that some have ineffective or no treatments available. Added to this, individual patients may respond differently to the same treatment, and patients who previously responded well may go on to develop resistance to the treatment, rendering it ineffective.

Our work aims to use animals, in combination with experiments that take place in the lab, to support the development of new treatments for cancer and address the unmet clinical needs of some cancers. The use of animals is an essential part in the process of developing new treatments for cancer. Animal studies enable us to replicate complex aspects of the disease in the entire biological system, including the interaction of cancer cells with other cells and organs in the body. They enable us to test the effect of new treatments on the disease (for example tumour growth) in a relevant and intact biological

system. This work will enable key decisions to be made regarding which treatments should continue to be developed, and ultimately, which will make it into clinical trials, where they are tested in humans. Our approach to the work ensures that this is done in the most efficient way possible, and that the benefit gained from every animal is maximised.

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

Work carried out under this licence may involve the development of new animal tumour models (creating incidences of cancer in animals, similar to the human condition) and potentially the improvement of existing animal tumour models. Collectively these will allow us to test the ability of new drugs to treat specific cancers in a manner that more closely replicates the human condition. As part of this project we hope to develop or improve 3-6 animal tumour models (for example using different types of cancer cell) and to test the effects of at least 5-10 new anti-cancer drugs using these models, or currently available models.

This project may provide important information to progress new cancer treatments through the phases of drug development. New test agents will be evaluated for their ability to treat cancer in animal models that replicate the conditions in human cancer. The information gathered will enable us to identify the most appropriate treatments to take forward to human clinical trials and, importantly, quickly determine which drugs should not be progressed any further.

In addition, this work will increase our knowledge of how new drugs work and will help us to identify changes in the body that occur in response to the drug. We can use this further down the line to monitor responses in humans during clinical trials.

Data from studies under this project may be used to support patent applications and applications by clients for additional funding. Data produced may also support the design of regulatory studies for clients.

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

This project will generate important data in the development of new drugs. To assist with this, we will utilise our test agent dosing project licence to allow us to understand how drugs distribute in the body after dosing, how quickly they are eliminated, and the dosages that are well tolerated in rodents. This will aid in the design of dosing strategies for studies under this licence. Ultimately, work carried out under this licence is expected to result in the progression of new cancer treatments through various stages of drug development and ultimately into the clinic to treat patients with various forms of cancer.

Our focus on a science-led approach will enable key decisions to be made at each development stage on whether a test agent is likely to become a successful drug. This allows unsuitable drugs to be abandoned at an early stage and enables us to use the fewest number of animals possible per drug development programme. The identification of test agents as unsuitable for use in humans at an early stage of development will ensure a better success rate in the drug discovery process than has been seen previously in the pharmaceutical industry. Ultimately, this project will contribute to the successful development of new anti-cancer drugs, which may benefit patients with various forms of cancer who may otherwise have died from their condition.

The development of new or improved cancer models will enable us to test new drugs in as close an environment to the human condition as possible. This will increase the likelihood of patients benefiting from these new drugs.

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

All studies are designed such that the outputs from each animal are maximised. Expert knowledge is gathered not only from within the preclinical (animal) team performing the animal studies, but from other teams within our company, or our clients' companies. This ensures that all relevant work that has been performed in the laboratory is taken into consideration when designing animal studies. The in vitro (in the test tube) team at our company are experts at analysing tissue and blood samples collected from animals, and they help with details of sample collection and storage to ensure that the samples are collected and stored in the best way possible. They are also experts at working with small quantities of samples, particularly small volumes of blood samples, meaning that they can often analyse lots of different biomarkers (a measurable indicator of a disease state or other physiological state) and test agent levels from each animal.

In addition, we will seek expertise from our established networks, to ensure that we make use of any new knowledge or incorporate better methods of performing animal studies. We will also use these networks to provide information and training to others on the models and techniques we use in our research. We will maintain good communication with managers of the animal facilities to ensure that any tissues from animals being killed that are not required for our work can be made available to other researchers if suitable.

