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NON-TECHNICAL SUMMARY

Investigative & Enabling Safety Assessment Studies

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

Key words

drug development, safety assessment, toxicities

Animal types	Life stages
Rats	Juvenile, Adult
Mice	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?

This project aims to gather data to aid in developing new medicines by focusing on three key areas.

1. Assessing the effects and risks of specific agents/compounds to help choose the best drug candidates and improve drug discovery methods.

2. Explores drug development strategies to address unforeseen toxicities and enhance drug tolerability and effectiveness, including using new dosing methods.

3. Supports new medicine development and animal welfare through method development and special studies to improve research accuracy and refine animal care.

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?

Studies performed under this project licence will contribute invaluable data to support and progress the development of potential new medicines where there is a clinical unmet need including cancer, asthma, chronic obstructive pulmonary disease (COPD) and heart disease.

In drug development, a safety risk refers to the possibility that a new medicine might cause harmful effects or side effects in people who take it. Before a drug is approved, scientists study its potential safety risks to ensure it is as safe as possible for patients when used as intended. Studies supported by this work will be fundamental to the understanding of safety risks.

By assessing the safety risks of agents/targets early in the drug discovery process, enables a scientific assessment of an agent/target safety risks, alongside a strategy to mitigate observed toxicities whilst still delivering benefit to patients. Additionally, early assessment can lead to decisions leading to early removal of unsuitable agents/targets from further research.

Furthermore, it would be unethical to administer novel substances to humans before making every effort to ensure that they were safe. In all disease therapy areas, developing novel agents with any associated toxicity into a new medicine can ultimately limit the benefit to the patient. This is particularly relevant to cancer patients where such toxicities may be dose limiting, i.e. they cannot tolerate higher doses of agents to improve efficacy because of adverse effects.

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

Data generated from studies performed in support of discovery and early candidate selection will be used internally to allow decision making on whether to develop drug candidates and, with other

preliminary studies may help to define doses for subsequent studies. Data from studies will enable the investigation of safety liabilities identified through *in silico* (computer program simulated tests) and/or *in vitro* (in cells) methods and will be pivotal to the progression of novel anti-cancer agents and other therapeutics. Data from experimental studies will also be used to develop ways to mitigate, monitor or manage unwanted effects in the clinic, for example, by changing dosing regimen, previously intolerable adverse effects can be reduced, allowing a patient to continue with a beneficial treatment. Some studies will typically be performed to provide data in support of dose selection, typically tolerability and/or toxicokinetics (levels of drug in the blood or tissue), or to build/validate a new or different methodology thereby enabling other parts of the safety evaluation programme to proceed with best possible outcomes. Data specifically generated from investigations using humanised models will enable effective and translatable safety assessment of immunotherapies on the immune response.

For all of the above, data will feed back into programmes for future compounds to refine discovery and development.

For compounds which progress, these studies may be included in regulatory submissions as supporting studies. In drug development, a regulatory submission is when a pharmaceutical company gathers all the research data and information about a new medicine and presents it to health authorities, such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA). This process is essential for gaining approval to market and sell the drug. The submission includes details on how the medicine was made, how safe and effective it is, and any studies or tests that were done. The authorities then review this information to decide if the drug should be made available to patients.

Data from studies may be included in peer review publications or presented at internal and external meetings and conferences.

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

Early indications of safety and toxicity liabilities, this may include measuring body functions including for example impact on functioning of the heart or motor coordination where appropriate, will allow early detection of unsuitable molecules and thus support decision making with respect to the progression of the a novel molecule, ultimately supporting the development of only the best new medicines towards the clinic where patients with cancer, asthma, COPD, kidney and heart disease will benefit from improved clinical outcome and quality of life (cure, prolonged life span, less side effects).

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

Data from studies performed under this licence will feed back into research programmes for future compounds to refine discovery and development strategy and thus avoid unnecessary animal use.

For compounds which progress, these studies may be included in regulatory submissions as supporting studies where they have informed for example a more effective dose selection for regulatory studies.

