



Home Office

## NON-TECHNICAL SUMMARY

# Ischaemia Reperfusion Injury and Organ Transplantation

### 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

Transplantation, Ischaemia-reperfusion Injury, Rejection, Treatment

### Animal types

### Life stages

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|      |  |
|------|--|
| Mice | adult, embryo, pregnant, juvenile, neonate |
|------|--|

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|      |       |
|------|-------|
| Rats | 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 examine the mechanisms of organ injury in transplantation and to develop new therapeutic strategies, by studying ischemia (restriction in blood supply), the immune response and to investigate the efficacy of therapeutic interventions to improve acute and chronic organ function.

**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?**

Organ transplantation is a life-saving treatment for end-stage organ failure, but there is a growing disparity between the number of patients in need of an organ transplant and the number of suitable donor organs available. In January 2020, there were 6183 people in the UK currently waiting for an organ transplant. Conversely, the total number of patients whose lives were saved or improved by an organ transplant fell by 2% in the same year. The total number of transplants performed also fell, ranging from a 3% decline for pancreas transplants to 20% for lung or heart-lung transplants. This is in spite of a 2% increase in the referral rate of potential donors, as well as a 1% increase in the overall consent rate for organ donation.

In the UK alone, approximately 1000 patients die or are removed from the transplant waiting list every year due to the shortage of suitable organs for transplantation. This is a gross underestimation of the need for transplant organs, because a large proportion of patients with end-stage or organ failure are never offered transplantation, a decision itself driven in large part by the lack of available organs. This shortage of organs has necessitated in the usage of less-than-ideal organs, including from donors in whom the organs are exposed to a period of ischaemia prior to transplantation. This results in relative impairment of the function of these organs after transplantation. This initial injury can result in delayed function of the organs after transplantation, as well as augmented immune response (the immune system is working harder than usual to fight off a disease or infection) to the transplanted organs; this also leads to reduced long-term organ function and organ failure, exacerbating the shortage of organs for transplantation. The most important anticipated benefit of the proposed work, therefore, is to improve the quality and quantity of organs available for transplantation, thus improving both the quality of life of transplant patients and reducing the number of patients dying on the transplant waiting list.

This project aims to improve our understanding of the cellular mechanisms that underlie ischaemia-reperfusion injury (tissue damage caused when blood supply returns to tissue) and test the safety and effectiveness of promising novel therapeutic agents. New treatments that reduce cellular injury and organ dysfunction will help overcome the national and international organ shortage by increasing the availability of organs suitable for transplantation. Improved rates of organ transplantation would have

an enormous societal impact by shortening waiting lists, improving the quality of life of patients, and reducing complications and death from organ failure.

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

This project will generate academic knowledge, communicated in publications, that will support future research, new treatment products, and the basis of clinical studies which will have health and economic implications.

It is expected that the proposed project will ultimately enable novel therapeutic interventions (that may include drugs, cells or artificially created tissues) to improve acute and chronic organ function in transplantation. The work carried under the same project, that will continue in the new Project Licence, has already advanced one therapeutic agent from mouse and rat models to pig and human models, resulting in design of a human clinical trial. Importantly, because the underlying mechanisms of ischaemia-reperfusion injury in organs transplantation are shared with other common conditions such as myocardial infarction (supply of blood to the heart is suddenly blocked - heart attack) and ischaemic stroke (supply of blood and oxygen to the brain is blocked), the insights generated from this work will make a significant contribution to other clinical disciplines beyond transplantation.

The results of these experiments will be published in peer-reviewed journals and presented at national and international conferences. The treatments that are validated in the project may include new intellectual property. From our experiments, we hope to learn what the best ways to deliver the treatments are, and how often and how much of the treatments need to be given. Altogether, these will generate a blueprint for clinical studies, which will bring successful new treatments to patients.

