Home Office

NON-TECHNICAL SUMMARY

Determining mechanisms of tumour development and relapse following treatment

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, Chemotherapy, lymphoma, neuroblastoma, drug resistance

Animal types	Life stages
Mice	adult, juvenile, pregnant, embryo, neonate, aged

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?

This project aims to understand why cancer develops and how it changes in response to treatment, in particular, to determine why some cancers become resistant to therapy.

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 one of the major causes of death worldwide with at least 1 in 2 people developing this disease in their lifetime and only half of these surviving. It is therefore clear that we must conduct more research to understand not only why cancer develops in the first place but also why treatment is not successful for many.

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

This research will lead to new information regarding the mechanisms of cancer development as well as the reasons for resistance to therapy. This work will be published in the scientific literature as well as presented to both lay and scientific/medical communities at research conferences and meetings. In particular, through interactions with clinical communities as well as pharmaceutical companies to enable the translation of the research findings into real clinical impact. This could be in the form of the development of new therapeutic targets and drugs and/or clinical assays that can be used to determine whether a patient will have a good outcome following treatment. Alternatively, this research may facilitate clinical decisions such as whether their treatment needs to be altered due to a lack of response to the therapeutic protocol.

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

In the short term, the outputs of this research will benefit those in the medical and scientific communities to facilitate future research activities. In the medium-term, we these findings will be applied to the design of clinical trials and associated biological studies to learn more about cancer in the real world, outside of the lab. In the long term, it is hoped to develop new treatment strategies that may include new drugs or different combinations of existing drugs to improve cancer outcomes.

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

This research is part of many ongoing collaborative research activities, including Europe-wide and international research initiatives. In particular, both established research networks and clinical trials groups will be engaged whereby these data will be presented at network-wide meetings towards clinical application and to inform future research opportunities. In addition, via a Europe-wide training

network, this research will also contribute to the training of fledgling research scientists and clinician scientists in order that this future generation will be better informed and will be able to take forward the findings of this research to real clinical application. The research conducted under this licence will also be applicable to patient groups globally including those residing in low-income countries that are affected by the cancers that will be studied. Important collaborations with such populations will enrich the work.

In all cases, the work conducted will be published in the scientific literature, disseminated to both lay and scientific audiences at meetings and conferences, and communicated to interested parties such as pharmaceutical organisations to initiate collaborative research activities.

Species and numbers of animals expected to be used

- Mice: 3000

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.

Our research uses mice to understand why cancer develops, in particular mice that have been genetically modified to express proteins suspected to cause cancer. This work is necessary as a mechanism to prove that certain changes to the DNA code drive cancer development. Furthermore, to determine whether inhibition of the activities of these proteins will be effective in the treatment of people with cancer. We also use mice that have no immune system so that we can grow human tumours in the lab. If the mice have an immune system, they will reject the human tumour. Adult mice with no immune system host human tumour cells and allow them to grow. We can try to cure them of the disease.

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

We will breed mice that have no immune system so that they do not reject the human tumours. However, an immune system is necessary to enable some drugs to work, in particular drugs called 'immunotherapeutics'. To study these drugs, we will develop 'humanised' mice. Humanised mice are mice that do not have their own immune system are instead injected with human blood cells to create a human immune system. Mice are reconstituted with human immune system cells (humanised mice) by intravenous (iv) injection. Tumour cells are grown under the skin (sub-cutaneous) of these mice so that tumour growth can be easily monitored by measurements with a type of ruler called a calliper, and by feeling the mouse at the site of injection. On occasion, the sub-cutaneous environment cannot support tumour growth and so we also attempt to culture the primary tumour cells at other sites in the body such as in the abdomen (intraperitoneal) or in the blood (intravenous).

