Home Office

NON-TECHNICAL SUMMARY

Provision of an outsourced Drug Discovery platform for diseases with an inflammatory component

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

Out-sourced drug development platform, Inflammation

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

Discovering new and better medicines has become increasingly challenging and sadly many potential new drugs have failed when tested in man. Improved understanding of how diseases start in the body (including the role of faulty genes) and the development of new technology has come together to provide new ways of looking to treat disease. Under this project licence our aim is to provide a service to smart thinking clients by working with them on novel targets they have identified, and who pay for this service to achieve their scientific objectives. Combining our knowledge of animal models (and what their limitations are) with their understanding behind the target and the disease it is aimed to treat, we should help achieve a better clinical outcome. One such disease we have a real hope in a new treatment being successful for is osteoarthritis (OA), a condition that affects your joints causing pain and stiffness. Our focus is on looking at how other novel treatments for this disease have not been as successful as predicted and on how by teasing apart the progression of disease in the animal models used, may make them more clinically relevant for testing the next generation of related medicines. Secondly, some targets have the potential to treat more than one illness. For example stimulating a mechanism that helps the body rid itself of unwanted molecules may be beneficial in treating both lupus, which is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue in many parts of the body leading to symptoms such as a feeling of tiredness (it is often associated with a red rash on the face), and in a serious liver disease, that is not caused by over consumption of alcohol but by a build up of fat in liver, called non-alcoholic steatohepatitis (NASH). This disease may be associated with our unwitting desire to consume a fast food diet but once the damage has been done it is difficult to reverse. In these cases of potential multi therapies we will focus on the disease with the biggest un-met clinical need first (NASH) and if we find a potential drug that has a positive outcome in our animal model of that disease then we will go on to evaluate that compound's effects in models of other relevant diseases, such as lupus. The third area we hope to make a difference in is an example of where genetic mutations have identified a link with a lung disease called pulmonary arterial hypertension (PAH). Here the small arteries in the lungs become narrow, making it harder for blood to flow through so the heart has to work harder, eventually becomes weak and may even fail. There is a chance that understanding the outcome of this genetic alteration and how to moderate it with a biological agent could be of benefit to these patients.

These are just three examples of where we are providing a service to our clients to work towards a common goal of developing new and better drugs. However, because we want to do more we have other collaborations in the early stages of drug discovery that may lead to the treatment of additional diseases such as Alzheimer's disease and Alpha-1 Antitrypsin (A1AT) disease, eosinophilic airways diseases and cancers that are difficult to treat. Because targets for these diseases are developing we are mostly focusing on helping the client with proof of concept studies, developing cell based assays to test their compounds in and then looking at how well these compounds enter the blood stream so we know that drug will reach the right part of the body before going on to test them in the relevant animal model of disease. In some cases, better understanding of complex mechanisms that help keep our bodies healthy, and how abnormal activation of these mechanisms can lead to some diseases, generates new ideas on how to treat these diseases. For example, one way our body rids itself of unwanted cells, such as those that are damaged, is to send signals to these cells to induce them to undergo a form of 'cellular suicide' called apoptosis. It is now thought that some diseases of the brain, such as Alzheimer's disease or Huntington's disease may be caused or made worse by apoptosis "going wrong". A family of enzymes (proteins that speed up reactions) called caspases play an

important role in starting and advancing apoptosis and selected inhibition of some of these caspases has the potential to generate new medicines.

Whilst drugs are available to help treat all the above diseases none are perfect meaning there is still an unmet clinical need to find better medicines. Animal models that were developed in the past have been invaluable in taking current drugs in to the clinic but they may no longer be the most appropriate ones in which to test the effectiveness of drugs with a novel mechanism of action. Our aim is to provide animal models of disease that will be able to answer the question of whether a drug with a novel mechanism of action will be able to treat that disease when tested in humans.

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.

What are the potential benefits that will derive from this project?

Advances in our understanding of the science behind diseases that have an unmet clinical need such as OA, lupus and PAH will lead to new and better treatments. By 2050 there will be around 130 million OA sufferers, 40 million of whom will be severely disabled by the disease. There are significant unmet needs in the early diagnosis, monitoring and treatment of the disorder that could bring relief to suffering patients. Similarly, whilst the management of lupus has progressed enormously in the last 10 years, there are patients who do not respond to the most widely prescribed drugs and addressing this major unmet need by developing new drugs remains a priority. Non-alcoholic fatty liver disease (NAFLD) has reached epidemic proportions and is becoming the most common cause of chronic liver disease. NASH, a severe form NAFLD, is most likely to become the primary reason why patients will require a liver transplant over the next 10-20 years. An improvement in diet and increasing physical exercise is the first line taken to help these patients but this is known to be of limited effect. Hence, there is an urgent need for new and safe drugs that successfully reverses or prevents progression of liver injury in patients with NASH. The addition of studies in the huZ mice (formally referred to as PiZ) offers a potential treatment for patients with a worse prognosis for development of liver cirrhosis, due to the heterozygous presence of the gene for the Z form of A1AT. Once diagnosed with PAH a patient has a 30% chance of dying within three years. Despite improvements in the diagnosis and management of PAH over the past two decades, with the introduction of targeted medical therapies leading to improved survival, current treatments only manage the symptoms and the prognosis remains poor. By understanding and refining our animal models of OA, lupus and NASH we can learn more about progression of disease and how to design experiments in which to test new medicines in the most appropriate way for each target, and advise on how best to run a clinical trial in humans with the disease. Likewise developing preclinical models of complex inflammatory disorders of the airways or the brain and those cancers that are difficult to treat will help with evaluating novel therapies for these diseases. The leading cause of death in the UK in 2018 was dementia and Alzheimer’s disease accounting for 12.7% of all deaths registered (ONS) and this is expected to rise year on year.

