



Home Office

NON-TECHNICAL SUMMARY

Modelling fibrosis in multiple organs to understand 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

Key words

Scar, Fibrosis, Diagnosis, Therapy, Chronic disease

Animal types

Life stages

Mice	adult, juvenile, pregnant, neonate, aged
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Rats	adult
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Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 greater understanding of how scarring (or fibrosis) occurs in chronic diseases that can affect any organ in the body. In some instances fibrosis can even lead to multiple organ failure and/ or cancer. The goal is find better ways of diagnosing fibrosis early enough to reverse the disease process and develop new medicines to reduce or remove the scar and improve disease outcome.

A retrospective assessment of these aims will be due by 05 April 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

Fibrotic diseases (or scarring) are increasing and a major cause of death worldwide. In some cases, end-stage diseases can be treated by transplantation; however, there is a huge shortage of donor organs; significant side-effects from medicines (to suppress rejection by the body); and focus on end-stage disease is too late. Urgent development of novel diagnostics to determine stage of disease and anti-fibrotic medicines are needed. This requires a better understanding of the underlying process of fibrosis to develop hypothesis based approaches to newly identify factors that are present specifically during certain stages of disease, allowing highly targeted strategies for therapeutic intervention.

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

We anticipate new information will be published in scientific journals. However, we also expect some experiments will lead to novel discoveries for patient benefit. We hope much of our work will lead to new products (such as diagnostics) or for development as new treatments to improve scarring in disease.

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

Short term benefits of this project will be to the broad scientific community investigating chronic disease with new data on how fibrosis occurs. Long-term benefits will see our data being applied to health care as has been the case already for our studies. We have recognised that the transition from discovery in cells, through to animal models and toward human / patient benefit can take up to 10 years. However, we have found this to be a reality and our work is already being applied as novel tests to diagnose liver disease in the clinical setting. Our goal is to ensure all organ fibrosis we investigate as part of this project is eventually translated to human disease and patient benefit.

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

We collaborate broadly with the national and international scientific community. This will continue with personal dissemination of knowledge. More broadly our work will be published in high quality scientific journals as happens now to capture the widest readership. Even where our work is at preliminary stages, we present this at scientific meetings (with published abstracts) meaning that even approaches that may not be successful in our hands will be of use to the scientific community in this area. Through progressing our work for patient benefit we are also engaged with patient and public involvement (PPI) groups currently for liver fibrosis, lung fibrosis (including from COVID19 infection) and multimorbidity fibrosis (affecting any organ).

Species and numbers of animals expected to be used

- Mice: 30450
- 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.

This project intends to use rats and mice. For experiments with a primary focus on the use of ex vivo tissues or cells, the use of rats will be considered where this will significantly reduce the number of animals needed to generate sufficient material. The ability to use material originating from a single animal across several different conditions will provide increased statistical power to experiments by limiting variability and allowing paired approaches to analyses. Moreover, the use of rat where possible provides a closer genetic link to human - i.e. rats share more DNA in common with humans than mice do, so represent a better match. However, the use of mice is unavoidable as it provides a well-recognised species for work involving genetic alterations and there are standard protocols, methods and reagents used that have been optimised for this species with acknowledged benefits for use. As an adult disease - we will use adult animals to induce fibrosis by various methods depending on the organ under study.

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

In any organ, fibrosis (or scarring) occurs through tissue damage. Depending on the organ under study, this will be achieved through injections or inhalation of tissue damaging compounds, through dietary changes or by surgical procedures. To understand the initial phase of disease duration of experiments may only last up to 72 hours. However, to understand the long-term progress of disease experiments may last several months. For example some chronic fibrosis models can take 12 weeks to fully replicate the human disease and for progression to cancer (which can occur in the liver) this can take up to 60 weeks.

In some cases, where we have discovered new ways to treat and improve fibrosis, we will use medicines to treat the animals with fibrosis. From our previous experience this usually takes place in the middle of the disease model so that we see the real effect of medicines in halting or reversing the scar.

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

In most instances, following completion of each project animals will be humanely killed and tissue from animals will be removed for studies in the laboratory. In some instances, animals will be treated with agents that cause fibrosis. Although transient discomfort may occur at the time of administration the animals appear normal soon afterwards. Similar to 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 the animal will be humanely killed to ensure the animal does not exceed the severity limits set out in the project. Some animals will undergo surgery to induce fibrosis, but these are not life-threatening procedures. For surgical induced models animals receive post operative care including pain reducing agents. For some chronic disease models associated with scarring, the most common adverse effect is weight loss usually toward the end of the experiment. However these are typically within the shorter time periods of experiments (up to 2 weeks) and generally, aside from this, animals typically appear well. In lung fibrosis, shortness of breath can occur but is unlikely in the timescale of our experiments (up to 28 days). For the liver, liver fibrosis commonly leads to liver cancer in human. We have an experimental model to investigate this in mice using an injected chemical. These animals will develop tumours specifically in their liver typically towards the end of the experiment.

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

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

The fibrosis models have been selected as they are generally well tolerated in rodents. The expected overall severity for the majority of this project is moderate and animals will be closely monitored for any adverse affects.

Approximately 50% of rats and mice involved in this project will experience mild severity limit, 40% moderate severity limit and 10% severe.

What will happen to animals at the end of this project?

- Killed

A retrospective assessment of these predicted harms will be due by 05 April 2027

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

Despite progress in understanding the biology of scarring (fibrosis) in chronic diseases, many discoveries have been unsuccessfully translated for patient benefit.