Collaboration with external scientific groups and organisations enables us to explore the use of developing technologies and capabilities such as advanced scientific tools used to study proteins and

small molecules in living organisms, allowing us to extend data sets further from within an individual study, which increase understanding of scientific mechanisms thereby maximising the studies outputs.

Data from studies may be included in peer review publications or presented at internal and external meetings and conferences to share new knowledge and learning in the wider context of drug safety assessment and development.

Species and numbers of animals expected to be used

- Rats: 9000
- Mice: 6000

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.

Rats and mice have a well characterised pathophysiology, and are a widely accepted species to measure safety/toxicity effects.

Typically adult rodents are used, and occasionally juveniles this enables the safety of molecules across different age demographics to be assessed where required.

Rats and mice are used in safety assessments because their genetic and biological traits are similar to humans, which helps predict how drugs will work in people of a similar life stage. They have consistent responses to drugs, making it easy for scientists to compare results across studies. Their short lifespan and quick reproduction allow researchers to conduct long-term studies more efficiently. Additionally, there is a lot of existing research on these animals widely referenced in A Comprehensive Guide to Toxicology and Preclinical Drug Development and Principles and Methods of Toxicology, and regulatory agencies worldwide including the European Medicines Agency (EMA) and US Food and Drug Administration (FDA) trust and recommend their use in research to predict human effects.

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

Studies will be conducted by dosing routes similar to those used in man, e.g. by mouth (orally) or by injection. The animals are then observed regularly to monitor changes in appearance and behaviour. Procedures carried out during these studies include:

a) Weighing: as a loss in body weight is often an early sign of harmful effects in animals. Weighing causes very minimal harm, i.e., the stress associated with handling.

b) Blood sampling or collection of urine for measurement of different components, as changes in these may serve as early indicators of toxicity. Doctors, for similar reasons, often take blood and urine samples from humans. Blood maybe sampled from a small peripheral vein (e.g., tail vein); a larger

blood vessel using a surgically implanted canulae; or under non-recovery anesthesia from the heart of central blood vessels (e.g., vena cava).

c) Electrocardiography (ECG) monitoring to assess changes in heart function (e.g. number of heartbeats per minute) animals are anaesthetised to implant a device with sensor as mentioned below in (d), which following recovery allows remote recording. ECG is used by doctors to assess heart function in humans.

d) Animals may undergo surgical procedures, for example to implant telemetry devices for non-invasive, longer term, cardiovascular monitoring; for implantation of vascular cannulae, or for non-recovery investigations. Surgical procedures are always performed under general anaesthesia,

e) A degree of restraint or confinement may be required for some of the various dosing, sampling or assessment procedures such as those required to assess motor co-ordination (balance), muscular strength (ability to grip) or visual assessments which may be impacted by a novel drugs effects.

f) For the purposes of collecting cells for analysis, some animals may undergo vaginal flushing.

g) Some animals may also have food withheld for no more than 16 hours for mice or 24h for rats.

h) Occasionally, if an animal's temperature is required a rectal probe will be used.

At the end of the study, the animals are humanely killed by an overdose of anaesthetic. Where appropriate animals may be re-used for subsequent investigations. Samples of various organs are taken and examined under a microscope to ascertain whether the potential new medicine has caused changes that would rule out administration to humans.

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

Some animals are expected to have mild adverse effects such as slight weight loss. Animals may occasionally show more significant (moderate) adverse effects e.g. more marked weight loss for up to 1 week, or changes in appearance (e.g. ruffled fur) or behaviour (e.g. reduced activity, changed body posture), changes in faeces consistency/appearance, altered breathing patterns, transient short term (for minutes) involuntary muscle movements. Humane end-points are applied should such moderate effects become prolonged in incidence beyond 24 hours, with veterinary guidance as necessary.

A small proportion of NSG mice 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 Named Animal Care and Welfare Officer (NACWO) and/or the Named Veterinary Surgeon (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: 70% Moderate, 30% Mild

Rats: 70% Moderate, 30% Mild

What will happen to animals used in this project?

