Home Office

NON-TECHNICAL SUMMARY

Treatment of short and long term outcomes of viral lung infection

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

Lung, Virus, Bacteria, Immunity, Pathology

Animal types	Life stages
Mice	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?

Lung viral infections cause devastation globally every year as evidenced by the last coronavirus pandemic. We will discover new treatments for lung viral infections and the ongoing complications that arise.

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?

Understanding the role of the immune system in lung viral infection is key to the development of therapies that alleviate severe disease before vaccines are available. Some of our discoveries are being tested in patients already, but not all people respond in the same way. Our aim is to define a suite of treatments that will cover the majority of those severely affected.

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

Outputs will include:

1. A greater understanding of how the immune system is affected by changes in structure during infection.

2. Publications disseminating our findings to global scientists.

3. Development of concepts with the pharmaceutical industry

4. As demonstrated in our last project license, transfer and testing of our discoveries in relevant patient groups using new products.

5. Dissemination of understanding to the general public via articles in, for example, the public journal The Conversation and interaction with Public and Patient groups

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

Mid- to long-term the beneficiaries will be the pharmaceutical industry (new targets for development), and particularly the patients who are already benefitting from our discoveries. The health care system will also benefit. For example, our recent trials of new therapeutic reduced the length of hospital stay, reduced the number of patients requiring admittance to the intensive care unit and decreased long term outcomes. This reduces the stress on the NHS and the financial burden of lung infection.

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

We routinely use a Patient and Public involvement group from the British Society of Immunology to capture facets of disease that most concern the patients. This disseminates new knowledge.

My team hold regular REDDIT sessions, which is an online forum for anyone to ask questions on the topic we propose. The last one was on COVID-19 where in two hours we had over 1000 questions to answer. This platform also drives interactions between all participants, even when the session has finished.

I regularly contribute opinion pieces to "The Conversation" that has an online reach to thousands of subscribers. In the last year my pieces have made the top number of interactions in my establishment.

I regularly respond to National and International media interview requests regarding viral infections. I have been inundated during COVID-19.

I have a policy to publish all research, not just those that produce a positive outcome. Negative data is equally as important to prevent others going down the wrong path. Negative data will be published as per other findings in peer reviewed journals and on the Wellcome Trust open access site. The data is also placed onto the group webpages and raw data files shared

Species and numbers of animals expected to be used

- Mice: 6000

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 reason we use standard laboratory mouse strains is because over many decades they have proven to be readily infected with respiratory pathogens via the natural route by intranasal or aerosol administration. As in humans the pathogen replicates in cells lining the airways. Even viral strains that are not mouse pathogens induce a pneumonitis that, similar to humans, is reduced by early treatment with antivirals. The virus divides with similar speed to that in man. Symptoms of disease, like that in man, are dependent on the virus strain and the amount of virus administered. The route of infection, site of replication, replication kinetics and the specific cells recruited to combat that infection are identical to that in man.

We use adult mice, typically over 8 weeks of age as they yield enough cells to perform many experiments per mouse which reduces the need for separate experiments to address different questions. We have tried to replace animal use by using cell lines, but unfortunately a complex system is required to understand long term consequences of lung infection.

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

In a typical experiment mice will be housed initially in groups of 5. To induce a viral lung infection mice are first anaethetised by inhalation of anaesthetic. Virus solution is applied to the nostrills, which is then inhaled by natural breathing. Mice recover within a few minutes. It takes a maximum of 10 days to recover from the infection. Occasionally, during this 10 day period mice are given treatments by injection. At time points after infection, mice are killed by excess anaesthetic and multiple organs taken to assess pathology and the effect of any interventions. In a few experiments, mice will be left to recover from the viral infection for up to 6 month (typically 3 weeks) and then given a bacterial infection to see the impact of previous viral infection to general immunity to infection. Other than infection, we may also breed mice that are not available commercially.

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

About 50 % of the mice will not have an infection and so will not experience any adverse events. In those mice given a single infection with viruses respiratory inflammation and weight loss may occur. Weight loss appears gradually from day 3, peaks on day 6-10 and full recovery to their original weight occurs by day 10 after infection. Approximately 50 % of animals are likely to experience weight loss. This is because they have been given an intranasal infection that causes inflammation with influenza like symptoms. The remaining 50 % of mice have mild or no symptoms from the infection per se as the infection does not cause weight loss or they have been treated with control substances or therapeutics that reduce inflammation. At the peak of illness mice may show a hunched posture and reduced mobility, but in the main, all mice recover. Transient pain may occur on injection, though in most instances they are anaesthetised when this procedure occurs. Some weight loss may occur in mice given bacteria alone.

