Home Office

NON-TECHNICAL SUMMARY

Preclinical evaluation of cancer therapeutics

Project duration

5 years 0 months

Project purpose

- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Cancer, Therapy, Immunotherapy, Pharmacodynamics, Efficacy

Animal types	Life stages
Mice	adult
Rats	adult

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The purpose of this project licence is to provide a high quality demand led service, testing the efficacy and tolerability of novel anti-cancer therapies in validated rodent models of cancer.

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?

This will provide data to support IND (Investigational New Drug) submissions for Sponsors, and assist earlier drug development strategies (go/ no-go decisions based upon pre-clinical efficacy).

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

There is an unmet need for new cancer therapies given relapse rates, the fact that the disease may become resistant to treatment and the percentage of patients that experiencing long-term treatment side effects. The aim of this project is to provide data from preclinical models of cancer to biotech and pharma in order to identify those compounds most likely to have the greatest clinical potential and highlight those with limited efficacy or serious side effects for rejection or reformulation as early in the development stage as possible.

In early stage drug discovery, therapeutic candidates are generally subjected to a series of in vitro screens to identify activity on a cellular level, without the need to use animal subjects. Testing the toxic potential of a drug on cells in vitro (screening against cell lines and primary cells from various tissues) provides some indication of potency and specificity compared to current therapies, but ultimately the most promising drugs need to be tested in an animal model that is as predictive as possible of the human condition. Animal models allow us to establish how a potential drug may be absorbed by the body and/or tumour, observe any beneficial effects and if/when they cease becoming beneficial and what interventions are needed. This means we can determine appropriate dosing routes and optimise the schedules with which treatment is given. We can then analyse the success of the intended drug-related effects on the disease. The most frequent and well characterised models are those in rodents, where one can demonstrate a degree of efficacy relative to a current standard of care drug. They also highlight those drugs that are either ineffective or have serious side effects (filtering out those potential drugs with little benefit or limiting side effects as early and as accurately as possible).

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

The initial benefits will be to the pharmaceutical industry. The predictive models we have developed and continue to refine provide efficacy and mechanism of action data that will enable informed decision making on both drug development selection/prioritisation and on optimal dose routes/scheduling in the clinic. By reducing the risk of later stage failures, there is cost/speed benefits for clinical trials. The

benefit for them is therefore a reduced number of unproductive human volunteer studies (and a reduced risk of adverse effects due to dose scheduling errors) and most importantly the development of improved – more effective – cancer therapeutics.

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

The information we provide to the industry is as extensive as we can provide, from in-life parameters showing efficacy of the drug, to analyses of tissues post-mortem that will help confirm the mechanism of action of the drug and perhaps identify biological molecules that may represent a response to therapy. With this data, we aim to support our clients to progress their drug into clinical trials and, in the longer term, be of benefit to patients as a standard of care. Because of the in-house experience and expertise we have with efficacy testing, it is conceivable that this will help minimise the number of animals that would have been needed, reduce time points and drug concentration variation but also acquire the most information out of an experiment without the need for repeats. Thus, our work certainly helps implement the 3Rs in practice.

Species and numbers of animals expected to be used

- Mice: 5650
- Rats: 1250

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.

The programme requires that models are used which closely mirror the human disease situation. Adult rodents (rats and predominantly mice) are suitable for these studies as the work cannot be conducted in lower vertebrates, invertebrates or cell lines due to the poor resemblance of these options to the clinical setting.

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

Animals that have been obtained from a supplier will be acclimated to their new housing for at least 1 week. After this period, for tolerability studies, 1-2 animals will be dosed once or multiple times, using the same or increasing concentrations of test item. Careful monitoring of the animals will take place immediately after dosing to assess any dosing/drug-related effects, and periodically thereafter (typically twice a day).

Pharmacokinetic/pharmacodynamic (PK/PD) studies are used to determine how a drug is absorbed by the body, where it goes to and how long it stays there, and what effects it might have on the body. For PK/PD studies in non-tumour bearing and tumour bearing animals, small groups of mice or rats will be

dosed with a drug (or the same solvent without any drug, if using controls) via a known route, and at a volume, concentration and schedule that is known to be well tolerated.