Although there are times where we will not be able to share animal model information (for example, where it would put us at a competitive disadvantage), we aim to publish or share our findings (especially control data) wherever possible.

Species and numbers of animals expected to be used

- Mice: 3900

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 are the most common type of animal used for developing new cancer treatments. Mice are well characterised, meaning that a lot is already known about how the body works in mice, and a large selection of different tumour cell types are already available for use in mice. This means that for the most common types of tumours, such as, lung, skin, breast and bowel cancer, cells are already available allowing us to easily evaluate new drugs designed to treat these kinds of tumours.

Adult mice will be used for the work outlined in this project licence as we want the biology of the animals to be fully developed as this will better represent the patients we aim to treat.

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

The majority of animals will be part of studies that aim to test whether new drugs can alter the growth rate of tumours. Typically, tumour cells will be implanted by injection under the skin on the flank of the animal, or, less commonly, cells will be injected into the mammary fat pad (breast) tissue, or directly into a vein, the left ventricle of the heart (intracardiac), into the liver (intrahepatic), or into the brain (intracranial). Except when injected into a vein or into the left ventricle, tumour cells will grow into tumours at the site of injection. When injected into a vein, tumours cells will travel via the blood and form tumours around the body, most commonly in the lungs. When injected into the left ventricle, tumour cells will travel around the body via the systemic circulatory system and form tumours, most commonly in the long bones. The size of the tumours will be monitored as part of the experiment. Tumours that grow just under the skin (flank and breast tumours) can be measured using callipers or simple hand-held imaging devices. Tumours will be measured a minimum of twice weekly, and the frequency of measurements will be increased during the faster growth phase. Tumours growing internally require monitoring by non-invasive imaging and by following the overall condition of the animal closely. For non-invasive imaging, animals will be anaesthetised as it is important that they remain still to produce the image. Animals will be imaged at least weekly when tumours are present, with frequency increasing during the faster growth phase. Tumours will typically be allowed to develop over a 4-12 week period, however, actual study duration will be dependent upon the type of cancer cell injected and how quickly they grow. From previous experience, mice with bone metastases do not appear to show obvious behaviours of pain, but we as a team will monitor the animals very closely for early signs of pain, such as analysing facial expression (slightly closed eyes, pinched cheeks). Animals will be monitored closely using clinical scoring sheets, body weight trends will be tracked, and non-invasive imaging will be used to determine tumour burden levels. From the pilot studies, a surrogate endpoint will be determined based on bioluminescence data, ensuring maximum scientific data can be collected and the model is clinically relevant, whilst minimising the suffering of the animal. Any animals showing signs of pain will be provided with nutella buprenorphine.

As part of a study, animals will typically be dosed with anti-cancer agents (e.g. chemotherapies) over a period of several weeks or months and dosing will be initiated typically when subcutaneous or mammary fat pad tumours reach a volume of 100 mm^3. In the case of internal tumours this will be based on tumour-associated bioluminescence (production and emission of light from the tumour cells in the animal; TABL) which will be determined by non-invasive imaging under gaseous anaesthesia, or observation of tumour engraftment/progressive tumour growth guided by pilot data for each model where, for example, it is not possible to tag the tumour cells to emit light for imaging. In these cases endpoints earlier than the expected point where tumour burden would cause suffering will be used in a small group, to determine if the cells grow into tumours at the expected speed. Dosing will often take place on a daily basis, but this may vary depending upon the test agent. Drugs will be administered more commonly by the intraperitoneal (inside the body cavity), subcutaneous (under the skin) and oral (by mouth) routes and less frequently by the intravenous or intranasal or intratumoural (into the tumour) routes. For intraperitoneal, subcutaneous, oral and intratumoural administration, mice will be held securely by a trained researcher for the dose to be administered. For intravenous dosing, mice will be placed briefly in a specially designed rodent restrainer, the tail will be warmed using warm water or a heat lamp to dilate the blood vessels and make them easier to see before administering the dose.

