Title

Exploring Bacteriophage Therapy Against *Pseudomonas aeruginosa* in Paediatric Cystic Fibrosis: Early-Phase Clinical Trial Implementation

Submitted by

Jagdev Singh, MBBS, FRACP

A thesis in fulfilment of the requirement for the degree of

Doctor of Philosophy

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For my Nanaji and Naniji

A picture of bacteriophage drawn by a child with CF enrolled in this clinical trial (reproduced with permission)

Contents

Statement of authorship

This is to certify that to the best of my knowledge; the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged in the respective publications.

Signature: *signed*

Name: Jagdev Singh

Date: 31st October 2024

Acknowledgement

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Publication and relevant achievements

Published manuscripts

1. Singh, J., Hunt, S., Simonds, S. *et al.* The changing epidemiology of pulmonary infection in children and adolescents with cystic fibrosis: an 18-year experience. *Sci Rep* **14**, 9056 (2024). https://doi.org/10.1038/s41598-024-59658-4
2. Singh J, Robinson P, Pandit C, Kennedy B, Weldon B, Bailey B, *et al*. Factors influencing treatment response of pulmonary exacerbation in children with cystic fibrosis. Minerva Pediatr 2024;76:245-52. https://doi.org/10.23736/S2724-5276.23.07221-X
3. Jagdev Singh, Eugene Yeoh, Dominic A. Fitzgerald, Hiran Selvadurai. A systematic review on the use of bacteriophage in treating *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections in cystic fibrosis, Paediatric Respiratory Reviews, Volume 48, 2023. https://doi.org/10.1016/j.prrv.2023.08.001
4. Singh J, Fitzgerald DA, Jaffe A, *et al* Single-arm, open-labelled, safety and tolerability of intrabronchial and nebulised bacteriophage treatment in children with cystic fibrosis and *Pseudomonas aeruginosa* BMJ Open Respiratory Research 2023;10:e001360. https://doi.org/10.1136/bmjresp-2022-001360

5. Jagdev Singh, Stephanie Lynch, Jonathan Iredell, Hiran Selvadurai, Safety and Tolerability of Bronchoscopic and Nebulised Administration of Bacteriophage. Virus Research, 2024, ISSN 0168-1702. https://doi.org/10.1016/j.virusres.2024.199442

Conference abstracts

6. J. Singh, D. Subedi, F. Gordillo-Altamirano, R. Patwa, H. Selvadurai *et al.* Isolating and characterising phages against *Pseudomonas aeruginosa* and *Staphylococcus aureus* in children with cystic fibrosis, European Cystic Fibrosis Conference 2020

7. Singh, J., Hunt, S., Simonds, S. *et al.* The changing epidemiology of pulmonary infection in children and adolescents with cystic fibrosis: an 18-year experience. Thoracic Society of Australia and New Zealand Conference 2021

8. *J.A.S. Singh, S. Lynch, H. Selvadurai, and J. Iredell.* Viability and Infectivity of Bacteriophage PBPA103 After Nebulization With a Vibrating Mesh Nebulizer. American Thoracic Society Conference 2024.

9. J.A.S. Singh, S. Lynch, J. Iredell, and H. Selvadurai. Successful Eradication of Chronic Pseudomonas Aeruginosa Infection in Cystic Fibrosis. American Thoracic Society Conference 2024.

Authorship attribution statement

I certify that these publications and presentations stem from my substantial contributions. The details of attribution of published manuscripts are as follows:

The changing epidemiology of pulmonary infection in children and adolescents with cystic fibrosis: an 18-year experience

Jagdev Singh, Hiran Selvadurai conceived the research question. Jagdev Singh, Hiran Selvadurai, and Dominic A. Fitzgerald designed the study and analysis plan. Jagdev Singh, Sharon Hunt, Sharon Simonds, Christie Boyton, Anna Middleton, and Merilyn John collected the data. Jagdev Singh performed the statistical analysis. Hiran Selvadurai, Dominic A. Fitzgerald, Paul Robinson, Susan Towns, and Chetan Pandit reviewed the data. Jagdev Singh drafted the initial and final versions of the manuscript. All authors critically reviewed early and final versions of the manuscript.

Factors influencing treatment response of pulmonary exacerbation in children with cystic fibrosis

Jagdev Singh and Hiran Selvadurai have given substantial contributions to the conception or the design of the manuscript. Jagdev Singh, Brendan Kennedy, Merilyn John, and Brooke Bailey contributed to the acquisition, analysis, and interpretation of the data. All authors have participated in drafting the manuscript. Paul Robinson and Dominic A. Fitzgerald revised it critically. All authors read and approved the final version of the manuscript.

A systematic review on the use of bacteriophage in treating *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections in cystic fibrosis

Jagdev Singh and Eugene Yeoh registered, designed the study and performed the systematic review and drafted the manuscript. Hiran Selvadurai and Dominic Fitzgerald critically reviewed the manuscript. All authors read and approved the final version of the manuscript.

Single-arm, open-labelled, safety and tolerability of intrabronchial and nebulised bacteriophage treatment in children with cystic fibrosis and Pseudomonas aeruginosa

Hiran Selvadurai and Jagdev Singh conceived the research question. Jagdev Singh, Hiran Selvadurai, Anna Middleton, Jeremy Barr, Sharon Hunt, and Jonathan Iredell designed the study and analysis plan. Jagdev Singh drafted the initial and final versions of the manuscript. All authors critically reviewed early and final versions of the manuscript.

Safety and tolerability of bronchoscopic and nebulised administration of bacteriophage

Jagdev Singh contributed to the writing of the original draft, validation, project administration, methodology, investigation, formal analysis, data curation, and conceptualization. Stephanie Lynch assisted with writing – review & editing, resources, methodology, and investigation. Jonathan Iredell was involved in writing – review & editing, validation, supervision, and resources. Hiran Selvadurai contributed to writing – review & editing, validation, supervision, resources, project administration, methodology, and conceptualization.

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Isolating and characterising phages against Pseudomonas aeruginosa and Staphylococcus aureus in children with cystic fibrosis

Jagdev Singh designed and performed the experiment based on the instruction by Dinesh Subedi, Fernando Gordillo-Altamirano, Ruzeen Patwa. The abstract was critically reviewed by Hiran Selvadurai, Amaneh Khatami and Jeremy Barr.

Viability and Infectivity of Bacteriophage PBPA103 After Nebulization With a Vibrating Mesh Nebulizer

Jagdev Singh and Stephanie Lynch designed and performed the experiment. The abstract and results of experiment was critically reviewed by Hiran Selvadurai and Jonathan Iredell.

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2. Speaker at TEDxSydney Ideas Exchange 2021
3. Speaker at Cystic Fibrosis Conference 2024 (Scientific)
4. Speaker at Cystic Fibrosis Conference 2024 (Lay)

Media coverage

1. The treatment that could end antibiotic-resistant bacteria, The Sunday project September 2023, https://www.youtube.com/watch?v=bOheG_U5zVQ

Executive summary of the CHIP-CF study

Introduction

Pseudomonas aeruginosa is a major respiratory pathogen in cystic fibrosis (CF) patients, causing significant morbidity and mortality. Although its prevalence is decreasing in high-income countries due to effective therapies, it remains a persistent issue and is classified by the WHO as a critical drug-resistant organism. This has spurred interest in alternative treatments like bacteriophages, which target and kill bacteria. This thesis explores bacteriophage therapy for treating *P. aeruginosa* in children with CF, focusing on its safety and tolerability. This work aims to establish a clinical trial protocol that delivers bacteriophage therapy to children with CF and chronic *P. aeruginosa* infection, outside of a compassionate access scheme design.

To achieve this aim, several studies were undertaken addressing specific research questions.

Research questions

1. *What is the incidence and prevalence of P. aeruginosa within one of the largest paediatric CF centres in the country?*
   This study reviewed 18 years of microbiological and prescription data of patients with cystic fibrosis at the Children's Hospital at Westmead and found a 26.2% incidence of *Pseudomonas aeruginosa*, increased broad-spectrum antibiotic use, and a rise in

non-tuberculous mycobacteria among school-aged children. This study is presented in **Chapter 2** and has been peer-reviewed and published in Nature Scientific Reports (https://doi.org/10.1038/S41598-024-59658-4)

2. *What are the baseline characteristics of children with CF presenting with a pulmonary exacerbation, and what impact does P. aeruginosa have?*

This study reviewed four years of admission data involving 184 CF admissions which revealed the presence of *P. aeruginosa* in 73% of admissions, with only 44% of patients returning to baseline FEV1% and 41% requiring readmission within 6 months, further lowering FEV1% (75% vs. 88%, $p<0.001$). This study is presented in **Chapter 3** and has been peer-reviewed and published in Minerva Pediatrics (https://doi.org/10.23736/S2724-5276.23.07221-X)

3. *To what extent have bacteriophages been used in treating P. aeruginosa infections, particularly in CF?*

This systematic review identified 5 case studies (23 subjects, including 3 humans) meeting the study criteria. This analysis incorporated CF animal models to enhance understanding of bacteriophage therapy, addressing gaps left by the limited number of human studies. These studies demonstrated outcomes such as a

reduction in bacterial density, a decline in the number of exacerbations, and improved clinical outcomes. This study was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO registration: CRD42020207622) and reported per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting checklist. This study is presented in **Chapter 4** and has been peer-reviewed and published in Paediatric Respiratory Reviews (https://doi.org/10.1016/j.prrv.2023.08.001).

4. *Can bacteriophages be isolated and purified for therapeutic use by the candidate/clinician to better understand the process of bacteriophage isolation and purification?*

   The PhD candidate underwent training on bacteriophage isolation and purification to better understand the laboratory processes involved in therapeutic bacteriophage production. **Chapter 5** of this study demonstrates the successful isolation of bacteriophages using three clinical isolates of *P. aeruginosa*. This early work has enabled the isolation and purification of bacteriophages specifically derived from *P. aeruginosa* strains of patients with CF, forming the foundation of this PhD. By mastering isolation and purification methods, the candidate was then able to design a clinical trial

protocol that facilitated the implementation of this novel therapeutic approach.

The result of this experiment was presented at The European CF conference in 2020 with the conference abstract published in the Journal of Cystic Fibrosis (Reviewed by the scientific committee of the conference). (https://doi.org/10.1016/S1569-1993(20)30334-9)

5. *How can the integrity and infectivity of bacteriophages be assessed following nebulisation for therapeutic use?*

   A specific protocol was developed to evaluate the integrity of the bacteriophages to be used in the study. This experiment demonstrated minimal loss of bacteriophages following nebulisation using the selected nebuliser. By designing an experiment to assess individual bacteriophages undergoing nebulisation, this study establishes a framework to evaluate their viability and ensure they remain effective after nebulisation. It is crucial to establish this assessment to confirm that bacteriophage delivery achieves its therapeutic purpose. Furthermore, this framework will enable future researchers to use this technique to evaluate bacteriophages delivered via nebulisation, a commonly used method in respiratory medicine therapeutics. The results of this study in

Chapter 6 was presented at the American Thoracic Society Meeting and the conference abstract published in the American Journal of Respiratory and Critical Care Medicine (Reviewed by the scientific committee of the conference). (https://doi.org/10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A4125)

6. *Can a clinical trial be implemented outside the confines of compassionate access schemes?*

   Based on the paucity of clinical trials in this area, a protocol for the production and delivery of bacteriophages was designed and underwent the necessary and rigorous round of ethics review. For the regulation of 'unapproved' bacteriophage therapy, the Clinical Trial Notification (CTN) scheme was used to notify the Therapeutic Goods Administration (TGA). Approval was granted through the Sydney Children's Hospital Network (SCHN) sponsorship, following rigorous review by the Human Research Ethics Committee (HREC) and the Research Governance Office (RGO) of the institute. This process ensured compliance with ethical and regulatory standards for conducting the trial. **Chapter 7** contains a summary of the protocol which has been peer-reviewed and published in BMJ Open Respiratory Research (https://doi.org/10.1136/bmjresp-2022-001360). The full protocol is presented in the appendix of this thesis and has been registered with the Australia and New Zealand Clinical Trials Registry. (ACTRN12622000767707)

7. *What is the outcome of this therapy in terms of tolerability and safety?*

   Following the approval of the protocol to use bacteriophage therapy in this cohort of patients, two adolescents treated with this protocol had no side effects, with both showing improved lung function and one achieving eradication of P. aeruginosa. The outcome of this therapy presented in **Chapter 8** has been peer-reviewed and published in Virus Research (https://doi.org/10.1016/j.virusres.2024.199442). A third patient has been treated following publication and has tolerated the therapy well with *P. aeruginosa* no longer isolated from subsequent airway samples.

FIGURE LEGENDS:

Figure 1: Historical images showing; Left: three toddlers with cystic fibrosis of the pancreas described by Dorothy Andersen in 1938. Right: changes demonstrated within the airways, in particular, bronchiectasis. (*Source: Am J Dis Child. 1938;56(2):344-399. doi:10.1001/archpedi.1938.01980140114013*)

Figure 2: The typical structure of a bacteriophage (*source: https://letstalkscience.ca/educational-resources/stem-explained/phages-vs-antibiotics)*

Figure 3: Diagram representing the two different cycles of bacteriophage replication. Lytic phages are used for their antimicrobial properties [*Source: C.E.D Rees et al, Encyclopaedia of Food Microbiology (Second Edition, 2014.*]

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Figure 6: Factors contributing towards response to treatment. Values of >1 show response to treatment. OR: odds ratio, CI: confidence interval. Magnitude of decline = baseline FEV1%-FEV1% on admission

Figure 7: PRISM flow diagram

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Figure 9: Critical appraisal checklist for case reports selected.

Figure 10: Risk of Bias in animal studies selected.

Figure 11: Clockwise from top: Clear circles (plaque) produced on a *Pseudomonas aeruginosa* bacterial lawn by bacteriophage 509 PA L; Clear circles (plaque) produced during spot testing of bacteriophages (labelled L,S and C); Lysis demonstrated by Phage 01 on a *Staphylococcus aureus* bacterial lawn; Sets of bacterial lawn awaiting plaque formation; The final

highly purified distillate of PA 509 S with under 5 EU/kg/hour of endotoxin unit (based on the weight of a child of 30kg).

Figure 12: Demonstrating the growth of the bacteriophages in the latent, rise period and bacterial cell lysis phase of the five bacteriophages that were isolated. Bacteriophage 1 to 3 were isolated using *Pseudomonas aeruginosa* isolated while bacteriophage 4 and 5 were isolated using the *Staphylococcus aureus* isolates.