In addition, fibrotic disease and cancer are complex processes that progress and resolve over many weeks. This involves not only the affected organ, but also the immune system, production of factors that promote the disease process and cell-cell interactions during the periods of tissue damage and repair involved in fibrosis. For this reason, it is not possible to study these events in an in vitro/ ex vivo system as studying various cell types in isolation in vitro (cells / tissues in a dish) would not provide us with any insights into complex cell interactions within the body system during fibrosis.

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

There are several possible non-animal alternatives that we have considered. These include:

- Established cell lines as a useful starting point for assessing the efficacy and tolerability of therapeutic interventions and genetic manipulation. These can also be used in initial molecular studies prior to animal work.
- Human tissue obtained from people undergoing invasive diagnostic sampling (e.g. biopsy tissue). Tissue can be used as a pre-screen prior to animal studies.
- Computer modelling using published datasets.

Although some aspects can be investigated directly in cells and tissues, unfortunately we cannot understand fully the complex process of fibrosis. Current models in human tissue are typically limited to taking the tissue or specific cells into a dish. We use this but it is very limited to a short time frame and cells/tissues rapidly lose function when taken from the body.

Moreover, where specific genes have been identified as critically important in driving the disease process we are unable to manipulate these well enough in the currently available systems in human.

Despite these limitations with the current non-animal alternatives, where possible we will use the above as partial replacement as much as is practically possible

Why were they not suitable?

Immortalised cell lines deviate from the normal behaviour of cells over time and are therefore not a robust model in isolation. They also do not capture the complex interplay between different cell types. Where appropriate, preliminary investigations will be performed using established cell lines in vitro before progressing into use of primary cells or tissues ex vivo and through to in vivo studies.

The currently available models that use humans cells or tissues cannot fully recapitulate the whole body environment involved in disease progression. This involves a complex interaction of different cell types and the damaged tissue / scar environment. The models are also short lived so do not replicate the process involved in fibrosis which occurs over many years in human.

We will continue to ensure all animal studies and discoveries are translated to human tissue as we already have in the past.

A retrospective assessment of replacement will be due by 05 April 2027

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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 have carried out statistical calculations for our models and have a great deal of experience having published in peer reviewed journals of the numbers required to ensure significant results are produced using both in vitro (cells in a dish) and in vivo (in the animal) experiments. For the majority of our work this typically requires 5 animals in 4 groups (to include control measure for both those undergoing fibrosis induction).

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

In designing our project we have used expertise from statisticians who are part of the NC3R's Experimental Design Assistant (EDA). For any new models that require experimental design we will seek similar advice and in addition consult the NC3Rs EDA.

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

From our current (and previous) project licences we have gained a lot of experience and information to instruct our experiments. This includes detailed calculations and real experimental outcomes for number of animals required. In many cases we have archived control tissue from previous experiments so we can reduce animal numbers by only carrying out the treatment (for example only inducing fibrosis). For many of our experimental models we can only use male animals (many female animals are protected from fibrosis in several organs). However, in this broad project we can now use females for many of our experiments including all cell preparation and even some in vivo fibrosis experimental models. Where possible we make use of human cells and tissue to further reduce the need for animals. In addition, we are very well organised as a group and routinely make use of several tissues from one animal such as liver, lung, kidney and heart. These structures already in place in the group ensure we limit the number of animals used for this project.

A retrospective assessment of reduction will be due by 05 April 2027

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

In the case of cellular studies, we will use rats as this allows a greater analysis of the mechanisms associated with the disease process compared to mouse, due to closer genetic proximity to human. However, for in vivo studies, mice will be necessary based on the use of genetically modified strains.

To investigate the therapeutic potential of our findings in fibrotic disease in different organs from multiple insults, it is necessary to use more than one model of injury. We have chosen established models of organ fibrosis that have good comparison with the human disease and have been refined over many years in labs worldwide. In all cases the models used are well tolerated.

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

The experiments proposed will use the simplest possible animal system. The mouse and rat are the only systems we can use as they have good replication of the human disease process (induced by fibrosis) and the mammalian system provides all cellular interactions (for example with an intact immune system and endocrine system). Moreover, the mouse is the only mammalian system that allows broad genetic manipulation of specific discoveries as potential therapies in disease. The use of terminally anaesthetised animals would not allow the course of disease to be studied.

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

As evidence of limiting animal experimentation through refining our models, improved technical skills and post-operative care we have reduced the mortality of a surgical model to induce liver fibrosis (bile duct ligation) from 30% to ~10-15%. We have also successfully implemented warming cabinets into any post operative care regimes to greatly improve welfare from any surgery induced fibrosis. We will seek to refine all other protocols wherever possible (most are much less severe). Such measure have included adding non-invasive monitoring of lung function during models of lung fibrosis to ensure animals welfare.

We are also part of an extensive national fibrosis network on broad organ fibrosis where all involved are open to discussion over how to improve methodologies in practice. This has often resulted in shortened experiments in recognition that the same outcome can be achieved in a much reduce time span. This will continue and is an excellent platform.

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

Prior to any animal experiment, details are described in detail and discussed with our unit to ensure best practice is taking place within the licence. We have standard operating procedures in place for complex experiments such as those requiring anaesthesia and surgery. We will follow LASA guidelines (<https://www.lasa.co.uk/>) including animal welfare, administration of substances and aseptic technique. We will also ensure our personal research areas are up-to-date through monthly literature searches to refine any experimental models we use.

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

Our unit provides weekly newsletters that include updates from the NC3R website. This includes specific insight into any specific areas researchers have an interest in. However, we will also ensure we consult the 3Rs website regularly for any advances relevant to our work that can be implemented. This is a requirement for any individuals carrying out animal research.

A retrospective assessment of refinement will be due by 05 April 2027

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?