Treatment of the mice with investigational drugs starts once the tumours reach a certain size (as determined by calliper measurements, or in case of intravenous/intraperitoneal growth, by looking for tumour cells in blood samples and using imaging techniques such as magnetic resonance imaging (MRI) scans). In order to better mimic the human scenario, whereby tumours are generally diagnosed when they are relatively large (preventing normal bodily activities leading to symptoms and subsequent diagnosis), treatment commences when the tumours reach 0.5-1.0 cm at their widest point. Mice are humanely killed at the pre-defined study endpoint, which is either the end of the treatment regimen, or when the tumour reaches the maximum recommended size of 1.5 cm in any one dimension. For up to 50% of mice, the tumours are allowed to reach 2 cm when more cells are required for downstream analysis (particularly when analysing tumour cell subsets such as the very small populations of cells that remain after therapy, so-called treatment persister cells, or cancer stem cells). In these cases, the mice for these studies are clearly identified before commencing the project and are monitored closely for side-effects of tumour growth (e.g., paralysis). However, animals will be collected earlier than the 2cm limit if they achieve the scientific value that is needed for the experiment at an earlier stage of development. Drugs are administered by the most appropriate route according to how drugs are metabolised by the body (so-called pharmacokinetic/pharmacodynamic parameters). This can be through the mouth, intraperitoneal (into the abdomen), intra-tumoral, sub-cutaneous (under the skin) or intravenous (in the blood) or via a mini pump implanted under the skin. In the past we have dosed mice with the drugs for a maximum of 30 days, but it has become apparent that this is not enough time – when the drug treatment has completed, despite it looking like the tumour has disappeared (even when using high resolution devices such as MRI scans), the tumour returns. As such, we would like to have more flexibility in drug delivery schedules allowing us to give the drug with a variety of treatment schedules (e.g., metronomic dosing (on, off, on, off drug etc), drug holidays (on, off for a long period of time, on etc.) and co-administration of different drugs over longer periods of time. In all cases, mice are closely monitored for any clinical signs that might indicate that the drugs are toxic, for example by monitoring the weight of the mice daily.

Genetically altered (GA) mice (mice that have had their DNA code altered) will largely be used for ex vivo (outside the body) studies of tumour cells. These mice express cancer-causing genes, so-called oncogenes, in certain parts of the body, and develop tumours at these sites. For the most part, the GA mice we use produce tumours of the immune system and therefore develop tumours at sites of immune system components such as the thymus (an organ that sits above the heart), spleen and lymph nodes. Tumours are detected by regular palpation (massage) of the mice, and we will incorporate imaging, such as MRI scans, at key timepoints and on presentation of clinical signs to allow early tumour detection to minimise suffering. To allow tumour growth, the GA mice are aged, sometimes as long as 15 months of age. The GA mice are also used to examine how tumours develop and, in these cases, the mice are killed by a humane method shortly after genotyping (checking the DNA code) for analysis of tumour cells outside of the body. We may breed GA mice to incorporate other genetic elements into our studies. For example, clonally expressed T cell receptors (e.g., TCR transgenic mice), the 'flags' on the surface of immune cells that recognise invading pathogens, to see if these also contribute to tumour growth.

To assess the effects of potential secondary events on tumour development, i.e., environmental factors that might contribute to the development of cancer, GA mice might also be administered stimulatory agents to assess the rate of tumour development and incidence in comparison to control groups. For example, TCR transgenic mice expressing oncogenes in immune cells might be dosed with agents that activate the immune cells. This might include ovalbumin protein or components of this, called peptides.

We will also examine the role of other environmental agents such as microplastics that are shed from a range of plastics and polymers. These agents may be delivered by the routes previously described for drug administration but might also include delivery through the nose, perhaps in an aerosol.

Typically, work conducted under this licence allows mice to live under standard conditions until tumours develop. As soon as tumours are detected, the mice are humanely killed, and the tumours harvested for studies conducted in the lab and outside of the body (ex vivo).These tumours do not grow without a host biological system and the mouse provides the least sentient being that can facilitate tumour growth. However, at all times we attempt to grow the tumour cells in the lab in plasticware and as technologies evolve that facilitate this, we will incorporate this more into our research approach.

In the case of human tumours grown in mice with no immune system, the tumour cells are typically injected and grown under the skin on the hind flanks of the mouse. In some cases, for example if we are studying leukaemias, cancers of the blood, the cells may be injected into a blood vessel or at another site that is more representative of where the tumours grow in humans, such as the abdominal cavity. As some new cancer treatments involve activating a patient's immune system to recognise and kill tumour cells, we may also 'humanise' the mice by first establishing a human immune system. In these cases, immunodeficient mice are first injected with human immune cells derived from blood to establish a human immune system before the tumour cells are injected. The mice are monitored continuously for their well-being, and should the tumours prevent their normal activities such as feeding and moving, the mouse is humanely killed, and the tumour harvested for ex vivo studies or re injection into a new host mouse. In some cases, we treat the mouse bearing the tumour with different drugs to determine if these are good options to cure the tumour and prevent its continued growth. In these cases, the mice are again closely monitored for any clinical signs, not just those that might be caused by the physical presence of the tumour, but also those that might be due to the drug being administered. If any of the mice show clinical signs, they are immediately humanely killed. However, this is a rare occurrence as we monitor tumour growth not just by eye, but also using imaging modalities such as MRI scans and ultrasound so that tumours are harvested before they impact the wellbeing of the mouse. Drug treatment experiments are typically conducted over periods of months with the initial months being the period in which the tumour grows and in the subsequent time, the mouse is being treated. If the treatment approach is not successful and the tumour continues to grow, the mouse is humanely killed before the tumour reaches a size that would impact on the wellbeing of the mouse. On some occasions, when we think the mouse has been cured as we cannot detect any tumour cells in the body, the mouse is allowed to survive in case the tumour relapses or returns, so that we can study the tumour cells at this point to understand why they have grown back. To prove that the cells which grow back are truly resistant to the drugs, we may also treat the mice again with drugs to see how the tumour responds.

