Home Office

NON-TECHNICAL SUMMARY

The development of rodent cancer models and their pre-clinical use as a platform for drug development and drug evaluation.

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, Anti-tumour

Animal types	Life stages
Mice	Adult, Aged animal
Rats	Juvenile, 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 identify potential drugs / therapies that reduce, inhibit or prevent the growth of tumours leading to new and improved cancer treatments.

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?

The benefit of this project will ultimately be the introduction of new and improved treatments for the management of cancer.

There were 9.6 million deaths from cancer worldwide in 2018. Lung, liver, stomach, and bowel are the most common causes of cancer death worldwide, accounting for more than four in ten of all cancer deaths. Lung, liver, stomach and bowel cancers have been the four most common causes of cancer death since 1975.

Based on our past record we would expect to deliver 10-15 potential new drugs into clinical development during the period of this licence. Whilst not all of these will be successful, as we move to more novel and sophisticated approaches to cancer management, it seems reasonable to predict that approximately 20% of the nominated compounds will reach people living with cancer and provide significant benefit to cancer sufferers. Since most of our approaches here are applicable across a broad range of tumour types it is likely that these could have wide utility. These may not completely cure cancer patients but it is expected that they will enable a significant number to live longer with improved quality of life following the diagnosis of this devastating disease.

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

The benefit of this project will ultimately be the introduction of new and improved treatments for the management of cancer.

We will share pre-clinical animal data with principal investigators to influence clinical trial designs. Publication of both successful and unsuccessful data in high impact journals, with the aim to publish approximately 15 scientific papers in high impact journals each year.

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

Since our approaches are applicable across a broad range of tumour types it is hoped that the drugs developed from this project licence will have wide utility across different cancer patient populations. These may not completely cure cancer patients but it expected that they will enable a significant number to live longer with improved quality of life following the diagnosis of this devastating disease.

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

New information will be disseminated at key national and international conferences via poster sessions and seminars. Publication of both successful and unsuccessful data in relevant journals. We will share pre-clinical animal data with principal investigators to influence clinical trial designs. We work in collaboration with many scientists and groups in which we are able to share data and learning meaning that important data generated from our animal studies can be shared more widely to avoid others repeating work and to help influence other areas of animal use.

We will share good practice with AWERB and also internal in vivo community groups.

Species and numbers of animals expected to be used

- Mice: 106500
- Rats: 10500

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.

We use animal studies in mice and rats alongside many other experimental approaches and they are crucial in building up a complete picture of cancer biology. Our research using animals has helped drive advances in cancer treatment that are benefiting people with cancer all over the world today.

Our work mainly uses mice (90%), which can grow tumours which mimic those of human cancer patients. Studies of cancer in mice mimic the complex way tumours grow and spread in people with cancer. The majority of our work involves implanting a tumour on the side of the animal. Although this is unlike the patient situation the use of these models over the years has demonstrated utility in selecting viable drug candidates.

Mice can be easily genetically altered to allow us to study the genetic causes of cancer and reproduce tumour types which naturally occur in humans in the correct tissues and body systems.

We also conduct some studies in rats (10%). Some compounds that we want need to test may not have sufficient levels in the blood to have an effect on the tumour in the mouse and therefore we need to use the rat as an alternative species. The rat is also usually the species of choice for toxicity studies. These studies would be conducted under a different project licence but it may be necessary to directly compare the dose level of a drug that causes an effect on the tumour to the dose level that produces unwanted side effects. This is to ensure that there is a big enough margin between activity and safety in order to progress to the clinic.

In some instances cancer growth can be influenced by hormones, these are classed as hormone-sensitive tumours. It may be necessary to run some experiments in rats that do not have high levels of circulating hormones and in these instances rats that have not yet become sexually mature will be used. These are classed as juvenile rats.

Earlier life stages of vertebrates such as zebrafish is an option, and early studies of human xenograft models do show promise of tumour inhibition studies . However the small size and difficulty of collecting meaningful blood samples makes pharmacokinetics essential in our work, currently impossible in that model, meaning that rodent models remain the most effective model at present.