For intranasal dosing, mice will be briefly anaesthetised and placed on their backs to allow the dose to be administered directly into the nostrils.

Less commonly, as part of some studies, animals bearing tumours may be given radiation treatment directed at the tumour either alone or alongside other anti-cancer treatments. Animals will be anaesthetised throughout the treatment and are expected to make an unremarkable recovery.

Blood samples, or small samples of tumour tissue, may be collected during some studies to measure levels of the test agent or to assess how well the drug is working. Blood samples are usually small in volume and are taken from a superficial vein such as the tail vein. Larger volumes of blood may be collected at the end of a study when an animal is under deep terminal anaesthesia. Small samples of tumour may be collected from live animals under anaesthesia using a very fine needle. Other non-regulated procedures such as passive urine collection (allowing the animal to pass urine naturally on to a clean surface so that it may be collected) may be performed.

At the experimental end point, animals will be humanely killed and tumours will be dissected and typically examined to look for evidence that the test treatments have worked.

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

The most common adverse effects in the studies described in this project licence will arise from the tumours themselves or from the test agents. We are developing a scoring system that will enable us to monitor and ultimately minimise the level of suffering an animal experiences on our experimental protocols. Our scoring system will take into account the animals body weight, activity level, general body condition, posture and the condition of any visible tumours and will be used to classify the degree of suffering an animal experiences. We also take into account the duration of adverse effects and the cumulative experience of the animal. Therefore, an animal that experiences, for example, several short periods of reduced activity following dosing may experience mild suffering, but an animal that experiences several longer periods of significantly reduced activity that are accompanied by another clinical sign such as weight loss, may be experiencing more moderate suffering.

Metastases (cancer cells that have spread to other parts of the body) resulting from mammary fat pad tumours, and as a result of systemically (intravenously or intracardiac) administered cancer cells, can be difficult to detect and may cause the animals adverse effects as their size and number increases. Signs that an animal may have a number of internal tumours that are starting to affect normal functioning include reduced activity, weight loss (greater than 15% from starting body weight), poor body condition or poor posture. Again, we will use our in-house developed scoring condition to minimise the level of suffering an animal may experience. Animals will be killed before they exceed moderate suffering.

In the orthotopic (in the target organ) models (intrahepatic and intracranial), the tumour will develop in the organ of initiation and metastases (secondary tumours) may develop. Organs that tumours may spread to over time include the lungs, spine, or the abdomen, increasing tumour burden and potentially impacting normal bodily functions. Signs that tumour burden is starting to impact the normal function of an animal include reduced activity, weight loss (greater than 15% from starting body weight), excessive weight gain due to the development of ascites (fluid in the abdomen), jaundice (yellowing of the skin or visible membranes), impaired motor function, poor body condition or poor posture. Ascites may occur

either as a result of cancer spreading to the peritoneum causing fluid leakiness, or as a direct result of the liver cancer raising the pressure within the liver and nearby blood vessels and forcing fluid out into the abdomen. Jaundice may occur due to liver damage affecting normal liver function, or due to the blockage or narrowing of a bile duct, preventing drainage of bile from the bloodstream into the intestines.

Determining the tumour burden is vital to ensure the study will end before the animal begins to suffer beyond an acceptable level. Where possible non-invasive optical imaging (the use of light as an investigational imaging technique to obtain detailed images of organs, tissues, cells, or molecules, used by researchers and clinicians for disease diagnosis and treatment) will be used to characterise patterns of spread of different tumour types. We will use this in conjunction with our in-house developed scoring condition to define humane and scientific endpoints in order to minimise the level of suffering an animal may experience. Imaging will also allow animals to be removed from study at an early stage if they are found to have zero tumour growth or tumours growing in the wrong site, and will prevent animals undergoing unnecessary procedures.