Kidney disease accounts for 5% of all deaths, but progression of the disease could be managed with the right treatments early on. By studying early signs of the disease and developing preventative treatments for people at risk a real difference could be made to the prevalence of the disease and the suffering caused by it.

By researching on new targets for such related diseases is a clear unmet clinical need. Targets in the early phase of drug discovery our scientific driven approach to the service we provide will help prove whether these targets are worth pursuing by our clients. In all cases we will enable milestones to be met more efficiently for our sponsors (academics, the Pharmaceutical industry, clinicians, venture capitalists) so key ‘go’ / ‘no go’ decisions can be made using the minimal number of animals possible and assure a better success rate in the drug discovery process than has been seen previously.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will use normal mice and rats and mice that have been genetically altered to investigate the disease of interest or the role of a specific gene in the development of that disease. We anticipate we will use 12450 mice and 8250 rats during the five years of this licence.Rats (50) have now been added to Protocol 9 and a new protocol using 50 rats has been included so the total number of rats during the course of this licence has now increased by 1000 to 9250. The numbers of rats and mice in our existing protocol of osteoarthritis has been adjusted down so that the addition of three protocols to study respiratory disorders and cancer, and the addition of rats to an existing protocol, will not increase the total number of animals we expect to use. In January 2021, an additional 1800 mice were added to protocol 5 and 500 rats were added to protocol 20, bringing the total numbers to 14250 mice and 8750 rats. In August 2021, a new research objective in kidney disease added a new protocol containing 1000 mice, and an additional of 500 mice for breeding of some of the studies, bringing the total number to 15,750 mice. Also in August 2021, a new set of experiments to address an existing objective for cancer added a further 200 mice, bringing the total to 15,950 mice.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The animal models involve either rodents that have a predisposition to exhibit disease or those that undergo a regulated procedure to induce disease. For example we breed mice that have been genetically modified as a model of lupus. These mice spontaneously develop antibodies to their own tissues in the same way as humans with lupus do but they appear outwardly normal. We measure levels of these antibodies in the blood by taking a small sample from a vein in the tail, which causes minimal pain or distress. As these mice age they develop kidney damage, which we can detect by measuring how much protein is excreted in their urine by allowing the mice to urinate onto a special stick that measures protein. Animals are humanely killed before the disease is likely to cause anything other than moderate distress.
To model osteoarthritis we either inject an agent into one knee of a rat or mouse that causes inflammation or we carry out a surgical procedure that mimics a tear in the knee caused by a sports injury. Gradually the architecture of the knee is destroyed, as is seen in humans with OA, and as a

consequence the animal may experience mild to moderate pain. We monitor this using the same techniques as used in humans. For example by measuring how much weight an animal puts on its affected limb compared to its healthy limb. We know from our experience of looking at histology of the knees how the disease progresses and we do not allow any experiment to go beyond a certain point to ensure that no animal experiences severe pain or distress.
In our model of fatty liver disease we mimic the human condition by feeding normal mice with a diet that is high in fat and putting sugars in their drinking water. This “fast food diet” is similar to that many humans consume who have an unhealthy life style and who eventually develop fatty liver disease. Whilst some of these patients do become very sick none of our mice experience anything other than mild or moderate distress before they are humanely killed and the degree of liver damage assessed.
To reproduce PAH in our animal models we either, expose them to air containing low levels of oxygen for up to four weeks and then put them back into normal air or we inject them with a plant extract. In some cases animals are pre-treated with an agent called Sugen that exacerbates the effects of low oxygen levels when they are returned to normal air. These procedures result in changes in the lungs, similar to those seen in humans with the disease, which we measure at the end of a study when the animal is anaesthetised and from which it is not allowed to recover. In some cases we make measurements using non-invasive imaging techniques so we can follow the course of the disease with time. Prior to the non-recovery step in the protocol most animals will suffer no more than mild to moderate distress. However, of those animals that are pre-treated with Sugen, some may suffer a sudden cardiac arrest and prematurely die, just like some humans who are diagnosed with PAH. Death in this way is not anticipated to be particularly painful for the animals.
We use drugs or other substances to try and prevent or reverse disease. Our cancer models may use human cells to induce cancer in mice, making them more relevant to studying the human condition. We monitor the development of tumours using calipers and mice are killed once the tumour has reached a pre-defined size or if they show any signs that the animal is no longer able to tolerate the presence of the tumour, such as poor coat condition, reduced movement and/or social interaction. To mirror diseases of the airways in an animal models we expose them to allergens or pathogens that initiate an inflammatory response in the nose or lungs causing the animal to develop non-life threatening asthma or increased mucus secretion, which in some cases is associated with nasal polyp-like lesions. To study the role of caspases in diseases of the brain, we may interrupt the blood supply to the brain or inject agents that damage specific areas of the brain, which we can measure by sectioning the brain, once the animal has been humanely killed, and using tools to study the brain’s pathology under the microscope. These insults may result in subtle changes in how animals behave that we can assess by testing their ability to do certain tasks. For example, how well they can reach and grasp food rewards; how well they stay balanced on a rod that is slowly rotating; or what their pattern of movement of the limbs (gait) is when they walk or are encouraged to run. The behavioural changes we see can then be looked against the changes we find in the brain to see how well they correlate. The techniques used for dosing with test agents and sampling to measure outcomes are chosen to cause the minimal pain and distress to the animal, whilst achieving the desired outcome. At the end of any programme of work, animals are humanely killed and in most cases, tissues taken and analysed to answer questions around that programme of work.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