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)?

This project works to a maximum severity of Moderate. This status is determined by a number of factors including degree of weight loss (maximum of 20 %), mobility, posture and overall condition. Mice are weighed daily and observed. In some cases mice have neared maximal weight loss but are still alert and in good condition. Instead of culling the mouse, we monitor the trajectory of weight loss (the steepness), breathing and general condition, twice daily. This prevents unneccesary termination of the experiment which would require another experiment to be set up. 70 % of mice in our protocols only experience up to 15 % weight loss

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

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

Viral infection causes complex problems, and much of the disabling effects exist long term. The life-threatening illness to infection is caused by the interaction of many body systems. The illness also depends on cross-talk between immune cells and structural components of the lung. During our last project grant we discovered that immune cells are influenced by alterations in tissue structure and have taken this idea into patients and the treatment of those patients. Our discoveries will reduce the need for animal experiments in the future as we focus more on patients. However, we still need to discover more treatment options as one size does not fit all.

Cells cultured in the laboratory do not take into account blood and air flow or the impact of the nervous system. Nor do they show drug distribution. Replacements would be ideal in the current economic climate as animal experimentation is costly and time consuming.

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

I strongly support the use of non-animal alternatives and have considered the following:

1. Matrigel systems that contain 2-3 different cell types ,

2. Organ on a chip,

3. Tissue slices,

4. Human lung resection, and

5) Human transplant lungs

Why were they not suitable?

Alternatives 1 and 2 still do not represent the lung microenvironment . Nor to they replicate the long term build up of complications over time. Furthermore they do not show how a virus infection leads to bacterial complications in the future, which is a critical medical issue in many patients with underlying lung conditions. Cellular systems also do not allow analysis of repair and the impact of altered repair.

In 3 and 4 we routinely use material collected as part of lung resections in patients with cancer, focussing on "normal" lung 4 cm from the cancer mass. This tissue has effectively reduced the range of experiments performed in mice and we will continue to use this approach. However, the tissue is highly criticised for its suitability and we cannot address bacterial super-infections or the repair process using it.

In an attempt to make the lung resection more representative we initiated discussion with lung transplant teams at our local hospital. Despite long negotiations, transplant material not used in patients is still unavailable. This tissue would still not replicae bacterial super-infections or the repair process well.

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?

There is an extensive literature using in vivo models of respiratory infectious disease and the applicant has greater than 20 years of experience. The overall number of animals is considerably reduced from the previous license due to the progress made. The estimated numbers in each of the three protocols is based on using 4-5 mice per group, which we have found to provide strong statistical power that reduces the necessity for multiple repeats. Initial therapeutic testing used 5 mice per group which is reduced to 4 mice per group depending on the reproducibility of the effect in the first study. We will not rely on historical precedent however to determine sample sizes as this may lead to serious over- or under-estimation of animals required. Data collected in preliminary experiments will be used to compute the sample size needed in follow up studies.

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

The estimated number of animals to be used has reduced dramatically from the last licence since I am no longer supervising the early career academics that used my license for their experiments. The experimental design used for the remaining protocols has reduced animal use in the following ways:

1) Sharing: With correct timing and consultation, we have determined that multiple scientists, asking different questions can use the same mice. Mouse number are based on 2-3 scientists sharing mouse use. In practice we have reached seven sharing.

2) Reducing repeats: It is possible to repeat observations whilst also progressing the research to the next question. Direct repeats are discouraged.

3) Confidence in reproducibility: Over the years of experience the reproducibility of the model has become established. In this license we can therefore be sure of a statistical significance with 4-5 mice per group.

4) Use of NC3R resources: There are online resources available to test null hypotheses (where you take an educated guess that something is true) or the alternative hypothesis (the opposite of the null hypothesis). These resources tell you how many comparisons need to be made within experiments and between experiments. Relevant resources are also present for randomisation, blinding and allocation. These resources have been used to assess the number of animals in proposed experiments

All personnel using this licence will make optimal use of the experimental design assistant (EDA)and the scientific implications of poor design choice.

