



Home Office

## NON-TECHNICAL SUMMARY

# New therapeutics for inflammatory bowel conditions

### 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

Inflammation, Intestine, Therapeutics

### Animal types

Mice

### Life stages

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 the project is to facilitate drug discovery and development for the treatment of inflammatory bowel diseases (IBD) in humans.

**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?**

The incidence of inflammatory bowel conditions, such as Crohn's disease and Ulcerative Colitis (UC), is increasing in the developing as well as the developed world. This increase may be driven by changes to the types of bacteria that live in the gut, as a result of the increasing amounts of processed foods that we eat, and other environmental factors that we are exposed to, such as chemicals. These changes may trigger an unwanted inflammatory response in the bowel. An individual's genetic make-up is known to influence their risk of developing long-lasting inflammation.

Inflammatory bowel diseases can be socially-debilitating due to the discomfort, pain and diarrhoea that they can cause; they can also get worse with time and can lead sufferers to develop other life-threatening diseases, such as bowel cancers. As the disease gets worse, patients require more powerful drugs to keep their disease under control. The drugs themselves have unwanted side-effects, such as immunosuppression, leaving patients vulnerable to infection. As a result of damage caused by the inflammation, the bowel may become narrow or stick to itself, or stick to the inside of the body wall (adhesions). Narrowing of the bowel may result in blockage and the adhesions can result in holes forming between different parts of the bowel, or between the bowel and the outside of the body; these issues require patients to have surgery to have the damaged bowel removed.

There is, therefore, an unmet clinical need for new treatments which are more effective, with less side effects.

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

The principal output from the project will be decisions on the potential for new drugs to treat inflammatory bowel diseases in humans. The identification of new drug targets may also be possible.

Where appropriate and permissible, data will be shared with the wider scientific community, through presentation at relevant scientific meetings and publication in peer-reviewed, open-access journals.

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

Primarily, the scientists who have developed new therapeutic strategies and agents for IBD will benefit from being able to make decisions about research priorities. Other researchers pursuing similar goals may benefit from what we find out, by making appropriate choices of experimental models or therapeutic approaches; for example, based on findings that may be presented or published by ourselves or our collaborators. Ultimately (although not within the lifetime of the project), it is hoped that people suffering with inflammatory bowel diseases will directly benefit from the output of the studies that we have been part of, with the availability of new treatments.

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

Work output is dependent on using personnel who are trained and competent in all the experimental techniques required to deliver the aims of the project.

Planning and management of the studies undertaken are also critically important, particularly with regard to the choice of the correct experimental model and design of experiments, so that studies are fit for purpose.

Finally, data must be analysed and communicated effectively in order to ensure that their meaning is fully appreciated and that appropriate conclusions are drawn, and decisions made.

Underpinning all of the above, is the maintenance of animal welfare and the application of humane endpoints, in order to maximise the quality of the data obtained from a study.

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

- Mice: 3750

## **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 are using young adult mice in this project. This reflects the age at which first diagnosis of IBD is most common in humans. Adult mice will have a fully mature gut, including the presence of established populations of microorganisms within the gut. The use of animals allows us to investigate whether drugs can prevent disease and reverse established disease. These models provide essential information for making important decisions during the drug development process. Mice are the animal of lowest sentience in which we can perform these investigations.

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

In this project, mice will develop colitis (inflammation of the large bowel), that is similar to Crohn's disease and ulcerative colitis in humans. Induction of colitis is achieved in one of three ways: a

chemical may be used to cause the inflammation; the mice may have been bred to have a mutation which results in them spontaneously-developing intestinal inflammation; finally, inflammation can be induced in mice which lack a fully functional immune system by injecting them with certain types of white blood cell from a normal, healthy mouse. The mice with colitis will then be treated with drugs to see if they can prevent the development of the disease, or if they can reverse the disease process. These drugs may be administered by injection or given by mouth; they can also be administered in the animals' food and drinking water. Sometimes it may be necessary to perform small studies so that we can gain better knowledge of how and when we should give the drug to treat the disease, and what dose of the drug we should use. Blood may be sampled during the study. Mice can be administered compounds, in addition to the candidate therapeutics being tested, in order to measure changes associated with inflammation; subsequently, these compounds may be measured in blood samples. At the end of the study, mice will be humanely killed and tissues and blood taken for analysis.

