



Home Office

NON-TECHNICAL SUMMARY

Provision of an outsourced drug discovery platform for metabolic and cardiovascular diseases

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

Metabolism, Cardiovascular

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 develop new and improved treatments for metabolic and cardiovascular disease. As part of this work we will optimise experimental models of disease to ensure they are fit for purpose.

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, generating important information that cannot be found without the use of animals. The clients that we work with are developing test agents (potential new drugs) which will hopefully contribute to helping people with various metabolic and cardiovascular diseases where current treatments are not available or need improving upon. For example, Pulmonary Arterial Hypertension (PAH) is a rare but severe cardiovascular disease where 40% of patients die within 3 years of diagnosis. To be able to test new drugs for diseases like this, it is necessary to use rodent 'models' which mimic areas of the disease to provide proof-of-concept data and to determine which drugs are likely to work well in humans as a treatment for the disease.

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

This project will generate important data in the preclinical development of potential new drugs for metabolic and cardiovascular disease. We aim to use this licence to test potential therapeutics in each of the disease areas listed, and to progress the most promising treatments which reduce disease severity through various stages of drug development and into the clinic. We are currently working on a series of drugs for PAH which have shown promising results in our rats that have a form of PAH. We aim to support the progression of these drugs through to the stage that they are able to enter clinical trials within the duration of this licence. We also aim to work with several other clients in each of these disease areas to offer a similar service.

These studies will determine which test agents are suitable for further preclinical development and for progression to clinical trials. They will also identify test agents which are not suitable for further development. This is an important benefit, as it will streamline research and ensure that animals are not used unnecessarily in developing compounds that will ultimately fail further down the development pathway. We prioritise ensuring that our models are designed such that unsuitable test agents are identified as early as possible in the testing process. One particular example of this is in our PAH work,

where a class of new drugs are known to have the potential to cause an unwanted side effect of heterotopic ossification (bone formation in inappropriate locations). This project includes a protocol to allow us to rule out test agents with this side effect early in the development process.

Data from studies performed in this project may also be used in applications by our clients for further funding or as part of applications to allow test agents to be progressed into clinical trials.

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

This project will generate important data in the development of potential new drugs. Work under this licence is expected to result in the progression of new treatments for cardiovascular and metabolic disease through the stages of drug development and ultimately into human clinical trials.

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 been seen previously in the pharmaceutical industry.

Ultimately, this project will contribute to the successful development of new drugs for cardiovascular and metabolic diseases, which will benefit patients who currently have limited treatment options available.

PAH is a disease with an estimate global prevalence of 1%. This disease can affect both sexes and all age groups, with a higher risk in females. PAH has a high mortality rate, with patients surviving on average only 3-5 years from diagnosis. Effective treatments would therefore have a significant impact on the lives of patients diagnosed with PAH.

Non-alcoholic fatty liver disease (NAFLD) has a global prevalence of 25%, and it is estimated that up to 5% of the UK population may be affected by the more serious form of the disease, non-alcoholic steatohepatitis (NASH). These diseases are closely associated with obesity, and therefore the number of patients suffering from them and who would benefit from new treatments is likely to increase. The average age of people with NASH is 40-50, with this age decreasing due to the increasing problems with obesity in society. The mortality rate is higher for patients with NASH than the general population, with liver damage from NASH increasing risk of liver failure and cancer.

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 utilised not only from within the preclinical (animal work) team performing the animal studies, but also from other teams at our company and our clients' companies. 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 very low volume blood samples, meaning that they can 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: 2500
- Rats: 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.

Both mice and rats are well established 'models' for use in both preclinical drug research and more generally in research into metabolic and cardiovascular diseases. 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. Adult rodents will be used for the work outlined in this project as we wish the biology of the animals to be fully developed to better represent the patients of the diseases the test agents are designed to treat.

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

The majority of animals used in this project will be part of studies that aim to test whether new drugs can improve or prevent development of symptoms associated with metabolic or cardiovascular disease. This will include investigation of any potential unwanted side effects that could be caused by the new drugs. Drugs will most commonly be administered 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) and less frequently intravenously (into a vein) or intramuscularly (into the muscle). On occasions a slow-release device will be implanted for the subcutaneous or intraperitoneal administration of drugs. At the end of all studies blood and/or tissues will be collected. These may be

frozen or preserved using a fixative. Tissue may be placed into fixative after death or the fixative may be injected into the bloodstream or directly into the heart of an anaesthetised animal who will not regain consciousness between the injection of fixative and death.