In cancer studies, animal models have been developed that show the disease at the relevant site, e.g., lung cancer models that develop cancer in the lung, as opposed to subcutaneous models where the cancer cells are engrafted subcutaneously even if this is not where they would normally grow. These are described as orthotopic models.

For efficacy or PK/PD studies in animals with locally growing cancer, tumours will be established by subcutaneous injection of tumour cells. Careful monitoring of the animals and the implantation site will be performed, as well as normal health and well-being observations. Efficacy will be determined by achieving a desired effect on the disease with little to no unintended affects to health or well-being. This may include calliper measurements that can be performed on conscious animals with minimal distress. Test Item (or the same solvent without any drug, if using controls) will be administered via a known route, and at a volume, concentration and schedule that is known to be well tolerated.

For efficacy or PK/PD studies in orthotopic models, or animals with cancers that affect the entire body, tumours will be established by intravenous, intrafemoral, intraperitoneal (through the abdominal wall), intracranial, or intrathoracic injection of tumour cells. If the implantation technique requires aseptic surgical intervention, animals will be anaesthetised, and analgesia may be given during and after surgery. Careful monitoring of the animals and the implantation site will be performed, as well as normal health and well-being observations. This may include calliper measurements that can be performed on conscious animals and/or in vivo imaging of anaesthetised animals. Test Item (or the same solvent without any drug, if using controls) will be administered via a known route, and at a volume, concentration and schedule that is known to be well tolerated.

Blood samples may be collected periodically via a tail vein bleed in conscious animals. We will always follow the NC3Rs guidelines/limits for peripheral blood sampling.

Animals may be subject to in vivo imaging. This most often involves an intraperitoneal injection shortly before anaesthetising the animal for the imaging procedure. Animals will always be allowed to sufficiently recovery from anaesthesia (return to normal eating and drinking and peer interaction) before the next imaging session. Imaging is generally performed 1-2 times per week. For studies where we are trying to determine which organs/tissues are affected by the disease, which only a small proportion of animals will experience, optical imaging may need to be more frequent. The imaging sessions are about 5 minutes and as animals are anaesthetised with inhalable anaesthesia they recover quickly. Recovery may be aided by keeping the animals warm and giving them mashed food.

Animals may receive radiotherapy, either locally or whole-body irradiation on one or several occasions, under anaesthesia. Animals will always be allowed to sufficiently recovery from anaesthesia (return to normal eating and drinking and peer interaction) before the next imaging session.

The endpoint for a study will usually be a specified timepoint, tumour size/burden (based on previous data and standard of care for the cell line) or if a humane endpoint has been reached. At this time a final terminal bleed may be performed under terminal anaesthesia and tissues harvested after euthanasia for analysis. The length of the studies will vary, but generally falls between 1 and 3 months.

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

Frequent adverse effects after test item administration may be weight loss and diarrhoea. Less frequent adverse effects may be bruising, anaemia, loss of appetite, reduced movement, grooming and postural changes. Infrequent adverse effects may be abnormal breathing, tremors, convulsions and reduced peer interaction.

Animals will experience stress due to restraint and transient discomfort from needle insertion and/or anaesthetic injection or inhalation of gaseous anaesthetics (100% incidence).

Repeated intravenous injection can result in irreversible damage to the vein and very occasionally, a haematoma/bruising may develop.

Intraperitoneal injection (through the abdominal wall) is likely to be painful if the needle injures an abdominal organ or if the substance being injected is an irritant.

Oral administration (gavage) is associated with minor discomfort. Very occasionally damage to the oesophagus may occur or substances may enter the lungs resulting in dyspnoea.

Slow-release pellets and osmotic minipumps are small and cause no appreciable effects. Animals may experience a lowered body temperature during anaesthesia. Animals may experience pain on recovery from anaesthesia.

Animals will experience stress due to restraint and a transient discomfort from blood collection (100% incidence). Animals may become overheated or dehydrated from spending too long in the hotbox.

Subcutaneous injections of tumour cells are associated with a momentary discomfort. Very occasionally, a haematoma or bruising may develop. Very rarely, tumours may grow to a size that could cause discomfort or interfere with animals ability to satisfy thirst or hunger. Rapidly growing tumours may ulcerate.