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

The outcomes from work carried out in this proposal will be of significant benefit to researchers in the fields of transplantation, ischaemia-reperfusion injury, immunology and beyond. We anticipate this will also result in direct benefit to clinicians and patients by enabling the clinical translation of these technologies and therapeutic approaches. Specifically, we anticipate the findings from this work will directly inform the design and conduct of clinical trials in transplantation by academics and pharmaceutical organizations. Throughout the project, researchers both inside and outside the field of transplantation will benefit from the above outputs. IR (ischemia-reperfusion) injury occurs in many human diseases, including myocardial infarction (heart attack) and stroke (when the blood supply to a part of the brain is cut off). The processes that occur in mitochondria (membrane-bound cell organelles), cells and organs are applicable to all of these diseases. Because the cellular processes underlying injury in transplantation, heart attacks and strokes are similar, we anticipate that our findings, including new drug treatments developed for use in transplantation, could potentially also be of benefit in treating heart attacks and strokes. Importantly, our raw data, publications and presentations will disseminate new knowledge to other researchers, who can then build on the discoveries.

In the short-term (1-3 years), the primary beneficiaries of the proposed project will be other researchers who are also developing new therapies in the fields of transplantation, ischaemia-reperfusion injury, immunology and beyond. We anticipate that the findings of this study will be of broad relevance to the research community in this field. In the medium term (3-5 years), we anticipate

that industrial companies involved in the manufacture of therapies, who are essential for the ultimate production of these therapies, will also benefit from the findings of this study. In the long-term (5-7 years), this project will benefit patients. The benefit will initially be limited to those patients enrolled in clinical trials. We hope that ultimately (7-10 years) large numbers of patients will benefit from the findings of this study.

More specifically, we expect to generate at least one new therapy and advance it to a stage that it can be investigated further in large animal models. In the long-term (5-7 years), we expect that the findings of this study will result in the design of at least 1 human clinical trial to test the safety and efficacy of a novel therapy developed in this project.

During our work under the preceding licence, we have already started extending the findings of our studies on the mechanisms of ischaemia reperfusion injury to pig and human models. We have demonstrated that these findings are relevant to human hearts and kidneys, thus confirming that therapeutic interventions have a high chance of success in humans. Importantly, we have identified 2 drugs that reduce ischaemia-reperfusion injury in mice. We have already started testing these agents using pig and human organs.

The surgical and scientific techniques used in this project are very specialised, and therefore limited to a few experts who are able to carry them out. As this project progresses, more researchers will be trained in these specialist skills. They will go on to apply them in collaborative or new studies in the future, ensuring the dissemination of good experimental practice and model refinement.

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

We will disseminate all findings of our studies, including unsuccessful approaches, through publication in peer-reviewed journals, presentation at scientific conferences, and through meetings with other researchers. All publications will be open access, including through platforms such as F1000Research. This project includes collaborations with a large number of researchers with expertise in complementary areas, and this network will be utilised to maximise the dissemination of the new knowledge gained through this project.

### **Species and numbers of animals expected to be used**

- Mice: 5375
- Rats: 100

## **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 project will utilise normal wild type or genetically modified mice. Mice are the lowest species with a comparable physiology that enables the study transplant organ injury induced by ischaemia and the immune response (reaction which occurs within an organism for the purpose of defending against foreign invaders) and to investigate the efficacy of therapeutic interventions to improve acute and chronic organ function. Importantly, genetic strains of mice are available that allow studies to be designed that can generate valuable information about specific therapies. In one of the experiments we may use rats as their larger size makes the complex surgery easier and more likely to be successful.

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

Animals are typically expected to undergo only 1 or 2 surgical procedures under general anaesthesia from which the animal will be recovered. If >2 invasive (ie, laparotomies and excluding re-suturing of wounds, injections or bleeding) general anaesthetic procedures are planned (excluding procedures under terminal anaesthesia), the additional invasive general anaesthetic procedure will only be undertaken if the animals do not display signs of ill health and after discussion with the named veterinary surgeon.

