Home Office

NON-TECHNICAL SUMMARY

Mechanisms and treatment of pulmonary vascular 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
- (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

Cardiovascular, Endothelium, Genetics, Therapy, Pulmonary hypertension

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant
Rats	adult

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?

This project aims to determine the mechanisms of genetic forms of a rare disease, pulmonary arterial hypertension (PAH). We will use this knowledge to develop and test new treatment strategies for pulmonary arterial hypertension and other related conditions.

A retrospective assessment of these aims will be due by 04 September 2026

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?

Pulmonary arterial hypertension is a rare disease with a relatively poor chance of survival once the disease is diagnosed. Despite the availability of existing licensed treatments, the average rate of death at 3 years after diagnosis is 40%. We aim to develop new approaches to treatment that target the pathways identified from human genetic studies. Targeting pathways identified from genetic insights are more likely to lead to successful treatments that improve survival as well as symptoms. The use of rodent models of PAH, including genetic models, provides important preclinical proof-of-concept for these new treatments.

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

Outputs from this project will include new knowledge, which will be captured in high impact publications in internationally recognised scientific journals. In addition, we will provide proof of concept for the use of new drug approaches in pulmonary arterial hypertension.

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

The major beneficiaries of these outputs will be scientists, clinicians and patients with PAH. In the short term, validation of human genetic findings in genetically modified animals provides important evidence for a causal role of mutations identified in patients with PAH. This evidence will support genetic testing and counselling advice to patients. In addition, in the longer term, drugs shown to be effective in our project can be advanced into the clinic for testing in patients with PAH.

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

We will disseminate new knowledge and findings by publishing our results in internationally recognised scientific journals. In addition, we will publicise our outputs via the communications teams of our institute and funders. We will also use social media to publicise our outputs. We will attend meetings of PAH patient groups and disseminate our research findings in their newsletters. We collaborate widely with international experts, when necessary. This is particularly important when working on a new gene or pathway that might be unfamiliar to us. We collaborate with commercial partners when possible to hasten the translation of our findings into the clinic for the benefit of patients. For example, our previous work has led to a University spin out company that is developing bone morphogenetic protein 9 as a new treatment for PAH. Whenever possible we will publish and disseminate any negative research findings, for example via the Faculty of 1000 Open Research Platform.

Species and numbers of animals expected to be used

- Mice: 6000
- Rats: 900

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 adult rats and mice for the majority of our proposed studies. Typically, mice carrying human PAH disease-causing mutations only develop minor changes in their lung blood vessels at 3 months of age. We have found that mice at 6 months of age, or with some mutations 1 year, develop an age-related pulmonary hypertensive phenotype. Mice remain our preferred species for assessing the impact of disease-causing mutations on the disease-associated changes in the structures and responses of the lung blood vessels and heart, and we have adapted our invasive and imaging protocols for this species. For the study of non-genetic forms of PAH, the rat is a more robust model than the mouse. For example, the Sugen-hypoxia protocol in rats, representing the combination of a drug (Sugen) and low oxygen levels similar to those experienced by people at a high altitude (hypoxia), leads to the development of marked and sustained PAH and disease-associated changes in the lung blood vessels that, of existing rodent models, most closely resembles human PAH. Mice do not respond to the same

extent and often do not develop thickening of the inside surface of the lung blood vessel wall to the same extent as is seen in rats and humans. Therefore, the use of rats and mice is necessary and complementary to the objectives of this project.

In a small number of experiments, we may need to use young mice (5-8 weeks of age) that have already been weaned. This will happen with mice carrying gene mutations that only appear when a drug, such as Tamoxifen, is injected. This will be carried out in cases when we need smaller mice to test the effects of expensive drugs, or if we need to isolate lung cells, which grow more successfully if isolated from mice between 5-7 weeks of age.

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

Injections and procedures will be kept to a minimum to achieve the required results of the experiments. Pilot and pharmacokinetic studies will be performed on new molecules and drug candidates in order to determine dose levels and dosing frequencies required to assess efficacy. The most appropriate route of administrations will be determined by pilot and pharmacokinetic studies or taken from appropriate published literature.

Animals will be kept under anaesthesia for the minimum time possible to achieve the required results. Typically, the catheterisation procedure takes approximately 30-40 minutes to complete, depending on the severity of disease. While under anaesthetic animals will be maintained at an appropriate temperature by the use of a heat mat or similar heating device. Following aseptic recovery surgery animals will be kept in a pre-warmed recovery environment until fully recovered and freely moving.

