PROGRAMME DOCTORAL
Institut Pasteur/Sorbonne Université – Projet de recherche doctoral

Establishing the efficacy of phage therapy for multi-drug resistant *Klebsiella pneumoniae* urinary tract infections

Rationale:

Antimicrobial resistance (AMR) is a major health care and societal concern and was the cause of up to 4.5 million deaths, worldwide, in 2019. *Klebsiella pneumoniae* contributes significantly to the acquisition and transmission of antibiotic resistance and is the 3rd cause of death associated with AMR in the world [1]. In France, *K. pneumoniae* is the 3rd leading cause of urinary tract infection (UTI), with prevalence increasing every year [2].

Among healthy individuals, 60% of women and 5-14% of men will experience UTI in their lifetime, with up to 44% recurring within six months [3]. In people with bladder dysfunction due to peripheral or central neurological lesions (neurobladder), UTI account for almost half of healthcare-associated infections with a major negative impact on morbidity and mortality [4]. In this population, *K. pneumoniae* is one of the two most prevalent multi-drug resistant (MDR) uropathogens [5]. Critically, MDR neurobladder UTI are associated with an increased risk of treatment failure and kidney failure, prolonged hospital stays, and higher morbidity and mortality [5-7]. Thus, uropathogen drug resistance is a major clinical challenge that will only become more difficult to manage as antibiotic resistance spreads. One way to address this challenge is by the development of non-antibiotic-based therapies to manage recurrent UTI in these vulnerable patients. The use of therapeutic bacteriophages represents one such promising alternative. However, data from *in vivo* UTI models and use in humans is limited.

Objectives:

The global objective of this study is to demonstrate the efficacy of bacteriophages targeting clinical MDR *K. pneumoniae* strains from neurobladder UTI in a preclinical model of UTI, as a crucial step to validate their use in patients. Specifically, the PhD student will (1) select, sequence, analyze the *in vitro* interactions of phages with *Klebsiella pneumoniae* strains isolated from neurobladder patients with recurrent UTI, to define the best phage cocktail that kills *Klebsiella pneumoniae*, (2) optimize a preclinical mouse MDR *Klebsiella pneumoniae* UTI model to assess the efficacy of the phage cocktail, and (3) assess immune responses to phage therapy *in vivo*.

Methodology:

In Aim 1, isolated bacteriophages targeting MDR *Klebsiella pneumoniae* clinical strains will be characterized by sequence, burst size, efficiency of plating, pH, and temperature stability. Mutant bacteria resistant to the bacteriophages will be isolated and their genomes sequenced to identify the receptors of these bacteriophages. Together these analyses will define cocktails of active bacteriophages targeting different bacterial receptors. Cocktail efficacy will be increased using *in vitro* targeted evolution by the Appelmans protocol [8]. The stability and efficiency of the optimized bacteriophages will be tested in urine and in an *in vivo Galleria mellonella* larvae infection model. This work will be performed in the CIMI laboratory (Dr Eckert) and is estimated to take 12-18 months.

In Aim 2, to establish an MDR *Klebsiella pneumoniae* UTI model, strains from neurobladder patients will be intravesically instilled via catheter into female and male mouse bladders, using a protocol routinely used in the Pasteur lab [9]. Infection efficacy will be assessed by determining bladder bacterial load at specific timepoints post-infection (e.g., 1, 2, 7, 14, 28 days post-infection). Resolution kinetics will be determined by urine sampling. Up to 5 MDR *Klebsiella pneumoniae* strains will be tested to identify strains that achieve at least 10^5 CFU per bladder at 24 hours to enable assessment of bacterial burden reduction after phage therapy. Phage activity will be assessed by treating mice at different time points (e.g., 1, 2, 7 days post-infection). Phages will be

given by intraperitoneal or intravesical injection, alone or with antibiotics and bladders will be collected 24 hours post-treatment to determine bacterial burden and number of phages in comparison to untreated groups.

For Aim 3, bladder homogenates will be collected for analysis of cytokines by multi-analyte assays (e.g. Luminex). Flow cytometry will be used to determine whether phage therapy alters the cellular immune response. Aims 2 and 3 will be performed in the Institut Pasteur lab (Dr Calin) and is estimated to take 18-24 months.

Expected results and perspectives:

While Aim 3 depends upon Aim 1 and 2, we are confident this project is feasible as Dr Calin already established that at least three *Klebsiella pneumoniae* strains isolated from neurobladder patients infect mice to levels that will allow assessment of whether phage therapy is efficacious. Additionally, at least 40 phages that target *Klebsiella pneumoniae* strains have been identified and will be tested against patient MDR *Klebsiella pneumoniae* strains (CIMI lab, Dr Eckert). We expect that this work will identify and validate a phage cocktail with efficacy against MDR *Klebsiella pneumoniae* in a robust preclinical model, and establish whether this approach synergizes with host immune responses, providing a foundation for the development of a clinical trial to treat neurobladder patients with phages. Both the CIMI team and the Institut Pasteur team investigate new therapeutic approaches to fight antibiotic-resistant bacterial infections. Dr Eckert is a clinical microbiology specialist with extensive expertise in antimicrobial resistance and *Klebsiella pneumoniae* phage therapy. Dr Calin is an infectious diseases physician with extensive clinical expertise in the management of relapsing UTI in complicated clinical settings, such as neurobladder. Dr Calin is currently working on mouse UTI models in Dr Ingersoll's team "Mucosal Inflammation and Immunity" at Institute Pasteur and developed the MDR *Klebsiella pneumoniae* UTI mouse model. The PhD candidate will learn diverse skills and techniques in the CIMI and Pasteur environment, working with BSL2 pathogens and performing imaging with spinning disk microscopy, flow cytometry, mass spectrometry, and bioinformatics in the two labs and in technical core facilities.

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