Two experimental models will be used to study ischaemia-reperfusion injury (IRI) in the kidney. In the first model, blood vessels to one or both kidneys will be clamped under general anaesthesia for up to 90 minutes, followed by reperfusion. Animals will be recovered and maintained for up to 9 months to investigate the long-term impact of IRI on kidney function. The duration of general anaesthesia will not exceed 6 hours. In the second model, recipient mice will first undergo removal of one kidney (nephrectomy) under general anaesthesia. A donor kidney that has been exposed to various periods of ischaemia, will then be transplanted into recipient animals during the same operation and reperfused. Animals will be recovered and maintained for up to 9 months to examine long-term organ function. In some kidney transplant recipient animals, the remaining native kidney of the recipient animal will be removed under general anaesthesia no less than 5 days after transplantation. The aim of the removal of the second native kidney is to enable the function of the transplanted kidney to be monitored independently of the native kidney. The second native nephrectomy will only be performed if the animal has fully recovered from the transplant operation. By transplanting donor kidneys to genetically identical or mismatched recipients, the impact of IRI on the immune response to transplanted kidneys can be studied. Blood sampling and imaging may be performed to assess injury to the kidney.

To examine IRI in the heart, donor hearts will be transplanted into the abdomen of recipient animals and reperfused. Heterotopic heart transplantation (heart transplant to abnormal place) is an experimental technique that is optimised and routinely performed by our group. It enables the transplanted heart to re-commence beating in the abdomen after reperfusion. Importantly, because the heart is transplanted heterotopically, subsequent failure of the transplanted heart (for example through acute or chronic rejection) does not lead to ill health in the recipient animal. Moreover, simple palpation can be used to non-invasively assess the function of the transplanted heart through the abdominal wall. By transplanting donor hearts to genetically identical or mismatched recipients, the impact of IRI on the immune response to transplanted hearts can be studied. Animals will be kept up to 9 months after transplantation to examine long-term organ function. Blood sampling and imaging may be performed, in addition to palpation of the abdomen, to assess injury to the transplanted heart. Animals receiving a heart transplant may be treated with cells or drugs (for example, to modify the immune compartment), have blood sampling or non-invasive imaging prior to the heart transplant. The heart transplants will be performed under terminal anaesthesia if the period of reperfusion is less than 60

minutes and during which other manipulations such as imaging may be performed. The duration of general anaesthesia will not exceed 6 hours. Such a short period of reperfusion may be necessary to study organ injury soon after reperfusion.

To examine IRI in the liver, the blood vessels supplying the whole liver or part of the liver will be clamped under general anaesthesia for up to 90 minutes, followed by reperfusion. The duration of general anaesthesia will not exceed 6 hours. Animals will be recovered and maintained for up to 9 months to study the long-term impact of IRI on liver function. Blood sampling and imaging may be performed to assess injury to the liver. The liver IRI experiments will be performed under terminal anaesthesia if the period of reperfusion is less than 60 minutes and during which other manipulations such as treatment with drugs or imaging may be performed. Such a short period of reperfusion may be necessary to study organ injury soon after reperfusion.

To examine vascular rejection, donor or bioengineered aorta grafts may be transplanted orthotopically (same anatomic location as the original tissue) into the abdomen of mice or rats under general anaesthesia. Animals will be recovered and maintained for up to 9 months to study the long-term function and rejection of aortic grafts.

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

It is expected that most (more than 90%) of animals will recover rapidly post-surgery.

In some cases, due to the surgical procedure, some the animals may experience weight loss, reduced food intake, reduce movement or an abnormal coat (piloerection and/or wet ungroomed coat). In such cases, the animals will be humanely killed, if these clinical signs do not respond to treatment (such as high energy and easily digestible diet) and persist for up to 24 hours.

When rapid adverse effects may be expected, animals will be monitored very frequently (up to one hourly) during the initial period (approx.6 hours) when adverse effects are most likely to occur (based on data from previous animal experiments and clinical studies).

Animals will also be humanely killed if they experience clinical signs that approach the limits described in the project according to the Home Office guidelines and as stated in the protocols.

### **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 expected severities are: mild 25%, moderate 75%, 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?**

The definitive investigation of mechanisms and therapy of ischaemia-reperfusion and immune injury during transplantation requires study in intact animals. Transplantation of organs into animals are necessary to enable monitoring of acute and chronic organ function. Much of the proposed work is carried in the laboratory and using human tissue or organs only (including perfusion with blood on specialised machines), thus minimising the need for animal experimentation. Importantly, it is anticipated that this work using isolated human organs will lead to the refinement and optimisation of IRI models which can be ultimately used to replace experimental use of animals.