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

Animal reduction will be facilitated by group members sharing tissue and employing rigorous experiment design developed by our statisticians to calculate the least number of mice needed to address our questions. Where an experimental type is used more than once we will assess performance over time to ensure that it is continuing to perform well. The laboratory has archived specimens that can be used by subsequent researchers. Unfortunately storage processes can affect our results and so these archived tissues have limited use. As proof of principle however, they are adequate.

At our establishment we have instigated a community of researchers for sharing. As an example, we have a germ free mouse colony and when some are used in an experiment the details (age, gender, number per group and time points) are put onto a Teams platform. Members are therefore aware of the experiment and indicate whether they wish to collect additional tissues. This has been incredibly successful and in some experiment lung, gut, skin, bone marrow, kidney, blood and brain have been sampled from the same group of mice, reducing animal use by 7 fold compared to users working individually.

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 only use mouse models that have been refined over the years to minimise animal distress and replicate the human scenario better. Procedures involve administration of virus and bacteria by inhalation and injection of immune modulators to reduce disease severity.

Minimisation of animal suffering critically depends on close monitoring of mouse condition (body weight, mobility, the quality of mouse fur, presence of cyanosis and whether they cling onto the cage lid when weighed). Staff perform this inspection daily, or twice daily if concerned. See scoring criteria below. We try to avoid intraperitoneal injections where possible, using intradermal or subcutaneous where the alternative exists. We will refer to the Joint Working Group on Refinement. The latter, we feel is less intrusive than intraperitoneal. Where appropriate procedures are performed under inhalational anaesthetic that allows recovery within a few minutes. This prevents mice from losing body temperature whilst immobile.

Body weight	No weight loss	0
	0-5% weight loss	1

	>5 -10% weight loss	2	
	>10-15% weight loss	3	all experimental animals will be placed on mash
	>15-19% weight loss	4	If weight loss continues for greater than 72 hours, mouse must be sacrificed
	>19% weight loss		Cull Immediate action must be taken.
Ear positioning	Normal	0	
	Slightly retreated	1	
	Retreated completely back	2	
Coat and skin condition	No piloerection	0	
	Slight Piloerection	1	
	Marked piloerection	2	
Social interaction	Normal	0	
	Isolated or withdrawn from group	1	
Posture	Normal	0	
	Abnormal posture (hunched)	1	
Posture and mobility	Normal	0	
	Hypoactive	1	
	Lack or reluctance to move when stimulated	2	
	Inactive	Cull	Immediate action must be taken.

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

Standard laboratory mouse strains are readily infected with respiratory pathogens via the natural route of the nose. As in humans the pathogen replicates in exactly the same lung cells. Even viral strains that do not usually appear in mice induce an inflammation that, similar to humans, is reduced by early treatment with antiviral medicines such as amantidine and ribavirin. Symptoms of disease, like that in man, are dependent on the influenza strain and the dose of virus administered. The route of infection, site of pathogen growth and the immune cell response are identical to that in man. Unfortunately, none of this can be replicated in less sentient species. Mice of a younger age are technically difficult to infect and fail to represent the majority of infections that occur in adults and the elderly. Furthermore, early age responses to infection are different to adults.

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

An academic at the establishment and the named veterinary surgeon have developed a severity score monitoring system (see above). To minimise animal suffering and recognise when humane end points have been met, they have created an objective scoring system that will be applied to every experimental animal. To generate this scoring system, we have selected welfare indicators that are relevant to this scientific study, are practical to carry out and do not disturb animals during assessment.

Expected adverse effects

- Some animals (5-10%) may experience temporary (less than 24 hours) symptoms (erect fur, respiratory symptoms) following intranasal exposure. Mice are given general anesthetic and there is some risk of pain or death (less than 1%) during the procedure.

- During this time-period animal welfare will be monitored daily

- Weight loss remains the most accurate predictor of mortality, and so body weight will be monitored daily.

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

We will follow:

Joint Working Group on Refinement

The government animal testing and research: guidance for the regulated community (https://www.gov.uk/guidance/research-and-testing-using-animals)

The NC3Rs webpage https://www.nc3rs.org.uk/

Standard Operating Procedures developed with the University facility and the named veterinary surgeon.

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

All personnel associated with this project license will routinely interact with the NC3Rs online resource (https://www.nc3rs.org.uk/). Updates from the website will be a standing item on the group agenda where advances will be discussed.