Home Office

NON-TECHNICAL SUMMARY

New treatments for renal and liver disease

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
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

kidney, diabetic, treatment, renal, liver

Animal types	Life stages
Mice	adult, aged
Rats	adult, aged

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 licence is to develop novel treatments for patients with renal or liver disease.

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?

Renal diseases remain devastating illnesses with unacceptably high rates of mortality and morbidity worldwide. The kidney is a complex organ made up of over 26 different cell types and plays a critical role in toxin elimination, pH balance and hormone production. Acute kidney injury (AKI) is a sudden loss of kidney function whereas chronic kidney disease (CKD) is a progressive loss in kidney function over a period of months or years. When kidney function falls below a certain point, it is called kidney failure. Between 8 and 10% of the adult population have some form of kidney damage, and every year millions die prematurely of complications related to kidney diseases. Kidney dysfunction is a disease area that impacts many lives but where there are currently limited treatment options available. Once a patient reaches kidney failure, a kidney transplant is necessary to prevent death. As insufficient kidneys are available to meet demand only a small proportion of patients undergo a kidney transplant operation. The remainder must undergo dialysis, where a machine is used to perform the function of the kidney to clean the blood. Even with timely dialysis, the death rates in patients with kidney failure vary from 20% to 50% over 24 months.

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease worldwide and is often found in patients with type 2 diabetes. There is no approved treatment for NAFLD. Fatty liver disease and inflammation in the liver will eventually lead to cirrhosis (scarring of the liver). A patient with liver disease is also at a higher risk than the general population for developing a form of liver cancer called hepatocellular carcinoma (HCC).

Clearly novel approaches for the treatment of liver and kidney diseases are required to help the many patients suffering. In work under this licence we aim to identify new treatments for CKD, AKI and NAFLD. As we pursue our goal of finding new therapeutic approaches will also uncover new insights into disease progression and learn more about the process of repair from injury in the liver and kidney. Wherever possible scientific discoveries and developments arising from this licence that are not commercially sensitive will be published to the benefit of the wider scientific community.

Based on our past record we would expect to deliver 3-5 potential new drugs into clinical development during the period of this licence. Whilst not all of these will be successful, as we move to more novel and sophisticated approaches to clinical trials, it seems reasonable to predict that approximately 20% of the nominated compounds will provide significant benefit to patients with renal or liver disease.

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

The benefit of this project will ultimately be the introduction of new and improved treatments for the management of kidney and liver disease. This will be achieved by progressing novel candidate compounds to clinical trials and discontinuing ineffective approaches. Other benefits include publications, presentations and patents filed. Our research will also advance our basic biological understanding of the kidney and liver in health and disease.

For liver disease specifically, work carried out on this project licence will only support experiments that will be carried out in the laboratory using cells and/or tissues harvested from mice or rats. Drugs that look promising in those experiments will be taken further in work that will take place outside of this project licence.

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

In the short term other researchers within the field and the pharmaceutical industry will benefit from increased knowledge and learning in which therapeutic approaches might be the most effective and which are not. In medium term (10 years) patients and clinicians will hopefully benefit from the launch of novel therapies for renal and liver disease, that will provide therapies for diseases where there is currently no specific treatment such as NASH or AKI, or provide more effective therapies, or therapies with reduced side effects for patients with CKD. In the long term (20 years) society will hopefully benefit from the decreased health burden that will result from the development of new drugs and technologies.

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

Wherever possible scientific discoveries and developments arising from this licence that are not commercially sensitive will be published to the benefit of the wider scientific community through publication in open-access journals and presentation at scientific meetings. When not commercially sensitive resources, such as data, reagents, tissues will be made available to other researchers. For example detailed characterisation of animal models used for pre-clinical drug testing has previously been be shared within the renal community to accelerate drug development. We will aim to publish approaches to therapy that are ineffective or have developability challenges to prevent this work being repeated by other companies or researchers.

We will share good practice with the local Animal Welfare and Ethical Review Body (AWERB) and also within internal and external forums that bring together researchers utilising animals for scientific purposes from across different disciplines.

Species and numbers of animals expected to be used

- Mice: 7200

- Rats: 1600

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.

We will use rats and mice during this program of work. Some animals will be genetically altered to modulate a specific gene, or to produce a specific disease state. We have chosen rats and mice because 90% of the genes in rodents are shared with humans and there is extensive litterature characterising mouse and rat physiology. Many models of human disease have been developed in mouse and rat and share many of the characteristics seen in human disease. Because of these similarities we can be more confident findings that we make in mice and rats will also apply to humans.

