Home Office

NON-TECHNICAL SUMMARY

Animal models of fibrotic 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

Fibrosis, Liver, Kidney, Experimental therapeutics

Animal types	Life stages
Mice	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 provide a demand-led service for the delivery of new treatments for patients with fibrosis. This project will test the efficacy of antifibrotic drugs in validated rodent models, to refine and optimise the use of therapeutics for the treatment of fibrosis in humans. The data generated from our studies will impact the drug development process, support new drug applications, and allow drugs to progress to the clinical trials.

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?

Fibrosis occurs in response to injury when the tissue repair response becomes uncontrolled, leading to thickening and scarring of the tissue, and organ dysfunction over time. Fibrosis can affect any organ, including, skin, lung, liver and kidney. It is a major cause of death worldwide and it is often seen in chronic inflammatory disorders, such as metabolic syndrome and cardiovascular disease. Unfortunately, to date, only two drugs are available for the treatment of lung fibrosis, and no treatment has yet been approved for liver or kidney fibrosis.

Despite substantial progress in our understanding of the nature of fibrosis, a gap remains between the identification of antifibrotic targets and conversion of this knowledge into effective treatments in humans. Therefore, our preclinical data will pave the way to predict successful clinical trials and help pharmaceutical companies decide which drug(s) to develop.

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

This project will provide data from various pre-clinical models to biotech and pharmaceutical industries, to identify those compounds most likely to have the greatest clinical potential and highlight those with limited efficacy results.

The drug development in the field of fibrosis remains limited, hence offering this work as a service, represents an extraordinary opportunity to yield new discoveries and drive a new era of precision medicine in the treatment of chronic fibrotic diseases. In the long run, the project's potential outputs are human health improvement, thus reducing the burden on healthcare.

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

The principal benefit of this project is to identify promising next generation treatments for fibrotic disorders benefiting patients with multiple fibrotic conditions. The short-term benefit of the data generated from studies is to facilitate the drug development process. In medium-term the data generated is used to support new drug applications, and the long-term is to enable drug treatments to progress to the clinical trials.

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

To maximise the outputs of this work, we will share the results of the project through publications and preprints when appropriate and possible, in line with the establishment policy for the use of animals in research. We will also look into building new relationships, both in the field of fibrosis and beyond, as the knowledge generated through this project can be easily applied to other diseases.

We have an extensive in-house experience of drug testing *in vivo*. We can provide to our commercial sponsors our in-depth knowledge of fibrosis and *ex vivo* tissue analysis to identify biochemical markers that better correlate with disease outcome. This will maximise the quality of the data and provide a broad census of cells involved in fibrosis. Crucially, this data will support our sponsors with increased diagnostic accuracy and prognosis in clinical trials.

Species and numbers of animals expected to be used

- Mice: 2400

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 programme requires that models are used, which closely mirror the human biological system and can capture the potential therapeutic effects on fibrosis and inflammation. Adult mice are suitable for these studies as the work cannot be conducted in lower vertebrates, invertebrates, or cell lines due to the poor resemblance of these options to the clinical setting.

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

The bulk of the animals in this protocol will undergo at least one or two (100%), or up to four (50%) of the following procedures listed below:

1. Animals will be obtained from established providers and acclimatised for at least 1 week before studies are initiated.

2. Animals will be treated with agents that cause liver and kidney fibrosis. For all models, animals will be monitored daily and weighed at least 2-3 times a week, unless more frequent weighing is warranted.

3. Test Item (or the same solvent without any drug, if using controls) will be administered via a known route, and at a volume, concentration and schedule that is known to be well tolerated.

4. Blood samples may be collected periodically via a tail vein bleed in conscious animals. We will always follow the NC3Rs guidelines/limits for peripheral blood sampling.

5. We may collect urine samples so that we can measure changes in kidney function associated with fibrosis (e.g., presence of different chemicals in the urine and urine volume). We will employ the most refined method of collection of urine, to include urine droplet, but where collection over 24 hours is required, mice may need to be kept in 'metabolic' cages.

6. In some instances, tissues from animals may be collected for studies in the laboratory (*ex vivo* studies) under terminal anaesthesia.

Fibrosis of the liver may be induced by injection of carbon tetrachloride into the abdominal cavity. This chemical is metabolised in the liver and converted to a highly reactive form, that causes liver damage, resulting in inflammation and fibrosis. This model of advanced chronic liver disease is one of the most widely performed and can also be used to study fibrosis regression as the damage caused by the chemical is partially reversed when exposure stops. Mice may be given a high fat diet prior to chemical exposure since liver fibrosis often overlaps with obesity. This model is useful to study toxic-mediated liver fibrosis and mimics acute liver injury in humans.