Chronic hypoxia studies require the animal to be at a minimum oxygen concentration of 10% for at least 21 days to achieve the desired phenotypic response and for rats to develop the excessive growth of cells at the inside surface of the pulmonary blood vessel wall that narrows the vessel. Longer durations of hypoxic exposure up to 28 days may be required in some mouse strains. Following hypoxic exposure rats will be dosed for 6-8 weeks to determine efficacy of the molecule being tested. During the dosing period animals may undergo imaging in order to track disease progression.

Substances injected will be of known safe dose concentrations and administration routes. The administration of substances will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. Small molecule inhibitors and biologic reagents will be solubilised in solutions that have been tested and deemed safe.

Blood sampling via superficial veins (usually from the hind leg) will be kept to a minimum and safe volumes calculated based on estimated circulating volumes. Pharmacokinetic studies will typically require a single dose of a molecule or substance followed by a number of scheduled bleeds. The limits for the volume and frequency of blood sampling from laboratory animals, as defined according to Wolfensohn & Lloyd, 2003, Handbook of Laboratory Animal Management and Welfare, 3rd Edition and the National Centre for the Replacement, Refinement and Reduction of Animals in Research website, will be adhered to.

In a small number of animals, we will undertake pulmonary artery banding, where a small loop is tied around the pulmonary artery, between the right side of the heart and the lung, to reduce the flexibility of

the vessel wall and place some pressure on the right heart. In patients with genetic mutations, it has been observed that their right hearts increase in size, but do not become more muscular, so cannot cope with the increases pressures that are found in PAH patients. This leads to a more rapid failure of the right heart. In other models, where the lung blood vessels are also involved in the disease process, we cannot specifically study the changes in the right heart, or it's response to potential treatments. The pulmonary artery banding allows us to explore the response and effects of treatments on the right heart directly.

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

Genetically altered mice produced under this protocol are not expected to exhibit any harmful phenotype. Some animals will experience mild to moderate transient pain associated with an injection or blood sampling procedure.

From our previous experience, a subset of rats that are exposed to the PAH-inducing stimulus of a single injection of Sugen followed by 3-weeks hypoxia (Sugen-hypoxia) with subsequent maintenance in normal air during which a progressive pulmonary hypertensive phenotype develops may experience right heart failure. Right heart failure occurs rapidly without any obvious phenotypic symptoms. We have refined this model to minimise the number of animals experiencing this response.

In the pulmonary artery banding model it is anticipated that a small proportion of mice (<10%) will die under anaesthesia, during recovery from anaesthesia or within 2-3 weeks of surgery. Deaths under anaesthesia or during recovery from anaesthesia usually occur if the banding of the artery leads to very high pressures in the right ventricle leading to acute right heart failure. This will be apparent based on clinical signs during recovery from surgery and the animals will be killed by a Schedule 1 method if this is observed. Death in the period of 2-3 weeks after surgery may be due to the development of pericardial effusion. Animals will be monitored for evidence of clinical signs and will be killed by a Schedule 1 method if this is observed.

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)?

Overall, the expected severities in mouse studies will be mild. Some models may exhibit a moderate phenotype, but this is anticipated to be less than 5% of all mouse models. Mice on the PA banding protocol might experience a severe phenotype due to the nature of the surgery, though this is expected to be less than 1% of all animals.

Overall, the expected severities in rat studies will be moderate, primarily as the Sugen-hypoxia model is the model of choice with regard to relevance and refinement. The proportion of rats anticipated to experience a moderate phenotype is 25%.

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 04 September 2026

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?

Although we routinely use human cells and tissues, as well as molecular and biochemical approaches to achieve our research objectives, the biology of these systems is restricted compared to the complex compositions of the cell types involved in cardiac and vascular remodelling in the whole organism. For example, bone marrow-derived cells may contribute to the process of pulmonary vascular remodelling or protect against remodelling. It is not possible to incorporate the complexity of the animal situation into these cell based models. In addition, different regions of the lung may influence each other, such that there may be influences from the alveoli or lymphatic system on pulmonary vascular remodelling, and complex interactions between cell types in the vascular wall. Moreover, distant organs influence the lung vasculature via factors circulating in the blood. For example, changes in liver function influence the function of the pulmonary circulation, as evidenced by the occurrence of PAH in patients with liver disease.