Figure 13: Structure of a PBPA103 (similar to *Pseudomonas* bacteriophage M6), under the genus of Yuavirus. *Source: Swiss Institute of Bioinformatics (ViralZone 2014*

Figure 14: Transmission electron microscopy demonstrating the damage that can occur during aerosolization of bacteriophages. The bacteriophage shown here is PP01; (A) Aerosol containing one damaged phage with other body parts; (B) aerosol containing 13 phages' head; (C) aerosol containing 6 phages head and 1 full phage; (D) aerosol containing 2 damaged phages with other body parts; (E) aerosol that contains one full bacteriophage; and (F) biosampler control of an undamaged bacteriophage. *Adapted from: N. Groulx, et al 2016, Aerosol Science and Technology*

Figure 15: The nebuliser used during the experiment and for therapy. Vibrating mesh nebulisers use mesh deformation or vibration to push the liquid through the mesh. *Source: Product Manual, Omron Healthcare*

Figure 16: The NCBI taxonomy of PBPA103 (based on the 2022 taxonomy update of the ICTV bacterial viruses subcommittee) which closely resembles *Pseudomonas* bacteriophage M6, sourced from Lyse N Tech in South Korea, database: http://www.phagebank.or.kr/

Figure 17: The bacteriophage undergoing aerosolization using a mesh nebuliser in a biocontainment cabinet. Flowtrough is collected with the test tube connected to the mouthpiece using a silicon tube. Bacterial lawn plates were exposed to the aerosolized effluent and showed complete clearance of the bacteria.

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Figure 19: Timetable of schedule, footnote: [@]= sputum on day 1 to be performed before the first dose of nebulised bacteriophage, Blood investigations are timed to coincide with standard blood taking practices; admission, day 2 to review tobramycin level and day 14 during removal of the central line. [a]= This clinical review will be conducted prior to performing the bronchoscopy.

Figure 20: Spirometry trend over three years leading up to bacteriophage therapy. ▲ ; commencement of bacteriophage therapy, ■

; commencement of Elexacaftor-Tezacaftor-Ivacaftor. BT; bacteriophage therapy.

Figure 21: Chest X-rays of M17 taken 6 months before BT (Before) and 6 months after completion of bacteriophage therapy (After).

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Chapter 1: The CHIP-CF study: Targeting Pseudomonas aeruginosa in Children with Cystic Fibrosis

A brief history of cystic fibrosis

In 1938, Dorothy Andersen was evaluating children with malnutrition secondary to celiac disease when she discovered that a subgroup initially thought to have celiac disease had fibrotic changes in their pancreas, which was the potential reason for their pancreatic insufficiency. She coined the term 'cystic fibrosis (CF) of the pancreas' for this condition. Through post-mortem examinations of 49 children with pancreatic fibrosis in the cohort she studied, 45 were diagnosed with cystic fibrosis of the pancreas, highlighting its prevalence. While her seminal paper focused on the histopathological significance of these findings, she also described 44 children under 15 years of age who presented with complications from respiratory infections and evidence of suppurative bronchiectasis. [1]

Paul di Sant'Agnese contributed to the early understanding of cystic fibrosis (CF) in the late 1940s when he discovered that a cohort of patients with heat exhaustion had CF and significantly elevated sodium and chloride levels in their sweat.[2] This disease became known as a "generalized exocrinopathy," characterised by malabsorption of fat, resulting in steatorrhea and growth failure, the presence of thick, sticky mucus clogging the mucus glands throughout the body (termed "mucoviscidosis"), and elevated sweat chloride levels, which became an

important diagnostic test for CF. [3-5] The focus on the relationship between sweat chloride and CF continued over the next few decades, and the defect in chloride transport within the sweat ducts was described just over 40 years ago. [6]

While Dorothy Andersen recognised that the aetiology of CF was hereditary with an autosomal recessive origin,[7] it was 40 years later that the first CF-causing gene was discovered using cells derived from the sweat duct. [8,9] This gene, which encodes the cAMP-regulated chloride channel known as the CF transmembrane conductance regulator (CFTR), is expressed in many epithelial cells throughout the body. This explains the manifestation of CF in various systems, such as pancreatic insufficiency, biliary cirrhosis, congenital bilateral absence of the vas deferens, and lung disease.[10 11]

The discovery and understanding of the CFTR gene have significantly impacted the management of CF.[12,13] More than 2,000 sequence variants of the gene have been discovered, with about 700 of these responsible for the disease. The identification of the CFTR gene paved the way for the development of highly effective modulator therapies that specifically target the defective CFTR protein, correcting its function at a molecular level. These advancements have transformed the treatment landscape for CF, offering improved health outcomes and quality of life for many patients. [14,15] Building upon the progress made by these remarkable

individuals, it is important to continue learning, adapting, and developing new treatments for CF until a cure is discovered.[16,17]

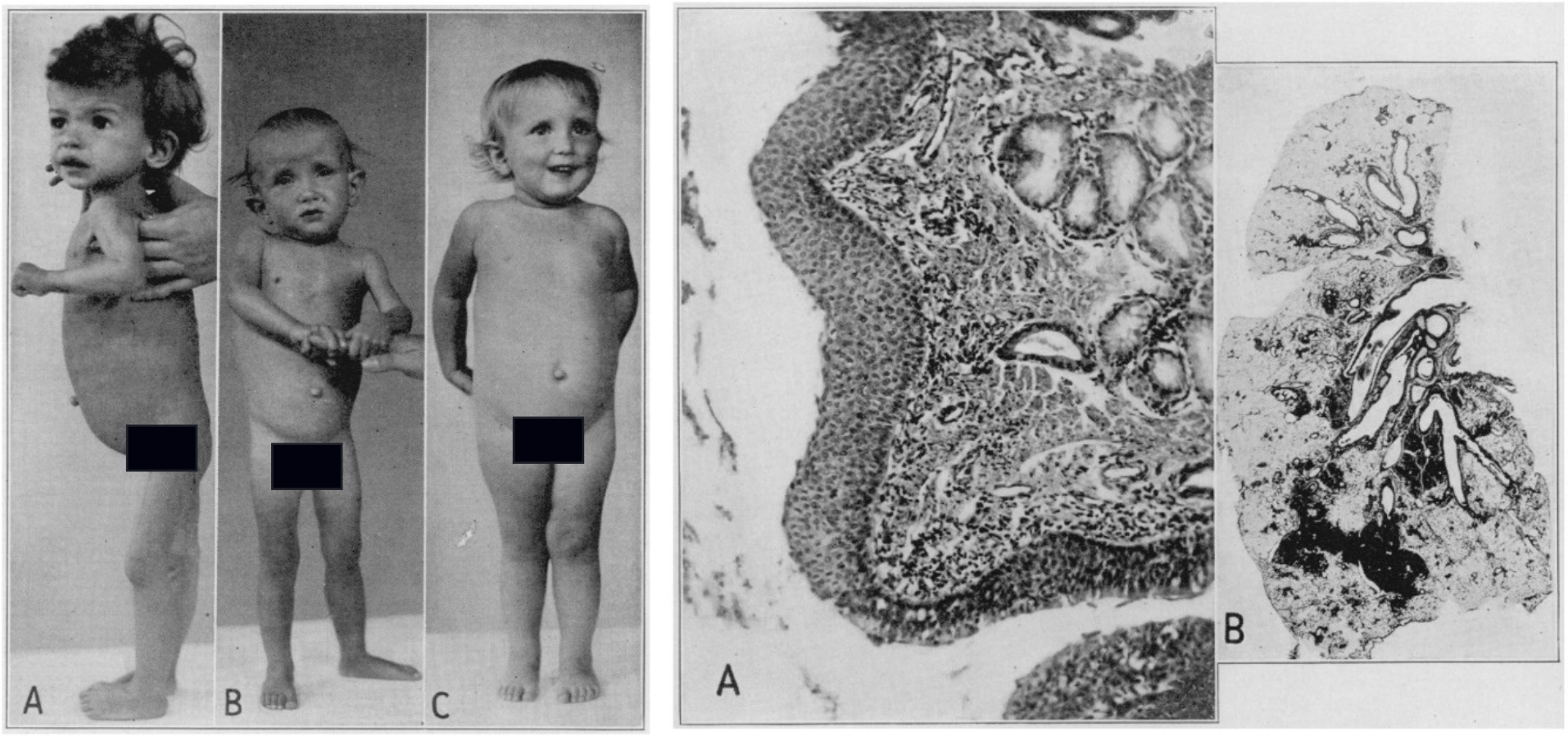

Figure 1: Historical images showing; *Left:* three toddlers with cystic fibrosis of the pancreas described by Dorothy Andersen in 1938. *Right*: changes demonstrated within the airways, in particular, bronchiectasis. *(Source: Am J Dis Child. 1938;56(2):344-399. doi:10.1001/archpedi.1938.01980140114013)*

The epidemiology of cystic fibrosis

It is estimated that there are more than 150,000 individuals living with CF across 94 countries.[18] In Australia, there are close to 4,000 individuals living with this condition.[19] Of these, 90% have at least one F508del variant in their CFTR gene. Almost half of Australians living with CF are under 18 years of age with a third living in New South Wales. Close to 90% of CF diagnoses in Australia occur in the paediatric age group, with the majority (82.4%) occurring in the first year of life. [20]

Cystic fibrosis and the lungs

CF is a life-limiting genetic condition of the lungs, exocrine organs, and gastrointestinal tract that occurs in 1 in 2,500 births, affecting mucus, sweat, and digestive enzyme production.

Morbidity and mortality in cystic fibrosis (CF) are largely attributed to early or recurrent pulmonary exacerbations. [11] Therefore, the management of chronic pulmonary infections remains critical in the care of individuals with the disease.[21] Despite an increase in the median survival age over recent years, chronic pulmonary infection and concomitant airway inflammation leading to respiratory failure still account for 80 to 95% of deaths in individuals with CF. [22,23] This vicious cycle of infection and inflammation begins early in life, resulting in a decline in lung function, poorer nutrition, and structural lung abnormalities. [24]

The impact of pulmonary exacerbations and its impact in CF has been subject to a number of studies. [25]In children, a pulmonary exacerbation in the first 2 years of life is associated with the presence of bronchiectasis, a decline in lung function, and a poorer nutritional outcome during their preschool years.[26,27] Modelling has shown that the number of annual pulmonary exacerbations is a predicting factor of survivability at 3 to 5 years.[28]

Airway pathogens in CF airways

A variety of organisms can infect the airways of individuals living with CF. Over the years, and particularly in CF registries worldwide, much of the focus has been on *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Haemophilus influenzae*, *Achromobacter* species, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus*. Patterns of airway infections suggest an age-related predilection for certain pathogens.

Notably, longitudinal data indicate that the bacterial prevalence observed in older cohorts of people with CF provides insight into the likely *future* microbiological landscape of younger cohorts. [29] While trends in respiratory infections have evolved with advancements in CF management, these pathogens remain clinically significant due to their persistent prevalence and impact.

Interestingly, a recent study of 1,200 children and adolescents found a strong interconnection between bacterial infections. The study demonstrated that each prior bacterial or fungal infection incrementally increased the risk of *P. aeruginosa* acquisition.[30] This underscores the complex microbial dynamics in CF airways and highlights the importance of early and targeted infection control strategies.

Why is *Pseudomonas aeruginosa* a problem in CF?

Pseudomonas aeruginosa is one of the most common respiratory that affects people living with CF, leading towards significant morbidity and mortality. It is estimated that up to 25% of children and a higher percentage of adults living with CF will be infected by these organisms in their lungs. In Australia, almost half of people living with CF have this organism detected in their airway samples.[20] Chronic P. aeruginosa remains a significant challenge in the management of CF, with its definition remaining a subject to debate amongst the CF research community. [29,31]

The presence of *P. aeruginosa* in the airways is associated with a 2.6-fold increase in mortality rates within eight years, as well as lower baseline weight, a decline in lung function and higher CF-related hospitalizations.[27,30,32,33]

While the prevalence of these organisms is declining in high-income nations during the era of highly effective modulator therapies,[34] only 12%

of people living with CF globally have access to these medications, primarily due to their costs.[35] Additionally, prescribing criteria in high-income nations also exclude 1 in 5 people living with CF.[36,37]

The presence of *P. aeruginosa* is expected to continue playing a significant role in the CF lung in the forthcoming years. Treating respiratory tract infections caused by *P. aeruginosa* with prolonged and repeated antibiotic therapies has significant side effects, including sensorineural hearing loss, hypersensitivity, and impaired renal function. Additionally, there is the added risk of a change in the airway microbiota allowing for opportunistic infections and a rise in resistant organisms. Furthermore, this organism has been classified as a critical drug-resistant organism by the World Health Organization and remains a challenging organism to treat.[38] This has sparked a race to uncover alternatives to antibiotics, with growing interest in bacteriophages.

What are Bacteriophages?

There has been growing interest in using bacteriophages, viruses that replicate within bacteria and lead to selective bacterial cell death. Bacteriophages have been used widely in Eastern Europe for decades and have gained popularity in the Western scientific community in recent years.[39] However, there is still much to learn about the safety, delivery, and effects of bacteriophages, particularly in CF lungs, which differ from healthy lungs, notably having impaired mucociliary clearance. Bacteriophage (phage) therapy may offer a solution to this problem.

Phages are viruses that replicate within bacteria, causing highly selective bacterial cell death and reducing the impact on the healthy microbiome compared to antibiotic therapy. In the face of increasing antimicrobial resistance and dwindling prospects for the discovery of new antibiotics, there has been a resurgence of interest in phage therapy. However, such research has generally excluded children who potentially stand to gain the most from this treatment if it is shown to be safe and effective

The landscape of bacteriophage therapy

While this thesis primarily focuses on Pseudomonas aeruginosa in the CF airways, it is important to acknowledge the broader scope of bacteriophage research targeting other clinically relevant bacterial pathogens. Bacteriophages have been investigated in clinical trials against organisms such as *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus*, non-tuberculous *Mycobacterium* and *Acinetobacter baumannii*. [40,41]

These studies have explored bacteriophage therapy in a range of infections, including urinary tract infections (UTIs), ventilator-associated pneumonia (VAP), chronic respiratory infections, burn wound infections, and osteomyelitis.[42] Many of these trials, primarily in phases 1 and 2, have demonstrated the feasibility and safety of bacteriophage therapy, though challenges such as phage resistance, standardisation of dosing, and regulatory approval remain key areas of ongoing research. [43-45]

This growing body of evidence highlights the potential for bacteriophages to complement or even replace antibiotics in difficult-to-treat infections, paving the way for future clinical applications beyond P. aeruginosa in CF.

