Home Office

NON-TECHNICAL SUMMARY

Development of Live Bacterial Therapeutics to treat diseases with intestinal dysbiosis

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

Microbiome, Immunology, Dysbiosis, Therapy

Animal types	Life stages
Mice	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?

To identify mixtures of beneficial bacteria which have the potential to be used as bacteriotherapies (bacteria used to treat human disease) to treat diseases with intestinal dysbiosis.

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?

Imbalances of the intestinal microbiota, termed “dysbiosis” can cause intestinal diseases such as inflammatory bowel disease (IBD) and has further shown a role in the development of other diseases related to diet such as obesity and type 2 diabetes. Ulcerative colitis is the most common type of inflammatory disease of the bowel. It affects about 1 in 400 people in the UK. Crohn's disease affects about 1 in 700 people in the UK.

This work will help us to understand the mechanisms whereby healthy gut bacteria species (microbiome) are able to influence immune responses during intestinal dysbiosis. Specifically, the research will aid in the development of new bacterial therapies to treat diseases associated with imbalances in gut bacteria.

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

The long-term output of this programme will be to identify the best bacterial therapies for the treatment of IBD, obesity and type II diabetes.

In the shorter-term this will involve making new discoveries on the types and behaviour of common gut bacteria found in people and how these interact with the immune system.

Discoveries made on new bacterial species will be published and available to other researchers

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

The significant shorter-term output of the programme of work will be to generate data to demonstrate which bacterial cocktails can be used to progress to pre-clinical development in preparation for clinical studies in humans.

In the longer term, we expect our lead novel bacteriotherapies to increase the effectiveness of current therapies for intestinal diseases such as immunosuppressants (drugs that inhibit or prevent activity of

the immune system) and immunotherapy (biological therapy for the treatment of disease by activating or suppressing the immune system).

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

The results of the research will be published in scientific journals and presented at scientific conferences. New techniques may be patented and shared with other researchers.

Publication of unsuccessful data or techniques will also be considered where possible.

Species and numbers of animals expected to be used

- Mice: 8000

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.

This project will use mice to develop colitis or diabetes. Mice are widely used for this type of work to predict the mechanisms of action and the effectiveness to treat disease of new medicines. Importantly, mice are also able to be bred without gut bacteria (germ-free) to enable us to measure the impact of bacterial therapies. All mice used will be adult mice as these will have a fully developed immune system.

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

The majority of mice on this project will be administered human microbiome or specifically isolated bacteria. Subsequently the gut of these mice will be colonised with these particular bacterial species either using samples obtained from human patients or specific bacteria grown in the lab. Sometimes in order to help these bacteria grow we first need to give the mice antibiotics either in drinking water or by injection into the abdomen to eliminate the bacteria that already live in their digestive tract. At various times they will be treated with our candidate bacterial cocktails given by the mouth which requires putting a small tube into their throat for a very short period of time to deliver the bacteria into the stomach (oral gavage). We will use the least invasive way possible to administer the antibiotics, some of which can be in the food and drinking water but occasionally due to the type of antibiotic we need to do this by injection.

In general the bacterial cocktail will be administered orally under anaesthesia. A subset of these mice will be used in our therapy studies and will be administered with anti-inflammatory drugs by injection to the abdomen. These are treatments that are currently used in hospitals to treat patients. All these procedures will cause minimal suffering to the mice. All the mice will be checked daily by qualified animal care staff.

Mice that have been given bacterial therapies above will then go into a challenge model. These models are DSS colitis which is a chemical given in drinking water that causes an irritation to the gut of the mice. The second model is the development of diabetes where mice will be given high fat diet compared to a normal diet.

When the mice reach the endpoint of the study, defined by a humane endpoint such as weight loss or gain, they will be humanely killed prior to tissue collection. Some mice could have small blood samples collected while they are alive so that we can track the effect of a treatment and a further terminal blood sample under non-recovery anaesthesia.

