Home Office

NON-TECHNICAL SUMMARY

Discovery and development of treatments for immunological 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

Autoimmune disease, Rheumatology, Dermatology, Immunological disease, Gastroenterology

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 develop and validate animal models that can further our understanding of basic mechanisms involved in immunological disease and enable us to test new therapeutic agents.
- To use the models developed above to identify novel treatments that will impact human immune diseases

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?

Despite decades of research, millions of people live with serious immune mediated disease such as colitis, arthritis, systemic lupus erythematosus (SLE) and atopic dermatitis. For example rheumatoid arthritis, which is an autoimmune disease resulting in painful swollen joints and loss of movement, affected nearly 20 million people worldwide in 2017 (Safiri 2019) with more than 1 million new cases being recorded each year. Other autoimmune diseases such as lupus (SLE) can have devastating effects on patients lives, including debilitating symptoms which impact their ability to work. Allergic inflammation can also lead to the development of serious immune mediated disease such as atopic dermatitis, where the inflammation and pruritus (itch) present significant unmet medical need. Current treatment options are not effective in treating all patients, and many have significant dose limiting side effects (e.g. steroids). We believe we can identify novel treatments to address the symptoms and underlying mechanisms which cause these conditions, and addressing this unmet clinical need is a high priority for the medical research community.

Safiri S, et al. Ann Rheum Dis 2019;78:1463–1471

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

This Licence will enable us to investigate potential new mechanisms involved in multiple immune mediated diseases and test our novel treatments in simple and complex models, with the ultimate output being a new medicine progressing through to human clinical trials. Simple mechanistic models will be used to demonstrate the role of a particular pathway or system, for example using a treatment known to activate a specific pathway, and then using a novel treatment to block the effect. The more complex models, which engage multiple interacting systems but do not necessarily replicate a human disease, will be used to provide additional information about the likely effect of our treatment on a

disease. For example, we may gain more information by seeing how a new treatment blocks the development of skin inflammation in an immune driven model, having previously tested it for activity using a selective stimulus. The outputs of these studies will contribute to active decisions to progress projects and novel treatments along the development path towards clinical testing, or to cease work on pathways or therapies which appear to lack benefit. These studies occur very early in the research pipeline and it can be difficult to publish due to concerns about patent life and intellectual property, but we would expect to share our findings as much as we can with the scientific community.

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 enabling us to test new therapeutic hypothesis in vivo relevant to new treatments for immune mediate diseases. Along the journey to delivering that clinical goal, we will also generate new scientific findings, and build our knowledge of the pathways and biological systems involved.

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: 8000
- Rats: 1500

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 diseases 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 responses of the immune systems in aged animals, which for mice and rats equates to 1 year or older. This will only be done where there is a clinically relevant question or scenario that requires it.

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

In a typical simple immune challenge 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 a set time after dosing, they may have an immune stimulating substance injected into the skin under general anaesthesia. Once the animal has recovered they would be returned to their home cage. At some time later the animals may be 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. Blood samples may be analysed for signals such as immune cell activation, or for drug content. The final blood sample is usually taken under deep non-recovery anaesthesia, when a hypodermic needle is placed directly into the heart to take a large volume of blood, followed immediately by killing the animal. At this time, after confirmation of death, the skin would be dissected to allow analysis of the injected region.

Variations on this design may include the injection or topical application of substances which can cause an itch response, and we will monitor the animals to observe the scratching frequency and other parameters in order to determine if our treatment has modulated the response. Alternatives to injection into the skin may be topical application to provoke an immune response and local inflammation.

Some studies may involve another body site such as a subcutaneous air-pouch, which is generated by injecting sterile air under the skin on the back of the animal. Once the pouch is formed, inflammatory cells can be recruited and activated locally in the pouch following injection of an immune stimulating substance.

Stimulation of a pathway present in circulating cells or a specific organ can be achieved by an injection into a vein, or abdominal cavity. Activation and inhibition can then be monitored by peripheral sampling of blood or terminal sampling of body fluid and tissues.

Treatments in the studies above will typically be given by injection or through dosing into the stomach, but in a minority of cases we may also use a surgically implanted drug delivery device (pump) to do this.

In some studies we may inject cells from another animal or which have been grown in a test tube, to enable us to track the movement of cells during immune responses, or to have a role in the immune response itself (e.g. using cells which respond to a known allergen).

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, the abdominal cavity or air-pouch. 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. Injection into the skin (intradermal) is performed using general anaesthesia to reduce discomfort and allow greater control of the injection.

The sensitisation (or passive immunisation) and challenge models we propose are expected to produce local inflammatory responses, which are likely to include swelling and increased sensitivity (hyperalgesia), and in the case of pruritic responses, irritation that evokes scratching (itch). Overt pain is not an intended consequence of these models and animals will be monitored to limit the severity experienced. Animals may exhibit clinical signs associated with inflammation and illness such as reduced grooming (and/or over grooming of inflamed or challenged areas), reduced social interaction and lowered body temperature. These effects may last over several days, for example following sensitisation.