Bacteriophages

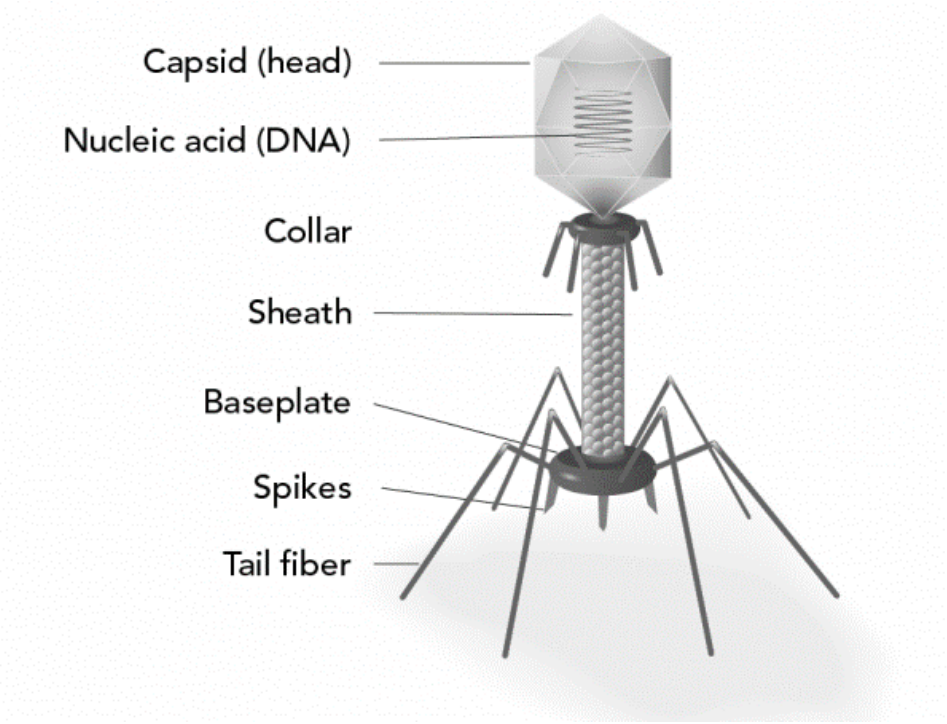

Figure 2: The typical structure of a bacteriophage (*source: https://letstalkscience.ca/educational-resources/stem-explained/phages-vs-antibiotics*)

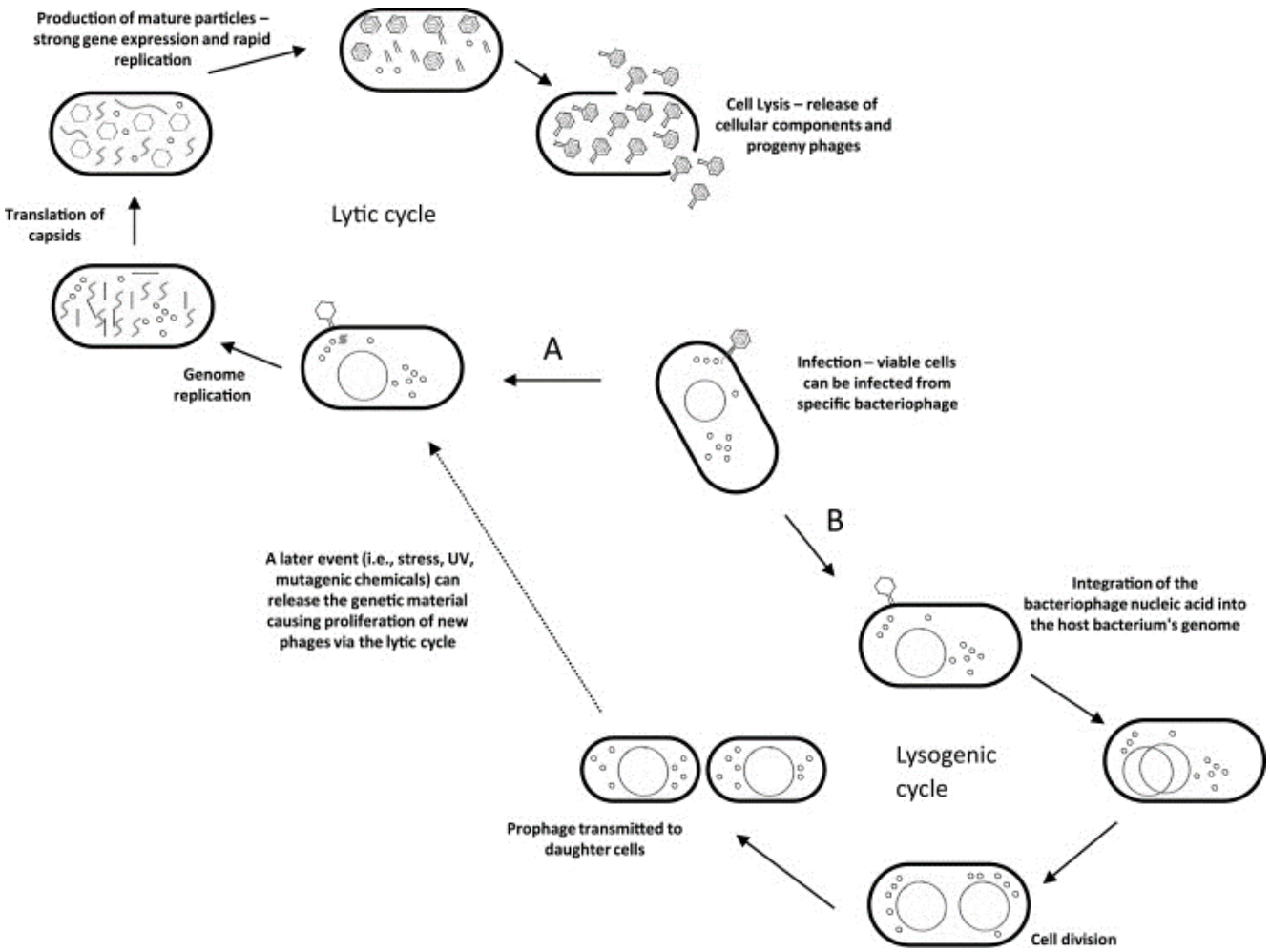

Figure 3: Diagram representing the two different cycles of bacteriophage replication. Lytic phages are used for their antimicrobial properties [*Source: C.E.D Rees et al, Encyclopaedia of Food Microbiology (Second Edition, 2014.]*

Factors that influenced the design of this therapeutic trial of bacteriophages for treating *Pseudomonas aeruginosa* in the lungs of children with CF

Phages have been utilised in studies in different countries mainly within the context of compassionate access schemes.[46] Phages have low natural toxicity as they target specific bacteria which are largely strain-specific without disrupting the host's normal flora or human cells.[47] Although not persistently observed, phages have been shown to reverse antibiotic resistance and restore susceptibility to various classes of antibiotics in *P. aeruginosa*.[48] Phages have been mainly used intravenously in the treatment of children with CF with no local infusion site reactions, anaphylaxis, seizures, abnormal vital signs, or gastrointestinal disturbances.[46,49] The youngest child who received intravenous bacteriophage therapy was two years of age.[50] Only two children have previously received bacteriophage via nebulisation and none have received it through bronchoscopy. [46]

Dose, duration, and route of administration may influence this with topical, enteral, and inhaled routes of administration considered less immunogenic compared to intravenous and intraperitoneal administrations.[51-53] Optimum dosing remains unclear, however, based on previous pre-clinical studies that include in-vitro and animal models, the Plaque forming unit (PFU)/mL to achieve treatment effect is commonly between 10^6-10^{10} PFU/mL.

As for the innate immune response, studies have shown that purified phages (as opposed to raw phage lysates) do not expose pathogen-associated molecular patterns (PAMPs) effectively and therefore are not able to induce an inflammatory response. [54,55]

Phages that are non-genetically modified organisms are used as a surrogate for viruses in aerosolization studies to evaluate the efficacy of viral filters. [56]

While various methods of administering bacteriophages have been explored, it is hypothesized that localized administration of these viruses can promote bacteriophage replication in the presence of highly concentrated pathogens, thereby enhancing the efficacy of phage therapy. However, nebulising bacteriophage solutions can subject them to mechanical stressors such as vibrations, heat stress, and shearing forces, potentially compromising phage viability and infectivity. Phages have shown some potential to remain intact following nebulisation in a murine model.[57-59]

The importance of this trial

This study hypothesises that phage therapy is a safe and well-tolerated adjunct therapy in paediatric patients with CF with *P. aeruginosa* infection in their lower airways. The research questions are:

1. Are endo-bronchially instilled and nebulized phages safe, easily administered, and well-tolerated?

2. Are phages delivered directly into the bronchial tree via bronchoscopy followed by nebulised bacteriophage therapy effective in reducing the load of P. aeruginosa within the sputum?

This trial is particularly significant because it is the first clinical trial that has moved from compassionate access to an early-phase clinical trial, demonstrating a critical step forward in the potential widespread application of bacteriophage therapy.[46] Furthermore, it is the only clinical trial designed specifically for children and the only one that is exploring bronchoscopic delivery of bacteriophages directly into the airways. [60,61]

Achieving the narrative: from audits, review, and pre-clinical studies to a therapeutic trial

The CHIP-CF study has been designed to address the above research questions. To achieve this, a few questions needed to be answered or addressed. This included:

8. *What is the incidence and prevalence of Pseudomonas aeruginosa within one of the largest paediatric CF centres in the country?*
9. *What are the baseline characteristics of children with CF presenting with a pulmonary exacerbation, and what impact does P. aeruginosa have?*
10. *To what extent have bacteriophages been used in treating P. aeruginosa infections, particularly in CF?*
11. *Can bacteriophages be isolated and purified for therapeutic use by the candidate/clinician to better understand the process of bacteriophage isolation and purification?*

12. *How can the integrity and infectivity of bacteriophages be assessed following nebulisation for therapeutic use?*
13. *Can a clinical trial be implemented outside the confines of compassionate access schemes?*
14. *What is the outcome of this therapy in terms of tolerability and safety?*

Chapter 2: The Changing Epidemiology of Pulmonary Infection in Children and Adolescents with Cystic Fibrosis: An 18-Year Experience

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Introduction

The management of pulmonary infections is critical in the care of individuals with cystic fibrosis (CF). Despite an increase in the median survival age over recent years, chronic pulmonary infection and concomitant airway inflammation leading to respiratory failure still account for 80 to 95% of deaths in individuals with CF.[22,23] This vicious cycle of infection and inflammation begins early in life, resulting in a decline in lung function, poorer nutrition, and structural lung abnormalities.[24]

Assessing long-term epidemiological trends in CF among children poses significant challenges, with studies often limited to registry reports, of a limited timeframe,[62] involve a small number of children and adolescents,[63] focus on specific organisms of interest,[64,65] or are derived from results obtained from bronchioalveolar sampling alone.[66,67] Furthermore, larger studies conducted before the year 2000 may not reflect recent advancements in CF treatment,[68-72] highlighting the need to evaluate any changes in the incidence and prevalence of CF bacterial pathogens to establish a reference point for future therapeutic interventions.

To this end, we conducted a study to investigate the trends in the incidence and prevalence of respiratory pathogens among children and adolescents

with CF since the turn of the new millennium. By evaluating long-term longitudinal data within a clinical setting in the modern era of eradication therapy,[73] we would like to determine the changes that may have occurred in different age groups over time.

Methodology

Study population

Children and adolescents with CF between birth to 18 years of age who were managed within a large CF centre in Australia between January 2002 and December 2019 were included in this study. Universal newborn screening of cystic fibrosis had been well-established before the study period.[74] Data collected from their existing electronic medical record included; the microbiological culture result (method of collection, date during which sample was collected with the corresponding age of the child or adolescent), and hospital pharmacy-based medication prescription data. This study was approved by the Ethics Committee of the Sydney Children's Hospital Network (2020/ETH00815) and was conducted based on local guidelines and regulations. Exemption from consent was obtained from, and approved by the same committee.

Clinical routine during the study period

In our centre which encompasses a large region in New South Wales, outpatient (CF clinic) reviews occur four times a year, with infants or those who are clinically unwell reviewed on a more frequent basis. During these

visits, airway samples are routinely collected regardless of the presence or absence of symptoms either through spontaneous expectoration (typically in older children), oropharyngeal suctioning performed by a trained CF nurse (typically in younger children), or via bronchoalveolar lavage (BAL). Airway samples microbiological cultures are ordered based on either BAL culture order label (samples obtained via BAL) or sputum CF culture order label (samples obtained through either spontaneously expectorated sputum or airway sample obtained from oropharyngeal suctioning).

All infants less than one year of age have been prescribed oral flucloxacillin or occasionally amoxicillin and clavulanic acid from diagnosis as part of our CF clinics' routine *Staphylococcus aureus* prophylaxis approach for over 20 years.

In terms of the microbiological practices which has remained consistent during this study period, sputum specimens have been set up on 1) MacConkey agar for gram-negative bacteria e.g., coliforms, *Pseudomonas aeruginosa,* and *Inquilinus limosus*, 2) Anaerobically incubated chocolate agar with Bacitracin for *Haemophilus influenzae*. 3) Mannitol salt agar for *S. aureus* 4) Horse blood agar e.g., *Streptococcus pneumoniae* and *Moraxella catarrhalis*. 5) Cepacia agar for *Burkholderia cepacia* and incubated for 7 days. 6) Non-tuberculous mycobacteria (NTM) testing is performed in an external Mycobacterium Reference Laboratory (MRL) using the automated blood culture system (BD BACTEC™) and testing occurs annually. Matrix-assisted laser desorption/ionization-time of flight (MALDI-

TOF) mass spectrometry (MS) has been used since 2015 for the rapid identification of organisms.

The microbiologist's report on the results of the collected airway samples is routinely reviewed by the CF team within 5 to 7 days after the samples are obtained. Treatment, where applicable following discussion with the primary CF physician is then prescribed. The treatment strategy includes; admission for parenteral antibiotics, a course of oral antibiotics, and/or nebulised antibiotic treatment.