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

We would emphasise that our animal studies are greatly informed by large scale human genetics studies in patients in PAH. Our animal research is conducted only when we have a very high degree of certainty of the importance and impact of the research based on observations from human genetics. We strive to use human-derived material wherever possible to undertake mechanistic studies and to screen for drug effects. For example, we use human stem cells in which we introduce mutations in specific genes to examine the effect of PAH causing mutations on cell function. In addition, we have developed techniques to isolate blood outgrowth endothelial cells from patients and controls to undertake studies of specific gene mutations. We employ extensive cell-based approaches to validate the roles of specific genes or pathways prior to embarking on animal studies. Thus, the cell- and animal-based approaches are used in a complementary manner to achieve our research objectives, but animal studies are only embarked on once we have reason to believe that a particular gene or pathway is likely to be central to a disease and could be tackled by using suitable drugs in patients.

Why were they not suitable?

It is not that non-animal alternatives are not suitable; it is rather that in isolation they are not sufficient to achieve our objectives. The combination of non-animal and animal approaches is required to generate the confidence to move forwards into clinical studies in humans and patients with PAH.

A retrospective assessment of replacement will be due by 04 September 2026

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?

The number of animals has been estimated based on our extensive experience over the past 15 years with animal (mouse and rat) models of PAH, allowing for the improvements in experimental design and refinement of our protocols that have taken place over that time. For example, we know approximately how many mice it takes to breed a colony ready to undertake a full phenotyping assessment. In addition, we know how many mice or rats are needed to address a specific question to achieve our objectives. We have assumed that each gene or pathway (we anticipate investigating 4-8 such pathways) under investigation will be pursued to conclusion in estimating these numbers. In reality, we may terminate a programme of work early if the emerging results do not justify continuation. This is kept under continuous review.

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

We use historical research data from our laboratory databases to appropriately power our studies depending on the desired endpoint. The endpoint might be measurement of blood pressure within the lungs, for example to determine whether a genetically altered mouse has a significantly higher pressure than wild type littermates. Alternatively, the endpoint might be measurements of the degree of thickening of the walls of blood vessels within the lung, or the response to a drug intervention. In the latter case we decide beforehand what represents a meaningful reduction in pressure or increase in heart function and power the experiments based on this.

We have significantly reduced the numbers of animals used in our phenotyping studies by using ultrasound to track the progression of pulmonary hypertension longitudinally in genetically altered mice. This means we can study the heart function of the same mouse twice by ultrasound at two different time

points. With new genetic mouse models, we do not know at what time point animals develop PAH. In the past we would have needed to undertake terminal catheterisation of the right side of the heart in two groups of animals at, for example, 3 and 6 months to determine this. With ultrasound we can track pressures over time and can use half as many animals. For example, in a recent study in genetically altered mice we measured heart function by ultrasound at 4 month, 8 months and 11.5 months of age. In this study we used 45 mice, as opposed to the 135 mice that would have been required for pressure monitoring by catheterisation at each point. This approach has more than halved the required animal numbers in these protocols.

Where appropriate we will use the web-based Experimental Design Assistant available from the National Centre for Replacement, Refinement and Reduction of Animals in Research to ensure that we use the minimum number of animals consistent with our scientific objectives and undertake appropriate statistical analysis. We will also follow the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) Guidelines for planning animal research and testing.

We will follow the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) during the conduct of our experiments to support publication of data of maximal quality, reliability and reproducibility.

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

Mating will be set up according to the requirements for specific experimental designs to reduce the risk of overproduction of animals. Mice will be genotyped rapidly to permit early assignment to experimental protocols and enable the separation of breeding pairs once numbers that are sufficient to achieved power have been reached. Multiple organs will be harvested to allow for future analyses that may not be relevant to the specific experimental aim. Tissue and tissue extracts will be stored long-term to enable future analyses for new targets or sharing with other researchers.

A retrospective assessment of reduction will be due by 04 September 2026

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.

We will use mice carrying changes in their genes that are similar to the human disease. The human disease develops slowly and only affects about one in five people who have the damaged gene. We believe that the effect of the gene damage is often only revealed if something else happens in the lung, such as an infection or exposure to pollutants. We may need to expose mice with the damaged genes to a factor that increases their chance of developing disease. The disease that develops in the lung blood vessels does not cause any obvious signs that these animals are developing disease.

