Home Office

NON-TECHNICAL SUMMARY

Profiling of test agents in rodents in a drug discovery platform

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

Pharmacokinetics, Drug discovery, Tolerability, Biomarkers, Safety

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 aim of this project is to generate data on the pharmacokinetics, pharmacodynamics and tolerability of test agents for the disease areas listed in this licence, and the effect of test agents in rodents over a longer period of dosing.

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 project will generate crucial data in the preclinical (before human dosing) development of potential new drugs for the treatment of cancer, cardiovascular diseases and disorders of haemostasis, metabolic diseases, inflammatory diseases, neurological diseases and musculoskeletal disorders. All of the diseases we work on are diseases where current treatments are not available or need improving upon. These studies will generate important information that cannot be found without the use of animals. All clients that we work with are developing test agents (potential new drugs) which will hopefully contribute to helping people with various diseases where current treatments are not available or need improving upon.

This project investigates both how the test agents are processed and removed by the body (pharmacokinetics, or PK), and how the test agents affect the function of the body (pharmacodynamics, or PD). This requires information from a whole animal system and cannot be replicated in a cell or a test tube. The project will determine important characteristics of test agents to decide the best dose levels, dose routes (how the drug is taken) and patterns of dosing (how often the drug is taken), whether the test agents have early signs of unwanted side effects, and whether the test agents are likely to work as an effective treatment for disease. We will also study how the test agent distributes itself around the body. This information will be key in decisions regarding which test agents should continue to be developed, and which are unsuitable for further work. Ultimately, this will feed into which test agents make it into clinical trials, where they are tested in humans, which will ultimately benefit humans through new treatments for diseases. Our approach to the work ensures that this is done in the most efficient way possible, and that the use of every animal is maximised.

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

As part of this project, we expect to test the PK (what the body does to the drug) of at least 30 test agents, of which the majority will be new test agents. We expect at least 10 of these to move onto further work either under this project or other projects. In addition, we expect to run a minimum of 20

longer term studies looking at the safety and tolerability of test agents as well as how they change body function as part of this project.

Data produced by studies under this project may be used to support patent and funding applications by clients. They will also support the design of regulatory preclinical (animal) studies for clients and may be included in applications for regulatory approval. We will also provide valuable information from this project that will halt the further development of several test agents, preventing unnecessary use of animals in studies using test agents that would not be suitable for use in humans. These test agents will be for potential use as a therapeutic in the following disease areas:

- Cancer
- Cardiovascular diseases
- Disorders of haemostasis
- Metabolic diseases (including inborn errors of metabolism such as alpha-1 antitrypsin deficiency)
- Inflammatory diseases
- Neurological diseases
- Musculoskeletal disorders
- Liver disease

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

This project will generate important data in the development of potential new drugs.

The PK analysis which determines how much drug gets into the body and what the body does to the drug will enable our clients to select the best test agents (drugs) for further development. These studies show how long it takes for drugs to get into the blood and the length of time they stay in the blood or tissues (parts of the body) before the body gets rid of them. As well as providing information for key decisions as to whether test agents are likely to make suitable drugs, PK studies provide vital information required to design the longer-term studies needed in drug development. Ultimately, PK studies also feed into the design of clinical trials.

The safety and tolerability work will check that test agents are suitable for repeat or continuous dosing over longer time periods and look to see if test agents have any early side effects. These studies will help with working out the best dose for potentially treating disease. They also make sure that we identify any unwanted side effects that mean that they would not be suitable for use as a drug for humans. These studies also help us to design longer term studies in the best way possible to provide the information required about the way the test agent is working.

The longer-term dosing studies in this project will allow us to investigate the effects of the test agents on biomarkers, which are measurable levels of key molecules in the body which tell us whether body function has been altered by the drug. This will provide us with information on whether the test agents

are able to alter the levels of biomarkers that are most relevant to the disease that the test agent is designed to treat. If a test agent does not alter the important biomarkers, this may mean it will not be a suitable treatment for disease.

Biodistribution studies will give important information on which areas of the body test agents target, which can be key to how a test agent performs as a treatment for disease. For example, harmless viruses can be used to target specific organs to alter expression of a gene in that organ. Therefore, it is important to determine where the test agent is accumulating. This will be done through the use of molecules attached to the test agents that light up (for example through fluorescent or bioluminescent tags) and can be seen in live animals using imaging machines.