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

Before starting studies in this program, we use a lot of internal and external data, research, and test results. This includes lab tests on cells and computer simulations to identify early signs of safety issues and risks related to a target or new agent. These may include risks to health or how well a person is likely to tolerate a drug. These methods, although powerful, cannot fully predict the safety risk. We can only fully understand how these findings might affect living organisms and their potential negative effects on humans by incorporating animal studies into a carefully designed experiment.

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

We are continually investigating and applying alternative cell based (non-animal) systems to assess potential adverse (unwanted) effects and thus reduce the number of animal studies required. For example, specific types of hazards, predictive of potential to changes in blood pressure and heart rate, can be detected using human cell based systems and these assays are performed early in the safety assessment programme. “Organ-on-Chip” technology is extensively used, particularly to assess liver and bone marrow adverse effects. These are self-contained units containing living human cells. The human cells recreate the physiological functions of organs without using animal models.

Why were they not suitable?

In silico and in vitro approaches are becoming increasingly powerful tools in the design and development of new medicines and are used extensively to understand potential adverse effects. These approaches can help in understanding various isolated aspects of a new drugs safety profile and these methods are used to screen out those that clearly do not have the desired properties. However, because the safety profile for a given drug is multifactorial, it is not always possible to mimic the entire breadth of physiological responses and any interactions as seen in a whole animal (in vivo) system and to this end, such studies performed under this licence remain necessary.

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 have been estimated based on animals numbers used for the current licence/programme of work over a 4.5 yr period. These numbers are not definitive and could change in relation to the target/therapeutic disease area needs.

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

The numbers of animals used for each experiment will be set after consultation with a professional statistician using and aligning with principles laid out in the PREPARE guidelines and the NC3Rs experimental design assistant to ensure all animal studies use the most appropriate design to answer the primary objective and to avoid bias. Animal numbers are kept to the minimum required for the scientific objectives, and endpoints being measured effectively.

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

The following approaches will optimise the number of animals used, avoiding further studies being performed as stand-alone studies under this licence:

- Where possible, additional sampling (e.g of tissues at the end of a study) may be included in planned studies thus deriving more data from one experiment.
- Samples may often be stored awaiting the development of a suitable assay, for examples the use of samples to obtain a measure which is a similar measure to that used in patients.
- Where possible, control or vehicle (placebo)-dosed animal tissues/samples may also be used for other experiments.
- If a new technology or approach is to be used within this Project, small pilot studies will first be performed to validate equipment/methods and ensure scientific rigor, expertise and experience, prior to committing to larger studies.
- Studies often employ a dose escalation design where treatments have staggered starts to prevent unnecessary suffering and animal losses; in the event a maximum tolerated dose is reached, further dosing will cease.

Other steps taken to ensure minimal animal use include the use of databases and computer modelling. Data generated under this project will be uploaded to a central database. This is critical to reducing the number of animals used and allows:

- Rapid access to data by all scientists and avoids requests and repetition of studies.
- Other research scientists carrying out evaluation of the same test compound may design their studies at appropriate dose levels, routes etc.
- The refinement of computer models to predict pharmacokinetic (PK) properties utilises software to simulate multiple dose PK (level of drug in the blood or tissue), rather than repeat dosing animals, and is critical to selection of appropriate doses for toxicological evaluation.

Other approaches that reduce animal numbers used include:

- Randomisation and blinding are both used to ensure the data obtained is the most robust as possible. They do this by reducing bias and therefore improving validity and reliability.
- Crossover study design may enable all dose phases to be performed in one animal – typically used for studies measuring heart rate and blood pressure where re-use enables the same animals to be used for multiple evaluations.
- Increased assay sensitivity and the development of microsampling (low blood volume) has enabled serial sampling within the same animal allowing measurement to be generated from a single animals rather than the multi animal approach utilising 3 animals per time point for example. This approach reduces animal numbers used and reduces data variability across cohorts.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

The species, models and methods used within this licence are widely accepted and have a proven record in creating safety data that can be applied to humans. There is a wide knowledge and understanding of the response of rats and mice to various chemical and biological agents and the related safety data in the pharmaceutical industry. Typically, the rat is the rodent species of choice unless it is known to be an inappropriate model for man for the test substance or target-related toxicity. Mice may be used where they are considered a more appropriate species than rats e.g. for kinetic reasons and/or presence of pharmacological target.

