|  |
| --- |
| **Establishing preclinical safety and efficacy profiles of bacteriophage Kara-mokiny 3 targeting *Pseudomonas aeruginosa*** |
| Renee N Ng1,2\*, Barbara J Chang3, Stephen M Stick2,5,6, Anthony Kicic2,4,6, WAERP1,2,5,9, AREST CF2,5,7,8 |
| *1School of Biomedical Sciences, University of Western Australia, Australia*  *2Wal-Yan Respiratory Research Centre, Telethon Kids Institute, Australia*  *3The Marshall Centre for Infectious Diseases Research and Training, School of Biomedical Sciences, University of Western Australia, Australia*  *4Occupation and the Environment, School of Population Health, Curtin University, Australia*  *5Department of Respiratory and Sleep Medicine, Perth Children’s Hospital, Australia*  *6Centre for Cell Therapy and Regenerative Medicine, School of Medicine and Pharmacology, University of Western Australia and Harry Perkins Institute of Medical Research, Australia*  *7Murdoch Children's Research Institute, Australia*  *8Department of Paediatrics, University of Melbourne, Australia*  *9St John of God Hospital, Subiaco, Australia* |
| **Introduction/Aims:**  Acute bacterial respiratory infections are extremely challenging to treat since bacteria readily become antibiotic resistant. Hence, alternative treatments including bacteriophage (phage) therapy are currently being explored. This study hypothesised that the exposure of primary airway epithelial cells to a purified phage does not induce airway cytotoxicity or stimulate inflammation and reduces viable bacterial counts.  **Methods:**  Phage Kara-mokiny 3 was propagated and endotoxins removed via tangential flow filtration. Primary airway epithelial cells (pAEC, n=7; 5 males; mean; 4.2±2.4 years) cultured at air-liquid interface (ALI) were then exposed to Kara-mokiny 3 and *P.* *aeruginosa* PAO1 for 24 h. Inserts were stained with haematoxylin and eosin and alcian blue to assess macroscopic structural modifications post-exposure. Collected basolateral supernatants and apical washes were measured for inflammatory cytokine production (IL-6 and IL-8) and cytotoxicity (LDH). Bactericidal activity of Kara-mokiny 3 was measured through enumeration of viable *P. aeruginosa* post-treatment.  **Results:**  No disruptions to the pseudostratified layers of the airway were observed. There were no significant differences observed across the measured alcian blue-stained sections. pAECs exposed to Kara-mokiny 3 did not directly induce airway cell death (LDH fold change (FC); -0.6±0.3). Post-infection, IL-6 production at the apical surface (representative of luminal inflammation) although elevated was not significantly different (FC; 7.3±15.8) compared to controls. A similar observation was seen in the basolateral supernatant (representative of systemic inflammation, FC; -0.5±0.7) measured. Similarly, IL-8 was not induced when pAECs were exposed to Kara-mokiny 3 (apical FC; -0.1±0.3; basolateral FC; -0.2±0.2). There was a significant 105-fold reduction in viable bacterial counts observed post-treatment with Kara-mokiny 3 (1.2x108±1.3x108 CFU/mL vs 1.4x103±1.4x103 CFU/mL).    **Conclusion:**  This study generated preclinical safety data on use of phage Kara-mokiny 3, namely no macroscopic disruption to the airway cellular architecture, no induction of airway cell death and no significant airway inflammation, while demonstrating strong bactericidal effects.  **Grant Support:**  Department of Health WACRF Project Grant  Perpetual Impact Grant  Perth Children’s Hospital Research Fund  The University of Western Australia RTP Scholarship  CFWA Golf Classic Top Up Scholarship  Wesfarmers Centre for Vaccine and Infectious Diseases HDR Top Up Scholarship  Stan and Jean Perron Research Excellence Award |