Our focus on using a science-led approach will enable key decisions to be made at each development stage on whether a test agent is likely to become a successful drug. This allows test agents which are not suitable as drugs to be abandoned at an early stage using the fewest number of animals possible for each test agent. The identification of test agents as unsuitable for use in humans at an early stage of development will also ensure a better success rate in the drug discovery process than has previously been seen in the pharmaceutical industry. Ultimately, this project will contribute to the successful development of new drugs, which will benefit patients with the diseases being treated.

Health conditions linked to inflammation are very common and half of deaths worldwide can be linked to diseases involving inflammation. Cardiovascular and metabolic diseases lead to a third of deaths worldwide. Disorders of haemostasis contribute to these deaths where abnormal clotting occurs. In the case of the rarer diseases where clotting is reduced, such as Haemophilia, the diseases reduce life expectancy. Cancer is also sadly a leading cause of death worldwide, with more than a quarter of deaths in the UK involving cancer. With both an aging population and the increasing problems with obesity in society, the occurrence of these diseases is likely to increase. Neurological diseases are also increasing, with Alzheimer’s and dementia the leading cause of death in the UK in 2018, and with prevalence of Parkinson’s disease set to increase by nearly a quarter in the next ten years. Musculoskeletal disorders affect 1 in 4 adults in the UK. Liver failure can affect patients acutely from drug overdose (such as paracetamol) or soon after birth, or chronically from a number of in-leading diseases (such as liver disease). There is a lot of overlap between the diseases covered by this project, for example a rise in cardiovascular and metabolic diseases such as heart disease and diabetes increase the risk of stroke and the associated life-changing neurodegeneration. Therefore, it is clear that investigation into potential new drugs for all of these diseases is required to improve chances of survival, but also to improve the quality of life for patients living with the diseases.

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

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

In addition, we will seek expertise from our established networks both within our establishment and further afield, to ensure that we make use of any new knowledge or better methods of performing animal studies. We will also use these networks to give information to others about any ways in which we can help via our research. This will include sharing information about unsuccessful approaches in addition to sharing information on any refinements and improvements. We will maintain good communication with managers of the animal facilities to ensure that any tissues from animals being killed that are not required for our work can be made available to other researchers.

Due to the nature of the work undertaken in this project we are unlikely to be able to publish data, as this would put ourselves or our clients at a competitive disadvantage. However, where advances are made in study design, we will publish or share these wherever possible.

Species and numbers of animals expected to be used

- Mice: 5250
- Rats: 2400

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.

Both mice and rats are well established 'models' for use in studies determining the PK and tolerability of new test agents and the effect of test agents on biomarkers. This is due to their similarities to humans, with these 'models' mimicking aspects of human physiology and disease. They are very well characterised, meaning that there is a lot known about how the body works in mice and rats, both when healthy and with diseases. This helps in the interpretation of results from studies using test agents. We are using adult animals in this project as this will reduce the variability in the results and is the life stage most relevant to the majority of test agents. This project will look at test agents for the following disease areas:

- Cancer
- Cardiovascular diseases
- Disorders of haemostasis
- Metabolic diseases (including inborn errors of metabolism such as alpha-1 antitrypsin deficiency)
- Inflammatory diseases
- Neurological diseases
- Musculoskeletal disorders

- Liver disease

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

In the majority of studies, test agents will be administered to animals. This dosing will be by a number of different methods. Test agents will be administered intravenously (into a vein), intraperitoneally (into the abdominal body cavity), subcutaneously (under the skin), orally (either using a dosing tube or via adding the test agent to the food or drinking water), intranasally (inhaled into the nose), intracolonically (into the colon) and/or intraduodenally (into the small intestine). Where dosing is performed intranasally or intracolonically, this will be in animals who are anaesthetised. Intravenous injection may also be performed under anaesthesia but will usually be performed in conscious (not anaesthetised) animals. On occasions a slow-release device will be implanted for the subcutaneous or intraperitoneal administration of drugs. In some cases, where it is important to determine how a drug directly affects the brain, the test agents may be delivered straight into the brain using a very small needle via a tiny hole in the skull. A frame is used to direct the injection very accurately to the exact brain region needed. This will involve surgery under deep anaesthesia whilst the injection is done. Sometimes the same must be done with the liver; a small incision will be made into the abdominal wall to access the portal vein (this will deliver drugs straight into the liver). This will be done on one occasion.