With all surgical procedures there is some risk of development of infections or wound complications (<1%). Use of genetically altered or immunocompromised animals may exacerbate the frequency of infections.

In cancer studies, animal models have been developed that show the disease at the relevant site, e.g., lung cancer models that develop cancer in the lung, as opposed to subcutaneous models where the cancer cells are engrafted subcutaneously even if this is not where they would normally grow. These are described as orthotopic models.

Orthotopic or metastatic tumour models may have various adverse effects, dependent on the site of implantation. Metastasis is clinically relevant, and there are a number of cancers that will initially grow at a primary site, but cells will break away, travel around the body and then develop as secondary (or metastatic) tumours elsewhere in the body. Common sites of metastatic disease after intravenous implantation include the lungs, liver, ovaries, kidneys and spleen.

For orthotopic models, such as leukaemia, animals may gradually become anaemic, weak, lethargic and lose body weight. Infiltration of the spleen or liver of disease can lead to enlargement of these organs which may be palpable.

For orthotopic/metastatic models implanted intraperitoneally, tumour formation may occur in one or several organs in the intestinal cavity. This may also lead to the formation of ascites (collection of fluid in the abdomen) which could cause discomfort, affect behaviour, and mask weight changes.

For orthotopic models in bone, animals may experience pain on recovery from anaesthesia and tumour formation may affect integrity of bone, invade surrounding muscle tissue, or hinder movement.

For orthotopic models in specific regions of the brain, animals may experience pain. Tumour formation may affect the physical condition and general behaviour, including displays of repetitive or uncontrolled movement, fitting, head tilting or abnormal responses to stimuli.

For orthotopic models of the thorax, tumour formation may cause discomfort and laboured breathing.

Animals receiving whole body irradiation, may experience a reduced appetite and hence weight loss and possible loss of condition. Radiation sickness evidence by diarrhoea could develop (less than 5%).

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)?

The expected severity for mice and rats under all protocols under this licence is moderate. Based on the previous 5-year licence period, 85% of mice and 55% of rats are expected to experience the moderate severity.

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?

This project requires that the models used are ones which closely mirror human disease. Animal models address issues which current in vitro tests cannot accurately determine such as the therapeutic index of a new agent, whole body pharmacokinetics (PK) and pharmacodynamics (PD). PK/PD studies are used to determine how a drug is absorbed by the body, where it goes to and how long it stays there, and what effects it might have on the body. Use of animals is needed to address the development and treatment of disease in an environment which is the closest to the microenvironment found clinically. There is not currently an alternate representative of these systems that can be accurately studied that does not involve the use of an animal model.

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

We have, and continue to, develop complex *in vitro* predictive models. We have validated 2D and 3D tumour culture models, including those that mimic the response of cancer stem cells vs a more hierarchical tumour tissue. However, to-date none are sufficient to replace the animal models themselves.

Why were they not suitable?

All compounds tested will previously have been screened using *in vitro* models to determine those candidates suitable for *in vivo* testing. These include studies that demonstrate the mechanism of action (eg. regulation of proliferation, induction of cell death, target cell specificity). Their limitation is, that although they allow assessment of test items on specific aspects of the disease process, they are insufficient in modelling the complex interaction of the different cellular systems present within an *in vivo* tissue.

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 numbers are based on the number of animals used during the previous 5-year licence period.

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

The most important aspect of the proposed programme of work, which will reduce the overall number of animals used, will be a careful selection of only those targets that offer a realistic prospect of therapeutic exploitation. This will be done on the basis of *in vitro* target validation studies and any available *in vivo* data, and work may be turned down if there are insufficient *in vitro* data available. *In vivo* models will not be used for random compound screening and all experiments will be hypothesis led. All experiments will be conducted following standard operating procedures and include appropriate controls, so that the efficacy of test items can be accurately assessed. The number of animals used will be the minimum number required to test the experimental hypothesis at the required level of statistical power and significance. We regularly consult academic and commercial statisticians. For example, data produced under the previous licence have been reviewed and the suggestions for improved analysis have been included in this licence application.

