NON-TECHNICAL SUMMARY

Development of Bacteriotherapies to Treat Intestinal Dysbiosis

Project duration
5 years 0 months

Project purpose
- (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
Bacteriotherapy, microbiota, dysbiosis, infection, inflammatory bowel disease

Retrospective assessment
The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits
Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?
Mammals are colonised by diverse and abundant microbial communities of bacteria fungi and viruses, termed microbiota. These microbiota are required for normal immune system development and response to pathogens. Imbalances in the composition of the microbial communities, termed dysbiosis, can cause diseases to develop and poorly understood syndromes, such as IBD and infection susceptibility.

The aims of the project are to design and test bacteriotherapies - defined mixtures of beneficial 'health-associated' bacteria - to correct dysbiosis and treat Clostridium difficile infection and Inflammatory Bowel Disease (IBD) in murine disease models.

Amendment March 2021

This licence required an amendment as the current facility is closing. Therefore we require a new establishment to transfer animals, set up the breeding of mouse colonies and continue research.

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.

What are the potential benefits that will derive from this project?

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

In the longer term, the work outlined in this proposal is expected to lead to novel bacteriotherapies to treat intestinal imbalances in bacteria linked to Clostridium difficile infection and Intestinal Bowel Disease (IBD) in humans to reduce disease severity.

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

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Over the 5 year period of the project, we anticipate to use 6300 mice.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?
We will give mice different mixes of bacteria or a substance which causes inflammatory bowel disease by inserting a tube into their mouths and throats, by injection, by using infected bedding or housing the mouse with another infected mouse. We will then take blood samples, faecal samples and surface tissues and check the health of the animals. Some animals which will have been given the mix of bacteria, will then be given disease-causing microorganisms to see whether they are resistant to infection. Animals used for these procedures are expected to experience disease symptoms such as weight loss and inflammation. In some mice, compounds which are similar to clinically approved drugs will be administered via injection through the wall of the abdomen to see whether they protect the mice from developing disease. Mice will be humanely killed at the end of the experiments.

Animals are expected to experience mild to moderate intestinal disease and inflammation and will be treated with bacteriotherapies with the goal of curing disease. Mice will be humanely killed at the end of the experiments.

The breeding of animals in itself is not expected to exhibit any harmful effects.

Germ-free mice can develop abnormalities within the gut such as an enlarged caecum (pouch connecting the small and large intestine) as they age. In turn, the size of the caecum may trigger a torsion of the intestine (twisted caecum). This causes chronic diarrheal status and death of the mouse in up to 3% of the animals.

Mice will be checked at least twice per day and those observed to be less active, showing poor coat condition, abdominal distension, or other clinical signs that in any way compromises normal behaviour will be humanely killed.

Gnotobiotic mice, containing a known microbiome, do not develop the same intestinal issues as germ-free mice.

Breeding of animals is not expected to produce any adverse effects. For natural breeding issues, such as littering problems, advice will be sought from a Named Animal Care and Welfare Officer or the Named Veterinary Surgeon. The age of breeders, number of litters and breeding performance will be monitored to minimise the impact of the enlarged caecum with age.

**Replacement**

State why you need to use animals and why you cannot use non-animal alternatives.

The complex environment of mammalian surfaces and organs cannot be accurately modelled *in vitro* (in laboratory conditions) and, as a result, the immune response and pathological features linked to host-microbe interactions cannot be replicated.

Using human cells from blood samples for laboratory assays does not fully replicate the complexities of the gut barrier and the interactions which occur between gut bacteria and the immune system (white blood cells) within the gut.

**Reduction**
Explain how you will assure the use of minimum numbers of animals.

We have established infection protocols with reproducible disease courses and defined outcomes using pathogens with clinical features that are relevant to human diseases. During this period we have also established statistical measures linked to biologically relevant outcomes to ensure we employ the minimal number of animals per experiment.

The results of the research will be published in scientific journals and presented at scientific conferences.

New mouse models may be patented and shared with other researchers.

The use of a range of human laboratory assays to complement this research will increase our understanding of the behaviour of particular bacterial species. This will subsequently help to reduce the number of animals required for this project.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We use mice for select experiments as they represent an ideal model to study host-microbe interactions, and serve as an invaluable pre-clinical model for therapy development. Mice can be genetically manipulated to mutate genes relevant to human disease susceptibility and there are immunological reagents available to monitor the host response to microbe interactions. We also closely monitor mice on a daily basis for signs of illness and suffering, scoring for physical signs of illness such as piloerection, hunched gait and mobility along with weight loss. Our animal facility uses a sophisticated database to track the health status of every animal.

High quality source materials/agents will be used, e.g. therapies already used to treat human diseases. When determining what concentration and frequency to use for antibodies or anti-inflammatory agents this will be guided by information available in the literature or internal expertise.

To reduce the likelihood of the enlarged caecum with age in germ-free mice breeding will be limited to 12 weeks of age for males and 18 weeks for females (if pregnant at 12 weeks).

Mice will have two 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.

For the 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.