In PK studies animals will usually be given one dose of test agent and blood samples will be collected multiple times after this dose is given. In some PK studies, we will use animals with a cannula, which is tubing allowing direct access to either a blood vessel or to the gut. These cannulae require surgery to put them in place. This surgery will either be performed by scientists at our company using our in-house expertise, or more commonly performed by an external supplier who are experts in these surgeries. When using animals with a cannula into a blood vessel, they may be given the same test agent twice, once straight into the blood (intravenous) and once by a different route, with blood samples taken after both doses. There will be enough time between the two doses to make sure that no test agent is left in the body before the second dose. This will allow calculation of how much of the drug given by an extravascular route (not straight into the blood, for example given orally) makes it into the blood.

For safety and tolerability studies, animals will be dosed with test agent(s) over a period of several days or weeks. In some safety studies the dosage will be set at a level predicted to be effective when used as a treatment in animals to check if any side effects are observed. For tolerability studies, the concentration of test agent(s) may be increased during the study to test if higher doses have early side effects. Blood samples may be collected during the study and blood and tissue samples will be collected at the end of the study to make sure as much data as possible is generated from each animal. During safety and tolerability studies, blood samples may also be taken to investigate the PK of the test agents being used, and whether this is changed after the animals have received the test agent for several days.

For dosing studies, animals will be dosed with test agent(s) over a period of several weeks or months. Dosing will usually be daily, but this may vary depending upon the test agent. Blood samples may be collected at regular intervals during the study for the measurement of biomarkers or test agent levels. Other non-regulated procedures such as urine collection may also be performed. Blood and tissue samples will be collected at the end of the study to maximise the data generated from each animal.

For biodistribution studies, animals will be dosed with test agent(s) once or several times. The animal will then be put under brief anaesthesia (typically for 20 minutes, maximum of one hour) in order to take images of the whole animal to visualise test agent distribution.

Where animals are used only for the collection of blood or tissue samples for *ex vivo* work (work outside of a living body in the laboratory), all procedures will be performed under non-recovery anaesthesia where possible, such that the only procedure the animal experiences is the process of being anesthetised. They will then be kept unconscious under deep anaesthesia whilst blood and/or tissue(s) are collected. On some occasions, animals may be dosed with a substance prior to humane killing, if required for the *ex vivo* work. Again, where possible this will be performed under non-recovery anaesthesia, but on some occasions it may be necessary to dose animals over a period of weeks prior to blood collection. Terminal blood and tissue samples will be collected immediately before death and rodents humanely killed. Additional tissue samples may be collected after death.

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

Animals used for PK studies, longer-term dosing studies, biodistribution studies and those used for the collection of blood samples for *ex vivo* work should not experience more than transient (short-lasting) pain and discomfort. Those animals with a cannula for PK studies will go through a surgery before the PK study is performed. The surgery will be performed under general anaesthesia, such that the animal will remain in a state of unconsciousness throughout the surgery. Recovery from surgery is expected to be quick, with recovery from anaesthesia within 2 hours. In most cases, the cannulation surgery will be performed by an external supplier who have extensive expertise in these surgeries and reliably perform the surgery without problems. Animals will be allowed to recover for a suitable period determined by the supplier (currently 7 days) prior to shipment to us. For vascular cannulation, we often use vascular access buttons located in the interscapular region. These vascular access buttons connect to the end of the cannula and have a magnetic metal cap to cover the access point, meaning that animals are able to be group housed without the risk of the cannula being damaged by cage mates. In all cases the cannulae do not cause problems with the ability of the animal to move freely and scratching at the cannula site is only observed on very rare occasions. Where a slow-release device is used in longer-term dosing studies, these animals will undergo surgery under general anaesthesia, with quick recovery expected. The location of the slow-release device is not expected to have any effect on the animal’s ability to move freely.

Animals that need to have drugs delivered directly to the brain or liver will undergo surgery. The surgery will be performed under general anaesthesia, such that the animal will remain in a state of unconsciousness throughout the surgery. Recovery from surgery is expected to be quick, with recovery from anaesthesia within 2 hours.