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

The rats and mice we use are bred at specialised facilities and are then transported to our facility. We primarily use breeding facilities within the UK but on very rare occasions specialised strains may be required to be imported from within the EU or USA. Animals will be transported in safe enclosed boxes that contain bedding, food, water and are maintained throughout the journey within temperature and humidity levels appropriate for the species. Upon arrival to our facility the mice or rats are transferred into clean cages. In our facility these cages are Individually Ventilated (IVC) and each cage has its own clean air, food and water supply. In addition bedding and nesting material and numerous forms of enrichment are included. Enrichment provided such as paper houses, tunnel and chew sticks allow the rats and mice to have improved welfare and demonstrate natural behaviours such as sheltering, nesting, climbing and gnawing. The cages are maintained at the appropriate temperature and humidity for the species. Cages are cleaned at least once a week and water and food is checked daily. Animals are left to get used to to their new home surroundings for at least 7 days before any experimental procedures are performed.

Mice and rats are typically housed in small groups as they are social animals. On occasions animals may be singly housed e.g. on occasions male mice naturally fight with each other so may be housed singly for welfare reasons.

In a typical study animals have a microchip for identification inserted under the skin while under anaesthesia, and are injected with tumour cells under the skin which grow into tumours. A wide variety of tumour types may be used within this licence. Our work is not focused on one area of cancer but across all cancer types with focus on lung, prostate, breast, ovarian, pancreatic and bladder. The majority of studies use cells that are derived from human tumours. As the human tumour tissue is foreign to the animal, the animals immune system would reject the tumour tissue and therefore we need to grow human tumours in either mice or rats that have an impaired immune system. This allows the tumour tissue to grow and not be rejected.

Tumours are usually injected as cells using a needle, this is done on the lower left or right side of the back (flank) of the animal. In some cases the tumour is not available as cells and therefore a very small piece of tumour tissue needs to be put into this area surgically. The mice or rats would be anaesthetised as this requires a very small cut in the skin so the tumour tissue can be placed under the skin. The cut is then closed using either stitches, special glue or clips. Having tumour cells injected or surgically placed under the skin allows the grown tumour to be easily monitored and measured and does not affect the animals ability to move around. Sometimes tumour cells require the presence of hormones, such as oestrogen, in order to grow. When required, we supply these hormones to the animals through small pellets that we place under the skin via a minor surgical procedure or in their drinking water.

In some cases tumours may be grown within the organ of interest e.g lung, breast, prostate, pancreas. This may either be via direct injection of cells into the organ or the use of genetically engineered mouse models. The injection of cells into the organ of interest may require surgery, injection into the tail vein or direct injection into the organ of interest. The least invasive way of administering the cells will be used. Genetically engineered mouse models use mice where mutations seen in the clinic are induced in the organ of interest. Where tumours are grown within the organ of interest, tumour growth may be monitored by non-invasive imaging methods e.g bioluminescence or MRI. Animals will be closely monitored for any clinical signs that may relate to the tumours growing e.g changes in breathing pattern.

All tumour studies will be run in a small number of animals to determine the growth characteristics of the tumour before proceeding into larger studies looking at the effect of potential anti-cancer drugs. In these small pilot studies the animals are closely observed every day and body weights and condition of the animals are recorded to ensure that the animals are healthy. The tumour is measured at least once a week and the condition of the tumour is observed daily. If the tumour grows in a consistent manner this can then be used in subsequent studies to determine the effect of potential anti-cancer drugs.

In these studies once the tumour has started to grow the animals will start to receive doses of a potential anti-cancer drug. Anti-cancer drugs are usually dosed orally via the mouth, or into the abdomen (intraperitoneally), and the animal may typically receive up to two doses per day for 28 days. The animals are closely observed every day and body weights and condition of the animals are recorded to ensure that the animals are not unwell. Animals will be closely monitored for any effects that may relate to treatment dosing e.g. change from normal clinical condition. The effect of the potential anti-cancer treatment on the tumour growth is compared to the tumour growth in an animal which does not receive the potential anti-cancer drug. The hypothesis is that the anti-cancer drug will significantly reduce the growth of the tumour. For some drugs, we are able to asses their effect by looking at specific changes in blood and/or other tissues/organs after dosing, which means we can test these agents in animals without implanting a tumour.

During the study blood samples may be taken from a vein. The blood sample is analysed to measure the level of the potential anticancer drug or other substance in the blood.