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

Upon arrival to our facility the mice or rats are transferred into clean Individually Ventilated Cages (IVC). The cages are maintained at the appropriate temperature and humidity for the species. Cages are cleaned at least once a week and water and food is checked daily. Animals are left to get used to to their new home surroundings for at least 6 days before any experimental procedures are performed.

Mice and rats are typically housed in small groups as they are social animals. On occasions animals may be singly housed e.g. on occasions male mice naturally fight with each other so may be housed singly for welfare reasons.

Mice and rats will be used either to harvest cells or tissues or to evaluate novel drugs that are potential new treatments for kidney disease.

In efficacy studies a disease state will be induced so that the effectiveness of novel drugs in improving kidney disease can be assessed. Disease will be induced by either :- genetic alterations, modified diets, administration of drugs with the potential to damage the kidney or surgical procedures under general anaesthesia (i. removal of one kidney, ii. obstruction of urine flow, iii. temporary blocking of blood flow to the kidney). The surgical procedures will be carried out as follows i. For the removal of one kidney, a 1-cm incision will be made in the flank of the animal, the kidney will be exposed and surgically removed, and the incision will be closed with sutures. The surgical procedure is expected to take approximately 20 minutes. ii. For the obstruction of urine flow, an incision will be made in the flank of the animal and the ureter will be tied off using a fine piece of silk, the ureter is the canal that carries urine from the kidney to the bladder. The incision will be closed with sutures. The surgical procedure is expected to take approximately 20 minutes. iii. For the temporary blocking of renal blood flow an incision will be made in the flank of the animal and a small clip put on the vessel supplying blood to the kidney. Blood flow will be stopped for typically 25 minutes (and never longer than 60 minutes) while the mouse is maintained under anaesthetic and body temperature is maintained by a heating pad. After that the clamp will be removed and the incision will be closed with sutures. For all the above, no experiment will be performed until full recovery of the animals.

Genetically modified animals are used that display phenotype that mimic many of the characteristics of patients with diabetic kidney disease. Namely these animals may experience weight gain, high blood sugar, excessive urination in combination with high blood pressure, depending on the genetic modification.

Irrespective of the method of inducing kidney damage the characteristics of the kidney injury will be determined in a small number of animals if not already known before proceeding to larger studies looking at the effect of potential kidney drugs. In these small pilot studies the animals are closely observed every day and body weights and condition of the animals are recorded to ensure that the animals are healthy. If kidney damage has been induced in a consistent manner this model of disease can then be used to test the effectiveness of novel drugs. Drugs can be dosed through the mouth, into the peritoneum (body cavity), in the fatty tissue, just under the skin or alternatively into the vein. Direct administration into the blood stream may require the surgical placement of a permanent cannula into a blood vessel. Where administration is required for prolonged periods, animals may be surgically implanted with slow release devices such as a mini-pump. The surgical subcutaneous implantation of a mini pump is carried out under general anaesthesia and involves making a small incision on the back of the mouse, typically between the shoulder blades, a small pocket is created under the skin and the mini pump inserted with the catheter tubing extending out from the incision site. The incision is closed using sutures or tissue adhesive. In some circumstances substances will be administered directly into the kidney - this procedure will be carried out under general anaesthesia with a surgical procedure to isolate the kidney by making a 1-cm incision in the flank of the animal and exposing the kidney. After the micro-injection into the kidney with a fine needle is completed, the surgical incision is closed. Another method to administer a treatment into the kidney is to use ultrasound to visualise the kidney in an anaesthetised animal and then use a needle to pass through the skin and inject directly into the kidney. In all surgical procedures the animals are given post-operative pain killers and monitored closely for any signs of pain, infection, or other complications.

The effect of the potential treatment for renal disease on renal function is compared to function in an animal which does not receive the novel treatment. Renal function can be assessed by tests performed in urine or blood collected from the animals. Urine collection will be done in specially designed cages in which animals may be kept for up to 72 hours, alternatively a spot sample maybe collected through non-invasive means by placing the animals on a type of sand that does not absorb the urine and allows it to be easily collected, following spontaneous urination by the animal. Blood samples may be collected from a vein. Other measures used to assess the effectiveness of the drug treatment and in some circumstances understand how it is working include blood pressure and imaging under general anaesthesia.

At the end of the study the animal is killed, and the kidney and other tissues may be taken which can then be used for further investigation. This procedure is done under non-recovery anaesthesia where the animals will only be aware of the anaesthetic being administered and may experience mild distress and no pain.

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

Animals will experience mild, transient pain and no lasting harm from administration of substances by injection using standard routes and from blood withdrawal. The total number of injections an animal

can receive is limited. The amount of blood that can be collected is limited in relation to the animals blood volume.