Case Definitions and Stratification

Incidence was defined as the first time a respiratory pathogen of interest is isolated from the sputum of the child or adolescent with CF. Once the child or adolescent is an incident case for that particular pathogen, they were excluded from the denominator for the subsequent years.

Prevalence was defined as a child or adolescent with a respiratory pathogen isolated from their sputum in a specific year. Once the child or adolescent is a prevalent case for that particular pathogen, any further positive culture of the same pathogen isolated from the same child or adolescent was excluded for the remainder of that year.

Nine organisms of clinical interest in CF were selected for analysis. This includes; *S. aureus, P. aeruginosa, H. influenza, Aspergillus fumigatus, Serratia marcescens, NTM, B. cepacia, Achromobacter xylosoxidans*, and *Stenotrophomonas maltophilia.*[75]

The cohort was divided into four age groups: <2 years, 2 to 5 years, 6 to 11 years, and >12 years. The rationale behind this age group includes 1) biological variability in terms of differences in microbiome composition, immune system development and environmental exposure e.g. home or pre-school 2) management approaches such as methods of physiotherapy, lung function testing or the availability of medications such as dornase alfa 3) to align with existing clinical trials in CF transmembrane conductance regulator (CFTR) and CF registry reports.

In terms of medications prescribed and obtained from the hospital pharmacy, prescription of oral antimicrobials (including amoxicillin and clavulanic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, flucloxacillin, and itraconazole), nebulised antimicrobials (including amikacin, colistin, and tobramycin), and other medications (including dornase-alfa, hypertonic saline nebules, and CFTR modulators and correctors) were reviewed.

Statistical analysis

We used descriptive statistics to summarise the data, reporting organism incidence and prevalence as n (%). To assess changes over time, we calculated the annual incidence and prevalence of each organism based on individual airway samples and used regression analysis to evaluate these measures. Based on the coefficients obtained from the regression model, the average change in incidence and prevalence was presented. Prescription trends were also analysed on an individual basis. Results are

reported as % change (with 95% confidence intervals) for incidence and prevalence and as number of prescriptions/year ± standard deviation for medications prescribed. Changes in the mean age of the first organism isolation were assessed using analysis of variance. All statistical calculations were performed using the SPSS Statistic Data Editor (IBM Version 28, New York, USA, 2021). Statistical significance was defined as $p < 0.05$.

Results

Study population and bacterial samples

During the study period, 419 children and adolescents with CF were followed up with 206 (49.2%) born on, or after 1st January 2002. A total of 18,339 airway samples were collected during the study period with 401 (2.2%) collected via bronchioalveolar lavage, with the remaining samples obtained from expectorated sputum or oropharyngeal suction.

Out of the total airway samples that were collected, 724 (3.9%) samples met the criteria for incidence and 15,332 (83.6%) samples met the criteria for prevalence as defined in the methodology of this study were included in the analysis.

Incidence and prevalence of respiratory pathogens

Throughout the entire study period, *S. aureus* (25.1%), *P. aeruginosa* (26.2%), and *H. influenzae* (17.9%) exhibited the highest incidence among

respiratory pathogens. Together, these pathogens accounted for 70% of the overall incidence over 18 years. In contrast, *B. cepacia* (0.69%), *A. xylosoxidans* (2.1%), and NTM (3.7%) had the lowest incidence across the study period, collectively representing 6.5% of the overall incidence over 18 years. (Table 1)

Throughout the entire study period, *S. aureus* (47.8%), *P. aeruginosa* (34.5%), and *A. fumigatus* (8.4%) exhibited the highest prevalence among respiratory pathogens. Together, these organisms constituted almost 95% of the overall prevalence over 18 years. In contrast, the least prevalent respiratory pathogens were NTM (0.72%), *B. cepacia* (0.69%), and *A. xylosoxidans* (0.48%) throughout the study period. Collectively, these organisms represented less than two percent of the overall prevalence over 18 years. (Table 2)

Changes in age of first isolation of respiratory pathogens

The ages at which these pathogens were first isolated are as follows: *S. aureus* (3.35±2.1 years), *H. influenza* (4.28±2.7 years), *S. marcescens* (5.24±4.09 years), *P. aeruginosa* (5.27±2.9 years), *A. fumigatus* (7.31±2.85 years). This is followed by *S. maltophilia* (8.95±2.95 years), *B. cepacia* (9.055±2.3 years), NTM (11.38±2.06 years), *A. xylosoxidans* (11.71±2.86 years).

Over time, respiratory pathogens have shown an increase in the mean age of the first isolation: *S. aureus* ($p < 0.001$), *P. aeruginosa* ($p < 0.001$), *H.*

influenza (p <0.001), *S. marcescens* (p=0.006), *A. Fumigatus* (p= 0.02), *B. cepacia* (p=0.58), NTM (p=0.052), *S. marcescens* (p=0.308), *S. maltophilia* (p=0.47), *A. xylosoxidans* (p=0.80). The changes over years of these respiratory pathogens are illustrated in Figure 4.

Changes of overall and age-specific incidence and prevalence of CF organisms from 2002-2019

Amongst the organisms with the highest incidence, *S. aureus* showed the largest decline in incidence over time, followed by *H. influenza* and *P. aeruginosa*. Meanwhile, NTM and *B. cepacia* showed a non-significant increase in incidence. A similar pattern of change in prevalence was observed. (Tables 1 and 2)

With respect to age groups, incidence of *S. aureus, P. aeruginosa, H. influenza* and *A. fumigatus* in children <2 years of age have remained unchanged. A similar pattern of change in prevalence was observed. Meanwhile, NTM showed a significant increase in both incidence and prevalence in children 6-11 years of age.

Treatment

Throughout this study, a total of 29203 medications (oral antimicrobials n=18,367, 62.9%) were prescribed. The antibiotics that were increasingly prescribed include amikacin (3.09±2.24 prescription/year, p=0.003), amoxicillin/clavulanic acid (8.98±2.17 prescriptions/year, p<0.001), colistin (1.95±0.3 prescriptions/year, p=0.032),

trimethoprim/sulfamethoxazole (18.1±8.7, p<0.001). Flucloxacillin (-4.48±1.073, p<0.001), tobramycin (-8.46± 4.7, p<0.001) showed a decrease in prescription. Ciprofloxacin (-6.049±5.1 prescriptions/year, p=0.068) and itraconazole (-4.53±1 prescriptions/year, p=0.07) did not show any significant change over time.

Dornase alfa prescription increased by 16.74±4.1 prescriptions/year (p<0.001). The prescription of hypertonic saline nebulisation increased by 24±4.6 prescriptions/year (p<0.001). There were 7 children or adolescents on CFTR corrector or modulator therapy.

Discussion

This paediatric-focused study evaluates annual changes in the incidence and prevalence rates of respiratory pathogens across different age groups, while also comparing medication prescription trends over an 18-year period. This study provides valuable data from a real-world clinical setting where infants under the age of one receive universal antimicrobial prophylaxis and, standardised respiratory pathogen surveillance is conducted by qualified personals using consistent sampling and microbiological testing protocols. In particular, obtaining samples through sputum and oropharyngeal suctioning is considered to have the highest concordance with BAL samples, rendering them more representative of lower airway infections compared to other sampling methods like throat or cough swabs.[76] The findings contribute to our understanding of the long-

term trends in respiratory pathogens and associated clinical management in the paediatric population, particularly in the modern era of eradication therapy. [73]

Our study showed that together, *S. aureus* and *P. aeruginosa* make up the majority of respiratory pathogens both in terms of incidence (51.3%) and prevalence (82.3%). Data preceding 2000, report prevalence of these two respiratory pathogens to be higher at 95%.[72]

Registry data taken from 2018-2020 showed a prevalence of *P.aeruginosa* of 20.9%[75] and *S.aureus* of 55.26% in children and adolescents under the age of 18. In comparison, our data shows a recent prevalence of *P. aeruginosa* of 17.6% and *S. aureus* of 45.3%. Of the less frequent respiratory pathogens, NTM prevalence was 4.3% from registry data vs 3.7% from our cohort and BCC was 3.2% vs. 1.3% respectively.

In a recent publication by VanDevanter *et al*, a trend of decline in *P. aeruginosa* prevalence was observed, as evidenced by the examination and presentation of registry data within a comparable time frame. [77] Following this, Fischer *et al* raised a crucial question regarding whether the observed changes in *P. aeruginosa* over time were also apparent in other respiratory pathogens of interest in CF.[78] We have demonstrated that over the past 18 years, the incidence and prevalence of the most common respiratory pathogens in CF such as *S. aureus*, *P. aeruginosa*, *H. influenzae and A. fumigatus* have decreased steadily. This significant decline of between 2-4% of individual respiratory pathogens are observed both in the incidence

and prevalence. Meanwhile, less common organisms such as NTM*, B. cepacia* and *A. xylosoxidans, S. maltophilia* showed no significant change in terms of incidence and prevalence.

We also found that the incidence and prevalence of respiratory pathogens remain unchanged for infants up to 2 years of age across all respiratory pathogens. Additionally, we have found that our cohort of children and adolescents with CF are found to have a positive airway sample culture for these respiratory pathogens significantly later that the earlier years of this study.

Our centre has adopted the universal use of *S. aureus* prophylactic antibiotics in infants diagnosed with CF preceding this study period. In a systematic analysis performed which reviewed four studies, there was a weak indication that *P.aeruginosa* was isolated less frequently in children under three years and more frequently in children between three to six years in the prophylactic group. [79] In contrast, despite our universal use of prophylactic antibiotics in infants, our study shows 1) a decline in the incidence and prevalence of *P. aeruginosa*, 2) no increase in the incidence and prevalence of organisms such as NTM and *B. cepacia* 3) an increase in the mean age of first isolation of respiratory pathogens of interest, 4) no change of incidence and prevalence of respiratory pathogen < 2 years of age. A contributing factor in terms of improvements in infection control practices may have helped keep our incidence and prevalence lower than the national average. While being potentially circumstantial, these findings

suggest that the use of prophylactic anti-staphylococcal antibiotics is not associated with an increase in *P. aeruginosa* or increase in prevalence of other less common respiratory pathogen. Prospective studies such as the CF-START study in evaluating outcomes of prophylactic treatments will hopefully provide conclusive proof of its benefits and safety.[79]

By examining prescription trends, we have found that there is a rise in the use of anti-pseudomonal nebulised antibiotics such as amikacin and colistin. This suggests that *P.aeruginosa* is being more aggressively treated over time as both this antibiotics are considered as second line after tobramycin[80]. However, the increase in use of amikacin could also be attributed to an increase in NTM incidence and prevalence. Encouragingly, we have found that the emphasis on respiratory clearance has increased over time with the significant increase in the prescription of dornase alpha and hypertonic saline in our cohort.

Our study comes with certain limitations that warrant consideration. Firstly, the sputum and prescription data lack representation from external laboratories or pharmacies, potentially limiting the comprehensiveness of our findings. Additionally, we did not culture anaerobic bacteria and did not routinely test for co-infection with respiratory viruses, leading to an omission in addressing potential co-infections among these organisms in our study. Moreover, the annual frequency of NTM testing, as opposed to routine CF airway sample cultures, may result in an underrepresentation of NTM within our study cohort.

Thirdly, our data originated from a single CF centre in Australia, raising concerns about the generalizability of our findings to a broader population. Fourthly, our incidence calculation may involve a small number of children or adolescents intermittently found to have these respiratory pathogens in their airway samples. Finally, the relatively limited sample size of children and adolescents on CFTR modulators or correctors is noteworthy, as our study predates the widespread adoption that followed the approval and government funding of these medications in Australia. Current evidence suggests that while it may more difficult to obtain sputum samples in children on CFTR therapy, its' impact on the growth of specific bacterial pathogens needs to be closely examined.[81] The low number of children or adolescents on CFTR modulators or correctors is an important aspect of this study as it will enable future comparison in a post-modulator era in the management of CF.

Our study has several strengths. First, we analysed a large number of sputum samples, both overall and in different age groups, providing a longitudinal comparison of changes in CF treatment over the past 18 years. This is the first study of such magnitude in children and adolescents with CF, providing age-specific incidence and prevalence, as well as prescription trends. In particular, our review of incidences of these organisms and the age of first positive culture provides additional information towards our understanding of CF respiratory pathogens over the past two decades.

Second, our study includes a large cohort of children born on or after January 1st, 2002, when newborn screening has already been well-established, allowing us to assess the acquisition of respiratory pathogens from shortly after birth over the past 18 years. Third, the practice of using prophylactic anti-staphylococcus antibiotics universally has given us the opportunity to assess the outcomes of its' use over a significantly long period of time. While strong conclusions cannot be made without a non-prophylactic control arm it does provide insight into the long-term impact of its' implementation on respiratory pathogens in our cohort.

In summary, our study shows a change in the epidemiology of CF pathogens in a single large paediatric clinic that practices universal prophylaxis in children. First, we observed a decline in the incidence and prevalence of the most commonly found CF pathogens such as *S. aureus, P. aeruginosa, H. influenzae,* and *A. fumigatus*, as well as a delay in the first acquisition of these pathogens. However, less common pathogens such as *S. marcescens*, NTM, *B. cepacia, A. xylosoxidans*, and *S. maltophilia* did not show significant changes. Second, we found no change in the incidence or prevalence of respiratory pathogens in infants under 2 years of age over time.

