Rationale: Urinary tract infections (UTI) are the most common bacterial infection encountered in clinical practice. In neurologic bladder dysfunction (neurobladder), UTI account for 40% of healthcare-associated infections with major impact on morbidity and mortality. In these infections, the proportion of multi-drug resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae* (KP) bacteria is higher than in the general population, leading to a higher risk of therapeutic failure. Antimicrobial resistance (AMR) is a major health care and societal concern and the cause of up to 4.5 million deaths, worldwide, in 2019. KP contributes significantly to the acquisition and transmission of antibiotic resistance and is the 3rd cause of death associated with AMR in the world. Thus, there is an urgent need for the development of innovative therapeutics for the management of MDR UTI, to offer potentially life-changing solutions to patients in therapeutic impasse and reduce the consumption of antibiotics to limit antibiotic resistance. 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 KP strains from neurobladder UTI in a preclinical model of UTI to validate their use in patients. Specifically, the PhD student will (1) select, sequence, analyze the *in vitro* interactions of phages with KP strains isolated from neurobladder patients to define the best cocktail combination of phages that kill KP, (2) optimize a preclinical mouse MDR KP UTI to assess the efficacy of the pre-defined phage cocktail, and (3) assess immune responses to phage therapy *in vivo*.

Methodology: In Aim 1, isolated bacteriophages targeting MDR KP 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. The stability and efficiency of the optimised bacteriophages will be tested in urine and an *in vivo Galleria mellonella* larvae infection model. This work will be performed in the CIMI lab (Dr Eckert) and is estimated to take 12-18 months.

In Aim 2, to establish an MDR KP UTI model, strains from neurobladder patients will be intravesically instilled via catheter into female and male mouse bladders. 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 10 MDR KP 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 administered by intraperitoneal injection, alone or with antibiotics and bladders will be collected 24 hours post-treatment to determine bacterial burden in comparison to untreated groups. For Aim 3, samples will be collected for analysis of cytokines by multi-analyte assays (e.g. Luminex). Flow cytometry will be used to measure the cellular immune response, to determine whether phage therapy alters the cellular immune response. This work 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 we have already established that at least three KP strains isolated from neurobladder patients infect mice to levels that will allow us to assess whether phage therapy is efficacious. Additionally, at least 30 phages that target KP strains have been identified and will be tested against patient MDR KP strains. We expect that this work will identify and validate a phage cocktail with efficacy against MDR KP in a new 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. Dr Calin is an infectious diseases physician with clinical expertise in the management of relapsing UTI in complicated clinical settings, such as neurobladder. She works on mouse UTI models in Dr Ingersoll's team "Mucosal Inflammation and Immunity" at Institut Pasteur. 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.