



Home Office

NON-TECHNICAL SUMMARY

PK and PKPD studies to support Respiratory and Immunology research

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, PKPD, Respiratory disease, Immunological disease

Animal types

Life stages

Mice

adult, aged

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?

This licence has two aims:

- To enable us to decide what doses of new treatments we need to use in our respiratory and immunology disease models. We will do this by dosing animals and taking blood samples to measure how much of a new treatment is present at certain times after dosing (pharmacokinetics (PK))
- To enable us to understand how much of a treatment is needed to have an effect on the biological target. We will do this by dosing animals which have had a simple pretreatment or challenge, so that we can measure both the amount of treatment in the blood (PK) and the effect of the treatment (pharmacodynamics (PD)) and use both pieces of information to build a mathematical model (PKPD model). This can be used to inform both future animal disease studies and human dose selection for clinical studies.

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?

There is an ongoing need to discover and develop new treatments for respiratory and immunological diseases such as asthma, chronic obstructive pulmonary disease (COPD), arthritis and lupus. To bring these life changing medicines to patients takes many years of research and requires demonstration that these treatments are effective and safe. To understand how effective a new medicine could be in human disease we bring together a package of information that shows how it affects human and animal cells, how it interacts with tissues from patients, how it modifies relevant animal models of disease and how much we will need to give to patients to be effective. Before testing a new treatment in an animal model we need to know how much to give and how often to have an effect. This licence will enable us to test new treatments in mice and rats and predict how much to use in these models. This information also allows us evaluate the doses that we need to deliver to be effective in patients.

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

This Licence will provide key information about the properties of our novel treatments, such as how long they remain active in the blood of an animal after dosing, which is needed by our research teams to plan and deliver further studies. For example we will obtain an understanding of the peak concentration in the blood, which parts of the body it reaches and how long it takes to be eliminated. These findings along with information from human and animal cell studies will enable us to move

suitable novel treatments forward through our pipeline and into human clinical trials, and ultimately result in new medicines to treat patients. Pharmacokinetic data are often reported when we publish results in disease models.

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

As an organisation our focus is on delivering life changing medicines to patients, and this licence will contribute to that overall goal by generating specific information that is used to determine how much medicine needs to be dosed to have an effect. Without an understanding of how much to give or how often, we cannot conduct effective research in animal models of disease or predict how much we may need to give patients. Our studies are used to support specific research into new treatments for diseases that affect many patients and have life limiting impacts, including chronic lung disease and immune disorders.

Drug discovery and development can take years if not decades, so novel treatments tested under this licence are likely to enter human clinical trials after the 5-year lifetime of the licence. However, treatments tested under previous licences with the same goal are now in clinical phase testing and we are confident that this will continue.

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

We have an open culture and interactions between our scientists and those from other research organisations are encouraged. Whenever possible we regularly share our findings at academic conferences and symposia, but due to the commercially sensitive nature of the work we are unable to be open about our very early and novel findings until appropriate patent protection is obtained. We do share openly information regarding our animal study techniques and practices with our peers to maximise animal welfare, and are committed to supporting the 3Rs.

Species and numbers of animals expected to be used

- Mice: 4600
- Rats: 2600

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

The overwhelming majority of these studies will be performed in adult mice, as this is the species most commonly used in the disease model studies we will carry out. Mice are used in those models as they are a mammalian species with a similar (but not identical) organ anatomy and immune system as compared to humans. Although immunological and respiratory disease can affect patients at any age, the majority of rodent studies are performed in adult individuals (ie post-pubertal) as rodent

development is very rapid and animals become sexually mature in a matter of weeks. Rats will be used rarely, when the disease model is performed in the rat, or there is some other technical need (such as the target system in the rat being closer to the human in its properties).

Although it will be very rare, we may occasionally need to understand the pharmacokinetics of our new treatments in older animals, which for mice and rats equates to 1 year or older. This will only be done where there is a disease model that requires it.

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

In a typical pharmacokinetic experiment, adult mice would be implanted with identification microchips under anaesthetic and a few days later weighed and dosed (the dose given being related to the weight of the animals). The dose may be an oral liquid, delivered using a round ended dosing tube attached to a syringe which is placed into the throat of the animal to ensure all the material reaches the stomach. Alternatively, the dose may be given as an injection using a hypodermic needle under the skin, or into a vein or into the abdominal cavity. Animals are returned to their cages and then at set times (which could be over a single day or a number of weeks) taken out and restrained in a small tube for a few minutes while a blood sample is taken from the tail using a small hypodermic needle. Usually no more than 3 samples are taken from the tail vein in the lifetime of the animal, and only if the samples needed are very small. The final blood sample is usually taken under deep anaesthesia, when a hypodermic needle is placed directly into the heart to take a large volume of blood, followed immediately by killing the animal. Apart from these typical experiments we may occasionally dose using other techniques such as directly into the lungs, or injection into a leg muscle, or surgical implantation of a drug delivery pump under the skin.

Some studies may also include giving the animal stimulus (such as an injection of a substance that can trigger release of inflammation markers into the blood) so we can understand activity of the treatment as well as exposure (PKPD studies). These may look similar to a pharmacokinetic study but with the additional injection of the stimulus or other pretreatment.

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

Animal dosing may cause some brief pain, such as when a hypodermic needle is used to dose under the skin or into a vein or the abdominal cavity. This is brief in nature and animals are not expected to show any signs of ongoing discomfort afterwards. Similarly, the needle used to take a blood sample is likely to result in fleeting pain or discomfort but should not have a lasting effect. Direct administration of material into the lungs may be done under anaesthetic by introducing a narrow tube to the airway, and this has a small risk of touching the sides of the airway and causing irritation or injury.