Staphylococcus aureus	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	change by year (95% CI)	p-value
	n=13	n=6	n=12	n=20	n=11	n=11	n=16	n=11	n=14	n=12	n=10	n=5	n=10	n=22	n=14	n=13	n=6	n=7		
Overall	6.1	2.8	5.6	9.4	5.2	5.2	7.5	5.2	6.6	5.6	4.7	2.3	4.7	10.3	6.6	6.1	2.8	3.3	-3.861(-5.284--2.439)	<.001
0 to 1 year	10.9	4.0	8.9	7.9	5.9	7.9	8.9	4.0	5.9	5.0	5.9	2.0	4.0	5.0	7.9	3.0	1.0	2.0	1.186(-2.63-5.001)	0.543
2 to 5 years	2.7	2.7	4.1	16.2	6.8	2.7	8.1	8.1	8.1	8.1	2.7	1.4	4.1	10.8	1.4	6.8	1.4	4.1	-2.413(-4.689--0.137)	0.038
6 to 12 years	0	0	0	0	0	3.4	3.4	3.4	6.9	3.4	6.9	6.9	6.9	20.7	17.2	13.8	3.4	3.4	-1.087(-2.353-0.179)	0.092
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	11.1	33.3	0	11.1	33.3	11.1	-0.619(-1.851-0.614)	0.325

Pseudomonas aeruginosa	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	change by year (95% CI)	p-value
	n=6	n=4	n=10	n=10	n=12	n=10	n=13	n=8	n=11	n=7	n=10	n=3	n=19	n=15	n=9	n=16	n=14	n=13		
Overall	3.20	2.10	5.30	5.30	6.30	5.30	6.80	4.20	5.80	3.70	5.30	1.60	10	7.90	4.70	8.40	7.40	6.80	-2.803(-4.263--1.343)	<.001
0 to 1 year	2.40	7.10	14.30	14.30	14.30	7.10	7.10	9.50	0	2.40	2.40	0	4.80	7.10	0	4.80	0	2.40	0.127(-3.878-4.132)	0.951
2 to 5 years	7.10	1.40	5.70	5.70	8.60	8.60	12.90	5.70	7.10	4.30	5.70	1.40	7.10	4.30	1.40	4.30	1.40	7.10	-2.265(-4.561-031)	0.053
6 to 12 years	0	0	0	0	0	1.70	1.70	0	10.20	5.10	8.50	3.40	15.30	10.20	10.20	13.60	15.30	5.10	-0.459(-1.548-0.63)	0.409
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	15.80	15.80	10.50	15.80	21.10	21.10	-0.423(-1.529-0.683)	0.453

Haemophilus influenza	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	change by year (95% CI)	p-value
	n=1	n=1	n=0	n=8	n=14	n=7	n=14	n=10	n=7	n=8	n=8	n=1	n=15	n=11	n=10	n=8	n=3	n=4		
Overall	0.80	0.80	0	6.20	10.80	5.40	10.80	7.70	5.40	6.20	6.20	0.80	11.50	8.50	7.70	6.20	2.30	3.10	-3.456(-4.951--1.961)	<.001
0 to 1 year	2.30	0	0	9.10	11.40	6.80	6.80	9.10	4.50	6.80	2.30	0	9.10	9.10	13.60	4.50	2.30	2.30	2.354(-1.559-6.266)	0.238
2 to 5 years	0	2.00	0	7.80	13.70	3.90	19.60	2.00	7.80	5.90	5.90	2.00	13.70	3.90	2.00	9.80	0	0	-2.83(-5.174--0.486)	0.018
6 to 12 years	0	0	0	0	7.10	7.10	3.60	17.90	3.60	7.10	14.30	0	14.30	10.70	7.10	0	3.60	3.60	-2.562(-3.952--1.172)	<.001
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	0	28.60	14.30	14.30	14.30	28.60	-08(-1.373-1.214)	0.904

Aspergillus fumigatus	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	change by year (95% CI)	p-value
	n=0	n=1	n=1	n=1	n=2	n=3	n=8	n=8	n=11	n=8	n=2	n=0	n=7	n=5	n=13	n=11	n=6	n=6		
Overall	0	1.10	1.10	1.10	2.20	3.20	8.60	8.60	11.80	8.60	2.20	0	7.50	5.40	14.00	11.80	6.50	6.50	-1.693(-3.341--045)	0.044
0 to 1 year	0	0	33.30	0	0	0	0	33.30	0	33.30	0	0	0	0	0	0	0	0	-0.667(-7.338-6.004)	0.845
2 to 5 years	0	4.80	0	4.80	4.80	9.50	4.80	19.00	19.00	9.50	0	0	4.80	4.80	9.50	4.80	0	0	-3.109(-5.841--0.377)	0.026
6 to 12 years	0	0	0	0	1.80	1.80	12.70	5.50	12.70	9.10	3.60	0	10.90	5.50	14.50	10.90	7.30	3.60	-1.231(-2.38--083)	0.036
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	0	7.10	21.40	28.60	14.30	28.60	0.104(-0.964-1.171)	0.849

Serratia marcescens	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	change by year (95% CI)	p-value
	n=1	n=0	n=1	n=0	n=2	n=1	n=2	n=0	n=1	n=0	n=0	n=0	n=2	n=4	n=3	n=5	n=3	n=3		
Overall	3.60	0	3.60	0	7.10	3.60	7.10	0	3.60	0	0	0	7.10	14.30	10.70	17.90	10.70	10.70	-1.281(-3.375-0.813)	0.231
0 to 1 year	14.30	0	14.30	0	28.60	0	14.30	0	14.30	0	0	0	0	0	0	14.30	0	0	N/A	N/A
2 to 5 years	0	0	0	0	0	20	0	0	0	0	0	0	0	20	0	20	40	0	0.942(-2.5-4.385)	0.592

6 to 12 years	0	0	0	0	0	0	12.50	0	0	0	0	0	12.50	12.50	12.50	12.50	12.50	25.00	0.566(-1.232-2.364)	0.537
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	12.50	25.00	25.00	25.00	0	12.50	-0.859(-2.122-0.404)	0.182
Non-tuberculous mycobacteria	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**change by year (95% CI)**	**p-value**
	n=0	**n=0**	**n=0**	**n=0**	**n=0**	**n=1**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=1**	**n=1**	**n=3**	**n=6**	**n=6**	**n=9**		
Overall	0	0	0	0	0	3.70	0	0	0	0	0	0	3.70	3.70	11.10	22.20	22.20	33.30	1.591(-0.654-3.836)	0.165
0 to 1 year	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A
2 to 5 years	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	50	0	0	0.771(-4.942-6.484)	0.791
6 to 12 years	0	0	0	0	0	0	0	0	0	0	0	0	9.10	0	9.10	18.20	27.30	36.40	1.562(048-3.076)	0.043
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	0	7.10	14.30	21.40	21.40	35.70	0.331(-0.796-1.459)	0.565
Burkholderia cepacia	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**change by year (95% CI)**	**p-value**
	n=0	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=1**	**n=0**	**n=0**	**n=0**	**n=1**	**n=1**	**n=2**	**n=0**		
Overall	0	0	0	0	0	0	0	0	0	0	20	0	0	0	20	20	40	0	0.568(-3.603-4.738)	0.79
0 to 1 year	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A
2 to 5 years	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A
6 to 12 years	0	0	0	0	0	0	0	0	0	0	33.30	0	0	0	33.30	33.30	0	0	-0.46(-3.234-2.315)	0.745
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0.446(-1.219-2.11)	0.6
Achromobacter xylosoxidans	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**change by year (95% CI)**	**p-value**
	n=0	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=0**	**n=1**	**n=2**	**n=0**	**n=3**	**n=3**	**n=1**	**n=0**	**n=4**	**n=1**		
Overall	0	0	0	0	0	0	0	0	0	6.70	13.30	0	20	20	6.70	0	26.70	6.70	-0.142(-3.242-2.958)	0.928
0 to 1 year	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A
2 to 5 years	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A
6 to 12 years	0	0	0	0	0	0	0	0	0	11.10	22.20	0	22.20	22.20	0	0	22.20	0	-0.915(-2.833-1.003)	0.35
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	16.70	16.70	16.70	0	33.30	16.70	06(-1.453-1.572)	0.938
Stenotrophomonas maltophilia	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**change by year (95% CI)**	**p-value**
	n=0	**n=0**	**n=0**	**n=0**	**n=1**	**n=0**	**n=1**	**n=4**	**n=2**	**n=1**	**n=3**	**n=3**	**n=6**	**n=9**	**n=2**	**n=8**	**n=5**	**n=9**		
Overall	0	0	0	0	1.90	0	1.90	7.40	3.70	1.90	5.60	5.60	11.10	16.70	3.70	14.80	9.30	16.70	-1.281(-3.375-0.813)	0.231
0 to 1 year	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A
2 to 5 years	0	0	0	0	0	0	11.10	0	0	0	0	11.10	22.20	33.30	0	0	11.10	11.10	N/A	N/A
6 to 12 years	0	0	0	0	3.20	0	0	12.90	6.50	3.20	9.70	3.20	6.50	16.10	0	12.90	9.70	16.10	1.781(-1.375-2.813)	0.796
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	7.10	14.30	7.10	14.30	28.60	7.10	21.40	-0.067 (-2.452-4.897)	0.13

Table 1: Incidence rates of pathogens isolated according to consecutive years. Figures are represented in percentages, indicating the proportion of the respiratory pathogen's presence within each age group over the different years. n= number of children or adolescents with a positive airway sample culture for the corresponding year. The change by year (95% CI) represents the coefficient obtained from regression modeling, indicating the percentage change per year, along with its corresponding 95% confidence interval and p-value. N/A=not applicable.

Staphylococcus aureus	2002 n=94	2003 n=89	2004 n=102	2005 n=130	2006 n=138	2007 n=134	2008 n=141	2009 n=162	2010 n=161	2011 n=171	2012 n=157	2013 n=105	2014 n=152	2015 n=153	2016 n=154	2017 n=132	2018 n=130	2019 n=133	change by year (95% CI)	p-value
Overall	3.80	3.70	4.20	5.30	5.70	5.40	5.70	6.60	6.60	7.00	6.50	4.30	6.30	6.30	6.30	5.40	5.40	5.40	-2.571 (-3.502--1.64)	<.001
0 to 1 year	12.30	10.50	13.50	12.90	11.70	9.90	11.70	7.00	6.40	2.90	1.20	0	0	0	0	0	0	0	1.146 (-1.955-4.247)	0.469
2 to 5 years	8.20	8.00	8.00	9.40	9.80	7.50	7.30	9.20	8.00	7.80	6.70	3.60	3.20	2.30	1.10	0	0	0	-0.339 (-1.5-0.822)	0.567
6 to 12 years	2.80	2.80	3.70	5.30	5.90	5.90	5.60	7.00	6.40	7.80	6.60	4.90	7.20	6.90	6.50	5.40	4.80	4.50	-2.605 (-3.55--1.659)	<.001
≥ 12 years	0	0	0	0.70	0.90	2.40	3.40	4.20	5.80	6.30	7.30	5.20	8.80	9.90	11.40	10.70	11.30	11.80	-2.571 (-3.502--1.64)	<.001

Pseudomonas aeruginosa	2002 n=95	2003 n=89	2004 n=102	2005 n=105	2006 n=104	2007 n=95	2008 n=102	2009 n=87	2010 n=100	2011 n=96	2012 n=93	2013 n=35	2014 n=80	2015 n=71	2016 n=61	2017 n=68	2018 n=50	2019 n=45	change by year (95% CI)	p-value
Overall	6.30	6.00	6.90	7.20	7.10	6.30	6.90	5.90	6.80	6.40	6.30	2.40	5.40	4.80	4.10	4.60	3.40	3.10	-2.985 (-3.966--2.003)	<.001
0 to 1 year	16.50	13.70	14.10	12.00	10	8.80	9.20	6.00	4.80	2.80	1.20	0	0	0	0.40	0	0.40	0	0.811 (-2.281-3.904)	0.607
2 to 5 years	7.50	8.60	9.40	9.90	10.30	8.40	8.10	7.10	7.10	7.30	7.10	3.40	3.40	1.90	0.60	0	0	0	-0.513 (-1.682-0.656)	0.39
6 to 12 years	3.70	3.10	5.10	5.50	4.60	4.80	5.70	5.50	9.20	7.50	7.30	3.30	7.70	7.30	6.80	5.90	3.70	3.30	-2.828 (-3.819--1.838)	<.001
≥ 12 years	0	0	0	1.30	3.40	3.40	4.70	4.40	4.40	6.40	8.10	1.30	9.80	9.80	8.40	13.80	10.80	10.10	-2.985 (-3.966--2.003)	<.001

Haemophilus influenza	2002 n=8	2003 n=10	2004 n=7	2005 n=17	2006 n=36	2007 n=28	2008 n=44	2009 n=40	2010 n=33	2011 n=34	2012 n=26	2013 n=8	2014 n=46	2015 n=34	2016 n=27	2017 n=21	2018 n=12	2019 n=11	change by year (95% CI)	p-value
Overall	1.80	2.30	1.60	3.90	8.00	6.40	10	9.10	7.30	7.50	5.90	1.80	10.50	7.80	5.90	4.80	2.70	2.50	-3.931 (-4.95--2.912)	<.001
0 to 1 year	16.70	0	8.30	0	8.30	8.30	25.00	16.70	8.30	0	8.30	0	0	0	0	0	0	0	2.417 (-1.02-5.854)	0.168
2 to 5 years	5.30	9.20	5.30	5.30	15.80	11.80	10.50	5.30	7.90	7.90	6.60	1.30	3.90	3.90	0	0	0	0	-0.239 (-1.625-1.146)	0.735
6 to 12 years	1.40	2.00	1.40	6.80	10.80	8.80	11.50	13.50	8.10	8.80	4.10	2.00	7.40	4.70	3.40	2.00	1.40	2.00	-3.977 (-5.132--2.821)	<.001
≥ 12 years	0	0	0	1.50	3.00	2.50	7.90	6.90	6.40	6.90	6.90	2.00	15.80	11.90	10.40	8.90	5.00	4.00	-3.931 (-4.95--2.912)	<.001

Aspergillus fumigatus	2002 n=35	2003 n=37	2004 n=34	2005 n=33	2006 n=30	2007 n=32	2008 n=46	2009 n=38	2010 n=38	2011 n=45	2012 n=36	2013 n=14	2014 n=41	2015 n=36	2016 n=53	2017 n=45	2018 n=40	2019 n=31	change by year (95% CI)	p-value
Overall	5.30	5.60	5.00	5.00	4.50	4.80	6.90	5.70	5.70	6.60	5.40	2.10	6.20	5.40	8.00	6.80	6.00	4.70	-1.002 (-2.109-0.104)	0.076
0 to 1 year	10.70	18.70	20	12.00	8.00	9.30	6.70	8.00	4.00	1.30	1.30	0	0	0	0	0	0	0	0.6 (-2.535-3.735)	0.708
2 to 5 years	11.60	10.50	7.20	9.90	9.40	8.30	11.00	5.50	6.10	6.60	5.50	1.70	2.80	2.20	1.70	0	0	0	-0.895 (-2.134-0.345)	0.157
6 to 12 years	2.00	1.40	1.70	2.00	2.40	3.40	6.80	6.10	7.10	8.80	7.80	3.40	8.80	7.80	10.20	8.80	6.10	5.10	-1.231 (-2.267--0.194)	0.02
≥ 12 years	0	0	0	0	0	0	0.90	3.60	2.70	4.50	1.80	0.90	8.90	8.00	17.90	17.00	19.60	14.30	-1.002 (-2.109-0.104)	0.076