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

Mice typically do not show any altered behaviour when administered bacterial therapies by oral gavage. These bacteria have been extensively characterised within our laboratory assays (analytic procedures in laboratory medicine). Bacterial cocktails are administered orally under anaesthesia to reduce the movement of the animal and ensure all bacteria reaches the stomach. Thus reducing any likelihood of abrasion to the oesophagus and subsequent infection due to the bacteria.

DSS (Dextran sulphate sodium) is a chemical which irritates the lining of the gut leading to inflammation and the development of colitis. For mice receiving DSS to induce colitis this can give rise to clinical signs such as diarrhoea and weight loss. This will be closely monitored (twice a day or more if necessary) and when it exceeds predefined thresholds of either maximal weight loss of 20% or diarrhoea for 48 hours the affected mice will be humanely killed. Mice will be provided with a mashed diet in the bottom of the cage if weight loss over 10% or diarrhoea is observed.

In the model of diabetes, mice will gain weight over time. Mice will be weighed and have a cut off of 40% weight gain or if the blood glucose level is greater than a pre-set value of 25umol. Animals reaching these endpoints will be humanely killed.

Mice will be used to assess the impact of bacterial cocktails on the immune system of healthy mice in the absence of disease. These mice could experience some side effects from the treatments such as lethargy identified by a lack of responsiveness. All mice are carefully monitored and if they reach pre-set observations such as maximal weight loss or display signs of dehydration or changes to normal behaviour they will be humanely killed. We will provide extra bedding and additional support to the mice to minimise these effects.

The type of mice and experimental protocols that we are using are well established, widely used and designed to cause the least pain, suffering and distress to the mice.

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 - 20%

Moderate - 80%

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 immune system is extremely complex. During the development of IBD there is an interaction between bacteria and immune cells within the gut such as white blood cells. These interactions are highly regulated and can not be replicated in experiments carried out in the laboratory.

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

We are able to grow specific bacteria species from human gut within our laboratory. We then measure the basic interactions of these bacteria with immune cells, such as white blood cells, isolated from human blood samples. These non-animal alternatives allow us to determine the difference between good and bad gut bacteria without the need to use animals. We continuously consider their use and development in light of new experimental data.

Why were they not suitable?

It is not possible to replicate the full complexity of the interactions between bacteria and immune cells using laboratory experiments which do not use animals. Animals with an active immune system remain essential to understand these interactions under conditions within living tissues such as the colon.

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?

Based on our extensive experience with colitis studies in mice, we use 8-10 mice per treatment group. This is a sufficient sample size to allow for the differences in response we typically observe between individual mice. All experiments with a positive outcome are repeated at least once and a maximum of two times to ensure reproducibility.

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

We continuously evaluate our methods and experimental results to determine if we can further reduce the number of animals per experiment. We now believe that one of the major sources of variation is caused by the composition of the bacteria between mouse colonies and even between mice in different cages. The effect of mother-offspring proximity during the early stages of life has a strong influence of the composition of an individual's microbiota.

We will employ three measures to address this. First, we will inoculate germ-free animals with defined bacterial types (microbiota) or a purposely designed microbiota guided by our data. The second approach we will employ is to use littermates, who are genetically identical, such that the microbiota is controlled against cage to cage effects. A third approach is using wild type mice with a standard mouse microbiome that are treated to enable colonisation with human microbiome.

We believe these three approaches will lead to less variation between experiments and, as a result, the need for fewer animals to be used.

We also utilise the NC3R's experimental design tool The Experimental Design Assistant (EDA) (https://www.nc3rs.org.uk/experimental-design-assistant-eda) to aid in experimental planning and follow the most recent PREPARE Guidelines: https://norecopa.no/PREPARE to ensure all aspects are conducted to the highest standards. To ensure best practices we follow the updated ARRIVE guidelines [PLoS Biol 2020 18(7): e3000410]

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

All work is carried out to laboratory standard operating procedures (SOPs) written by Microbiotica and shared with staff within the facility to ensure standardisation throughout the studies.

Pilot studies will be carried out where any new substance is administered to determine variability in response. These data will aid in optimising the numbers of animals required per group to effectively determine any impact of treatment on the development of colitis or diabetes.