The majority of animals undergoing safety and tolerability studies will only experience transient pain and discomfort. However, some animals may experience more significant side effects. These could include weight loss (up to 15%, or 20% when using test agents designed for the treatment of cancer), pain and subdued behaviour. There are two main reasons when these studies may be required to cause more side effects. One of these is where the test agents are designed to treat fatal human diseases, where more side effects are acceptable in humans, for example in the case of chemotherapy for treatment of cancer. The other occasions are studies where it is important to find out what the maximum dose that can be given to animals is. Understanding the maximum dose that can be given is

important as it allows decisions to be made on the doses for all future work. In these studies the dosage will be gradually increased until side effects are noticed. Once side effects are observed the dose will be held at that level and the study typically completed within 72 hours. This allows us to see if the animals develop tolerance to the drug and the side effects normalise, or whether the side effects are sustained. Animals will be closely monitored to ensure that they do not suffer excessively.

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 - Mild: 60%

Mouse - Moderate: 31%

Mouse - Non-recovery: 9%

Rat - Mild: 51%

Rat - Moderate: 41%

Rat - Non-recovery: 8%

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?

The work to be performed in this project cannot be fully replaced by using models in a laboratory. Wherever possible, work is performed either using cells or tissues taken from an animal before moving into animal studies, to make sure that the animal studies are well designed and that the maximum amount of data is gained from each animal study. However, this project aims to investigate the PK, tolerability, biodistribution and effect on biomarkers after dosing with test agents, which cannot be determined without using animals.

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

Work can be performed in the laboratory to ensure that test agents are acting as expected before moving into animal studies. These may be using cell lines grown in the lab, or using tissues collected from animals. For example, we have previously performed these types of experiments by running

assays (laboratory experiments) looking at how quickly blood clots form in rodent blood, or by looking at the effect of a test agent on liver cells collected from a mouse. We use these studies before moving into animals to make sure that test agents that are unlikely to be useful to humans do not get tested in animals.

Why were they not suitable?

The use of animals is essential to determine how the body acts on test agents, and how test agents act on the body. It is not possible to determine how a drug gets into the blood and how it is broken down and removed from the body without using live animals. Similarly, it is not possible to determine whether a test agent will have side effects using cells or tissues in a laboratory, or to find out the effect a test agent will have on biomarkers.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

The number of animals to be used has been estimated by analysis of the number of animals used on previous projects, looking at the number of animals required for each type of study. This was then combined with a prediction of likely demand of future projects to give the numbers in this project.

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

We have extensive experience in the design of experiments of the types in this project, which has given us confidence in the number of animals required to ensure that no animals are used unnecessarily, but also that the data generated is reliable. Whilst through experience we do tend to use a standard group size for the majority of our pharmacokinetic (3 per group) and tolerability (5 per group) studies, we regularly refer to the PREPARE and ARRIVE guidelines and make use of the NC3Rs Experimental Design Assistant to ensure that we are using the correct number of animals for every study. When designing experiments for the effect of test agents on biomarkers, we also look at published literature to determine the variability observed in the biomarkers of interest where we have not tested these before. Where information is not available in published literature or from contact with other researchers, pilot studies in a small number of animals will be used first where appropriate to assess the variability of the biomarker to be tested and to determine the appropriate group size for future experiments. We can then use the NC3Rs Experimental Design Assistant to help determine the most appropriate group size.

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

Data from pilot studies and previous experience are used to ensure that the numbers used are both as low as possible, but also large enough to generate reliable data. Within our company, a member of the wider team has generated a tool for performing power calculations and can be consulted as necessary to assist with study design.

Where genetically altered animals are required, these will usually be provided by our breeding project, which will ensure that animals are bred efficiently using as few animals as possible by communicating need with colony managers. Where animals are obtained from external sources, only the number of animals required for the study will be purchased or imported.

Wherever possible, our *in vivo* (animal work) scientists will be blinded to the treatment status of an animal, reducing bias. This enables more reliable information to be gathered from a smaller number of animals. Those who carry out analysis on blood and tissues collected during the study are also blind to any treatment with test agent where possible.

Baseline data (e.g. bodyweight, biomarker levels) are recorded and animals assigned to treatment groups to minimise differences between the groups at the start of the study.

Good planning ensures that within any series of studies we can control for variability that might be introduced. To limit this variability we look at using animals of a similar age/weight range, testing different batches of test agent in the lab first, using the same source of reagents (chemicals used during the experiments), keeping records of observations made and standardising as many components of the *in vivo* model as is practicable.

We will also provide valuable information from this project that will halt the further development of several test agents, preventing unnecessary use of animals in studies using test agents that would not be suitable for use in humans.

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 both mice and rats during this project. Some of these will have a genetic alteration that will not cause them harm, but which provides a more appropriate model in which to perform experiments on test agents for particular diseases.