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

It is expected that most mice will develop diarrhoea (soft and unformed stool) to some extent, unless they are receiving a drug that prevents the disease. The diarrhoea may be associated with weight loss, or reduced weight gain relative to mice that do not have colitis. The diarrhoea can make the mice dehydrated; the chemicals used to induce the colitis may result in some blood being observed in the stool. It is expected that there will be some observable decrease in the overall body condition of the mouse. The mice can experience some discomfort because of the colitis and this may show in the way they move around. The mice can be less inclined to stand or climb to feed from their food hopper, so food intake may be reduced. Very rarely, there is the risk that a mouse may develop a non-resolving rectal prolapse. Mice may be expected to show mild clinical symptoms for a few days to a couple of weeks in some studies. The implementation of humane endpoints will limit the duration of more severe symptoms.

During the study, repeat injections may cause local irritation at the site. Mice may be restrained for the purpose of blood sampling from the tail vein, which may cause transient distress. Minor pain/discomfort may be experienced when blood is sampled. Very rarely sites of injection/sampling may become inflamed or infected. Discomfort from these procedures should be transitory and last for less than a minute.

Some strains of mice used are immunodeficient and so are more prone to developing infection, such as inner ear infection, although this is rare. Cells from a mouse with a normal immune system can be used to induce intestinal inflammation in the immunodeficient mice; very rarely, these cells may cause inflammation in another organ, such as the lungs. Symptoms of these adverse effects will be recognised in routine welfare checks and limited by the application of humane endpoints.

### **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)?**

mild or less: 20 %

moderate: 80 %

severe: not expected

### **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?**

The gastrointestinal tract is perhaps the most complex organ system in the body. It must provide a physical barrier and is responsible for processing food, absorbing nutrients and excreting waste – all of which are continuously trafficked through the gut. It can be regarded as the largest organ of the immune system and is a first line of defence for the body, against invasive microorganisms. It contains more than 100 million nerve cells. It is home to several hundred species of microorganism, of which not all have been identified; in addition, not all can be grown in a laboratory. Gut health depends on the complex interaction between the cells that line the gut (epithelial cells), the immune cells in the gut and the bacteria (the gut flora). Modelling the complex interactions between the intestine and its immune system and the microorganisms that live in the gut is very difficult; cell culture models are only able to partly recapitulate this. The mouse models of inflammatory bowel disease demonstrate similar features to the disease in humans, such as the dependence on gut bacteria, the types of immune cells involved and the response to a range of therapeutic agents. The animal models of colitis can also be predictive of drug action in humans; we have observed this with respect to a range of new drugs that have recently been licenced for treatment of IBD in humans.

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

Three dimensional cell culture models using human cells are currently being developed. Initially, the complexity of these models will be low, allowing only a direct assessment of how the intestinal epithelial cells (the cells that line the intestine, forming the physical barrier between the inside and the outside of the body) repair following injury and how experimental drugs can modulate the repair mechanism. Subsequently, it is hoped to introduce other components to the cultures, such as immune cells and bacteria (or molecules derived from bacteria). Other laboratories (both academic and commercial) are also developing similar models; we are collaborating with one such laboratory.

These culture models allow us to look at how molecules directly affect the intestinal epithelium, the single layer of cells that line the gut. For example, they can be used to determine if molecules have effects that may be beneficial to the regeneration of this epithelial cell layer, such as changes to how fast the cells divide, changes to the proportions of cells with different functions within this layer, and how good this layer of cells is in forming an effective barrier to microorganisms. The 3D cell cultures can also be used for toxicity screening, which can help in eliminating candidate therapeutics from a pre-clinical development programme.

There will be an initially slow, but progressive accumulation of data that will allow us to compare the outcomes we observe in the cell culture models to those we see in the animal models. The ultimate goal will be to replace, where possible, the need for animal studies.

### **Why were they not suitable?**

The three-dimensional cell culture models currently lack the complexity required to allow any definitive determination of the effectiveness of new drugs to treat IBD in humans. As mentioned previously, we are looking to improve these models and have a programme of cell culture model development in place.

The development of non-animal models depends on the availability of human donor material. The prospective acquisition of such material requires ethical approval and patient availability and so is a slow process (made slower by the current pandemic).

## **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 quoted is based on animal usage over the term of the current licence. The figure quoted is for the whole five year period.