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

In general, the mice do not experience any adverse effects from tumour growth as we carefully monitor their health status. We do not allow tumours to grow in size to the extent that they affect the wellbeing of the mouse. However, should the tumours affect the wellbeing of the mouse, it is normally due to growth of the tumour near to the hind limbs which can cause paralysis. Alternatively, tumours might ulcerate as they grow and in these cases, we will put in place close monitoring procedures. Affected mice are humanely killed immediately if they reach the pre-stated severity limit. As the mice are checked daily, at most, a mouse would experience paralysis for no more than 24 hours.

Some mice can experience weight loss due to the effects of the therapy, as do people with cancer undergoing treatment. However, whilst on the protocol, the mice are weighed daily to observe any changes that might trigger humane killing such as weight loss and lethargy. Should a mouse show any clinical signs, they are immediately humanely killed.

On rare occasions, a new drug being tested can have unexpected adverse effects. However, to minimise this possibility, we search the literature for existing and known toxicity data for a given drug before use. If such data does not exist, we start experiments with low doses of drug in one or two mice as a pilot project and scale up dependent on the results. As the mice are checked daily, at most, a mouse would experience paralysis for no more than 24 hours.

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)?

Mouse: moderate 20%

Mouse: Mild 80%

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

- Killed
- Kept alive
- Used in other projects

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?

Cancer is a complex disease that requires a number of host factors to be 'just right' for growth and propagation. For the most part, as a scientific community we do not know what these factors are, but they likely involve food and nutrients provided through the lymphatic and blood circulation as well as locally provided compounds such as growth factors and messengers produced by the immune system and the cells providing the structural support of the tumour. Until the conditions required for tumour growth can be emulated in vitro, we need to continue to use the mouse to host tumour growth and to be able to examine the tumour as essentially, a new organ. Secondly, how a tumour responds to treatment can vary depending on how the body as a whole reacts to administration of a drug, specifically, how the body processes the drug. Drugs are normally given to a patient orally or via the blood stream - these routes of administration can affect how a drug is metabolised and therefore its efficacy in the body. Therefore, to fully assess the activity of any given drug in affecting tumour growth, in vivo studies are ultimately required.

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

In vitro growth of tumour cells as single layers of cells or as organoids.

In vitro drug screens on established cancer cell lines.

Why were they not suitable?

Our experience shows that the tumour cells do not grow in these in vitro systems. It is clear that they require the environment of the mouse host to survive, likely due to the requirement for some, as yet unidentified growth factors.

For drug studies, whilst established cancer cell lines can be informative in determining the efficacy of a drug, the effects of bodily systems on the metabolism and, in some cases, activation of the drugs, must be determined in vivo. In addition, the established cell lines have evolved in the lab to become less representative of the tumours that were first removed from the patient to establish these cell lines, i.e., the cell lines have evolved and adapted to growth in a petri dish and no longer truly represent the genetics of the original tumour.

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?

In the previous project licence, we used 800 mice in the first 4 years of the project. I anticipate that we will continue to use mice at this rate as this current application is a continuation of the prior work. However, in the last project period, our research was inhibited by the pandemic, and we had not secured sufficient funding to complete all of the proposed work. We now have secured considerable research funding for all aspects of the research proposed and do not anticipate interruptions due to global pandemics. As such, I expect that are usage will triple over the 5 years of the project.

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

We follow PREPARE guidelines in our experiments. In particular, in the design of drug studies, we perform Power calculations to make sure we use the minimum number of mice to reach significant results and are assisted in this with the use of the NC3Rs experimental design assistant.

