Home Office

NON-TECHNICAL SUMMARY

Improving phage therapy to combat multidrug-resistant infections.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Bacteriophages, Phage therapy, Microbiome, Antibiotic resistance

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant 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?

This project aims to improve the therapeutic use of bacteriophages by investigating how bacterial defence systems and host immune responses impact phage effectiveness in the gut. By studying these interactions in vivo, we hope to design more effective, long-lasting phage therapies against antibiotic-resistant bacteria.

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?

Antimicrobial resistance (AMR) is a critical global health concern, with bacteria increasingly acquiring genes that render them resistant to all available antibiotics. This has made common infections harder to treat and has contributed to thousands of deaths annually in the UK alone. Among the many challenges AMR poses, one emerging concern is its role in intestinal inflammation. Recent studies, including my own, have shown that certain AMR bacteria can trigger gut inflammation in patients with Inflammatory Bowel Disease (IBD). These bacteria are often resistant to treatment and can persist even after phage therapy, likely due to poorly understood bacterial defence mechanisms that are active in the inflamed gut environment.

Bacteriophages (phages), viruses that specifically infect and kill bacteria, offer a promising alternative to antibiotics. Unlike antibiotics, they are highly selective and do not harm the surrounding beneficial microbiota or the host. However, despite their therapeutic potential, phage efficacy in vivo remains unpredictable. This is largely because we lack a detailed understanding of how bacterial defence systems and host immune responses shape phage success or failure in the gut.

This research is therefore essential to uncover how AMR bacteria resist phages in the context of inflammation and immune activity. By addressing this gap, we aim to generate the knowledge needed to make phage therapy a safer, more effective clinical tool for managing antibiotic-resistant, inflammation-associated gut infections.

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

We will understand how the immune system and the gut microbiome (the trillions of bacteria inhabiting the human gut, and playing a crucial role in maintaining health and regulating various biological processes) act in favour or against bacteriophages used as antimicrobial therapy against antibiotic resistant bacteria during gut inflammation.

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

This research will provide benefits across multiple sectors, with impacts ranging from short-term advancements to long-term public health improvements:

Short-Term Beneficiaries (During the License Period): Scientific and Medical Research: This work will enhance our understanding of bacterial-phage interactions, microbiome therapies, and personalized medicine, leading to new discoveries and innovations. The findings will also support the development of improved phage therapy protocols.

Medium-Term Beneficiaries (Translational Impact Post-Research): Patients with Antibiotic-Resistant Infections: Phage therapy offers a crucial alternative for patients with infections no longer responsive to antibiotics, potentially reducing disease severity and mortality.

Long-Term Beneficiaries (Broad Societal and Global Impact): Public Health: By providing an effective tool against resistant pathogens, phage therapy may contribute to controlling the global antibiotic resistance crisis, ultimately helping to preserve the efficacy of existing antibiotics and reduce the burden of drug-resistant infections.

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

To maximize the outputs of this work, we will prioritize collaboration, knowledge dissemination, and transparency, including sharing both successful and unsuccessful results. We have established collaborations with experts in microbiome dynamics, bacteriophage therapy, and infectious diseases, both within our institute and internationally, including clinical researchers working on antibiotic resistance. These collaborations will facilitate resource sharing, data exchange, and innovative approaches, accelerating research progress and increasing its robustness.

To ensure effective translation to patient benefits, we are engaging with clinical partners to explore the feasibility of integrating phage therapy into treatment strategies for antibiotic-resistant infections. Findings will be disseminated through conferences, workshops, and open-access publications to ensure accessibility. Additionally, we will work with healthcare practitioners and policymakers to support practical applications and policy discussions. By documenting and publishing both successful and unsuccessful methodologies, we aim to enhance transparency and provide valuable insights for future research. Negative results, which are often underreported, will be shared through open-access journals specializing in microbiology, infectious diseases, and experimental medicine. Additionally, they will be included in conference presentations, research data repositories, and preprint platforms to ensure accessibility. Where possible, we will integrate these findings into systematic reviews and meta-analyses to provide a broader scientific context and avoid redundant efforts.