Serratia marcescens	2002 n=2	2003 n=4	2004 n=3	2005 n=2	2006 n=5	2007 n=4	2008 n=3	2009 n=1	2010 n=2	2011 n=1	2012 n=0	2013 n=0	2014 n=3	2015 n=7	2016 n=8	2017 n=9	2018 n=9	2019 n=5	change by year (95% CI)	p-value
Overall	2.90	5.90	4.40	2.90	7.40	5.90	4.40	1.50	2.90	1.50	0	0	4.40	10.30	11.80	13.20	13.20	7.40	-1.35 (-2.872-0.171)	0.082
0 to 1 year	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0		
2 to 5 years	6.30	25.00	12.50	12.50	6.30	12.50	6.30	6.30	6.30	6.30	0	0	0	0	0	0	0	0	-2.325 (-4.424--0.226)	0.03
6 to 12 years	5.00	0	5.00	0	5.00	5.00	5.00	0	0	0	0	0	10	25.00	20	5.00	10	5.00	-0.346 (-2.513-1.822)	0.754

≥ 12 years	0	0	0	0	6.50	3.20	3.20	0	3.20	0	0	0	3.20	6.50	12.90	25.80	22.60	12.90	-1.35 (-2.872-0.171)	0.082
Non-tuberculous mycobacteria	**2002 n=2**	**2003 n=1**	**2004 n=1**	**2005 n=1**	**2006 n=0**	**2007 n=1**	**2008 n=0**	**2009 n=1**	**2010 n=0**	**2011 n=1**	**2012 n=0**	**2013 n=0**	**2014 n=2**	**2015 n=2**	**2016 n=3**	**2017 n=9**	**2018 n=9**	**2019 n=12**	**change by year (95% CI)**	**p-value**
Overall	4.40	2.20	2.20	2.20	0	2.20	0	2.20	0	2.20	0	0	4.40	4.40	6.70	20	20	26.70	1.131 (-0.799-3.062)	0.251
0 to 1 year	50	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-2.167 (-7.027-2.694)	0.382
2 to 5 years	25.00	0	25.00	0	0	0	0	0	0	0	0	0	25.00	0	25.00	0	0	0	1.05 (-2.671-4.771)	0.58
6 to 12 years	0	0	0	4.30	0	4.30	0	4.30	0	4.30	0	0	4.30	8.70	4.30	21.70	21.70	21.70	2.093 (0.046-4.14)	0.045
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.30	25.00	25.00	43.80	1.131 (-0.799-3.062)	0.251
Burkholderia cepacia	**2002 n=4**	**2003 n=2**	**2004 n=2**	**2005 n=3**	**2006 n=4**	**2007 n=3**	**2008 n=2**	**2009 n=2**	**2010 n=1**	**2011 n=1**	**2012 n=2**	**2013 n=0**	**2014 n=1**	**2015 n=1**	**2016 n=3**	**2017 n=3**	**2018 n=5**	**2019 n=4**	**change by year (95% CI)**	**p-value**
Overall	9.30	4.70	4.70	7.00	9.30	7.00	4.70	4.70	2.30	2.30	4.70	0	2.30	2.30	7.00	7.00	11.60	9.30	0.969 (-1.372-3.31)	0.417
0 to 1 year	25.00	25.00	0	12.50	12.50	12.50	0	12.50	0	0	0	0	0	0	0	0	0	0	-0.042 (-3.646-3.563)	0.982
2 to 5 years	12.50	0	12.50	12.50	18.80	12.50	12.50	0	6.30	6.30	6.30	0	0	0	0	0	0	0	-1.512 (-3.611-0.586)	0.158
6 to 12 years	0	0	0	0	0	0	0	11.10	0	0	11.10	0	11.10	11.10	22.20	11.10	11.10	11.10	1.465 (-1.609-4.539)	0.35
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	20	40	30	0.969 (-1.372-3.31)	0.417
Achromobacter xylosoxidans	**2002 n=0**	**2003 n=0**	**2004 n=0**	**2005 n=0**	**2006 n=0**	**2007 n=0**	**2008 n=0**	**2009 n=2**	**2010 n=1**	**2011 n=2**	**2012 n=5**	**2013 n=1**	**2014 n=4**	**2015 n=5**	**2016 n=6**	**2017 n=1**	**2018 n=9**	**2019 n=6**	**change by year (95% CI)**	**p-value**
Overall	0	0	0	0	0	0	0	4.80	2.40	4.80	11.90	2.40	9.50	11.90	14.30	2.40	21.40	14.30	0.469 (-2.718-3.656)	0.773
0 to 1 year	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N/A	N/A
2 to 5 years	0	0	0	0	0	0	0	33.30	16.70	16.70	16.70	16.70	0	0	0	0	0	0	2.717 (-0.389-5.823)	0.086
6 to 12 years	0	0	0	0	0	0	0	0	0	3.20	12.90	0	12.90	12.90	19.40	3.20	16.10	19.40	2.225 (0.404-4.047)	0.017
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	80	0	0.469 (-2.718-3.656)	0.773
Stenotrophomonas maltophilia	**2002 n=4**	**2003 n=3**	**2004 n=5**	**2005 n=5**	**2006 n=6**	**2007 n=6**	**2008 n=9**	**2009 n=10**	**2010 n=11**	**2011 n=4**	**2012 n=10**	**2013 n=4**	**2014 n=18**	**2015 n=20**	**2016 n=16**	**2017 n=22**	**2018 n=25**	**2019 n=20**	**change by year (95% CI)**	**p-value**
Overall	2.00	1.50	2.50	2.50	3.00	3.00	4.60	5.10	5.60	2.00	5.10	2.00	9.10	10.20	7.60	11.20	12.70	10.20	1.131 (-0.799-3.062)	0.251
0 to 1 year	0	33.30	0	33.30	33.30	0	0	0	0	0	0	0	0	0	0	0	0	0		
2 to 5 years	7.50	5.00	7.50	7.50	7.50	10	12.50	7.50	10	7.50	10	0	2.50	2.50	2.50	0	0	0	0.21 (-2.71-3.706)	0.854
6 to 12 years	1.00	0	2.10	1.00	2.10	2.10	4.20	7.30	7.30	1.00	6.30	2.10	13.50	10.40	8.30	12.50	11.50	7.30	1.993 (0.046-2.14)	0.063
≥ 12 years	0	0	0	0	0	0	0	0	0	0	0	3.40	6.90	15.50	10.30	17.20	24.10	22.40	2.341 (-0.469-2.982)	0.251

Table 2: Prevalence rates of pathogens isolated according to consecutive years. Figures are represented in percentages, indicating the proportion of the respiratory pathogen's presence within each age group over the different years. n= number of children or adolescents with a positive airway sample culture for the corresponding year. The change by year (95% CI) represents the coefficient obtained from regression modeling, indicating the percentage change per year, along with its corresponding 95% confidence interval and p-value. N/A=not applicable.

Figure 4: Mean age group of the first culture of CF organisms.

Chapter 3: Factors influencing treatment response of pulmonary exacerbation in children with cystic fibrosis

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Introduction

Morbidity and mortality in cystic fibrosis (CF) remains largely attributed to early or recurrent pulmonary exacerbations[82]. In children, a pulmonary exacerbation in the first 2 years of life is associated with the presence of bronchiectasis, a decline in lung function, and a poorer nutritional outcome during their preschool years[26,83]. Modelling has shown that the number of annual pulmonary exacerbations is a predicting factor of survivability at 3 to 5 years[28].

Studies have shown varying results of treatment outcomes with intravenous antibiotics (IV), with some studies demonstrating that up to 75% of patients respond to treatment [84-86]. The majority of patients enrolled in these studies were adults and extrapolating outcomes to the paediatric population may be problematic because of their differences in treatment adherence, treatment indications and outcome measures, co-morbidities, and the duration of the treatment [87-89].

This study was designed to determine the proportion of paediatric patients who do not recover following treatment of a pulmonary exacerbation or those who require readmission within 6 months from the last pulmonary exacerbation. As a secondary objective, the factors that

may contribute to treatment failure or success, and readmissions were reviewed.

Methods

Study Population

This was a retrospective longitudinal study conducted in The Children's Hospital at Westmead (CHW), Sydney, Australia between January 2015 and December 2018. This study was approved by the Ethics committee of The Sydney Children's Hospital Network (LNR/18/SCHN/380)

All children with CF who were admitted to CHW and treated for pulmonary exacerbations were included in this study. Children admitted for treatment of active non-tuberculous mycobacterium lung disease or those who had a lung transplant were excluded.

Clinical routines during the study

In CHW, admission is typically between 10 to 14 days which may be completely undertaken in an inpatient setting or through a hybrid setting with an inpatient admission followed by the continuation of treatment under the hospital-in-the-home (HITH) program. During the admission, the child will receive a minimum of twice a day of physiotherapy from a trained paediatric physiotherapist with regular clinical and dietetics reviews. The choice of antibiotics is determined by the treating clinician under the advisement of the hospital's antibiotic stewardship using the

most recent sputum or bronchoalveolar lavage sample (BAL) organisms' antibiotic sensitivity. Children who receive aminoglycosides as part of their treatment regime will undergo therapeutic drug monitoring on day 2 of admission and the necessary dose adjustment will be carried out in consultation with the infectious diseases pharmacist.

A decision is then made based on the progress of the child by the treating clinician with input from the multidisciplinary team of physiotherapists, dieticians, and clinical nurse consultants in regards to the duration of treatment, this may include; completing the admission following 10 days (or less) of treatment, completing the entire 14 days admission in an inpatient setting, transferring the child onto the HITH program for completion of the remaining treatment course, or prolonging the inpatient admission. For children admitted onto the HITH program, a minimum of 1 home-based physiotherapy is attended to daily while antibiotics will be delivered via infuser pumps over 24 hours/day for the duration of treatment.

Lung function tests were performed at a Thoracic Society of Australia and New Zealand (TSANZ) accredited respiratory function laboratory within CHW. Only lung function data that met the criteria of adequate testing were included[90]. All lung function measurements were performed using the Vyntus™ Pneumo Spirometer system (Vyaire™, Illinois, USA) and expressed as % predicted based on global lung index reference equations[91]. Anthropometric measurements were derived from a standard

weighing scale and stadiometer used in the laboratory before performing lung function.

Definitions

Baseline lung function was defined as the average of FEV_1% measured in the 6 months leading up to the admission. Response to treatment measured at the end of each admission was defined as the recovery to greater than or equal to 95% of the baseline FEV_1% [92,93].

Lung function severity (FEV_1%) was classified based on the European Respiratory Society classification; z-score (mild: -1.65 to -2.5, moderate: -2.5 to -4.0 and severe: <-4.1)[94] .

Sub-optimal nutrition was defined as having a body mass index (BMI) less than the 50th percentile [95].

Statistical analysis

Descriptive analyses of individual patients were performed to characterise these children. Continuous variables were described as mean± standard deviation. Chi-square statistical test was applied. Response to treatment was calculated based on individual admissions.

Subsequently, generalised estimating equations to adjust for the repeated exacerbations in individual patients and to evaluate factors influencing response vs. non-response to treatment was performed. Odds ratios derived from the generalised estimating equations were expressed as (OR

CI 95% lower limit-upper limit) for every unit of change in the explanatory variable.

Statistical significance in this study was considered to be a p-value < 0.05. All statistical analyses were performed using SPSS® Statistics software, version 25, IBM® Corporation, New York, USA.

Results

Patient characteristics

Between January 2015 and December 2018, 78 (52% males) children with CF were admitted for pulmonary exacerbation(s).

The majority of children were homozygous F508del. (n=51, 65%), 19(24%) were heterozygous for F508del. while another 8 (10%) were those with rarer or unknown mutations. Seven (8.9%) were not diagnosed during the routine newborn screening program. Sweat chloride on diagnosis was 95.5 ±15.5 mmol/L. Four (5%) children were pancreatic sufficient. Out of 78 children, 15 (19%) had or received a diagnosis of allergic bronchopulmonary aspergillosis (ABPA) while 19 (24%) had CF-related diabetes mellitus (CFRDM) during the period of this study.

Characteristics	***Category***	***Overall***	***Response to treatment****	***No response to treatment****
		(n=184)	**(n=108)** n(%)	**(n=76)** n(%)
Age group, *year*	6 to 12	80 (43.5)	50 (62.5)	30 (37.5)
	>12	104 (56.5)	58 (55.8)	46 (44.2)
Sputum culture	*P.aeruginosa*	65 (35.3)	40 (61.5)	25 (38.4)
	Non *P.aeruginosa*	119 (64.7)	68 (57.1)	51 (42.8)
Total days of admission, *days*	10-14	153(83.2)	91 (59.5)	62 (40.5)
	>14	31(16.8)	27(87.1)	14(45.2)
FEV_1% on admission	Mild	110(59.8)	78(70.9)	32 (29.1)
	Moderate	51(27.7)	23(45.1)	28(54.9)
	Severe	23(9.8)	7(30.4)	16(69.6)
FEV_1% baseline	Mild	175(95.1)	91 (52)	67 (38.3)
	Moderate	26(14.1)	17(65.4)	9 (34.6)
BMI centile on admission, *percentile*	<50	131(71.1)	73(55.7)	58(44.3)
	>50	53(28.8)	33(62.3)	20(37.7)

Time taken to readmission, *months*	<6	68(36.9)	32(47.1)	36 (52.9)
	>6	55(29)	35(63.6)	20(36.4)
	No readmission	61 (33.1)	41 (67.2)	20 (32.8)

Table 3: Characteristics of each admission during a pulmonary exacerbation. Percentages in columns marked with * are calculated based on proportions in the respective row.

Characteristics of individual admissions

Of the 78 children, 30 (38%) were admitted only once, while 48 (62%) had more than one admission (ranging from 2 to 12 admissions) during the study period.

This resulted in a total of 184 admissions throughout the study 4-year study period. These treatments were either completely undertaken in the hospital (n=107, 58%), completely at home (n=34, 18%) or using a hybrid setting (n=43, 23%).

The duration of these admissions ranged from 7 to 36 days, with a mean of 15 ±3 days. One hundred and fifty-four (84%) admissions received treatment for ≤14 days and 30 (16%) admissions received treatment of >14 days. (Table 3)

The mean age during admission was 11.7 ±2.7 years. There were 80 (43%) admissions which occurred between ages six to 12 years and 104

(57%) > 12 years of age. The duration of admission of children >12 years of age (15 ±4 days) were longer compared to admissions <12 years of age (14 ±2 days), p=0.016.