Any animals with tumours large enough to impede normal mobility or their ability to eat and drink will be humanely killed.

The treatment of animals with known anti-cancer treatments or test agents designed to treat cancer may cause adverse effects, similar to humans. Following some treatments, mice may display reduced activity, hunched posture and fur bristling. For most treatments these effects should be short lived and mild, however some animals may experience moderate effects, which may include sustained weight loss of up to 15% of starting body weight and/or longer lasting reduced activity.

A small incision in mammary fat pad tissue may be required in order to visualise the tissue fully to enable us to give an accurate injection of tumour cells into the tissue. Incisions will be performed as a surgical procedure under recovery anaesthesia, and animals are therefore likely to experience some pain as a result of the incision. Pain relief will be provided as appropriate.

Animals may undergo surgery under recovery anaesthesia for implantation of metastatic or orthotopic tumour models. It is likely that transient pain as a result of the surgery will occur. Appropriate pre-and post- procedure analgesia, as advised by the Named Veterinary Surgeon, will be employed.

Anaesthetic risk during surgery and imaging procedures will be mitigated by ensuring there is a suitable period of time between repeated anaesthetic events, to allow animals to recover fully. Animals undergoing prolonged anaesthesia are at increased risk of dehydration and a decrease in body temperature. Body temperature will be maintained by the use of appropriate warming, with monitoring to confirm that this is achieved. Repeated anaesthetic events will only be carried out when animals have returned to normal eating, drinking and mobility.

An estimated 20% of mice of strain NSG will 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.

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)?

Mice:

Mild 38%

Moderate 62%

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

- Killed

Replacement

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

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

Wherever possible we use cell-based experiments, however, even where cells are used it is still necessary to use animals to understand the complex nature of cancer. Animals enable us to study the whole body, allowing us to study both how cancer cells interact with other cells and organs within the body and how cancer cells spread throughout the body. It is not possible to fully study this in isolated cells and/or organs.

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

Our company regularly uses a range of in vitro (taking place in a test tube in the laboratory) methods utilising cells (typically cancer cells) to understand how a novel test agent might affect the cellular functioning of those cells. From these experiments we can prioritise test agents and only take forward those that have the desired effect, and therefore those that look the most promising for the treatment of particular types of cancer.

Why were they not suitable?

Cell-based methods are useful to test the impact that novel test agents have on a cellular level, for one particular type of cell, but they do not model how cancer cells interact with other types of cells and organs, or how they spread throughout the body. In addition, cell-based methods do not test the effects that the body might have on a test agent, for example, how it is absorbed, distributed and excreted from the body, all of which can alter how effective the test agent might be. Testing a new drug in an intact biological system, such as a well understood rodent system, enables us to study the complex interactions of cancer with other cells/organs in the body, as well as monitoring how the test agent performs in an intact biological system. None of the alternatives mentioned can replicate this, although

cell-based testing enables us to triage drugs and only take the best candidates forward for further development.

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 number of animals we will use throughout the five year lifespan of this licence has been estimated based on the number of cancer projects we have coming up in the next 12 months, combined with a prediction of likely demand of future projects for new clients over the remaining years of the licence.

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

We have extensive experience in the design of experiments of the types in this project, which has given us confidence in the number of animals required to ensure that no animals are used unnecessarily, but also that the data generated is robust and reliable. Through experience, whilst we do tend to use a standard group size for the majority of our tumour studies (6 mice per group), we regularly refer to the PREPARE guidelines (https://norecopa.no/PREPARE) and make use of the NC3Rs Experimental Design Assistant (https://nc3rs.org.uk/3rs-advice-project-licence-applicants-reduction) to ensure that we are using the correct number of animals for every study. When designing experiments to look at the effect of novel test agents on tumour growth, where the test agent has not previously been dosed before, we look at published literature or client data to determine the variability observed with similar test agents and/or similar tumour models. We can then use the NC3Rs Experimental Design Assistant, or our own in-house developed tool to help determine the most appropriate group size.

