Home Office

NON-TECHNICAL SUMMARY

Regulation of Basement Membrane Function in Health and 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

Key words

Kidney disease, Basement membrane, Glomerulus, Alport syndrome, Therapy

Animal types	Life stages
Mice	embryo, neonate, juvenile, adult, pregnant
Zebra fish (Danio rerio)	embryo, neonate, juvenile, adult, pregnant

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 overall focus of this PPL is to define the mechanisms that regulate the function of basement membranes in health and disease with a primary focus on kidney physiology and 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?

Basement membranes are sheet-like structures which line most tissues in the body and separate different types of cells. Basement membranes help to maintain the shape of tissues in the body and regulate different cell functions. We found that basement membrane defects in mouse kidneys occur before there is evidence of kidney disease and we propose that these early defects start a process that leads to kidney scarring. Diseases that cause kidney scarring can lead to chronic kidney disease, which affects 10% of the world population and there are no curative treatments. When kidneys fail, renal replacement therapy with dialysis or transplantation is necessary but costs are escalating and replacement therapies are not universally accessible. Strategies to improve early detection of chronic kidney disease and targeted therapy to prevent disease progression would have significant impact on improving human health. We aim to investigate how basement membranes are maintained in health and affected in disease. The primary focus will be on the kidney, but we also aim to study other tissues and organs to understand overall basement membrane regulation. We will conduct studies in human cells in culture, where we can investigate how basement membrane components are produced, but we are not able to properly test their function. Since it is necessary to understand how basement membranes function in the body, we will conduct *in vivo* studies in parallel using mouse and zebrafish.

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

We will disseminate our research findings in a variety of ways to reach broad audiences. For academic audiences we will publish in peer reviewed journals and to preprint servers to enable rapid access to our results. For clinical, academic, and commercial audiences, we will present our work at relevant conferences, both in person and with virtual presentation. We have established an online resource for basement membrane research, and we will continue to maintain this resource and post relevant findings from our studies. For patient and public audiences, we will maintain our strong connections with patient organisations and present at our public programmes events at the establishment.

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

This research programme aims to identify basic mechanisms of basement membrane regulation. It is anticipated that in the medium term, this will enable the identification of early biomarkers of basement membrane damage, which will eventually contribute to the development of new therapies and enable early intervention to rescue basement membrane damage in multiple tissues and organs. This could impact the early detection and treatment of diseases that are associated with basement membrane damage, such as genetic and acquired kidney disease. The knowledge and understanding generated by this work will benefit kidney researchers and will also have wider relevance for researchers studying basement membranes in other tissues. The work will also benefit clinicians by improving understanding of basic science, and it will ultimately benefit patients with kidney and other diseases characterised by basement membrane defects. It will also have impact on commercial organisations seeking to develop therapies for kidney disease and other researchers working in this field over the next 5 years.

The genetically altered mice we use in our experiments have a knockout of the Col4a5 gene. Defects in the COL4A5 gene are the most common cause of Alport syndrome, which causes kidney failure and deafness. Using our mouse model to study how this defect in Col4a5 causes disease, and to test possible therapies, has the potential to improve the detection and treatment of patients with Alport syndrome.

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

We have a good record of publication and aim to publish all research that could be of benefit to others. To increase the impact of our research we collaborate with groups across the world and we will continue to maintain these international collaborations. We will also publish negative results as these are important for progression in research. These can be published in a range of journals as short data notes or datasets.

Species and numbers of animals expected to be used

- Mice: 1,500
- Zebra fish (Danio rerio): 3,000

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.

In humans it is not possible to perform the mechanistic studies or the in depth analyses of disease tissue that I propose in this application. Mice and zebrafish will therefore be used in these studies as they are the lowest species in which suitable models of kidney disease exist. The genetically modified models across the lifecycle that we will use recapitulate many features of the human equivalents and therefore will provide valuable insights into mechanisms of basement membrane disruption during kidney disease. We have established new human cell systems including kidney organoids to

investigate our research questions. These new tools will reduce our use of animals in this programme of work.

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

Mice:

The severity of kidney disease can be measured by testing the urine and the blood of mice, and so in each study we will take samples from the mice to measure kidney function. Blood pressure is also affected by kidney function, so this will be measured either by a tail cuff, or by surgically inserting a small probe into the neck of the mouse while it is anaesthetised. In some mice kidney injury will be induced by removing kidney tissue surgically while the mice are anaesthetised, or by administering substances to cause injury. We can then study the changes that have occurred in the injured kidneys versus healthy control mice, which will help us to understand which therapies and treatments might work to prevent, slow down, or treat the kidney damage. We will also test therapies and use imaging techniques to visualise the changes in tissues. In experiments when the interventions are mild or moderate, we will follow the progress of mice. Should signs of ill health become apparent, the animal will be killed by a humane method.

Zebrafish:

We will genetically modify zebrafish so that they express genes which are 1) relevant to kidney diseases and 2) allow us to measure kidney function. We do this by injecting the zebrafish with chemicals and proteins, or by performing surgeries on the zebrafish. Injections and surgeries are conducted while the fish are anaesthetised so that they cannot feel any pain. When we edit genes, we also add fluorescent proteins which allow us to visualise what has changed in the kidney or the basement membranes of the zebrafish. Once the gene editing is complete, we can breed these genetically altered zebrafish and use the offspring to test potential new therapies which may treat kidney disease and basement membrane dysfunction. Any signs of ill-health in the zebrafish will result in the fish being humanely killed.

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

Since we are investigating early stages following injury or disease onset the animals will not have severe phenotypes, however some of the procedures we carry out can cause pain, discomfort or stress to the animals.