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

Several of our tumour models utilise imaging, which helps reduce animal numbers as the progression of disease can be followed in the same animal over time. We regularly seek statistical consultancy,

sometimes providing historical data, to ensure that our power analyses are correct for each model we offer. Internal expertise in tissue analyses, allows us to fully characterise the cellular changes associated with disease as well as the expression of key factors on the surface of tumour cells and surrounding cells. This information is utilised to refine existing/validated models so that the most appropriate model is chosen for clients' compound's mechanism of action.

Outsourcing work using these models to CROs such as ourselves, results in a greater level of assay standardisation, reducing the number of studies performed. We are exploring the possibility of sharing control data between studies to further reduce animal use, where Sponsors will allow.

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.

To test the efficacy of novel anti-cancer drugs it will be necessary to induce sufficient levels of disease. Some studies will be performed in immunocompromised mice, rats will only be used where suitable. More severely immunocompromised mouse strains may be required for some tumour models, and when they allow for the testing of human based immunotherapies (in this case mice may be carrying a humanised immune system).

We will predominantly use subcutaneous cancer models in mice. Animals will receive treatment delivered by an appropriate route of administration. Treatment response is determined by 3x weekly calliper measurements of the tumour. These types of models are a good way to determine if a drug has anti-cancer activity, however they do not allow for all types of disease. For this purpose, we have established and validated imaging models of cancer using labelled tumour cells implanted into the normal anatomical position. Since the cells are labelled, tumour burden can be monitored in the same animal over time using imaging methods. The endpoint for several of these models is now based on imaging signal rather than humane endpoints, reducing suffering.

On occasion, syngeneic tumour models in mice may be used. These models are developed by administering tumour cells derived from the equivalent strain of animal, i.e., a mouse receiving cells derived from the same strain of mouse. They are most useful for sponsor's who wish to test therapeutics in a fully functional immune system. For subcutaneous tumours, some treatments may cause transient inflammation/ulceration, which has been shown to resolve, and the mice then produce useful data with minimal residual distress from the skin. Hence, a transient window is needed to prevent premature termination due to a short-term effects. Ulceration is a clinical sign of a well-functioning immune response. However, tumours will be monitored and scored 3x weekly, and animals with increasingly significant damage, (tumour score = ulcerated), will be humanely euthanised by a schedule 1 method. The frequency will depend upon the cell type and the kinetics of tumour growth. Where possible, cell

lines that induce ulceration will be avoided. This is particularly relevant in therapies targeting the immune response to the tumour which may necessitate the allowance for ulceration.

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

Rodent models are the best characterised of all experimental oncology models, with mice being much more heavily investigated than rats. Furthermore, work cannot be conducted in lower vertebrates, invertebrates or cell lines due to the poor resemblance of these options to the clinical setting. Rodents provide a cost and time effective platform in general for most pre-clinical testing. For the purposes of oncology testing, the use of higher species is not required because there is a wealth of knowledge on different types of cancer in rodents, as well as decades of in-house expertise with such models.

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

Over the lifetime of our previous licence, we have established and validated several imaging models, where the disease burden can be monitored in the same animal over time, which means that less animals are used. The endpoint for several of these models is now based on imaging signal rather than humane endpoints, which means less suffering. Furthermore, we have refined the tolerability protocol and lowered the severity from severe to moderate for this protocol in this licence application. We have increased the number of different data read-outs that can be obtained from a single study.

We have introduced an ulceration score sheet to refine our tumour observation methods. This will help to ensure all staff are aware of what constitutes an 'ulcerated' tumour, and the endpoints are consistent. It will include descriptions of the appearance of the tumour; timelines based on historical data, i.e. time taken from implantation of cells to ulceration of tumour; and visual aids that can be updated for different cell lines and mouse strains. The frequency of monitoring will alter as the appearance of the tumour site changes, i.e. the frequency will increase with the severity of the observation.

We will continue to refine our models and anaesthesia and analgesia will be employed for any surgical procedures. Blood sample volumes and number of samples will be kept to a minimum.

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

Workman P et al. (2010). Guidelines for the welfare and use of animals in cancer research. Br J Cancer 102(11):1555-1577.

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

We are regularly getting informative emails from the NC3Rs and we make sure to attend seminars and workshops about the subject. We are keeping up to date with the literature within the field of pre-clinical cancer research and are actively looking to implement refinements of our models. Our standard operating procedures are reviewed on a regular basis to accommodate refinements.