Pseudomonas aeruginosa was isolated in 65 (35%) of the sputum samples provided during admission by each child. *P.aeruginosa* was present in 73% of these patients who were admitted within the last 1 year of the admission in >50% of samples collected routinely. *Staphylococcus aureus* represented 76 (41%) of the organisms isolated [methicillin resistant *S. aureus* was found in 6 (7.8%)]. *S.aureus* was present in 90% of these patients who were admitted within the last 1 year of the admission in >50% of samples collected routinely. The remaining organisms isolated in the sputum provided during admission included *Stenotrophomonas maltophillia, Hemophilus influenza*, *Pandoraea* species, *Scedosprorium prolificans, Burkholderia cepacia,* and normal respiratory flora.

The mean body mass index (BMI) centile on admission was 37.8±22.7 centile with 104 (73%) children presenting with a BMI of < 50th centile.

Spirometry results during admission and follow-up

The baseline FEV_1% was 86.15±15.6%. FEV_1% on admission was 72.31 ± 18.2%, at the end-of-treatment was 83.23 ±17.38% and at 6 weeks post-treatment was 80.22 ±17.8%. (Table 3)

Overall, FEV_1% on admission was 13.84 ±13.79% lower than the baseline FEV_1%. At the end of admission, the decline was 2.92±10.76%, p<0.001. At 6 weeks the decline was 9.42 ±20.03%, p<0.11.

Individual response to treatment and risk of readmission

Fifty-nine percent of the admissions resulted in a return to baseline to 95%. While 44% of these admissions achieved a return to baseline of > 100%.

Generalise estimating equations to account for repeated admissions/child was performed to illustrate the relevant risk factors.

The magnitude of the decline in lung function on admission in children who did not respond to treatment was 21.7±15.2% while the decline in children who responded to treatment was 8.3±9.4%, p <0.001. The remaining risk factors have been represented in Figure 5.

In terms of readmission, we found that 32 (41%) children were readmitted within 6 months after completing treatment with 43% of these children returning to baseline.

The overall time for readmission was 7.7±5.4 months. The mean time for readmission in children who responded to treatment was 8.9±6.4 months while those who did not respond to the treatment were admitted at 6.4±3.5 months, p=0.013.

Children who were readmitted within six months had an FEV_1% on admission that was 15.2 ±11.6% lower than the baseline FEV1% with an FEV_1% baseline of 81.5±16%, on admission of 66±17%, end-of-treatment of 75±17%, 6 weeks post-treatment of 72±17% In contrast, children who were not readmitted within six months had an FEV_1% on admission of 14.7 ±13.3% (p=0.68) lower than the baseline with an FEV_1% baseline of 88.3±14.7% (p=0.044), on admission of 74±17% (p=0.067), end-of-treatment of 88±16% (p<0.001) and 6 weeks post-treatment of 85±15% (p<0.001).(Figure 6)

Children over < 12 had a lower change of being readmitted within 6 months (OR -0.296 CI -0.52- -0.66)

No difference was observed between children representing within six months or those presenting after six months in terms of sweat chloride (OR -0.001 CI -0.009-0.007), sex (HR 0.96 CI 0.567-1.625), duration of admission (OR 0.027 CI -0.040- -0.093). No difference was seen in sex (p=0.631), presence of ABPA (p=0.579), CFRDM (p=0.09), treatment in hospital (p=0.152), treatment in hybrid setting (p=0.59) or at home (p=0.187), the presence of *pseudomonas aeruginosa* in sputum (p=0.145) or BMI centile < 50 (p=0.895).

Discussion

This study demonstrates that 2 out of 3 admissions occurring following a pulmonary exacerbation in a large, tertiary CF centre in Australia

managed to recover within 95% of the baseline FEV_1%. This study shows similar findings to a much larger, multi-centred US study conducted by Sanders *et al*, in which 75% of their study cohort returned to baseline lung function with treatment success in that said study defined as any FEV_1% in the three months after treatment was greater than or equal to 90% of the baseline FEV_1%[8]. In a small prospective study by Waters *et al*, 36 (51%) children that received treatment following a pulmonary exacerbation returned to baseline which was defined as a lung function that returned to >100% of their baseline lung function.

Nevertheless, despite returning these children to ≥95% of their baseline lung function, we found that the gains occurring at the end of the treatment were not sustained as was demonstrated by a drop in lung function within 6 weeks of discharge. This finding is corroborated by a prospective study conducted by Cunningham *et al* which demonstrated the FEV_1% increase was sustained for only 1 week following the completion of IV treatment[96].

Previous studies have shown lower baseline lung function[97], female sex[98], CF genetics[99], pancreatic insufficiency[100], poor nutrition[101], CFRDM[102], persistent infection with P.*aeruginosa*[83] were more likely to predict a poor response to treatment.

In our cohort, we found that children who recovered were typically those with better lung function values on admission and those who had a smaller drop in lung function from baseline at the start of their treatment.

We were not able to demonstrate any statistical difference in terms of sex, CF genetics, the presence of ABPA or CRFDM, BMI, or the presence of *P. aeruginosa* within the sputum.

In terms of duration of admission, we found no significant difference in regard to the response to treatment. This finding is particularly important as it has the potential for reducing disruption to routine activities and reducing healthcare costs by reducing hospital bed requirements. It also has the potential of reducing possible toxicity and resistance effects that may ensue from longer antibiotic courses [103]. In the literature, there have been conflicting results on the benefits of prolonging treatment with Collaco *et al* study suggesting a similar conclusion as ours while other studies showed continued benefits of prolonged antibiotic use[103,104]. More importantly, our study findings is consistent with a recent randomized controlled trial by Goss *et al* [105]. We have demonstrated that children >12 years of age during admission are admitted for a longer duration. Although the duration itself does not change the outcome, this may reflect the clinician's preference to keep older children in the hospital longer to consolidate their physiotherapy technique and also work on other issues such as compliance and weight gain.

We found that children who were readmitted within six months did not demonstrate any statistical difference in terms of sex, CF genetics, presence of ABPA or CRFDM, age during admission, BMI, or the presence of *P. aeruginosa* within the sputum.

These children were also not able to sustain the gains in lung function that was achieved during the admission when reassessed during their follow-up visits. We found that children who did not sustain their lung function gains at their six-week follow-up had a 5% chance of readmission for every 1% decline of FEV_1% away from their baseline. As a result, 1 in 3 of our children with CF were readmitted within six months. This could be due to two reasons namely planned admission (3 patients identified as planned admission) or recurrent exacerbations. This is a particular source of concern as we found that almost 1 in 2 children who were readmitted within 6 months of the previous admission did not respond to the subsequent treatment. These findings of a decline in lung function at the six-week follow-up review highlight the question about the adequacy of the existing post-treatment follow-up strategy.

While understanding the limitations of CF clinics to perform regular face-to-face reviews, it is important to consider and/or adopt other methods of tighter monitoring in these groups of children. For instance, the utilisation of home-based spirometry for a certain duration with notification to the CF team should there be a declining trend in lung function. This may help to trigger an earlier face-to-face review and institute pre-emptive strategies such as an increase in physiotherapy or a review of treatment adherence. The use of telehealth or online group physiotherapy may also be helpful in recently discharged children to ensure ongoing and optimal

respiratory rehabilitation under supervision from a trained CF physiotherapist.

The strength of this study is in that this is the first study in children to assess factors that may contribute towards non-response towards treatment following an admission for a pulmonary exacerbation. Additionally, our review of the reasons for readmission within a short time frame (six months) provides a novel insight that may help clinicians design follow-up strategies in children with CF who have recently had a pulmonary exacerbation.

The limitation of this study is that this is a retrospective longitudinal cohort study and therefore all the inherent flaws of this design are contained within this study particularly in inferring causality. Our data is also derived from a single centre which may not reflect the heterogeneity of patients and management in different centres (e.g. the availability of twice-a- day physiotherapy). In this study, we defined *a priori* the baseline lung function is defined as the best lung function within the last 6 months before exacerbation because there are no universally adopted criteria to define pulmonary exacerbation in CF[106,107]. This study only captures exacerbations that are deemed severe enough to require treatment and therefore did not include patients with exacerbations that are treated at home with oral or inhaled antibiotics and/or increased supervised physiotherapy sessions. Furthermore, this study was performed in the era that preceded effective modulators and the

consequent reduction of pulmonary exacerbations and admissions. However, this study may help to inform future studies comparing return to baseline between the period before and after modulators.

Based on our results, we suggest 1) scheduling earlier or more regular follow-ups than the routine 6 weeks and 3 months, 2) to consider and evaluate methods of monitoring following discharge from the hospital e.g. the use of home-based spirometry or the utilisation of ongoing supervised physiotherapy following discharge, 3) as the CF population is ageing, the role of primary healthcare providers in the follow-up of these patients after discharge should also be re-examined to provide a cooperative model of care and decentralisation of health provision.

Conclusion

We have identified factors that can contribute to poorer outcomes in patients. These findings can potentially help clinicians identify children who may not respond to treatment or may require readmissions. In particular, demonstrating that 1) the magnitude of the decline in lung function predicts the non-response to treatment and 2) the inability to maintain lung function gains made following discharge leading to readmissions and subsequent non-response to treatment are important findings in this study. This study suggests a need for closer monitoring which will provide the ability to plan for a more stringent post-discharge follow-up or timely intervention. This study underscores the importance of

scheduling more regular follow-ups post-treatment of pulmonary exacerbation

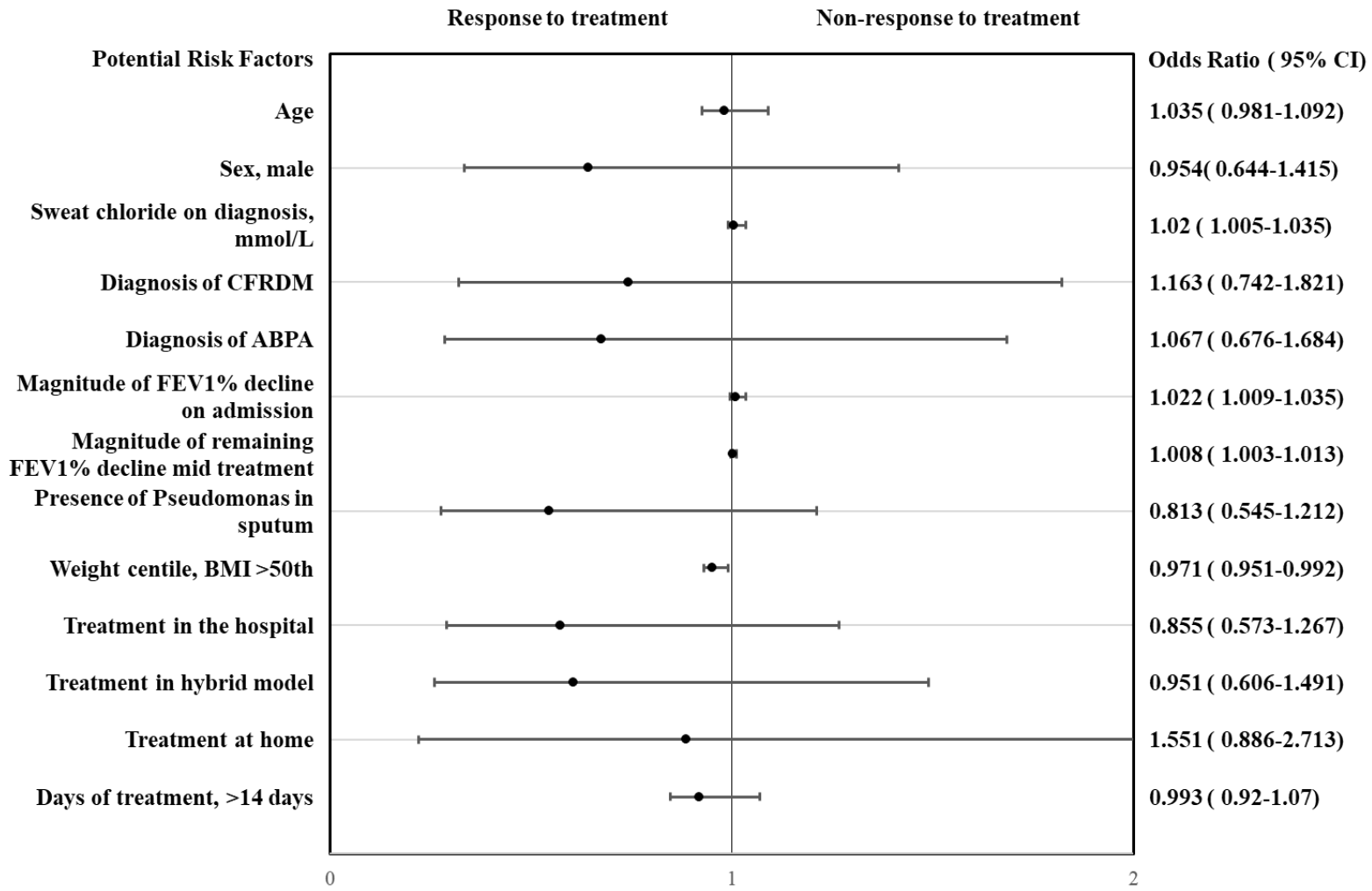

Figure 5: Risk factors and the associated response to treatment. Linear regression was performed controlling for the number of admissions in the individual child. CF-related diabetes mellitus, CFRDM; allergic bronchopulmonary aspergillosis, ABPA; body mass index, BMI