Mice will have surgery either to induce kidney damage or to administer drugs directly to the kidney or they may have minor surgery to implant a device under the skin that can release a medicine slowly. They are expected to recover quickly and will be given painkillers and post-operative care.

Some diseased animals will experience weight gain, high blood sugar, high blood pressure, increased drinking and excessive urination which will last for the duration of the experiment but will not reach a level that is likely to cause pain or discomfort to the animals.

Some animals may experience stress associated with isolation from single housing, such as when animals are housed in cages for urine collection.

Animals may experience transient stress associated with restraint for blood pressure measurement.

Animals which undergo changes in diet are not expected to experience distress but may result in obesity. Some diets may result in weight loss due to unpalatability. Animals will be placed onto normal diet should they lose 15% of their body weight.

Drugs maybe used to induce a disease state. Some drugs may damage cells within the kidney. Other drugs may be used to damage insulin prodcuing cells within the pancreas. The loss of these cells will induce diabetes and in this way we can study kidney disease than is caused by diabetes. The use of certain drugs, which are used to induce a disease state, may cause transient weight loss after administration. Animals will typically recover the weight lost due to drug administration within 1 to 2 weeks.

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)?

Mice   Non-recovery   2%

        Mild 23%

        Moderate 75%

Rats   Non recovery 2%

        Mild 23%

        Moderate 75%

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?

Whilst we use in vitro (experiments performed in cells in the laboratory) or in silico (experimentation performed by computer methods) where possible to short list candidate drugs, for many projects assessments of efficacy in disease for lead molecules will need to be assessed in vivo (in animals). There is currently no in vitro or in silico system capable of simulating complex whole animal physiology and the complexity of the kidney made up of at least 26 different cell types. Metabolic and kidney diseases have a complex origin with multiple components interacting to manifest the disease. Many therapeutic agents target specific biochemical responses or physiological mechanisms that in vitro systems cannot fully replicate.

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

As an organisation we invest heavily in human tissue and cell based technologies for our renal research. For example we use human kidney organoids to test drug activity in certain projects. An organoid is a miniaturized and simplified version of an organ produced in vitro in three dimensions that mimics the key functional, structural and biological complexity of that organ.i We also use mouse precision cut kidney slices and isolated rat glomeruli (a sub-structure within the kidney that acts as to clean blood passing through it). These systems are used extensively for mechanistic and pharmacological studies prior to in vivo studies. We have also evaluated other organ-on-a chip technologies that are commercially available from companies such as Mimetas and hope to add these systems into existing workstreams following validation of these platforms.

Why were they not suitable?

Individual mechanisms can be probed in cells or complex 3D models, and we conduct extensive studies to characterise these as far as possible before conducting experiments in animals. However processes such as filtration through the glomerulus are hard to replicate plus we need the interaction between different organs such as kidney and heart or the interaction between the kidney and the different cells of the immune system.

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?

We typically run on average 3 studies per month, each study may have 40-60 animals per study.

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

All studies are designed to ensure that the minimal numbers of animals are used to achieve the question being asked. This is done with help and guidance from a statistician who is a maths expert who uses huge amounts of data to figure out how likely it is that something will happen or not. We also use the NC3Rs EDA (https://www.nc3rs.org.uk/experimental-design-assistant-eda) a free online tool that helps to check the minimum number of animals is used consistent with the scientific objectives of the experiment. We also consult the NC3Rs website for general advice on reduction.

Pilot studies will be run for new models to understand effect size and variability and these data will be used to calculate how many animals are needed to subsequent experiments. Good experimental design principles such as randomisation are incorporated into all experiments. All study designs are approved by a statistician.

All experiments are performed in accordance with Good Laboratory Standards (GLS). This standard sets the minimum laboratory requirements for all our research and development. This ensures that procedures and results are accurate, reliable, traceable and reproducible and where appropriate,comply with the appropriate regulatory authorities' legislation.

All experiments are performed in accordance with the PREPARE guidelines - Planning Research and Experimental Procedures on Animals: Recommendations for Excellence.

All research that will be published will be published in accordance with the ARRIVE guidelines - Animal Research: Reporting of In Vivo Experiments.

Where possible mixed sex groups will be used in experiments.

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

We hope to optimise new imaging efficacy endpoints that will allow assessment of animals over time. This will mean several timepoints can be evaluated in the same animal and therefore less animals in total may be needed for a particular project. Where appropriate, samples from in vivo studies can be shared with collaborators to the maximise the scientific knowledge that can be gained from one study.