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

We have developed in vitro methods for tumour cell growth using feeder cell lines to conduct drug screens with patient-derived material. Where cells cannot be grown in vitro, we perform experiments with primary cells ex vivo. Ultimately, drug studies have to be conducted in vivo, particularly when in vivo drug activation is necessary.

We have also begun to use imaging techniques in our studies so that tumour development in vivo can be monitored more easily. In particular, we have used MRI to assess tumour growth and will apply this technique more in the future. We will also use ultrasound in this manner.

If a drug has been tested before in mice for the purposes we intend, we search the literature first for previously conducted toxicity studies, if available, to identify LD50 doses (the dose that has been shown to lead to the death of 50% of the animals in that study). We then conduct control experiments starting with low doses and incrementally increasing them whilst monitoring mice for any clinical signs before progressing to each higher dose. These pilot studies allow us to assess the tolerability of the drug and the amount of drug required to kill the tumour cells.

If a drug has been tested in vivo before, we assess the literature to decide on the best drug dose for our studies rather than repeating efficacy studies.

Mice are randomly assigned into treatment groups and are blindly administered drugs, the identity of which are unknown to the administering technician.

Allowing some mice to develop tumours of up to 2cm but not showing pre-defined clinical signs will reduce the number of mice required for an experiment, overall reducing total numbers of mice used.

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.

Mice:

NSG immunodeficient mice

Transgenic mice including strains expressing oncogenes predisposed to developing cancer

Techniques:

Sub-cutaneous, intraperitoneal or intravenous injections of tumour cells: these routes of tumour delivery are conducted by fully trained personnel without anaesthesia and have very minor and transient effects on the mouse. The mice are closely monitored after the procedure for any clinical signs.

Intraperitoneal, intravenous, oral and sub-cutaneous mini-pump delivery of drugs: these routes of drug delivery have transient and mild effects on the mice when conducted by trained personnel. They are conducted without anaesthesia but by fully trained individuals. Surgery to implant sub-cutaneous mini pumps is conducted under sedation or brief general anaesthesia from which the mouse is monitored closely for a full recovery. The mini pumps, when inserted correctly, have no effect on the wellbeing and normal activity of the mouse.

Imaging - MRI, ultrasound and PET/CT: allows us to monitor tumour growth when they are not detectable by palpation alone, preventing 'unseen' tumour growth to continue to the point where clinical signs ensue. Mice undergo sedation to improve their comfort during the procedure.

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

In order to study the development of cancer it is important that we consider the roles of the whole body in order to better model the scenario in humans. This requires components of the extracellular matrix, stromal cells, immune system components and a blood supply, factors that at present are not possible to fully mimic in vitro. In addition, a defining feature of cancer is the ability to invade surrounding tissues and to metastasise - these can only be truly modelled in vivo. As such our research to investigate origins and development of tumours will continue to be performed in vivo with GA mice. We will also continue to use mice as we have a plethora of GA mice that have previously been developed and characterised available to us. In order to conduct experiments assessing established tumours, we employ patient derived xenografts (PDX) as the least sentient system in which to grow primary human-derived tumours. With our PDX models we attempt to grow the tumour cells in vitro after each passage through the mouse but have had limited success even with 3D culture conditions. We continue to refine these in an attempt to establish non-animal alternatives and reattempt in vitro culture at each stage. When short term culture is sufficient, e.g., for determination of early chemotherapeutic sensitivity, we conduct initial experiments in vitro to determine active drug concentrations. However, due to effects of bioavailability, experiments are then conducted in vivo to predict the potential consequences in human patients.

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

We will add enrichment to the home cage (such as tunnels) and conduct non-aversive handling (tunnel and cupping), habituation and acclimatisation, e.g., if mice ordered in from an external supplier, will be allowed a habituation period of at least 1 week so the mice can settle into their new environment. When performing dosing, mice are first acclimatised to the handling technique leading up to the day of dosing, (such as scruffing), so as not to overwhelm the animal all at once.