Wherever possible, multiple experiments will be performed on the tissues collected from an individual mouse, including harvesting mouse tissues for experiments or cells that can be frozen and stored. For example, the spleen can be used to isolate mouse immune cells for further assays. Colon can be harvested for analysis of the immune cells within and DNA collected to measure changes in mouse genes with treatment.

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.

For this project our idea is that defined bacteriotherapies can prevent the development of or reduce the severity of colitis and diabetes in mice. We used peer reviewed literature to guide our colitis and diabetes model selection alongside in-house data. The chemical-induced colitis model is one we have extensively refined and the addition of the spontaneous colitis model in the IL-10 deficient mice are the most widely used models of colitis.

The chemical induced colitis model has been refined by Microbiotica to administer the lowest concentration of DSS that alters the gut but leads to less weight loss and other harms to the animals. The spontaneous colitis model develops gradually over time and therefore is a less severe model than an induced model.

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

Mice are used in this project as they share many of the same species of gut bacteria as humans. Animals with a more immature life stage do not contain these same bacteria and are not as easily bred and maintained in a germ-free 'bubble' within isolators.

The mice are required to carry out normal habits such as eating and drinking to enable us to measure the impact of orally ingested substances on the gut bacteria.

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

Germ-free mice are bred and housed in iso-cages within 'bubbles' or large contained isolators which prevent the entry of viruses or bacteria. The cages contain environmental enrichment and mice are typically and where possible housed within social groups of 4/5 animals.

Mice will have daily welfare checks which include observing that animals are healthy and can move freely in every cage, have sufficient food and water and the isolator temperature/humidity readings are appropriate. These details are recorded on observation sheets within a day book for assessment of an individual animal's health status.

We have reduced the maximum age of breeding germ free mice to reduce the risk of twisted guts commonly seen in older germ free mice.

Where novel agents are administered, the duration and frequency of monitoring including periods of continuous monitoring will be used. For example, following administration of novel agents a single animal will be dosed and monitored for one hour. Providing no unexpected adverse effects are observed the other animals on study will be dosed.

For mice which receive oral administration of a mammalian microbiome, this procedure is carried out under anaesthetic to reduce the likelihood of any harm due to movement of the mouse during the process and post procedure checks are carried out. We will continue to work with animal care staff to assess the requirement for anaesthetic use.

In our experiments, mice can receive up to the maximum oral dose of 20mg/kg of a mammalian microbiome which enables the optimal dosage and dosing regimen to be examined and determined in fewer experiments. In addition, we may also include specific control groups in our experimental designs to aid in data interpretation and analysis. Collectively, these specific refinements to our experimental procedures will provide more in-depth analysis the outputs from these experiments; and in doing so, they may also reduce our overall mouse numbers.

Active use of and continuous development of humane endpoints will allow us to continually refine procedures. We aim to test the use of the mouse grimace scale for each protocol (https://nc3rs.org.uk/grimacescales). For colitis models we have an in-house scoring sheet that monitors weight-loss and other signs such as diarrhoea over time.

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

To ensure best practices we follow the updated ARRIVE guidelines [PLoS Biol 2020 18(7): e3000410]

We also follow the PREPARE guidelines to ensure all best practices are followed by researchers who use animals on this licence. [Lab Anim. 2018 Apr;52(2):135-141. doi: 10.1177/0023677217724823. Epub 2017 Aug 3.]

In addition, we follow updates to best practice guidelines on The Laboratory Animal Science Association (LASA) and NC3Rs websites.

We will publish to ARRIVE and PREPARE guidelines and where possible data will be presented at scientific conferences.

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

We will use the NC3Rs website to gather up to date information about advances in animal research. This information will be disseminated to anyone involved with this licence including Project Licence Holder (PPLH), Personal Licence Holders (PILs), Named Animal Care and Welfare Officers (NACWOs), Named Information Officer (NIO) and Named Veterinary Surgeon (NVS) and implemented where appropriate.

We have subscribed to ATLA (Alternatives to Laboratory Animals) published by FRAME (Fund for the Replacement of Animals in Medical Experiments), in association with SAGE publishing to keep up to date with recent advances and alternatives in animal research.