In metabolic studies investigating NASH (non-alcoholic steatohepatitis) and NAFLD (non-alcoholic fatty liver disease), mice will be fed a high fat (or normal) diet to cause them to develop the liver problems associated with the disease. In these diseases, fat builds up in the liver, accompanied by liver inflammation in the case of NASH. Long-term, this causes damage to the liver and can reduce liver function. Animals will usually be fed the altered diet for 4 or more weeks and be administered with test agent throughout the study. Sometimes potential drugs may need to be given more than the typical maximum number of doses; this is because NASH studies tend to be over long periods for some purposes (typically 12 weeks), and a potential drug to prevent the disease will be given for the whole study, sometimes daily. Any animals given more doses than the maximum recommended will be done so with advice from the NVS, with additional checks for signs of damage from the more frequent dosing. Blood samples may be taken during the study to monitor the disease progression and effectiveness of the drug administration. At the end of the study terminal blood samples will usually be collected from anaesthetised animals immediately before death (meaning the animals are unconscious prior to blood collection and will not re-awaken) and mice humanely killed prior to collection of tissues.

Our PAH studies will use one of two methods to induce the disease in the animals. In the first, the sugen-hypoxia (SUHx) model, the majority of animals will be subjected to a hypoxic environment of 10% oxygen for 3-4 weeks (the equivalent of being at the top of a high mountain). A small subset of control animals will remain in a normoxic (normal air) environment throughout the studies. Animals may either be humanely killed immediately upon removal from the hypoxic environment or returned to a normoxic environment for several weeks prior to killing. Animals will be administered with a PAH enhancer by subcutaneous injection immediately prior to entering the hypoxic environment, and this administration may be repeated during the hypoxic period. They will also be administered with test agent at regular intervals. This will usually be during the hypoxic period in mice and after the hypoxic period during a period in normoxia in rats. Blood samples may be taken during the study to monitor progression. At the end of the study, the heart and blood vessels may be cannulated to measure pressure, prior to collection of a terminal blood sample. Cannulation is performed in terminally anaesthetised animals, and involves the insertion of a thin, flexible probe into the heart via a connecting blood vessel. Animals will be humanely killed prior to collection of tissues. The second method is the monocrotaline (MCT) method. This method will only be performed on rats, who will be injected with MCT via subcutaneous or intraperitoneal injection to induce a form of PAH disease which is similar to human PAH. The MCT only needs to be administered on one occasion to induce disease which is similar to human PAH. A small subset of control animals will be administered with vehicle (the liquid the drug is administered in but without drug). The animals will then be administered with test agent at regular intervals, with sample collection and heart cannulation as for the SUHx method.

In heterotopic ossification (HO) studies investigating the potential unwanted side effect of some test agents designed to treat PAH, animals will be subjected to muscle injury either via chemical or physical means. The reason for the muscle injury is that HO normally only occurs at the site of an injury, not in healthy tissue. Therefore, an injury must be caused to check if this side effect would be a problem if the test agent was used in humans. Muscle injury will be performed under anaesthesia and this will involve either injection of a toxin into the muscle or physical muscle damage by the dropping of a ball bearing onto the muscle. The injury is made to one of the rear limbs. Animals will be administered with pain

relief if needed. However, this is not usually required, with animals able to move normally after the injury. Animals will be administered with test agent on one or more occasions, often directly into the site of muscle injury. Blood samples may be taken during the study to monitor progression. At the end of a study a terminal blood sample may be collected immediately before death and all animals will be humanely killed prior to collection of tissues.

Where possible, we will make any tissues not required for our work available to other researchers after the death of the animal.