The scientific objective of this Project is to assess the early safety risk of novel medicines. To enable this assessment, agents will be administered at doses that may result in moderate bodyweight loss and clinical signs. The severity limits are those considered to be the minimum commensurate with

achieving the study objectives. In all studies conducted under the authority of this licence, the upper severity limit will be moderate.

Animals will generally be housed in groups and provided with specific materials to provide environmental enrichment.

Best practice, as established by published data, internal expertise, and veterinary guidance, for example, the use analgesics after surgical procedures will be employed to minimise suffering.

Blood volume taken for measures will be minimised by using microsampling techniques which significantly reduce the volume of blood removed from each animal, but still produce high-quality data.

Many factors are considered to refined substance administration, including working to guidance criteria defined from a number of best practice resources (NC3Rs; LASA; and published literature).

Carefully choose the route, dosage amounts, frequency, and duration to ensure they result in an optimal investigation which meets the scientific objectives, in the most refined way. Key examples of this refinement include;

- Administration of dye-like substances to enable imaging via the subcutaneous route (i.e., injection under the skin) is a more refined approach which negates the need to use of otherwise more invasive routes of either intravenous (i.e., into the vein) or intraperitoneal (i.e., into the abdominal cavity).

- A test substance is usually given in a way that ensures enough exposure to see the desired effects or detect general toxicity unrelated to its main use. Often, this is done through the intended clinical route, but other routes might be needed to reach the desired exposure levels.

Animals are regularly monitored and examined to ensure potential adverse effects will be kept to the minimum to achieve the objectives. This also allows for humane endpoints to be met.

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

More sentient species are used due to their closer biological resemblance to humans, which can provide more meaningful and applicable insights into potential human outcomes. Less sentient species like invertebrates or simple organisms have different physiological systems than humans. This can limit the relevance of study results, as their metabolism and responses to substances can vary significantly from humans. Rats and mice are more sentient species and have complex biological systems similar to humans, allowing for more accurate predictions of substance effects. For data from animals studies to be useful for understanding potential effects in humans, they must be conducted in models that can predict human responses reliably. Less sentient species often fail to provide this predictive power. For example, as part of safety assessment profiling a molecule may be assessed for effects on complex functional / behavioural observations such as visual effects, effects on the brain and nervous functions, or parameters related to the hearts function in telemetered animals. These tests evoke responses in rats and mice that can be readily translated to human patients

Generally, the rat is the rodent species of choice unless it is known to inaccurately reflect the human effect for a particular assessment. There is a wide knowledge of the response of rats to various chemical substances and the translatability of these responses to humans is more defined in the rat

than other species due to a wealth of background data in the pharmaceutical industry. Rats are big enough to provide repeated blood samples for scientific measurements, thus requiring significantly fewer rats than mice to achieve the same objective. However, mice may be used where they are considered a more appropriate species than rats e.g. for metabolic reasons and/or the presence of the pharmacological target.

Procedures are conducted under anaesthesia without recovery where it is possible e.g obtaining larger blood volumes at the end of studies is done via sampling from the vena cava under anaesthesia and without recovery. However, many toxicological responses and pathologies evolve over time with repeat dosing thereby requiring assessment using awake animals.

More immature forms of life (such as embryos or foetuses) are not suitable for this work because their physiological systems are incomplete and/or immature, and therefore would not be sufficiently predictive of results in the human species.

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

Where surgical procedures are conducted, best practice principles are followed, including the use of sterile/aseptic procedures carried in accordance with the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017), alongside the use of fluid and heat therapy during recovery. Additional animals are monitored daily with records of bodyweight, and body condition monitoring at least once daily post surgery for an appropriately defined (agreed with the NVS and NACWO) recovery period ahead of any subsequent procedure.