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

An initial experiment using reduced numbers of animals is often conducted first of all, which enables adjustments to be made before conducting the full experiment. Such experiments are referred to as pilot studies.

Data from pilot studies and previous experience are used to ensure that the numbers used are as low as possible, without compromising the robustness and reliability of the data. Within our company, a member of the wider team has generated a tool for performing power calculations and can be consulted as necessary to assist with study design.

Wherever possible, our in vivo scientists will be blinded to the treatment status of an animal, thus reducing bias. This enables more reliable information to be gathered from a smaller number of animals. Those who carry out analysis on samples (e.g. blood or tissues) collected during the study are also blind to the treatment status of the animal.

Baseline data (e.g. tumour size (where relevant), bodyweight) are recorded and animals are randomly assigned to treatment groups so there is no difference between the groups at the start of the study.

Good planning ensures that within any series of studies we can control for variability that might be introduced by external factors. To limit this variability we use animals of a similar age/weight range, test batches of test agent in the lab first, use the same source of animals and reagents, keep records of observations made and standardise as many components of an in vivo study as is practicable.

We will coordinate with other groups to share tissue including post-mortem tissues to reduce overall mouse numbers.

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.

Animals will be housed in a purpose-built, state of the art facility, that is free of disease-causing organisms such as bacteria, viruses and parasites. They will have access to food, water and items that enhance their environment, such as tunnels, chew sticks and mezzanine levels to climb on. Our company staff and the animal care staff are competent in rodent welfare and will ensure that animal suffering is minimised. We aim to house mice in groups to promote normal behaviour. However, aggressive behaviour can occasionally result in animals being singly housed to prevent injury.

The most common type of tumour model we expect to use is the subcutaneous tumour model, which involves injecting cancer cells just under the skin (in the subcutaneous layer) in the flank of the mouse. This results in tumour growth at the site of injection. This is the simplest method available for inducing tumours in mice and results in the least harm to the animal as the tumour is self-contained within the subcutaneous layer and therefore cannot interact with organs or spread to other regions within the body. This technique can be used for different types of cancer; allowing the search for effective treatments for different cancer types without needing to induce the cancer in the usually affected organ or tissue.

Other methods, such as orthotopic tumours and metastatic tumours will be employed when we need to answer more complex questions such as how cancer cells interact with different organs, how cancer spreads throughout the body, or how tumours respond to novel test agents in these different settings.

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

Adult mice are the lowest species of mammal that allow us to adequately study the complexities of human cancer. Due to the length of most studies, which span weeks to months to allow for tumours to grow, terminal anaesthesia is not a possibility. It is also important that we are able to monitor the behaviour of the animals in a conscious state as this allows us to monitor for adverse reactions to any test agents administered.

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

We have a demonstrable history of refining our procedures to minimise harm to our experimental animals. This includes our basic practice of always using the smallest needle possible for dosing to minimise any pain and distress to the animal. We also investigate the suitability of test agents to be delivered via a route that causes the least harm. For example, we recently implemented this for a test agent, allowing us to switch from twice daily oral dosing (where the dose is administered via insertion of a tube down the throat) to dosing via the drinking water. The palatability of drug in drinking water will be carefully considered, and if appropriate, we may consider flavouring the water to increase its palatability.