Species and numbers of animals expected to be used

- Mice: 4000

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.

In this project, we want to improve effectiveness of phage therapy by understanding how bacteria and bacteriophages interact in the mammalian intestine. One possible scenario is that bacteria activate anti-phage mechanisms to eliminate phages and survive virus infection. On the other hand, phages are also identified and eliminated by the gut immune system, although no existing literature clarifies which part of the immune system recognise and eliminates them. While these aspects need further study, the result is that phage therapy is hindered in vivo, unable to effectively eliminate infection. To address this, we will use two approaches: in vitro experiments, which focus on specific interactions between phages, bacteria, and immune cells, and in vivo studies in adult mice, which provide a complete view of how these interactions occur in a living gut environment.

In particular, mouse models allow us to study how gut inflammation, such as that seen in Inflammatory Bowel Diseases (IBD) like Crohn’s disease and ulcerative colitis, affects phage therapy. Unlike in vitro systems, living models are essential to simulate the complexity of inflammation and the gut's immune response. Overall, mouse model will enable a comprehensive understanding of the organism as a whole, which cannot be achieved solely through ex vivo or in vitro techniques.

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

If mice are germ free, they will be kept in germ-free conditions until colonization and/or infection with bacteria, in order to avoid contamination from other microorganisms. Germ-free animals will be colonised immediately upon removal from isolators. This should help avoiding the caecal enlargement sometimes these animals suffer. However, ex-germ free animals will be monitored for signs of abdominal distress as a precaution, and animals showing signs of discomfort will be promptly assessed and humanely culled if needed.

Mice may be infected and/or colonized with bacteria and bacteriophages through oral gavage. Colitis will be induced using various protocols, including:

- Immune cell transfer: Immune cells will be isolated from the spleens of humanely culled mice and transferred via intravenous injection into recipient mice with different gut microbiomes.

- Dextran sulphate sodium (DSS) administration: DSS, a chemical used to induce colitis and mimic inflammatory bowel disease, will be administered through drinking water.

- Chemotherapy-induced colitis: A single dose of a chemotherapy drug will be administered via intra-peritoneal injection to induce colitis.

Different protocols for colitis induction are necessary to model various clinical conditions in humans. Immune cell transfer and chemotherapy-induced colitis mimic chronic colitis, while DSS-induced colitis represents an acute flare-up. Both methods for chronic colitis are considered because, while immune cell transfer is biologically gentler and closely resembles human pathology, its efficiency in disease induction can be variable.

Following colitis induction, mice may receive injections as part of the treatment protocol, and blood samples may be collected at multiple time points via tail puncture to assess inflammatory and immune responses. Mice will also be weighed frequently to monitor body weight and inflammation onset. Stool samples might be collected at various time points, and broad-spectrum antibiotics may be administered via drinking water to deplete the gut microbiome.

For imaging purposes, mice may be anesthetized using injectable substances to ensure they remain immobile while still alive.

At the end of the experiment, mice will be humanely culled, and tissues such as the gut, lymph nodes, and spleen will be collected for further analysis.

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

Intestinal inflammation induced by adoptive immune cell transfer: there might be an acute phase where clinical symptoms such as weight loss, diarrhoea, and rectal bleeding may appear, and then subside after successful treatment initiation.

Intestinal inflammation induced by DSS administration: acute inflammation may appear, peaking within 7–10 days, with recovery typically occurring over the next 1–2 weeks. If applying repeated DSS cycles, inflammation and symptoms such as weight loss and diarrhoea might be longer, depending on the number and length of DSS cycles.

For some bacterial infections employed (e.g. Citrobacter rodentium), minor inflammation might occur. In some mice (~10%) some weight loss (5–10% of body weight) and diarrhoea might occur 1-2 weeks post-infection. However, the majority of mice clear infection, recover and become symptomless by 3-4 weeks post-infection.