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

In the past we have established the Biorepository for Translational Medicine which aims to provide fresh normal human tissue from deceased transplant organ donors for research, including for study ischemia-reperfusion injury in organ transplantation. During this project, we will continue to use human tissue and organs, exposed to a range of ischaemic conditions to study the impact of ischemia on mitochondrial, cellular and tissue viability. This enables the use of human, rather than animal tissues/organs. Additionally, this programme supports more than ~50 research projects locally by providing access to live human tissue/organs for research. Many of these research projects would have previously only been possible using animal tissue. This programme continues to make a significant contribution to Replacement.

Further to the above, to examine the ischaemia-reperfusion injury in organ transplantation, we will continue to use and further improve the ex vivo (outside of the living body) normothermic (normal body temperature) perfusion technology, developed in our lab, in which human organs are perfused with ABO blood-group-matched oxygenated blood. This technique enables the PPL holder to use human organs to study the IRI. This approach has enabled the clinical translation of our previous work towards human clinical trials and make significant contribution to Replacement of animals with human organs to achieve in some experiments, the same aims of this project.

In general this project has been preceded and informed by experiments using cells grown in special dishes in the laboratory (in vitro) and donated human organs that have been declined for clinical transplantation. Non-animal alternatives have, therefore, been extensively used as far as possible, in order to replace animal experiments.

**Why were they not suitable?**

We are using all of these alternative approaches to reduce the number of animals used in the proposed project. Use of cells does not enable the complexities of organ injury during transplantation to be assessed fully. While we have and continue to make extensive use of human organs, these are

very rare and access to them is very unpredictable. Moreover, because some of the human organs are of less-than-ideal quality (which is why they were not used for transplantation), they are not always able to be used to generate robust and reproducible data. Lastly, ex vivo experiments using human organs cannot be used to characterise the chronic aspects of organ injury and the efficacy and safety of novel therapeutics on a long-term scale. The definitive investigation of the mechanisms of organ injury in transplantation and to develop new therapeutic strategies requires an intact and functional immune system in an animal model.

## 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 have been estimated based on the range of studies that are planned, as well as based on the previous similar studies we performed during the last five years. Based on our previous experience, we are able to predict, for each study, the number of animals that are required to generate reliable and reproducible data. Using our previous experience, we are also able to predict how many studies we can perform in a given time period.

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

Our in-vivo experiments will continue to be designed to include the minimum number of animals needed per study, that can lead to reliable conclusion. The PPL holder monitors the number of animals used in each experiment and approves the study plans to meet the requirements of Reduction of animals. In order to minimize the number of animals used, the cohort sizes are determined with attention to the experimental variability in the data generated in each specific model, with aim of obtaining statistically and biologically-significant data using fewest animals as possible. We have a wealth of previous data to show that a group size of 5-6 individual animals are usually appropriate to allow definitive conclusions to be drawn using the minimum numbers of animals. When a new therapy is under investigation, we will first perform pilot experiments with small animal groups (typically 2-3 animal per group) to confirm the appropriateness of the experimental design and to generate pilot data to enable group sizes to be formally calculated. All experiments are conducted using randomisation and blinding as far as possible. Littermate animals will be randomised to receive experimental or control treatments to minimise bias from age, sex and weight. Data analysis is routinely conducted in blinded manner to minimise bias.

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



Many of the experiments will be designed to enable important data to be generated from individual animals about the impact of ischemia on multiple tissues, for example, when donor animals are treated with therapeutic agent, the kidney, heart and liver from the same animals are retrieved and tissues simultaneously analysed to generate important insights about injury to all tissues. This results in Reduction in the number of the animals used in this project by collecting more data from the animals that are already used and in order to maximise their use and reducing the need to use more animals.

Furthermore, in addition to the in vivo experiments (experiment on living organism), we have an advanced and comprehensive programme that uses cell lines and in vitro culture systems to examine the efficacy of agents to reduce the ischemia reperfusion injury during the transplantation. For example, these in vitro experiments are used to screen potential therapeutic agents and identify the likely therapeutic window of the drugs. This approach resulting of significant reduction of number of the animals been used.