Non-alcoholic steatohepatitis (NASH), is a condition where fat accumulation causes liver inflammation and scarring (fibrosis). In order to mimic this disease, mice may be fed with a nutrient-deficient diet (choline- and L-amino-acid deficient), high-fat and cholesterol diet for a period of 12-16 weeks. Control mice will receive a matching diet. This model is of great significance to understand how lifestyle and nutrition play a role in the development of fibrosis.

Renal fibrosis i.e., fibrosis of the kidney, may be induced by feeding animals a diet rich in adenine for 2-4 weeks. This model allows the study of mechanisms involved in renal dysfunction, similar to humans with chronic kidney disease. This model is relevant to understand renal fibrosis since inflammation and fibrosis markers can be detected during the progression or resolution of the disease when the diet is switched back to normal.

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

Like humans, animals can sustain fibrotic injury for a long period of time with no apparent symptoms. In the rare scenario that an animal shows signs of organ failure we will ensure that the animal does not exceed the severity limits set out in this project. Previous experience has shown that these procedures typically induce mild to moderate adverse effects:

- Animals will experience stress due to restraint and transient discomfort from needle insertion and/or anaesthetic injection or inhalation of gaseous anaesthetics (100 % incidence).

- Repeated intravenous injection can result in irreversible damage to the vein and very occasionally, a haematoma/bruising may develop.

- Intraperitoneal injection (through the abdominal wall) is likely to be painful if the needle injures an abdominal organ or if the substance being injected is an irritant.

- Oral administration (gavage) is associated with minor discomfort. Very occasionally damage to the oesophagus may occur or substances may enter the lungs resulting in difficulty in breathing.

- Slow-release pellets and osmotic minipumps are small devices implanted under the skin of the mouse and cause minimal effects. Animals may experience a lowered body temperature during anaesthesia required to implant these devices. Animals may experience pain on recovery from anaesthesia.
- Animals will experience stress due to restraint and a transient discomfort from blood collection (100% incidence). Animals may become overheated or dehydrated from spending too long in the hotbox.
- With all surgical procedures there is some risk of development of infections or wound complications (<1%).
- Animals fed with high-fat diet will develop obesity (up to 100%) and greasy coat (up to 100%), which may lead to over-grooming (~25%) and as a result possible skin inflammation/ulceration and infection (expected incidence of <5%).
- For a diabetic animal, if diabetes is ongoing, diabetic kidney disease, and high blood sugar will occur (up to 100%). For an obese animal without diabetes, weight gain can result in hypertension and respiratory depression.
- Animals with liver or kidney damage may lose 10-15% of body weight (up to 70%).
- Mice with kidney disease will show proteinuria (high quantities of protein in the urine) (100%).
- Mice with liver disease could show abdominal swelling, although this is rare and extremely variable.
- Animals may show cardiovascular or gastrointestinal system dysfunction (<10%).

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)?

Given the controls in place, the expected severity for animals under all protocols under the licence is moderate and may be experienced for up to 100% of animals used.

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?

Fibrosis is a highly complex process. Producing a non-animal alternative in the lab, which replicates all aspects of fibrosis, is currently impossible. Due to the complexity and ever-changing physiological environment during fibrosis, the use of mice is necessary as the tissue architecture plays an important role in influencing this process. Currently, it is inconceivable that we will be able to generate computer models that will allow us to study the cell-cell interaction in an ever-changing three-dimensional structure that is required for this study.

The animal models proposed for this study, have been carefully selected to allow effective drug development and reduce animal pain. Although these models are specific for kidney, and liver, other organs such as pancreas, gut, heart, bone marrow, and skin can be analysed in one model. Therefore, this approach can address the current scientific need and minimize the number of animals required in each experiment.

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

Non-animal alternatives such as drug testing on liver organoids and *in vitro* assays using fibroblasts, hepatocytes or cell co-cultures are being successfully established in the lab. We have invested heavily in *in vitro* model systems over the last years, purchasing new analysis platforms for the laboratory and fostering collaboration with other laboratories.

This technology has been considered for initial drug screening, which may help to partially replace the number of animals used. We have focused on developing 3D cultures, derived from tissue samples obtained from a range of species, including mouse and human. These cultures allow us to look at how drugs directly affect the digestive system and help us identify potential novel treatments.

Why were they not suitable?

Liver and kidney fibrosis alternatives from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) will be considered. However, cells studied *in vitro* do not offer a true reflection of inflammation and fibrosis that animals can provide. Furthermore, common variations in culture conditions can have large effects on cells or organoids and have to be taken into account when designing experiments and comparing results. Therefore, the use of animal models is vital to facilitate disease study and subsequent drug screening.