All the diseases we are working on have an inflammatory component. Inflammation is very complex with a web of interactions that unfold as the result of provoking an inflammatory stimulus. As such animal models of disease in which there is an inflammatory component are equally complex, with no one model being able to mimic the human condition and there is no *in vitro* model that recapitulates this complexity. Where they are available, we obtain human cell lines from patients with diseases to help support the hypothesis that a potential new target is likely to lead to the development of a new drug for that disease prior to testing in animals. We have also developed some new techniques in cells and tissues for measuring key biological processes that a target with a novel mechanism of action should affect. When testing new drugs, we can support the use of our animal models by applying these cell/tissue based techniques to the animal model of disease.

Reduction

Explain how you will assure the use of minimum numbers of animals.

The number of animals we use depends on the desired outcome, the study design, the number of groups needed (including appropriate positive and negative controls (both animals and test agents) and how large a difference we hope to be able to detect. Pilot studies that are used to refine procedures and to discover potential problems before the main study begins uses relatively small numbers of animals based on experience and judgement but numbers are large enough to provide needed estimates for future sample size analysis. We use experienced scientists with a range of skills to design experiments and interpret data. Their experience in the challenges of animal experiments helps with determining the minimum number of animals needed to generate data that is meaningful both biologically, and statistically for each measurement made within a study (e.g. the use of careful power calculations to ensure that a significant effect can be detected with the number of animals assigned to an experiment). Good planning ensures that within any series of studies we can control any variability that might be introduced. This includes using animals of a similar age/weight range; testing different batches of test agents *in vitro* first; using the same source of reagents; keeping records of observations made and standardising as many components of an *in vivo* model as practicable. Wherever possible our *in vivo* workers are blinded to any treatment thus reducing bias. Likewise those that carryout downstream analysis is blinded to the treatment. If an appropriate genetically altered animal is not available commercially then we manage our breeding programmes, as far as is reasonably practical so no animals are wasted. In most cases excess animals are used to provide blood or tissues to support target validation or are offered to others. If a programme of work on a particular GA strain is no longer required then the strain would be cryopreserved (frozen at very low temperatures) so they can be re-derived in the future.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

With any new programme of work we scrutinise the literature for information and work closely with our clients to identify which animal model is most appropriate for their target. When new models or experimental designs are being developed we use data from preliminary studies to guide us on refinements, points at which to intervene and what our humane end points should be. For example, our previous experience with a model of brain injury (similar to a mild stroke) where we assess the degree of injury by testing an animal's ability to grasp a food reward, suggested that dietary restriction is necessary to motivate the animal. To try and refine this we will investigate the minimum length of time food needs to be withheld to be effective, along with whether training the animal to recognise the reward as a 'treat' before testing removes the need for dietary restriction. Likewise, where signs of harm are difficult to predict, for example when testing novel compounds, we carry out preliminary studies at an expected therapeutic dose and route in small number of animals first (typically 3) and continually monitor them over a 2 hour time period. If acute adverse effects are seen then the dose is titrated down to a no effect level before pharmacokinetic or efficacy studies are conducted.

We seek input from others, such as clinicians and experts in other disciplines (such as toxicology); look to useful websites such as the NC3Rs, RSPCA and advisory bodies such as FELASA. We also follow the local rules for the animal facilities we use with respect to husbandry, housing, transport and acclimatisation periods.

We employ a consultant clinical histopathologist for assessing blind any pathology data in our models. This increases our knowledge of the models and where along the pathway of disease progression novel targets are most likely to be effective. This in turns supports the validity of our models relative to the human condition.

Amendment January 2021: During the course of the licence, we have optimised the formulation of a drug utilised in some of our long-term dosing studies in a strain of mice which are particularly skittish to allow dosing via the drinking water, rather than via oral gavage. This makes it less stressful for the animal to receive treatment.