All tissues obtained from animals will be, where possible, shared with other researchers to maximise data generated from the experiments. This will include tissue from experimental animals that have been killed, as well as animals used for breeding and surplus to requirement. Data generated from the experiments, including raw data, will be made available to other researchers in order to maximise the benefit of the experiments.

## 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.**

The experimental models and techniques that will be used in this project are:

Kidney or liver blood vessel clamping - ischaemia and reperfusion

Kidney or liver pedicle clamping - Non-recovery ischaemia reperfusion injury

Organ and tissue donor

Organ and tissue donor: Non-recovery

Kidney and Heart transplantation

Blood-Vessel Transplantation

All experimental models have been refined to ensure they cause the least pain and suffering. Importantly, none of the procedures are expected to result in severe clinical signs (such as persistent abnormal behaviour or persistent weight loss). Animals will be humanely killed if they display clinical

signs that do not respond to treatment (such as easily digestible food or pain relief medication). Animals will therefore not be permitted to experience lasting harm.

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

Mice are the least sentient animals that can be used to generate valuable data to investigate the transplant organ injury induced by ischaemia and the immune response and to investigate the efficacy of therapeutic interventions to improve acute and chronic organ function. As the immune response and the action of potential therapeutic strategies takes days to weeks to manifest, experiments cannot be performed exclusively under terminal anaesthesia. Due to the small size of mice, rats may need to be used for transplantation of vessels to ensure technical surgical feasibility.

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

We have refined all our surgical procedures, including transplantation of heart and kidneys. We have developed robust and effective methods to instruct and supervise new researcher in these methods and our expertise is regularly sought by other groups to perform procedures in order to reduce rates of technical failure. We have also provided the infrastructure and trained staff and supervision to ensure success of the technical procedures are as high as possible. All experiments are performed using aseptic techniques and we have not had documented cases of surgery-related infections in recent years. Experiments are designed so as to minimise the need to perform experiments out of normal working hours. The experiments in this project have been designed so that death of animals is never an expected endpoint. No animals will undergo procedures that are routinely expected to cause severe ill effects, thus limiting the distress and discomfort experienced by the animals.

In close collaboration with the staff at our animal facility, we have a robust mechanism for the post-operative monitoring of our experimental animals. Animals are also given routine post-operative pain relief medication, which has been proven to be effective in previous similar studies. When adverse effects may be expected, we readily increase the frequency of monitoring to identify animals that may be experiencing adverse effects. We also have our own dedicated animal technicians who ensures that our animals receive close attention if there are any concerns. We have achieved a number of significant refinements during the previous series of studies. These include enhanced environmental enrichment (such as extra card-board housing) and use of high-energy or tasty diets to prevent weight loss. Where adverse effects may be expected, we will perform particularly close and frequent monitoring of animals, including through the use of observation sheets and body weight records. We will continue to strive to develop new refinements.

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

All experiments will be conducted and reported in adherence to best practice guidelines including those published by the Laboratory Animal Science Association (LASA), such as guidelines for record keeping, performing surgery, education and training, and reporting of experimental results. We will also follow the PREPARE guidelines, so we can ensure that our animal experiments are conducted in a more refined way and that animal welfare is prioritized throughout the research process.

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

As a current project license holder, I am closely involved with the activities of the 3Rs committee at my institution, including the development of recommendations and dissemination of information relating to advances in 3Rs. I intend to continue with my activities, included but not limited to:

Stay up-to-date on the latest developments and best practices in 3Rs by regularly reviewing resources and guidance from organizations such as NC3Rs and Norecopa guidances: NC3Rs Resource Hubs, Norecopa Databases and Guidelines, NC3Rs Training and Norecopa Network

Incorporate 3Rs principles into the research design and planning process. This may involve considering alternative methods to animal testing, minimizing the number of animals used, and ensuring that animal welfare is a top priority throughout the research project.

Engage with other researchers, stakeholders, and experts in the field to share knowledge and best practices. Attend conferences and workshops, participate in online forums and collaborate with other researchers to stay up-to-date on the latest advances in 3Rs.

Continuously evaluate and refine our research methods to ensure that you are incorporating the most effective 3Rs practices. For example: assessing the effectiveness of alternative methods, and incorporating feedback from animal welfare experts and ethics committees.