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?

Data from previously published studies has been used to estimate the total number of animals in each protocol. To obtain the desired effect, the number of animals estimated has been calculated based on the accumulation of type I collagen. Where possible we will make use of organoids or archived tissue to reduce animal use.

Therapeutic compounds studied under this licence will have demonstrated *in vitro* efficacy and will have some supporting toxicological data. Only those compounds showing reasonable activity and specificity for their intended target will be considered for animal testing.

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

I have greater than 5 years of experience using mice as a model system to study fibrosis. I have used my experience together with statistical advice and online tools such as the NC3R's to minimise the number of animals used for testing our hypothesis. Furthermore, prior to the experimental design, I used the 3R's site (www.3rs-reduction.co.uk), the PREPARE guidelines (https://norecopa.no/prepare) and had input from statistical experts to minimise the animal used while ensuring high reproducibility.

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

Academic experts and independent companies are consulted to ensure the rigour of our experimental design and analysis. We are promoting multi-user studies to improve the efficiency of animal usage. We will stay informed about advances in the 3Rs (reduction, replacement and refinement), and implement these advances effectively during the project.

We will also have a fibrosis database to ensure that every organ collected will be recorded together with the relevant information (e.g., experiments conducted, age, sex) to ensure that it can be used when needed. In addition, the tissue collected can be used for pilot experiments. Furthermore, we will encourage clients to also share and publish negative results to avoid unnecessary duplication of studies. Together, this will reduce the overall usage of mice in our experimental procedures while sharing the information with everyone in the university when possible.

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.

Mouse models of fibrosis are well-characterised and demonstrate broad similarities with human disease, regarding symptoms and fibrotic changes. The models included in this project have been

selected based on the disease origin. Furthermore, the least damaging models with appropriate translation into human disease are being selected.

There are other models available such as the rodent cafeteria diet (feeding animals a choice of food items to stimulate energy intake) or nutrient-depleted diets (e.g., methionine- choline-deficient diet), but they have not been included in this licence application due to insufficient fibrosis or excessive body weight loss, respectively.

Surgical fibrosis models have not been included due to severe adverse effects and distress. Instead, more refined, and less harmful models have been selected in the interest of maintaining relevance to human pathology.

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

Mouse models are well-characterised and demonstrate broad similarities with human disease, regarding symptoms and fibrotic pathological changes, which non-mammal species do not adequately display.

Animal models have facilitated many discoveries that translated to humans, such as antifibrotic drugs and metabolic regulators of liver health. Examples are the glucagon-like peptide-1 (GLP-1) analogues, the farnesoid X receptor agonists (FXR) and the dual chemokine 2 and 5 receptor antagonist (anti-CCR2/CCR5) that currently are in development for the treatment of liver fibrosis (published studies).

Decades of research have provided substantial standard techniques to evaluate fibrosis in rodents, making mice the most suitable species for the studies proposed for this project.

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

All animal handing and care will be conducted in a manner that minimise stress whenever possible. Other welfare measures will be:

- Gavage: We use specifically designed gavage tubes, and may pre-coat our gavage needles with palatable lubricant, which has been shown to reduce the stress associated with the procedure and reduce gavage time.
- Injections: Repeated injections will be not performed on the same site. We will alternate between the right and left side to reduce pain and discomfort. Furthermore, localised administrations and manipulations will reduce the adverse effects that are typically associated with systemic manipulations where multiple organs and cell types are affected at the same time.
- Refinement can also be achieved in the type of mice we used. Animal genetic background will be considered to minimise harm. Very sensitive mice strains to the fibrosis model will not be used.
- Special care will be given to all animals after a surgical procedure or prior to any animal experimentation where they will be gently handled in order to minimise stress caused by the procedures. This will include increased monitoring after any surgical procedure which includes

post-operative care and analgesic if required. In addition, we aim to reduce the duration of all surgical procedures.

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

To ensure experiments are conducted in the most refined way, we will follow the Animals (Scientific Procedures) Act 1986, the Joint Working Group on Refinement 'Refining Admin of Subst' Lab Anim 2001, the PREPARE Guidelines, and the non-technical summaries found in: https://www.gov.uk/guidance/research-and-testing-using-animals. We are keeping up to date with the literature within the field of pre-clinical fibrosis research and are actively looking to implement refinements of our models.

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

We have signed up for newsletters from FRAME, NC3Rs, and www.nlm.nih.gov/toxnet, a cluster of databases on toxicology & hazardous materials, to support our research plan, reduce animal numbers and refine the use of toxic compounds to induce fibrosis. The NORECOPA portal and the PREPARE guidelines will be regularly consulted for design planning.