During the study, tissue samples may be taken from the tumour. The tumour sample is analysed to characterise the tumour or relevant treatment endpoint.

At the end of the study the animal is killed and the tumour tissue and other tissues may be taken which can then be used for further investigation.

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

An estimated 10-20% of mice of strain NSG 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.

Some animals develop small scabs at the site of microchip insertion on the scruff, however these scabs typically show signs of healing within a few days. The tumour is continually monitored and measured and although the tumour may continue to grow this does not appear to cause the animal any pain or discomfort and they continue to behave normally. The size the tumour can grow is limited by the use of a measurement/condition/size scoring system to ensure that it does not cause any pain or discomfort to the animal. Animals who are are given oestrogen to help tumour growth may experience skin lesions, which we monitor closely and treat with petroleum jelly.

The dosing procedure for dosing of the anti-cancer drugs does not usually cause any issues but the drug itself may have some side effects. Side effects may include weight loss, clinical observations or abnormal behaviours such as being less active or not socialising or interacting with their cage mates. Strict criteria are put in place to minimise any unwanted side effects to minimise any pain, suffering or distress to the animals. The side effects usually only last for a short period of time.

Very occasionally, some animals experience a minor and transient nosebleed during handling or restraint for procedures. These nosebleeds typically resolve quickly and generally do not have further impact on the animals.

Occasionally, as a result of fine needle aspiration or core needle biopsy, some animals may experience mild and transient bruising and/or swelling near the biopsy site. As a result of the procedure, the mice may also have a small wound from entry/exit of the gun/needle.

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)?

Approximately 90% of animals used within the licence will be mice and 10% rats. It is expected that 90% of both the mice and rats will be returned within the moderate category and approximately 10% within the mild category.

What will happen to animals used in this project?

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

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?

Animals are needed in our research to help us understand the mechanisms that underpin cancer, such as the growth and spread of tumours, and to develop new ways of diagnosing, treating and preventing the disease. Cancer is a very complex disease and animal studies are essential to understand these complexities within living organisms. They are also required by regulatory authorities before any trials of new drugs can be tested in humans. Animal studies are only performed after every feasible test has been conducted on cancer cells in the laboratory and where no alternative exists.

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

Multi-cellular 'organ on a chip' models are available, but as yet have not reached the reliability and integrated multi-system complexity of the rodent model, especially when shaping the treatment of patients in the clinic. There are no immune-competent in vitro models with functionality comparable to the rodent models. Non-animal alternatives are used in the identification and selection of compounds. These generally include measurements of the drug's activity on particular target cells. Activity in particular cell types however cannot predict the activity in humans due to a complexity of issues such as availability of the drug in the body and whether it is able to reach the target cancer cell.

Why were they not suitable?

Available alternatives are not currently as good as the existing rodent models and are not as suitable because they cannot mimic the living organism and the processes that under-pin cancer in a living organism.

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?

This licence reflects a well established cancer research program and the numbers of animals used within this project licence are based on the diverse areas of cancer that are being investigated.

We typically run approximately 20 studies per month, each study usually has ~100 animals per study.

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

All studies are designed to ensure that the minimal numbers of animals are used to achieve the question being asked. This is done with help and guidance from a statistician who is a maths expert who uses huge amounts of data to figure out how likely it is that something will happen or not. They ensure that all studies are designed to ensure that we are able to use the minimal numbers of animals to see an effect of a potential anti-cancer drug if there is an effect.

Good experimental design principles such as randomisation are incorporated into all experiments. All study designs are approved by a statistician.

All experiments are performed in accordance with Good Laboratory Standards (GLS). This standard sets the minimum laboratory requirements for all our research and development. This ensures that procedures and results are accurate, reliable, traceable and reproducible and where appropriate, comply with the appropriate regulatory authorities' legislation.

All experiments are performed in accordance with the PREPARE guidelines - Planning Research and Experimental Procedures on Animals: Recommendations for Excellence.

All research that will be published will be published in accordance with the ARRIVE guidelines - Animal Research: Reporting of In Vivo Experiments.

Where possible mixed sex groups will be used in experiments using genetically engineered mouse strains to reduce breeding requirements. Additionally cell lines will be generated from the genetically engineered mouse tumours models so that where appropriate the cell line derived models can be used, which reduces the requirements for breeding.