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

In studies investigating NASH/NAFLD mice will be fed a diet high in fat and/or given sugars in their drinking water. This may cause obesity due to the high fat content of the diets used. However, where the diet is less palatable (meaning that the animals do not like the taste as much as normal), animals may lose (or not gain) weight meaning that their weight will be lower than that of control animals at the end of the study. Diets will be made as appealing as possible to the animals, but sometimes the use of a less palatable diet can give better results in the studies and shorten the studies which reduces the overall impact on the animal. The fat in the diet may cause their coat to become greasy, which ultimately may lead to hair loss through over grooming.

During HO studies, animals may experience some pain associated with the induction of muscle injury. This will resolve within a few days of muscle injury occurring. In our experience, pain relief is not normally required; animals are able to move normally immediately after injury.

During PAH studies using the SUHx method, animals lose up to 10% of their body weight within the first 1-2 days of exposure to the hypoxic environment and demonstrate reduced activity. After this acclimatisation period, activity levels increase and eating behaviour and weight gain proceed normally. Animals who receive a PAH enhancer and exposure to the hypoxic environment and are then returned to the normoxic environment will develop a more severe disease. It is expected that some animals will be found dead in the rat model of this design. These animals suffer sudden death due to heart failure, and do not experience severe suffering. During PAH studies using the MCT method, rats similarly develop features of PAH as in the SUHx model and may also experience sudden death due to heart failure.

The test agents administered in all studies in the project are not expected to cause any lasting harm. Animals may lose some body weight initially whilst adjusting to them. Where a slow-release device is used, 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. Blood sampling is not expected to cause any lasting discomfort.

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: 38%

Mouse - Moderate: 62%

Rat - Mild: 14%

Rat - Moderate: 86%

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 undertaken under this project licence cannot be fully replaced with *in vitro* (conducted in a laboratory without use of animals) or *ex vivo* (conducted on animal tissue but not in live animal) models. Wherever possible, work is performed in *in vitro* or *ex vivo* models prior to *in vivo* (animal) studies, to ensure that the *in vivo* studies are well designed and that the maximum amount of data is obtained from each study. This can be validating the target of a test agent and/or testing the action of the test agent itself in a suitable cell line to ensure the test agent is acting as expected before moving into animal studies. This may also be performed in *ex vivo* samples, providing data not available from established cell lines, but without requiring full *in vivo* experimentation. Our *in vitro* and *ex vivo* work ensure that wherever possible we generate proof of concept data before moving into animal studies, thereby reducing the number of animal studies performed in total and preventing test agents which are unlikely to be viable therapeutic agents being moved forward to *in vivo* studies.

However, isolated cells cannot reproduce the complex nature of the entire system of a living animal. This is particularly true for the areas of research covered by this licence. Metabolism involves complex interactions between different organs in the body, which cannot be recapitulated in the lab. Similarly, the cardiovascular system and flow of blood around the body cannot be recreated in a cell-based system.

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. We have previously performed these types of experiments for example 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?

Whilst cell-based work in the laboratory can be used to test how test agents act on cells, they cannot model the effect on whole organs, or the interaction between different organs which is a crucial element of metabolic and cardiovascular diseases. In addition, cell-based work does not test the effects the body might have on a test agent, for example how it gets into the blood, travels around the body and how it is removed from the body. No alternative is available that can replace the need to test potential therapeutics in a live animal.

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 analysing the number of animals used on previous projects and 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 designing 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. Through experience we tend to use a standard group size for some of the studies under this project, using 6 per group for heterotopic ossification studies and 14 per group for PAH 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.

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 to assist with study design. This expert regularly tests the tool against peer-reviewed power calculators and can adjust as necessary.

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* scientists will be blinded to the treatment status of an animal, meaning that they do not know which treatment the animal is receiving. This reduces bias, enabling more reliable information to be gathered from a smaller number of animals. Where possible, those who carry out analysis on blood and tissues collected during the study are also blind to any treatment with test agent.

Baseline data (e.g. bodyweight, biomarker levels) are recorded and animals assigned to treatment groups to ensure there are no differences in baseline measurements between the groups at the start of the study. Once assigned to a treatment group number, the treatment status for each group will be assigned randomly.

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 an *in vivo* model as is practicable.

Whilst all studies in this project will require live animals, we will coordinate with other groups to offer post-mortem tissues for their work, where this is suitable for their work and does not affect the outputs from our own studies.