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

When determining the number of treatment groups (and, therefore, animals) required for a study looking at the effectiveness of a new treatment for IBD, the following groups must be considered are:

Untreated (non-disease, baseline) controls, a reference control (a drug known to reduce disease, which is used as a benchmark for the Test drug or drugs) and a relevant placebo control for the reference (e.g. an inactive molecule with a similar structure, or the medium in which the reference drug is formulated).

The remaining groups in the study will be for the Test drug and its placebo control. Careful consideration is given to the range of different doses of drug that may be used in order to reduce the number of treatment groups and, therefore, the number of animals. The range of drug concentrations used is often based on previous studies using the drug, either in another animal disease model or in cell culture experiments; this can also be based on results from studies using similar molecules. These studies may have been conducted by ourselves or others.

The size of the effect we are looking for in response to a drug will influence the number of animals that we use. This has to be realistic and can be based on previous work using similar drugs. The smaller the improvement in disease that we want to detect, then the more animals that need to be used. We commonly set a threshold for being able to detect a 25 % reduction in disease with reasonable certainty.

Testing more than one drug in a study is a good way of reducing the overall number of animals used. In this way, we can minimise the number of mice used for control groups, particularly if the drugs are administered in the same way and at the same frequency.

We continuously review the data that we obtain from our studies in order to monitor how our models and or reference drugs and controls are performing. These data reviewed by independent statistical experts in order to inform us of the correct number of animals to place in treatment groups.

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

We may perform small pilot studies in order to measure the concentration of drug that is achieved in the blood and in the intestinal tissue following its administration; this will be done with small numbers of mice per group (five or less), with one group per concentration of drug. Such studies can inform us of the most suitable concentrations to use in a bigger study to look at the effect of the drug on disease. The range of concentrations tested will be within the toxicity limits of drug; this will have been determined by previous work by other laboratories, based on animal studies and/or cell culture experiments.

We always seek to maximise the amount of information that we can obtain from each study. This is achieved by collecting clinical observations during the study and collecting as many tissue samples as possible, for analysis at the end of the study. We have available a wide range of analytical platforms to support our studies and give us access to multiple readouts that can inform us about different aspects of a drug's ability to suppress intestinal inflammation. In collecting as much data as possible from each study, we minimise the risk of having to run another study in order to gain further knowledge.

## **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 mouse models of inflammatory bowel disease. These models are well-characterised in terms of their biology and the onset of symptoms can be predicted and so a suitable frequency of monitoring animals for welfare purposes can be established and incorporated into study protocols. This also allows us to make informed decisions about when it is appropriate to end a study, according to the

question we are trying to answer (by running the study). Humane endpoints are employed. All mice are humanely killed at the end of each protocol.

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

Immature animals cannot be used as the intestine will not be fully mature and the population of microorganisms found in the intestine is also likely to be immature. These factors have a direct influence on immune responses in the intestine. The Zebrafish can be a useful model for studying genetic influences of intestinal inflammation, but testing candidate therapeutics can be challenging in such an organism and the skills and tools required to perform some of the analyses that are required are limiting. Our ultimate aim is to replace animal models where possible, so it is our opinion that our resources are best focused on this aim rather than establishing and validating another animal model. Mouse models are also the best-characterised and well-established models of IBD and employed by key opinion leaders in this field of research.

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

Mice are monitored at least once daily and clinical observations are recorded. Data are reviewed daily in order to assess if any intervention is required. There are defined points in studies when mitigation such as mash food and increased frequency of observations are introduced. Appropriate guidelines will be followed with regard to best current practice for blood sampling and administration of substances to the mice.

During the project, we will operate a process of continuous review to ensure that the models we run are fit-for-purpose. This involves looking at all the data that we collect and seeing how it may change with time. We can then make appropriate changes to ensure that disease is not too severe, or that models run no longer than they have to.

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

We will perform procedures according to our own standard operating procedures. We will also follow guidelines issues by the Joint Working Group for Refinement. *Lab Animals* (2001) doi: 0.1258/0023677011911345 and guidance from the NC3Rs, as appropriate.

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

We will stay informed about the 3Rs advances through attendance at lectures and seminars run locally, as part of a continuing professional development programme; through the 3Rs website; by keeping up with the latest scientific publications in the field.