Mice:

Almost all of the mice we use in our studies will experience ear-punching, where a small amount of tissue is clipped from the ear. This procedure causes momentary pain and discomfort, but has not been shown to have any long-term effects on animal wellbeing.

Surgery to remove kidney tissue or to place a probe that measures blood pressure will cause short-lived post-operative pain and discomfort, but once healed should not have adverse effects on the mice. The removal of kidney tissue will affect kidney function clinically but will be asymptomatic to the mice. Some mice will have a telemetry device surgically implanted to allow blood pressure measurements.

These mice will experience post-operative pain and discomfort which will be minimised as much as possible using pain relief.

Mice administered drugs via oral gavage will experience mild stress and discomfort during the administration procedure, but they are able to quickly recover. For urine collections, mice are housed separately from their cage mates for up to one hour. During this time, they will experience mild stress due to isolation, though this is resolved once the animals are returned to their home cage.

Fish:

Fish will be anaesthetised prior to receiving injections or undergoing imaging sessions. The fish may experience mild stress due to the addition of the anaesthetic agent to the water, but are expected to make a full recovery. Fish will also be anaesthetised prior to surgery, which may cause mild stress. Analgesia is used peri-operatively. Sterile technique will be used to minimise the chance of infection which could cause pain.

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 project has a ‘Moderate’ severity level.

Mice:

Subthreshold 50%, Mild 30%, Moderate 20%, Severe 0%

Zebrafish:

Subthreshold 50%, Mild 30%, Moderate 20%, Severe 0%

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

- Killed
- Used in other projects

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?

We aim to determine whether therapies can prevent or reduce the effects of diseases associated with abnormal basement membrane function. We have experience of using human cells to study mechanisms of disease. In addition to standard cell culture, we have also developed organoid cultures

as a more complex culture system, which mimics an in vivo environment more closely than standard cell culture. Although these studies are improving options for replacing animal studies, they still have limitations. Therefore, for our research to have greater impact on human health, it is necessary to use animal models for realistic preclinical experiments. The administration of treatments to whole animals will ensure that we can detect any side effects on other organs. The use of zebrafish will allow us to perform early screening experiments in embryos and therefore replace the need for some experiments in older fish or in mice.

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

Where possible, cell culture models using human embryonic kidney (HEK) cells and kidney organoids will be used in studies as an alternative to animals.

Why were they not suitable?

Cell culture models are a tool we use alongside our animal models, however cell culture models alone provide limited insights into mechanisms of kidney disease and response to therapies. Cell culture models enable us to study cell interactions and basement membrane production, but animal models are required to study the effects of therapies on organ function.

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 estimated the above numbers based on our work over the past 5 years and the anticipated increase in our work.

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

Sources of variability will be considered at all stages of the experimental design. For mice we will consider the genetic background and sex of the animal carefully when designing the experiment and choose animals that are appropriate to address the specific research question for a particular experiment. We will also consider the variability of experimental observers and where possible will allocate one observer to each animal experiment. We have experience of using the NC3Rs Experimental Design Assistant and we review the relevant literature to find data on effect sizes of interventions. To minimise the numbers of animals in our experiments we use a factorial experimental design approach with replication of optimised conditions.

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

We will regularly review our breeding strategies to ensure efficiency and we will consider strategies to reduce the number of animals overall by good experimental design. To do this systematically we have established a monthly animal research group meeting in my group. We also routinely perform pilot studies when we are using testing a new intervention. An initial sighting/pilot study with a small number of mice per cohort (<5) will enable estimates of variability, interaction effects and main effects that will be used in a subsequent formal statistical power calculations to determine sample sizes for contrasts of interest. Data analysis will be conducted according to a pre-specified statistical analysis plan drawn up in conjunction with statistical consultants. Important experimental results will be repeated or validated via an alternative follow-up experiment to minimise the likelihood of spurious nonreplicable results. We will continue to share tissue with other research groups.

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.

At present, the mouse represents the best or most refined species with which to test the efficacy of new therapies for basement membrane-associated kidney disease. It has a kidney of similar structure and anatomical complexity to human organs. However, our inclusion of zebrafish studies will allow us to refine the number of studies in mice and the nematode C. elegans is also a suitable in vivo model for studying basement membranes. Our experiments are proposed in mice during development and after birth when they will be closely monitored. Particular attention will be paid to their weights and behaviour. Should these parameters deviate markedly and/or persistently from normal, mice will be humanely killed. For all experiments in animals, we will use good experimental conduct with the appropriate use of peri-operative analgesia for surgical interventions and the appropriate species-specific management of animals during and post anaesthesia.

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

Where the scientific aim can be achieved using less sentient animals, such as zebrafish embryos instead of zebrafish, the less sentient animals will be used.

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

If we make changes to procedures we will ensure that these are approved for implementation and then we will increase monitoring, post-operative care, pain management, and training of animals as

appropriate.

Currently, the refinement procedures we have in place include: Use of peri-operative analgesics, frequent observations during post-operative periods, rotation of blood sampling/injection sites, altering cage enrichment tools (i.e. using tubes with larger entrance holes) to reduce risk of skin catching/rubbing post-operatively, and coating gavage tubes in sucrose solution.

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

There are no best practice guidelines for the use of animals in kidney research, however within an international group hosted by the International Society of Nephrology, I am preparing guidelines for the use of animals in kidney research which uses the PREPARE guidelines as reference. We expect to publish the guidance document in 2023.

For general best practice we will follow the Working Group refinement guidelines, 2001.

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

We will attend seminars hosted within our establishment and interact with the international research community and the published literature to keep up to date with practices that could improve our application of the 3Rs. We will regularly review updates in our monthly animal research group meetings.