General welfare monitoring is particularly important when conducting mice tumour studies. Animals undergoing tumour studies will be carefully monitored using our in-house body condition scoring system. Using this system, mice will be assigned scores based on body weight changes, changes in general condition (e.g. coat, posture and body condition), activity levels, condition of any visible tumours (colour and whether tissue necrosis or ulceration is present), as well as the number and size of any tumours (by non-invasive imaging such as the Peira TM900 handheld imaging device for measuring subcutaneous tumours or calliper measurements). Significant ulceration is not expected in the majority of animals as pilot work will be conducted to establish the rate of tumour growth that is least likely to result in ulceration at the tumour site. However, if ulceration develops, animals will be closely monitored and scored using our in-house developed scoring sheet, which will score ulcers as either dry or wet (exuding pus or clear fluids). Animals with wet ulcerated tumours persisting beyond 48 hours with no signs of improvement will be humanely killed. For dry ulcers, advice may be sought from the vet and minor interventions will be implemented if appropriate to allow the animals to continue the study. Mice with increasing scores will be monitored more frequently to ensure that no animal suffers more than is necessary during each study. In our experience, assessing the whole body condition of the animal gives us much more information on the degree of suffering an animal experiences compared to measuring tumour diameter alone. Animals can often tolerate large tumours (particularly subcutaneous tumours) without a negative impact on overall wellbeing, making measurement of tumour diameter alone unsuitable for understanding the true impact on the animal, or when to kill an animal. Therefore, while our scoring system does take into account the size of tumour(s), this is considered collectively with the above factors to assess the impact of the tumour on the animal as a whole, meaning that we gain a more accurate picture of the animal's health and can intervene early if welfare is significantly impacted. This in turn prevents the loss of valuable data and promotes maximum benefit from each animal that might otherwise have been lost when using a one-factor humane end-point.

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.

Mice will be group housed where possible and provided with an enriched environment in order to minimise stress. Should pain relief be required, we will consider adding the pain relief to a palatable substance such as Nutella to allow the animals to take it voluntarily.

For pilot studies in general, we will use previous experience from team members to determine the most appropriate injection locations and volumes. During pilot work to establish mammary fat pad tumours in mice, we propose to inject cancer cells into two mammary fat pads of the same animal. This will allow us to compare variables such as the delivery vehicle (Matrigel) used to deliver cancer cells and the number of cancer cells injected to determine how they affect tumour growth in the same animal. This will allow us to reduce the number of animals used, particularly in the early stages of studies where we aim to determine the best conditions for each new cancer cell line.

We will also investigate the use of ultrasound for guidance of the needle for intracardiac and intrahepatic injections. For the intracardiac metastasis models, ultrasound guidance will increase injection accuracy and confirm delivery of cells to the left ventricle, thereby minimising the number of animals that have to be removed from study early due to tumours growing in the wrong location. Ultrasound-guided injection will also be investigated as a replacement for traditional surgery in the case of the intrahepatic models. Ultrasound can also be used to identify blood vessels in the liver and will be used to ensure that the needle does not accidentally puncture a vessel leading to the animal suffering a haemorrhage (escape of blood from a damaged blood vessel).

In order to aid respiration and improve recovery from anaesthesia, animals with lung tumour burden (typically linked to the systemic metastasis models) will be provided with oxygen pre- and post-imaging events.

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

We use the Workman et al guidelines (Workman, P et al. Guidelines for the welfare and use of animals in cancer research. 2010. British Journal of Cancer. 102 p1555) to inform our experiments. In addition, we follow the PREPARE guidelines for the planning of studies (Smith et al., PREPARE: guidelines for planning animal research and testing. 2020. 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/.

In addition we will also refer to the following when conducting our work:

Prescott MJ, Lidster K (2017) Improving quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/laban.1217

LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. (E Lilley and M. Berdoy eds.). http://www.lasa.co.uk/publications/

Smith D, Anderson D, Degryse A, Bol C, Criado A, Ferrara A, Franco NH, Gyertyan I, Orellana JM, Ostergaard G, Varga O, Voipio H (2018) Classification and reporting of severity experienced by

animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. Lab Animal 51(1S): 5-57. doi: 10.1177/0023677217744587

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

When designing animal studies we consider the appropriate guidelines, including the guidance from LASA, the NC3Rs, and the PREPARE guidelines. This guidance will influence our study design.