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)?

30% of mice will experience moderate severity, 10% will experience mild severity and the remaining fraction will be sub-threshold.

What will happen to animals used in 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?

This study will investigate the interactions happening in the tripartite system bacteria-phages-mammalian host, to push phage therapy to clinical use. These interactions involve multiple cellular and molecular components, that cannot be fully recapitulated in ex vivo or in vitro. In vitro systems already

exist but they fail when recapitulating effects coming from the immune system. The intestine of an adult mouse model, our chosen model, provides a more holistic and realistic representation of complex biological processes. The study of the complex immune machinery and its response to bacteriophages during inflammation requires a comprehensive understanding of the organism as a whole, which cannot be achieved solely through *ex vivo* or *in vitro* techniques.

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

Several in vitro experiments will be conducted to assess bacteria-phage interactions and phage-immune cell interactions in detail. For instance, we will use cells that emit a particular colour when encountering viruses such as phages. This will allow us to characterize part of the immune response in vitro in a more humane and efficient way. Alongside these experiments, we will consistently review the latest literature to identify emerging models that could potentially replace animal experiments in studying immune responses. One area of particular interest is investigating bacteria-phage interactions in vitro (outside of living organisms). Our collaborators use a system of interconnected bioreactors, which are specialized laboratory devices that simulate natural microbial environments. These systems allow us to generate valuable data on how bacteria and phages interact under controlled conditions, helping to refine and reduce the need for animal studies.

Why were they not suitable?

While this study uses in vitro experiments to examine bacteria-phage and phage-immune cell interactions, these models alone cannot capture the full complexity of the bacteria-phage-host system. This research investigates whether bacterial anti-phage defences and the mammalian immune response act synergistically or antagonistically, impacting phage therapy efficacy.

The adult mouse intestine, our chosen model, provides a more realistic environment for these intricate interactions. Here, the immune system, microbiota, and molecular pathways respond dynamically to phages and inflammation—an interconnectedness that in vitro models lack.

Although ex vivo models like 3D organoids are promising, they currently lack the diverse immune and physiological elements of a whole organism. Therefore, while in vitro methods offer valuable insights, studying the live mammalian host remains essential for understanding phage therapy’s full potential and challenges.

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 required for this study was carefully estimated based on the experimental design, including the number of treatment groups, time points, and replicates needed to obtain reliable and reproducible data. Previous studies present in literature, and our own experience using these specific models, informed our estimates, ensuring our approach is consistent with standard practices and minimizes unnecessary replication.

We considered experimental groups, time points and technical repeats. We also included in prediction biological variation and we included sufficient animals per group to ensure robust conclusions while minimizing animal use.

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

The NC3R's Experimental Design Assistant (EDA) was utilized to refine our study design, ensuring that the experimental setup was optimized to reduce unnecessary animal use while addressing all research objectives. If no historical data is available on which to base the estimated variance, then we will use pilot experiments initially and use these to get provisional estimates. The sample size for decision making studies will be selected based on an understanding of a biologically meaningful effect and the variability in the primary endpoint. Randomization will be employed, and shared control groups across experiments are planned to optimize animal use. Scientific expertise and previous experimental data will be used to assess both aspects. Where variance estimates from previous experiments are available, power calculations to determine the minimum number of animals required per group will be performed, based on a statistical power of 80% and a significance level of 5%.

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

A thorough review of the literature was conducted to assess typical group sizes and outcomes for similar models. This ensured our estimates were realistic and aligned with existing knowledge, avoiding redundant experiments. Pilot experiments are planned to determine feasibility and refine methodologies, helping us avoid overestimating the number of animals required. We employed factorial designs to analyse multiple variables simultaneously, reducing the number of individual experiments. Whenever possible, we predict to perform multiple measurements from the same animal and sharing tissue with other researchers at the establishment. This approach reduces the need for additional animals. We carefully planned breeding schedules to ensure only the required number of animals will be bred for the study, avoiding surplus. Breeding pairs will be monitored for productivity, and surplus breeding avoided by aligning colony management with experimental timelines. Animals not used in experiments will be appropriately allocated to other ongoing studies whenever feasible, ensuring ethical use.