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

When different project groups want to investigate the effect of their compounds in the same tumour model wherever possible we run these within one study that share the same control group therefore reducing the requirement for multiple control groups if the studies were run independently, leading to an overall reduction in numbers.

To minimise any side effects associated with treatment of potential drugs, a small pilot study in typically 2- 3 animals is performed to ensure the treatment does not have any unwanted side effects before progressing into larger numbers of animals.

Wherever possible multiple tumour and/or tissue samples will be taken from the same animals and may be frozen down and used in other non-animal experiments. In rare cases where tumours fail to grow or grow atypically after induction on Protocol 5, it may be possible to re-use the animals under Protocol 4 to assess non-tumour-based endpoints for target engagement following treatment, which may reduce the need for further studies.

In some cases, administration of breast cancer cells directly into the mammary ducts via intraductal administration has been shown to improve the consistency of growth compared to tumours grown under the skin (subcutaneously). A more consistent model has the potential to reduce the number of mice required to achieve a scientific endpoint. Therefore this route will be used where it is felt that it has the potential to reduce animals required per study, without impacting on animal welfare.

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.

We will use a wide variety of different tumour models in both mice (90%) and rats (10%). The majority of the tumours are implanted as cells in an area on the animal (the lower left or right side of the back (flank)) which allows the tumour to be easily monitored and measured and does not affect the animals ability to move around. The size the tumour can grow is limited to ensure that they do cause any pain or discomfort to the animal. For some anti-cancer drugs, it may be possible to test activity in animals without a tumour using surrogate markers.

In some instances (<10%) it may be required to grow a tumour in the organ where the tumour was originally derived from, for example a breast tumour implanted into the breast tissue. The tumour may be monitored using imaging which can monitor the tumour growth.

The dosing procedure for dosing of the anti-cancer drugs does not usually cause any issues but the drug itself may have some side effects. Side effects may include weight loss or abnormal behaviours such as being less active or not socialising or interacting with their cage mates. The drugs are tested in a very small number of animals initially (typically 2 to 3 per group) and only drugs that do not have unwanted side effects can be used in larger numbers of animals.

It may be necessary to surgically neuter animals to reduce levels of hormones as the growth of some tumour models may be influenced by hormone levels (hormone-sensitive tumours). Any animals that undergo a surgical procedure will be provided with pain medication prior to the surgery (and after surgery where required) and maintained in a warm environment until full recovery to minimise weight loss. Pain medication may be administered after surgery within an edible jelly. The mice and rats will have access to a non-medicated form of the jelly prior to surgery to become accustomed to eating it.

All animals will be housed in specialised cages. These cages are Individually Ventilated Cages (IVC's) which filter the air and fully protects the animals from all micro-organisms. All food, water and bedding is also fully sterilised beforehand. All cages have various forms of enrichment included, for example a cardboard house, sizzle nest, tunnels, chew stick. The temperature and humidity is kept within a specified range that is optimal for the animals.

When establishing new tumour models in rats, where possible, we will use strains of rats that are sufficiently immune-compromised without the need for whole body irradiation to further suppress the immune system.

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

Using less sentient animals for example a non-mammalian species such as the fruit fly, is not possible since they lack a closed circulatory system and so you cannot replicate a number of the complex processes that underpin cancer such as the growth and spread of cancer.

Juvenile life stages of vertebrates such as zebrafish is an option, and early studies of human xenografts into zebrafish models do show promise of tumour inhibition studies (Xaio et al 2020). These models rely on protected life stages of zebrafish, and may need larger numbers, although moving to a vertebrate considered less sentient than a rodent may be a significant refinement in future (Costa et al., 2020). However the small size and difficulty of collecting meaningful blood samples makes the pharmacokinetics essential in our work, currently impossible in that model, meaning that rodent models remain the most effective model at present. Others have investigated embryo larval life stages of zebrafish that would be unprotected within the EU (since they work before the day 5 threshold of protection) with some success, but reliable quantification and exposure through internal concentration remains the barrier in this case as well (Hill et al., 2018).