The treatments we will dose are not expected to result in any adverse events, and for novel materials that have not been in animals before we will run a small pilot study (tolerability test) to check that this is the case. In studies where we will use a challenge or stimulus some animals may exhibit clinical signs associated with inflammation and illness such as reduced grooming, reduced social interaction and lowered body temperature.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority (>90%) of animals are expected to experience a severity of Mild. In our experience, simple PK and PKPD studies with dosing of a novel therapeutic, followed by tail vein sampling, do not result changes to animal condition or behaviour that are greater than Mild.

The remaining animals (<10%) may experience Moderate due to recovery surgery for implantation of drug delivery pumps under the skin, or in the event that animals on the tolerability testing protocol experience greater than Mild severity. Animals on the PKPD protocol may also experience Moderate changes such as reduced grooming or activity over a few days.

These proportions are estimates and may change in either direction if for example the projects we progress in the next 5 years require more or less drug delivery pump work.

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?

It is not yet technically possible to accurately predict the pharmacokinetic properties of novel molecules, so animal studies are required. Much effort is put into screening out unsuitable molecules during the early stages of research, for example using human and rodent liver cells to see if they are rapidly broken down, and other cell based assays to estimate how easily they might be absorbed from the stomach into the bloodstream. This greatly increases the likelihood of success, in terms of seeing good blood levels after dosing, in our animal studies. The complete characterisation of new molecules that is needed to calculate appropriate doses for use in animal models of disease, or to guide first time in human dosing schedules, still requires us to use animals.

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

There are multiple stages of non-animal screening of new molecules that are in place before testing in rodent. Depending on the type of new therapeutic agents will have performed tests (both using computer modelling and lab testing) to understand the physical properties which can affect drug absorption and how rapidly it could be broken down in the body. Cell based systems are used to assess how for example liver cells might process and break down a molecule.

Why were they not suitable?

The information gained from these non-animal tests cannot account for the complex interactions that take place inside a whole animal with multiple organs, cells and enzymes which could affect the way a drug molecule moves around the body, is processed or broken down and is eliminated from the body. As these molecules are going to be tested in animal models of disease, it is a scientific and ethical imperative that we select doses (how much treatment, how often to dose, which route to dose) which are likely to give us the necessary information. These studies can also be used to guide selection of doses for human clinical trials when combined with other information such as the way human cells or enzymes interact with the molecule.

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?

These estimates arrived at based on current and historical usage (how many animals per study, how many studies of each type per year) and allowing for anticipated changes in demand.

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

Pharmacokinetic studies are usually not hypothesis testing in a statistical sense, so we do not test for a significant difference in blood levels *per se*. Instead we are interested in obtaining parameters such as the peak concentration in the blood, and how much time it takes for the blood levels to drop (so we can understand how much drug to use and how often to dose). The number of animals per study is therefore a function of how many samples are required, and for how long, and the number of replicate samples required at each time-point, which is normally $n=3$.

We normally take more than one sample per animal (the limiting factor is blood volume, we do not want to impact animal welfare by sampling too much), so our analysis benefits from replicate samples taken from the same animals. Standard protocols normally require only 3 samples per time point, although this may be increased in certain situations for example when PKPD studies are performed and in these situations statistical methods are used. For some types of new therapeutics we may be able to dose a mixture of different molecules and measure them all simultaneously, which further reduces the number of animals. This cannot be done as a standard method as there is a risk of interactions or technical limitations on the measurement technique.

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

The animals we use for these studies are usually standard strains obtained from commercial suppliers which maximises the efficiency of production and animal usage. Occasionally we will use genetically altered animals bred for scientific use and the numbers are carefully monitored to ensure overproduction and wastage is minimised. New types of therapeutic agent which have not been tested in animals before are tested under a tolerability protocol on this licence, which uses very low number of animals (usually n=2) to confirm that these molecules are suitable for use in animals and do not induce any unexpected effects. This reduces the likelihood of us proceeding to a full study only to find a molecule causes an adverse effect that would stop the experiment.

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.

Pharmacokinetic studies normally consist of dosing animals (using a hypodermic needle and syringe, or special tube for dosing into the stomach), followed by taking blood samples, normally from a vein near the surface of the skin on the tail. Different dose routes may be required for different types of new molecule, or to determine if the molecule can be absorbed from the stomach, which may be important for some projects. Sometimes we may need to deliver a constant amount over a period of time, and in these cases we may use specialised miniature drug delivery pumps which we will position under the skin surgically. The use of these pumps may be needed to maintain a steady blood level or to avoid having to give multiple injections.

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

As these studies are used to help design experiments in animal models of disease we need to have high confidence that the doses we pick are relevant. Using species that are less sentient introduces a significant risk that the information we obtain is not relevant and could lead to wasted animals in the disease models. Terminal only studies would be of limited duration and risk giving information that is compromised as for example blood flow to the liver and other organs may be different under anaesthesia and could affect the measurements, and the presence of anaesthetic could alter the way material is processed by the body.

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

Animals are kept in modern well equipped facilities staffed by experienced and motivated scientific and welfare personnel. Animals are checked at least once daily when not on a study and at least twice daily once dosing and sampling have started. When surgery (under general anaesthetic) is used for implantation of drug delivery devices, peri-operative pain relief (analgesia) will be given as standard.

Hypodermic needles are always discarded after a single injection so that blunted needles (which can cause unnecessary tissue injury and pain) are not used.

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

LASA Good practice guidelines; AAALAC programme

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

As a Project Licence holder I am engaged with local and national 3Rs groups and events and am kept informed by my NIO of relevant new information.