Where possible, all animals will be group housed. The use of the Vascular Access Button for rodent infusion work enables group housing of surgically cannulated rats between infusion cycles where previously this was not possible.

Rats are housed in cages which offer a diverse environment divided over two levels, which encourages natural rat behaviours such as “rearing” and gives a greater floor area for exploration, alongside the recently introduced custom made hammocks, similarly mice are housed in cages systems with balconies. All animals are provided with specific materials to provide enrichment, including a selection of nesting materials, chew blocks, refuges/hiding places and foraging.

All animals will receive a minimum of 7 days acclimatisation before use in experiments.

We are fully committed to NC3R recommendations for single needle use for parenterally (non-oral) administered substances ensuring that undue pain and suffering linked to the use of blunt needles is not experienced.

As described above we are also heavily involved in non-aversive handling techniques for mice and have begun to ensure this is applied as "best practice". Training regimes to acclimate mice to “cupping/tube handling” techniques are underway to reduce stress in mice and therefore improve the integrity of data generated.

Blood volumes taken for measurements will be minimised by using microsampling techniques which significantly reduce the volume of blood removed from each animal but still produce high quality data.

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.

Continue to refine approaches to tumour monitoring with frequently reviewed tumour condition scoring and assessment and review alternatives to callipers, such as non-invasive imaging, which may have the potential to enable improved tumour burden monitoring and assessment, by reducing the time that the animal is handled.

Adverse effects will be kept to the minimum to achieve the objectives and will be closely and regularly monitored during and after regulated procedures. Frequency of monitoring will be adjusted should adverse effects be observed.

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

Best practice, for example the use of analgesics after surgical procedures will be employed to minimise suffering.

FELASA guidelines are used as a baseline to ensure experiments are conducted in the most refined way. Historically, guidelines such as those produced by FELASA have been used to define the upper limit for bodyweight loss at 20% in safety assessment studies. Our organisation has actively contributed to a global collaboration that used evidence to define the upper limit for body weight loss in maximum tolerated dose (MTD) studies (conducted at contract research organisations). We have extended this guidance to studies run in-house under this and previous Project Licences, meaning that a gradual bodyweight loss limit compared with baseline (pre-dose) and/or age matched controls, will be commonly less than 15% over a period of one week.

The dose selection principles outlined in the LASA/NC3Rs guidance on dose selection for pharmaceuticals will be used where appropriate.

Application of ARRIVE guidelines to study designs.

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

Advances in the 3Rs are regularly shared throughout our organisation and establishment network (NACWO, NVS and Named Information Officer (NIO)) via e-mail , or the internal communication systems or via professional networks.

Specific 3Rs based objectives are regularly included as part of an individual’s yearly performance goals and are assessed as part of that individual’s performance. In addition to this, we promote an annual presence at industry-focused meetings (IAT, LASA, FELASA, NC3Rs focused workshops) with the sole purpose of attaining and sharing 3Rs based knowledge with other companies and institutions.

The “cupping/tube handling” technique for mouse handling is such a technique that has been introduced from these activities and has been successfully implemented, validated and reviewed across as part of our continuous commitment to animal welfare improvements.

One of the functions of this licence application is to enable the introduction and review of new techniques or technologies that may be applicable to the 3Rs. the Instech Vascular Access Button (a medical device used to facilitate repeated access to the blood vessels) for intravenous infusion work enabled group housing of surgically cannulated rats and is one example of this protocol in use.

Furthermore, we have investigated and implemented enhancements to cage enrichment for rats by introducing hammocks into the double decker cage system, providing an additional area to shelter/sleep away from the main cage. For mice, we have deployed the application of enrichment rotation to encourage continued interest and positive behaviours, this has shown welfare benefit including increased nest building and reduced incidence of behaviours associated with potential welfare concerns. Both these examples have been recently presented externally in 2024, the rat hammock initiative was recognised at a 3Rs award event.