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.

Animals will be housed in a purpose-built, state of the art facility, that is free of disease-causing organisms such as bacteria, viruses and parasites. They will have access to food, water and items that enhance their environment, such as tunnels, chew sticks and mezzanine levels to climb on. Our company staff and the animal care staff are competent in rodent welfare and will ensure that animal suffering is minimised. We aim to house rodents in groups to promote normal behaviour. However, aggressive behaviour can occasionally result in animals being singly housed to prevent injury.

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 using test agents for particular diseases.

Our NASH/NAFLD, HO and PAH studies are all performed using well-established protocols, which reliably reproduce the important characteristics of the human diseases required for the testing of new drugs whilst minimising the harms to animals. We work to ensure that we introduce further refinements to our disease models wherever possible.

Test agents will often be tested first under our test agent profiling licence prior to testing in animals mimicking human disease. Where it is hard to predict the potential side effects of a test agent in the disease model, 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 gavage via food or water if possible), and where a needle is required using the smallest needle possible.

Amendment April 2022: For mouse PAH studies, the mice need to be under brief anaesthesia for an injection that requires them to stay still so that the drug doesn't touch the skin, potentially causing irritation. In most studies these injections need to be repeated several times. Therefore, under the guidance of the NVS we have seen some success in giving supportive subcutaneous saline to these mice to prevent dehydration and will continue to trial the use of it to gather more firm data on its effectiveness.

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

Adult rodents such as 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 complexity of metabolic and cardiovascular diseases requires the use of living animals, where the bodily systems are functioning and interacting with each other. Due to the length of the studies, which span weeks to months, terminal anaesthesia is not a possibility.

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 all studies, general animal condition and body weight will be monitored regularly. We are developing a detailed scoring system to monitor any side effects observed due to dosing. 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 when an animal within a cage demonstrates aggressive behaviour). Where anaesthesia is used, care will be given to support animals during anaesthesia and recovery, using heat mats to maintain body temperature and providing soft diet and hydrogel to aid recovery as appropriate.

Where an altered diet is used, we will ensure that animals are introduced to the diet over a period of weaning, allowing them to acclimatise to the diet before a full switch is performed. This will minimise

the risk of weight loss due to a sudden switch to an altered and potentially less palatable diet. For new diets which have not previously been used we will review these using a small pilot study to ensure that they are palatable before using them for large numbers of animals. Where animals are hyperglycaemic (have increased blood sugar levels), we will replace the bedding more frequently when an increase in urination is observed.

For PAH studies, we have refined the method of injection of PAH enhancers that can cause sores at the site of injection by ensuring that we pinch the site of injection as the needle is withdrawn. This has reduced the incidence of sores. For mice, the PAH enhancers are administered under anaesthesia to further reduce the incidence of sores, with subcutaneous saline also administered to assist with recover from the anaesthesia given the potential reduce activity levels whilst in hypoxia.

For HO studies, the level of muscle injury has been refined to cause an injury that does not significantly affect the behaviour of the animals or reduce movement, but is still sufficient to assess the potential for test agents to cause HO.

We use microvettes (small capillary blood collection tubes coated with anticoagulant 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.

Where possible, when animals are purchased from an external supplier these will be handled regularly during the acclimatisation period to reduce the stress for the animals when procedures commence. Where diet consumption is not crucial to the outcome of a study, we also provide treats such as sunflower seeds after handling and procedures to assist in habituation for the animals to the procedures and any restraint required.

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 Federation of European Laboratory Animal Science Association (FELASA) working group on pain and distress has also been used when setting the humane endpoints within this project. We use the resources on the NC3Rs website for guidance on the best practice for procedures such as blood sampling and for refinements to handling and husbandry. 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.

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 the Laboratory Animal Science Association (LASA), the National Centre for the 3Rs (NC3Rs) and the Royal Society for the Prevention of Cruelty to Animals (RSPCA). This will include the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellent) and ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines which provide guidance on

the design and reporting of animal studies. 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 (a resource of databases and guidelines from Norway) and Jax (a genetic animal strain resource providing a hub of research information), to ensure that we are using the most refined methods and are aware of any improvements to procedures that have been developed.