Where substances are being applied topically to skin we may use coverings to aid delivery and also reduce access to the site, for example using jackets or adhesive covers. Although these may impact movement and natural grooming behaviours they are intended to reduce systemic exposure of substances via grooming and ingestion.

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)?

In this Licence the severity experienced by an individual animal is a function of the immune modulating steps and the drug delivery steps. The majority (>60%) of both mice and rats are expected to experience a severity of Moderate due to the inflammatory and irritant challenges. We have run most of the proposed studies under a previous project licence (e.g. HDM induced ear inflammation, agonist evoked itch, air-pouch) and this estimate is based on the potential for therapeutic effect to ameliorate the inflammation, control injections/challenges which would be expected to have minimum impact on animal welfare and the use of drug-dosed, non-challenged controls which are required in many studies. These factors will bring many animals down into the Mild severity band.

A minority of animals (estimated <10%) may experience Moderate severity due to recovery surgery for implantation of drug delivery pumps under the skin.

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 eliminate all animal studies from our preclinical research programmes as the complex interplay of physiological systems including the innate and adaptive immune systems and the nervous system cannot be replicated using in vitro technologies. One reason for this is that we are working with highly novel mechanisms and need to ensure that in vitro findings can be extrapolated to our in vivo settings.

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

There are multiple stages of non-animal screening of new treatment strategies as well as novel molecules themselves that are in place before testing in rodents takes place. Depending on the type of new therapeutic agents we 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. The biological pathways we are studying are investigated using multiple human and animal cell based systems to ensure that the molecules we are going to test will modulate the target pathway or system.

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. In addition, the biological mechanisms and pathways we are seeking to modulate are both complex, involving innate and adaptive immune responses and interactions with whole organs and body systems, and highly novel and we want to ensure that the behaviour of these is physiologically relevant and not an artefact arising from cell or tissue culture conditions.

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 expectations (how many animals per study, how many studies of each type per year) and allowing for anticipated changes in demand.

For each protocol 1-4, the estimate is 2000 mice over 5 years, which is 400 mice per year per protocol.

For rats, the 1500 figure is composed of 250 rats over 5 years (ie 50 rats per year) for Protocol 1 and 2, and 500 rats over 5 years for Protocols 4 and 5 (ie 100 rats per year per protocol)

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

Before performing in vivo studies under this Licence, we will have conducted extensive in vitro research studies to confirm as far as possible that the agents we will use are pharmacologically active against the species specific target and have sufficient potency or affinity to have a biological effect in vivo. This reduces the risk of performing studies with agents that cannot be progressed. Prior to the studies described here, experiments are carried out to ensure that the dosing will be effective, by measuring the amount of treatment in blood after administration. This de-risks again the potential for using new therapeutic agents which are unlikely to achieve active concentrations at the target tissue or organ. Careful consideration is given to the number and size of all groups, and the overall goal is always to use the minimum number consistent with the scientific objectives. As we are testing novel materials it is necessary to include control groups to ensure the interpretation of the data is clear, which reduces the need for follow-up studies.

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. As described elsewhere in this application, pilot studies will be performed to inform our use of power calculations as well to determine the optimum effect window and kinetics of responses.

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 models described here are mechanistic and are designed to allow us to study particular pathways or systems which are involved in a disease. For example, by using a simple model of cell recruitment

into an air-pouch, we can study the movement of inflammatory cells (such as T-cells, monocytes and neutrophils) into the pouch and the mediators and enzymes they release, and look at the effect of blocking those mechanisms. Using this approach enables us to study pathways and pharmacological effects without having to use animal models of arthritis in the first instance. The animal welfare burden in arthritis is greater as the animals can experience pain and discomfort from swollen and inflamed paws, and this licence is not requesting permission for that model. The pouch cannot replace all arthritis studies but does allow us to study the biology and pharmacology of these mechanisms in a lower severity model, reducing the risk of proceeding into a arthritis study with an ineffective new treatment. In a similar way, we can use short/simple (hours-days) mechanistic studies of skin inflammation and itch prior to complex models of immune mediated skin inflammation (days-weeks) to focus our studies. This approach minimises the use of models with a higher welfare impact.

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

These studies are used to provide critical information to project teams regarding the efficacy of our new treatments, and will inform decisions regarding the progress of these treatments into more complex models of disease (which may not fall under the remit of this licence) and eventually into human clinical studies. 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 skin, liver and other organs may be different under anaesthesia and could affect the measurements, and the presence of anaesthetic could interfere with the mechanisms (or limit our ability to make measurements such as scratch behaviour).

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.