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.

DSS and adoptive T cell transfer are widely accepted protocols for inducing colitis, providing a reproducible and controlled model of intestinal inflammation. The dose and duration of DSS exposure are carefully titrated, both based on literature and advice with colleagues at the establishment, to induce mild to moderate inflammation, avoiding excessive or unnecessary severity. These methods avoid surgical interventions or invasive procedures, further reducing physical harm. Bacterial infections and phage therapy are administered orally or via non-invasive methods, avoiding surgical inoculations or prolonged interventions that could cause undue distress.

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

The use of specific animal models in research is determined by the scientific objectives and the biological relevance of the model to the research question. While less sentient organisms, such as invertebrates or cell culture systems, are valuable tools, they may not adequately replicate the complex interactions between eukaryotic host immune systems and pathogens observed in vertebrate models. For example:

- Complexity of Immune Response: Vertebrate models are often required to study systemic immune responses, which cannot be fully mimicked in simpler organisms.

- Specificity of Phage-Bacteria Dynamics: The interplay between bacteriophages, bacteria, and mammalian host organisms may depend on host-specific factors that only occur in vertebrates.

- Translation to Human Health: If the ultimate goal is to understand or develop therapies for human applications, animal models closer to humans physiologically are necessary for relevance and reliability.

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

Non-invasive techniques are prioritized for assessing disease progression and response to treatment, such as imaging of alive mice infected with chemiluminescent Citrobacter or faecal sample analysis, rather than invasive biopsies.

Minimisation of animal suffering critically depends on close monitoring of mouse condition (body weight, mobility, the quality of mouse fur). Staff will perform inspections twice a week, but daily if concerned (e.g., peak of infection, inflammation), to identify and address signs of pain or distress promptly. Weight loss, stool consistency, hydration, and general well-being are tracked, with humane endpoints strictly adhered to during peak infection. Supportive care measures, such as hydration and easily digestible food, will be provided to mitigate discomfort during colitis induction. For any optional procedures involving injection, we always aim to keep the numbers of administrations to the minimum

needed to achieve the aims of the experiment. Furthermore, optimal housing conditions, such as enrichment and group housing will be implemented in every experiment to ensure animal welfare.

Tissue collection will be performed at appropriate time points, ensuring that animals are euthanized humanely before inflammation becomes unmanageable or recovery becomes excessively prolonged.

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

We will use the NC3Rs Guidelines, the ARRIVE Guidelines, FELASA Guidelines and Guide for the Care and Use of Laboratory Animals.

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

Staying informed about advances in the 3Rs (Replacement, Reduction, and Refinement) and incorporating them into our project is a key ethical priority. To achieve this, we will:

1. Engage with Relevant Literature: We will routinely review publications, particularly in journals and conferences focused on animal welfare, the 3Rs, and experimental methodologies.

2. Collaborating with our institution’s Named Persons, and other 3Rs specialists will allow us to integrate expert recommendations into our experimental protocols.

3. My group will attend training sessions and workshops on 3Rs implementation, such as those offered by NC3Rs and other organizations dedicated to ethical research practices and innovative methodologies.

4. We will actively explore advanced alternatives, such as organ-on-chip models, computational modelling, and high-throughput in vitro systems, where applicable to reduce animal usage.

5. Regular Ethical Review: During the course of the project, we will work closely with our NACWO and NVS to review experimental protocols periodically. This ensures compliance with the Animals (Scientific Procedures) Act 1986 (ASPA) and identifies opportunities to further reduce animal use or refine techniques.