Costa et al 2020 EBioMedicine (The Lancet) https://www.sciencedirect.com/science/article/pii/S2352396419307881 Developments in zebrafish avatars as radiotherapy sensitivity reporters — towards personalized medicine.

Hill et al. 2018 F1000Res https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6234738/

Xaio et al. 2020 Trends in Cancer (Online 17th April) https://www.sciencedirect.com/science/article/abs/pii/S2405803320301217

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

All animals will be acclimatised for 7 days from arrival before they undergo any experimental procedure.

We closely follow and implement the latest welfare guidelines and therefore handle animals in a way that causes the least amount of harm or stress to them as possible while conducting these experiments.

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.

All surgery is performed in concordance with 2017 LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery.

The use of microchips provides unambiguous identification of mice and links directly to our data capture software to reduce typographical errors. When we use a microchip for identification, we will limit handling via the scruff during the week after the microchip is implanted to avoid disrupting the healing process and hopefully reduce the likelihood of small scabs developing at the site of entry of the microchip.

Explore if there are options to apply any palliative treatments to minimise any adverse effects of dosing. Addition of a bolus administration of saline for compounds known to cause dehydration to minimise associated weight loss.

Explore use of refined dosing applications in collaboration with the NVS, for example use of pin-port. When administering substances via the IP route using a daily or twice daily schedule, doses will be administered into alternating sides of the abdomen whenever possible. Additional welfare consultations with the NVS and/or NACWO may be implemented for IP dosing regimens approaching the lifetime limit.

For some compounds it is possible to administer then in the diet or drinking water and this has the potential to reduce animal handling in chronic dosing studies.

Explore the use of estrogen dependent breast cancer models that do not require estrogen administered by surgical implant of a pellet. For example, growing some breast cancer cell lines in the mammary glands, via intraductal administration, has been shown to remove the need for supplementing estrogen. For tumour models that still require estrogen supplementation to support growth, we are trying to supply the hormones via drinking water rather than via a pellet which requires a minor surgical procedure to insert it under the skin. Administering hormone in drinking water not only avoids a surgical procedure, it can also reduce the frequency of estrogen-related side effects (body scalding) in the animals. When we give the hormone via the drinking water, we will also add sugar or sugar-free cordial to the water to camouflage the taste of the estrogen so the animals continue to drink a normal amount and are less likely to experience dehydration.

Welfare scoring tables will be established for novel models where tumours grow in the organ of interest. This allows us to ensure our endpoints are the most refined possible. The aim will be to design the experiments to occur over a timeframe that avoids the animals developing clinical signs. Where possible we will include the use of imaging to provide a humane endpoint that can be applied before any clinical signs are observed.

We use a new needle for every individual animal injected parenterally. The same applies for when we implant cells.

Use of banked animal tumour and/or normal tissue samples taken from previous studies which can be used in non-animal experiments.

When serial core needle biopsy or fine needle aspiration samples are planned, we will ensure that any swelling has resolved, no open wounds are present, and any mild bruising is showing signs of healing before a subsequent sample is attempted. Serial samples will be collected from a new biopsy track whenever feasible. As we build our experience with different tumour models and mouse strains, we will look to develop guidance for biopsy/aspiration sampling suitability, in collaboration with the NACWO/NVS.

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

Guidelines for the welfare and use of animals in cancer research (Workman, P., Aboagye, E., Balkwill, F. et al. Br J Cancer 102, 1555–1577 (2010))

Animal research: Reporting in vivo experiments: The ARRIVE guidelines. Br J Pharmacol. 2010 Aug; 160(7): 1577–1579.

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18(7): e3000410.

https://doi.org/10.1371/journal.pbio.3000410

PREPARE: guidelines for planning animal research and testing (Adrian J Smith, R Eddie Clutton, Elliot Lilley et al. Laboratory Animals Volume: 52 issue: 2, page(s): 135-141

LASA Guiding Principles

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

I actively participate in the in vivo community, attending and presenting at conferences relevant for oncology preclinical models. I also follow the NC3R's website to keep myself updated on relevant 3R's initiatives (https://www.nc3rs.org.uk/news/using-award-scheme-promote-3rs-innovation). We also actively discuss and implement new 3R's initiatives and run a yearly 3R's competition, sharing information globally across different establishments. We actively set annual refinement goals, for example alternative mouse handling.