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.

Models are chosen based on the on the minimal pain and suffering to the animal in combination with the degree to which they faithfully replicate the disease process, or aspects of the disease process in humans. 90% of genes present in mouse and rat are also present in humans. Mouse and rat models of metabolic and kidney disease have been established by other groups and reported in the literature. The majority of drugs used in patients today to treat diabetes and kidney disease have the same effects in rodent models of disease. The inclusion of mice enables us to use mutant or genetically modified animals for early hypothesis testing, target validation and humanization of target as necessary.

Novel drugs are tested in a very small number of animals initially (typically 2 to 3 per group) and only drugs that do not have unwanted side effects can be used in larger numbers of animals.

Some methods to induce kidney injury require a surgical procedure. Any animals that undergo a surgical procedure will be provided with pain medication prior to the surgery (and after surgery where required) and maintained in a warm environment until full recovery to minimise weight loss.

All animals will be kept in cages that have various forms of enrichment included, for example a cardboard house, sizzle nest, tunnels, chew stick. The temperature and humidity is kept within a specified range that is optimal for the animals.

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

Mice and rats have a well-defined biology with a highly characterised immune system. In non-mammalian species such as the fruit fly, is not possible to replicate the complex processes that underpin metabolic disease and kidney and liver dysfunction.

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

Environmental enrichment is provided in every cage. Enrichment provided such as paper houses, tunnel and chew sticks allow the rats and mice to have improved welfare and demonstrate natural behaviours such as sheltering, nesting, climbing and gnawing.

Pilot studies will be conducted for new protocols to ensure the methods used provide for the maximum animal welfare in relation to the experimental objective. We will also aim to implement new ways, as technology evolves, to further improve the welfare of the animal over the course of these experiments (e.g. by embracing non-invasive measurements).

We now implement non-aversive mouse handling methods on all studies. This involves holding the mice using a tunnel or cupped hands rather than picking up by the tail to reduce any anxiety induced by handling.

All animals will be acclimatised for 7 days from arrival before they undergo any experimental procedure.

We closely follow and implement the latest welfare guidelines and therefore handle animals in a way that causes the least amount of harm or stress to them as possible while conducting these experiments.

All surgery is performed in concordance with 2017 LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. Animals will be given pain killers prior to surgery and afterwards.

For some compounds it is possible to administer them in the diet or drinking water and this has the potential to reduce animal handling in chronic dosing studies.

Use of acclimatisation to reduce stress response to restraint for example with blood pressure measurement.

To reduce male aggression some clean and dry (i.e. used but not soiled) nesting material is transferred from the old cage to the new cage during cage changes. It is known cage cleaning can disrupt social signals communicated through scent and thus disrupt the social hierarchy. Transfer of bedding material can prevent this and therefore decrease male aggression. Environmental enrichment such as partitions and tunnels can also reduce the prevalence of aggressive behaviour in group housed mice. The NC3Rs resource on reducing aggression between group housed males will be used to guide additional practices for reducing aggression.

In studies where the administration of tamoxifen to animals is required for the scientific purpose, the experimental design will be guided by "best-practice" resources produced through culture of care workshops organised within the scientific community.

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

We will use the PREPARE guidelines to assist with planning animal research. We will also use of web-based sources through the National Centre for the Replacement, Refinement and Reduction in Animals in Research (www.nc3rs.org.uk/experimental-design), ARRIVE (Animal Research: Reporting of In Vivo Experiment, guidelines for preparing publications; https://www.nc3rs.org.uk/arrive-guidelines) and Laboratory Animal Science Association (LASA) guiding principles documents for aseptic technique for any surgical procedures (https://www.lasa.co.uk/current_publications/).

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

Subscribing to publications such as ATLA (Alternatives to Laboratory Animals) Journal: https://journals.sagepub.com/home/atla; NORECOPA newsletter

Having regular discussions with the Named Persons and animal technicians within the facility to review current approaches and whether there are any new 3Rs opportunities and subscribing to the internal 3Rs enquiry list.

Attending NC3Rs events and workshops

3Rs resources are available on the University's in vivo SharePoint site. Frequent visits to the National Centre for the Replacement, Refinement and Reduction of animals in research's website. The following websites are also consulted for practical guidance Laboratory Animal Science Association (LASA), Jackson lab IMSR (international mouse strain resource) repository http://www.findmice.org/participate, LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery and the Royal Society for the Prevention of Cruelty to Animals (RSPCA).

We also actively discuss and implement new 3R's initiatives and run a yearly 3R's competition, sharing information globally across different establishments. We actively set annual refinement goals each year within the department.