We will employ immunodeficient mice to propagate human tumours and wild type congenic mice to propagate tumours derived from GA mice. Both models will be used to assess the impact of chemotherapeutic treatments on tumour response and clonal tumour evolution. The most appropriate site for tumour propagation in vivo will be determined based on experience and will largely constitute sub-cutaneous growth of solid malignancies and intraperitoneal or intravenous for liquid tumours. All of the techniques we use are already well established and result in minimal suffering to the mice. We perform all sub-cutaneous injections with two trained animal researchers so that the procedure is quick and accurate (one to hold the mouse, the other to inject). We also capture mice using non-tail handling

techniques prior to scruffing for injection (the NSG mice are very placid and respond well to handling). All trained animal researchers are also regularly assessed in their use of techniques by experienced animal technicians in the unit. We also attempt, when possible, to employ the services of animal technicians within the unit who regularly perform these techniques and are therefore very experienced resulting in minimal suffering to the mice. When performing injections, we suspend the reagents in the smallest volume possible to reduce the time taken to undertake the procedure although this is dependent on the solubility of the compound. Tumour growth is monitored by calliper measurement or palpation of internal tumours and liquid tumours by blood sampling. When attempting to grow a new tumour for the first time, the mice are carefully monitored using a staging system for clinical signs until we can establish the growth properties of that tumour. We also employ imaging techniques such as MRI and ultrasound where possible to monitor tumour growth. In this manner, tumours can be monitored, and the mice culled before overt clinical signs manifest. On some occasions tumour growth may be accompanied by ulceration. Mice that experience ulceration will be closely monitored to ensure that their overall wellbing is not affected. If it is, the mouse will be humanely killed. Towards this endpoint, we will develop a scoring system for ulceration based on experience, whereby photographs will be taken of ulcers at various stages and throughout the healing process to develop the system and guide licence holders as to experimental endpoints. We may also grow the human tumours in the mammary fat pad to better mimic the environment in which these tumours normally grow in humans, this is particularly the case for breast-implant associated lymphomas.

Pilot studies will be used to determine if adverse effects are expected when creating humanised mice and animals will not continue on study if they do. Pilot studies will be employed when first attempting growth of a new source of human immune cells.

Our mice are expected to develop tumours and therefore in order to minimise suffering we use an in-house designed Excel database to record experiments and severity levels reached. We also check our mice for tumour development by palpation and observation of clinical signs on a daily basis. Sub-cutaneous tumours, once established, are measured using callipers on a daily basis to ensure the size limits are not exceeded. At the same time, the mice are monitored for any clinical signs and any issues with the grafted tumour; in particular, tumours are monitored for ulceration, any growth that limits movement and general condition. Mice experiencing any issues with grafted tumour humanely killed.

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

Advice on experimental design will be sought from the statistical services unit and by using the NC3rs experimental design assistant (www.nc3rs.org.uk/experimental-design-assistant-eda). Factorial design of experiments will be considered where necessary to minimise animal usage and for quantitative experiments, power calculations will be used to determine sample sizes generally using a significance level of 5%, a power of 80% and a least practicable difference between groups of 25%. However, we will also plan experiments based on our prior experience of working with the described model systems and dependent on experimental limitations, for example, the number of cells that can be isolated from a human tumour and therefore propagated in the mouse; we expect treatment group sizes to consist of between 6-8 mice, but this may be less. Each experiment will be conducted following the development of a study plan whereby a statement of the objectives of the experiment, a description of the experiment, covering such matters as drug dose, frequency of dosing, number of mice per group, experimental endpoint, data analysis and the experimental material are described. Mice will be

randomly assigned to treatment groups and attempts will be made to blind studies. In essence, we will conduct all experiments according to the ARRIVE guidelines in accordance with NC3rs procedures in order that data can be disseminated by publication in the peer reviewed scientific literature. We will also consult the 'Guidelines for the welfare and use of animals in cancer research' published by Workman et al., British Journal of Cancer, 2010.

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

We will regularly monitor the scientific literature for advances in the modelling of human cancer, in particular paying attention to advances in the development of in vitro organoids and 3D bioprinting and will review journals such as ATLA (Alternatives to Laboratory Animals) Journal: https://journals.sagepub.com/home/atla. As these techniques improve and are better able to mimic the human tumour microenvironment, we will apply for funding so that we can adapt our research to these technologies. We will also regularly refer to the LASA Guidelines: https://www.lasa.co.uk/current_publications/ and to the RSPCA website (http://science.rspca.org.uk/sciencegroup/researchanimals).

We also subscribe to the local 3Rs email enquiry list that provides regular updates on advances in the 3Rs and their implementation and signposts the reader to important and relevant resources. We also regularly consult the local 3Rs search tool. We also subscribe to the NC3Rs webinar series and will attend these events as appropriate as well as consult the NC3Rs Gateway (https://f1000research.com/nc3rs).