PK studies are generally performed using a low dose of the test agent which reduces the chance of any unwanted side effects. However, when the test agent has not been dosed to animals previously, a small group will be dosed and monitored for 2 hours to ensure that there are no side effects before the test agent is given to the remaining animals. These animals will be further monitored up to 6 hours post dosing to ensure any side effects that occur several hours after dosing are also observed. PK studies involve taking blood at several different periods of time after dosing (time points) from as short as 2 minutes to as long as days after dosing. Where multiple animals are dosed with the same test agent, blood samples will be collected at different time points from the different groups of animals. The animals dosed first will be those for longer time points, with animals for shorter time points dosed subsequently. This means that side effects which are not seen until more than 2 hours have passed will not be relevant to animals dosed later in the day and that it is appropriate to begin dosing additional groups after only 2 hours. If any side effects are observed, the study would be stopped or the dose lowered. If side effects are observed, these would likely be subdued behaviour, piloerection (hair standing on end) or a hunched appearance.

Where it is hard to predict the potential side effects of a test agent, studies will be performed in a small number of animals first who will be monitored for at least 2 hours or overnight in the case of test agents where side effects are likely to take longer to be seen. Where side effects are observed, the dose may be altered to ensure no long-lasting side effects are seen before any longer-term studies are performed.

Dosing will always be performed using the least invasive route (e.g. oral dosing via food or water if possible), and where a needle is required using the smallest needle possible.

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

Mature mice and rats are essential for studying complex diseases, where all systems must be similar enough to humans to find the most effective drugs. The duration of most studies in this project are longer than can be performed under anaesthesia as they span several days or weeks. The anaesthetic drugs themselves may also affect the results of the studies.

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

We have a demonstrable history of refining our procedures and practices to minimise harm to our experimental animals. For example, we always use the smallest needle possible for dosing to minimise any pain and distress to the animal. We also investigate the suitability of test agents administered orally to be dosed via the drinking water rather than via oral dosing, where the dose is administered via insertion of a tube down the throat. We recently implemented this for a test agent, allowing us to switch from twice daily oral dosing to dosing via the drinking water. We will also sometimes use a slow-release device for dosing. Whilst this requires an initial surgical procedure, it reduces the need for the stress of regular injections.

During studies that involve repeat dosing, animal condition and bodyweight is monitored regularly, with body weight recorded prior to each dose as a minimum. We are developing a scoring system to monitor any side effects observed due to dosing, which will help us to ensure that these are tracked and acted upon as necessary.

Animals will be housed in social groups in the vast majority of cases, with animals housed alone only where this is absolutely necessary (for example in some animals who have a cannula inserted, where cage mates may dislodge the cannula). Where anaesthesia is used, care will be given to support animals during anaesthesia and recovery, using heat hats to maintain body temperature and providing soft diet and hydrogel to aid recovery as appropriate.

We use microvettes (small capillary blood collection tubes, coated with anticoagulant if appropriate to prevent blood from clotting) to collect small blood samples from animals. This allows us to take small samples in the most efficient way possible. We have also developed methods to be able to use a mixture of blood and water for some of the tests run on rodent blood, which allows us to collect a smaller volume of blood than would be required when using standard methods of preparing plasma from collected blood.

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

We will be using PREPARE guidelines for the planning of studies and follow the latest version (2020) of the ARRIVE guidelines for ultimate reporting of data. The LASA document ‘Guidance on dose level selection for regulatory general toxicology studies for pharmaceuticals’ will be used when planning studies, to help guide study design. This document, particularly the section detailing the report of the FELASA working group on pain and distress has also been used when setting the humane endpoints within this project. Where surgery is performed, we will refer to the LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. We will also regularly check the current list of LASA publications, to ensure that any relevant to work under this project are taken into consideration. Similarly, we will regularly reference the NC3Rs to ensure that we are implementing best practice for the techniques undertaken.

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

When designing animal studies we consider the appropriate guidelines, including the guidance from LASA, the NC3Rs and the PREPARE and ARRIVE guidelines. This guidance will influence our study design. One example of this is the use of the NC3Rs guidance on the number and volume of bleeds to be taken from rodents in the design of pharmacokinetic studies. We regularly check the NC3Rs website, along with other external resources such as Norecopa and Jax, to ensure that we are using the most refined methods and are aware of any improvements to procedures that have been developed.