There are no robust rat models with damage in the disease relevant genes, so we have to give them a drug and reduce their oxygen levels (similar to being on the top of a mountain for a few weeks) to start the disease process. Once this is started, the disease gradually worsens. We have changed the method to reduce the disease variability between rats and to make the process shorted by 4 weeks, so that the length of the total process until we test the level of disease is 7 weeks. During this period, the rats do not show any obvious external signs that they have disease, but their lungs will have developed disease. However, the amount of lung disease that is present in these rats is enough that we can test compounds that may represent future drugs for use in humans with the disease.

The major determinant of survival in humans with PAH is the response of the right ventricle to the increased pulmonary artery pressure. It is failure of the right ventricle that leads to early death. In mice, the available stimuli for the development of pulmonary hypertension result in adaptive hypertrophy of the right ventricle but not in right heart failure. In order to determine mechanisms and potential interventions for right heart failure, a model is employed that involves partially ligating the main pulmonary artery as it emerges from the heart. This causes a narrowing of the main pulmonary artery and greatly increases the pressure within the right ventricle, leading to right ventricular dilatation and failure. More than 90% of mice recover from and tolerate this procedure, whilst 10% die during the anaesthetic, immediately after during recovery or within 2-3 weeks of surgery as a consequence of pericardial effusion. This provides an opportunity to assess the role of specific genes and therapies that might benefit patents with PAH and right ventricular failure.

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

We cannot use younger mice for our studies as the pressure catheters we use are too large to insert into the blood vessels of younger mice. Rodents are the species of choice, because the cardiovascular systems of non-mammals do not have the same structure or responses. The disease we are examining takes time to develop, so shorter time points do not give a difference that is much different to non-diseased animals. Some of our protocols are non-recovery protocols; that is animals will undergo terminal procedures under a general anaesthetic and thus will not be sentient of the procedure.

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

We will always strive to minimise any potential suffering or harms caused to the animals by ensuring that our researchers are well-trained with good communication skills; researchers will be provided with relevant literature to ensure awareness of the best practices; staff will undertake training courses to

ensure their skills are updated and current and will be appropriately assessed. All researchers will adhere to the principles of refinement in accordance with NC3Rs.

For bleeding from superficial veins, animals will be placed in warming cabinets to ensure that vessels are dilated and therefore reduce bleeding time and stress.

In cases where recovery surgery is undertaken, animals will be maintained in warming cabinets and their recovery observed for any signs of post-operative distress. Appropriate pain relief will be provided.

Animals will be observed on a daily basis with the assistance of the technical staff in the unit and researches alerted to any problems as soon as possible to ensure that any health issues are dealt with appropriately, either through consultation with the NVS or schedule 1 killing according to the health issue in question.

Animal weights will be recorded and entered into the electronic MCMS database; this will be set to alert users to any weight changes that are defined according to humane endpoints.

In protocols of moderate or severe severity, scoring and monitoring methods will be implemented to ensure that animals do not exceed the adverse effects stated.

Where animals are not housed in their home cages, for example when they are housed in low oxygen chambers, the most appropriate environmental enrichment will be used. Staff will abide by the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery and pain management will be controlled by both before and after operation pain medication with the most appropriate dose for the species used.

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

The following general published guidance will be followed:

1) ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines version 2.0 (https://arriveguidelines.org/), published by NC3Rs to improve the reporting of research involving animals.

2) LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery, 2nd Edition 2017

3) The PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) Guidelines for planning animal research and testing.

Specific published recommendations for experiments in animals in the field of pulmonary hypertension will be designated as required reading for researchers conducting experiments under this licence:

Standards and Methodological Rigor in Pulmonary Arterial Hypertension Preclinical and Translational Research. Provencher S, Archer SL, Ramirez FD, Hibbert B, Paulin R, Boucherat O, Lacasse Y, Bonnet S. Circ Res. 2018 Mar 30;122(7):1021-1032. doi: 10.1161/CIRCRESAHA.117.312579.

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

We receive regular updates from NC3Rs, the EU NC3Rs ECVAM information network (including Norecopa, the Norwegian 3Rs centre.) and LASA, and alerts relating to new publications and studies. We also receive information from our institute and the named persons in our institute (for example the Named Vet), and our wider research community from scientists in the same field of research.

A retrospective assessment of refinement will be due by 04 September 2026

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?