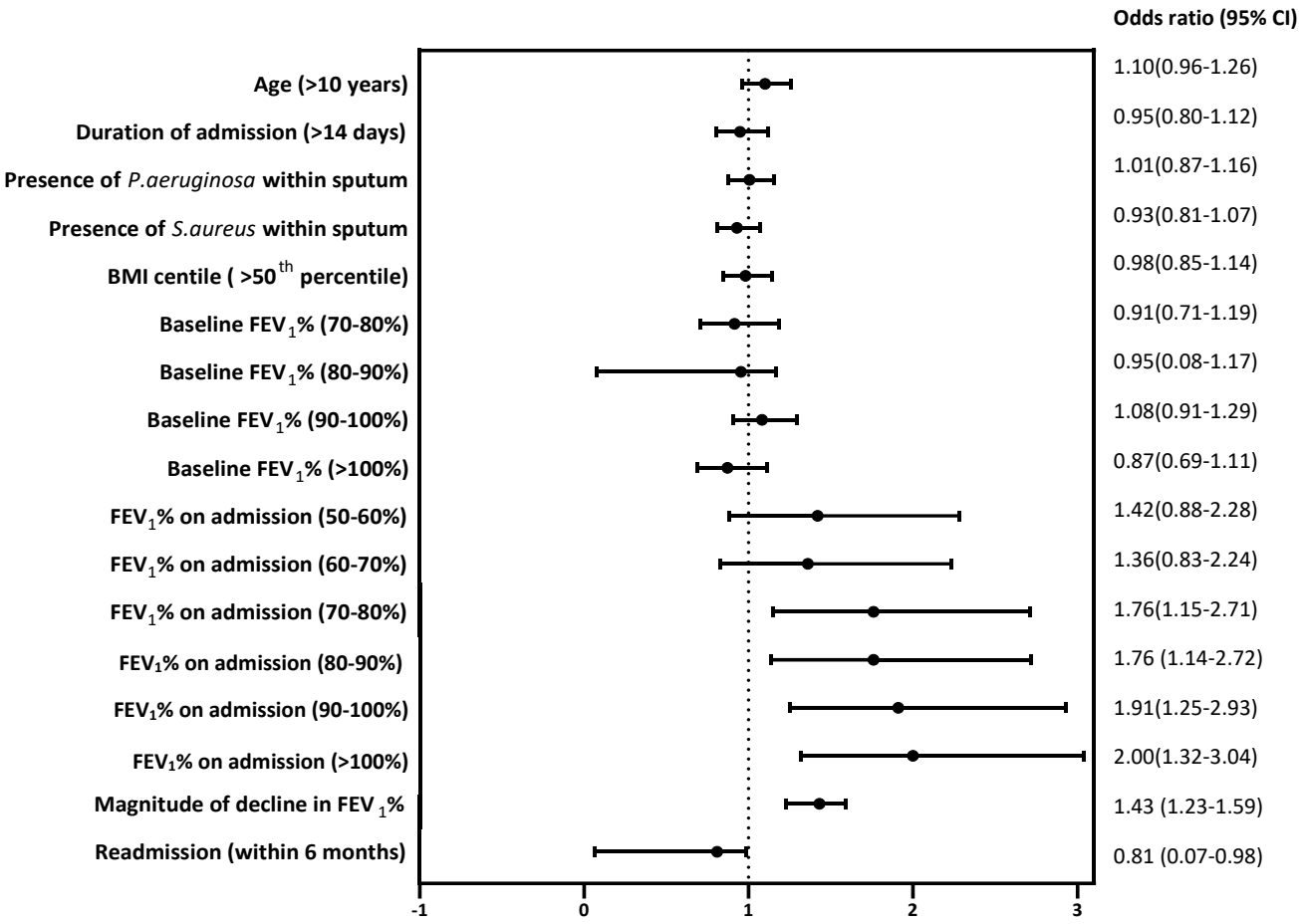

Figure 6: Factors contributing towards response to treatment. Values of >1 show response to treatment. OR: odds ratio, CI: confidence interval. Magnitude of decline = baseline FEV_1%-FEV_1% on admission

Chapter 4: A systematic review on the use of bacteriophage in treating *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections in Cystic Fibrosis.

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Background

Cystic fibrosis (CF) is a life-limiting genetic disorder leading to chronic pulmonary infections and a progressive decline in lung function. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the respiratory pathogens that most commonly affect people living with CF.[1] It is estimated that up to 25% of children and a higher percentage of adults living with CF will be infected by these organisms in their lungs.[108] The presence of *P. aeruginosa* in the airways is associated with a 2.6-fold increase in mortality rates within eight years, as well as lower baseline weight and higher CF-related hospitalizations.[27] Previous studies have demonstrated that *S. aureus* airway infection often begins in early life and is found to affect 80% of adolescents by 19 years of age.[109] Infections from *S. aureus* contribute towards an increase in inflammatory markers and lowered lung function.[110,111] While the prevalence of these organisms is declining in high-income nations during the era of highly effective modulator therapies, [34] only 12% of people living with CF globally have access to these medications, primarily due to their costs.[35] Additionally, prescribing criteria in high-income nations also exclude 1 in 5 people

living with CF. [36,37] Taken together, the presence of *P. aeruginosa* and *S. aureus* is expected to continue playing a significant role in the CF lung in the forthcoming years.

As antibiotic resistance continues to rise, treatment of respiratory tract infections is becoming increasingly difficult.[112] As such, there has been growing interest in using bacteriophages, viruses that replicate within bacteria and lead to selective bacterial cell death. Bacteriophages have been used widely in Eastern Europe for decades and have gained popularity in the Western scientific community in recent years.[39] However, there is still much to learn about the safety, delivery, and effects of bacteriophages, particularly in CF lungs, which differ from healthy lungs, notably having impaired mucociliary clearance.[113]

This systematic review seeks to consolidate results from animal and human studies, focusing on *P. aeruginosa* and *S. aureus* as the two most commonly isolated lower airway bacterial organisms in CF, and examining the effectiveness of utilising bacteriophages for treatment. Ultimately, this review aims to provide a comprehensive assessment of the potential for bacteriophages as a novel therapeutic approach in treating respiratory infections in people with CF, which can help guide future research efforts and inform clinical practice in the management of CF-associated infections.[114]

Methods

Protocol and Registration

The protocol for our systematic review was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO registration: CRD42020207622, 4th October 2020), and we adhered to the protocol without deviation. Our findings were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting checklist.

Eligibility criteria

This systematic review includes all original, journal articles published from 1942 to March 2023 in English, excluding conference abstracts. The research question was formulated based on the Population, Intervention, Comparison, and Outcome (PICO) framework, with a focus on patients diagnosed with CF and infected with *P. aeruginosa* or *S. aureus* within their respiratory tract. The intervention studied includes the administration of bacteriophages through intravenous, nebulized, inhaled, or intranasal routes, with no comparators required. Both *in vitro* and *in vivo* studies are eligible for inclusion. Only animal *in vivo* studies that involve the intervention performed on a CF transmembrane conductance regulator (CFTR) animal model have been included.

Primary outcomes include the eradication or suppression of these organisms within the sputum sample of the patient or CFTR animal model, and/or the loss of biofilm in *P. aeruginosa*. Secondary outcome measures include clinical improvements, such as a reduction in the number of

pulmonary exacerbations or admissions, length of hospitalizations, improvement of lung function, or patient-reported outcomes. Additional outcomes include the development of resistance towards bacteriophages or changes in the resistance profile of the organisms towards antibiotics. Side effects such as local reactions and inflammatory responses were also included.

Information sources and search

The literature search was conducted in July 2022, using the Excerpta Medica Database (EMBASE), Medical Literature Analysis and Retrieval System (MEDLINE), Scopus, and Cochrane Central Register of Controlled Trials (CENTRAL). Key search terms included phage (or bacteriophage), cystic fibrosis, *P. aeruginosa, S. aureus*, therapy, treatment, and/or application. A detailed database search criterion, including the search string, is presented in the supplement. All eligible articles were checked for references to identify any papers that may have been missed in the original searches. The searches were re-run just before the final analysis in March 2023, and additional studies that met the inclusion criteria were retrieved and included.

Study selection

The titles and/or abstracts of the retrieved studies were independently screened by two authors (JS and EY) to identify the studies that potentially met the inclusion and exclusion criteria outlined. The screening process included the removal of duplicates using reference management

software (Endnote X9™, Clarivate, Philadelphia, PA) followed by a manual review of potential remaining duplicates. The full text of potentially eligible studies was retrieved and assessed by two review members, with a third reviewer (HS) requested to resolve any disagreements regarding the eligibility of studies.

Data collection

The two authors (JS and EY) extracted data independently using a data extraction form based on the Cochrane Effective Practice and Organisation of Care Group data collection checklist (EPOC).

The following characteristics were extracted from each study: Administrative details such as author(s), year of publication, and journal published in; details of the study such as study setting, design, type and duration; details of the participants such as the number of participants or sample, sputum sample, bacterial sample, age of the patient, the sensitivity of organism, presence of biofilm; details of the intervention such as modality of administration, dosage, single strain bacteriophage or cocktail preparations, frequency and duration of administration. In studies with a comparator intervention arm (i.e., antibiotics), the type of antibiotics or treatment, duration, and mode of administration was recorded. Details of the outcomes are as mentioned above in the type of outcome measures. For case reports, the Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Case Reports was applied. [115] Risk of bias was not applied to the case reports. For the animal studies, the risk of

bias for each of the studies was determined using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE).[116] Any disagreement was resolved by consensus. The results are presented in the supplement.

Synthesis of Results and Analysis

The studies that met the inclusion criteria were summarised (Table 4). A narrative synthesis of the findings is provided based on the subject characteristics, the intervention and bacteriophage treatment (BT) regimen used, outcomes and adverse events.

Results

The database search yielded 318 records. After removing duplicates, 244 records were screened, resulting in 86 records being retrieved. Reports were excluded because they were conference abstracts, did not involve the specified route of administration for the intervention, or were conducted on non-CFTR animal models. Following the assessment of eligibility, 5 studies were included in the final analysis. (Figure 7)

Study and study subject characteristics

There were 23 subjects included in this systematic review, comprising 3 human subjects, 15 zebrafish with CFTR morpholinos, and 5 CFTR knockout mice. Of the 3 human subjects, one was in the paediatric age group while the other two were adults with CF. The interventions included oral, nebulized, and injection routes, and all bacteriophages used were

cocktails containing a combination of lytic phages. The human participants were given treatment based on compassionate access.

Amongst the animal groups, CFTR-deficient zebrafish and mice were used. The zebrafish were treated when the embryo was at the 26 hours post-fertilization (hpf) stage, during which blood circulation commences, allowing for systemic infection of Pseudomonas. Both animal groups were infected by PAO1-GFP, which is a widely used reference strain in research (National Centre for Biotechnology Information, Taxonomy ID: 1418248)

Type of bacteriophage used in treatment

In the human studies, all the interventions involved the use of cocktails of bacteriophages targeting *P. aeruginosa*. These cocktails included the widely used Pyo bacteriophage cocktail from Eastern Europe, produced by the Eliava Institute of Bacteriophages, Microbiology and Virology.[117,118] Pyo bacteriophage is a phage preparation that is effective against multiple organisms such as *Escherichia coli, Proteus mirabilis, Proteus vulgaris, P. aeruginosa, S. aureus, Streptococcus pyogenes*, and Streptococcus mitis. Another cocktail used was AB-PA01, which contains four lytic phages produced by AmpliPhi Biosciences Corporation (AMP).[119] AB-PA01 is active against over 80% of clinical *P. aeruginosa* isolates.[120] Only one study used a bacteriophage against *S. aureus*. This bacteriophage (Sb-1 phage, Eliava Institute of Bacteriophage, Microbiology and Virology) is a strictly virulent phage that has demonstrated virulence towards S. aureus, including antibiotic-resistant strains.[118] Another preparation used was the

Intesti bacteriophage, also a proprietary preparation by Eliava Institute, which was found to be effective against *P. aeruginosa* as well as intestinal-specific bacteria.[117]

A similar approach was seen in animal studies, in which cocktails of bacteriophages were used. Cafora *et al*. selected four lytic bacteriophages from a library of phages, while Aggarwal *et al* designed a cocktail of three to five lytic bacteriophages that were engineered into bacteriophage-loaded microparticles (MP).[121,122]

Dosing and duration of treatment

In the human studies, 8 mL of phage solution was administered orally with 2 mL nebulised daily for 20 days, followed by one year of daily nebulisation and three-monthly repeats of the oral regime.[117] Law *et al*. administered 5 mL phage solution intravenously every 6 hours over 8 weeks.[119] Kvachadze *et al*. administered bacteriophage solution (Pyo bacteriophage) nine times via nebuliser at intervals of 4 to 6 weeks, while Sb-01 (against *S. aureus*) was administered via nebulisation five times. For the animal studies, Cafora *et al* prepared a $5x10^8$ cocktail that was injected once into the duct of Cuvier, while Aggarwal *et al.* aerosolized their MP cocktail via endotracheal intubation once. (Table 4)

Primary outcomes

Microbiological outcomes

In the human studies, only one out of three subjects showed complete eradication of *S. aureus* and *P. aeruginosa* after BT. The bacterial load decreased from $1x10^2$ to $1x10^3$ CFU/mL over successive samples, resulting in eradication at 3 and 12 months for the respective organisms after completing BT.[118] One subject showed a decline in *P. aeruginosa* load by $1x10^1$ to $1x10^2$ CFU/mL.[117]

The resistance profile of two human subjects was presented. One subject showed a change in the multidrug resistance status of *P. aeruginosa* from demonstrating sensitivity only to colistin to demonstrating sensitivity to other antibiotics, such as ciprofloxacin, doripenem, piperacillin and tazobactam following BT.[119]

In the animal study, none of the 20 CFTR-deficient models showed complete eradication of *P. aeruginosa*. The mouse models showed a decline between 10^1 to 10^3 CFU/mL in *P. aeruginosa* bacterial load, while the zebrafish models demonstrated a 20% decline in bacterial load after BT. Additionally, a loss of biofilm was observed in the CFKO mice models.

Clinical outcomes

Out of the three human subjects, two were reported to have a reduction in pulmonary exacerbations. One subject did not have any exacerbations following 100 days post-completion of BT [120] while another demonstrated a decline in the need for antibiotics following BT, only requiring oral antibiotics once over a period of two years.[117]

One subject showed a decline in oxygen requirement within 8 weeks of treatment both in terms of concentration of oxygen and a stepdown of modality (high flow nasal canula to nasal canula). An improvement in white cell count was also observed (decline from 15/mm^3 to 7.7/mm^3). Furthermore, the subject showed an improvement of the acute kidney injury secondary to colistin administration (improved serum creatinine from 2.26 mg/dL to 0.83 mg/dL).[119]

In the animal models, it was found that the death of the *P. aeruginosa* infected zebrafish embryos reduced by a statistically significant 50% following the administration of BT.

Adverse events outcomes

Resistance of pseudomonas against BT was observed in 2 out of 3 human subjects. One subject demonstrated only a singular episode of resistance towards the selected phage during BT while another subject required a change from a cocktail-based BT to a bespoke based BT which resulted in the initial cocktail to regain the ability to cause bacterial lysis from resistant to intermediate resistance of cocktail-based BT following a bespoke BT). [117]

There were no complications towards BT reported in 2 out of 3 human subjects. One subject had normal liver function tests, complete blood count, electrolytes and did not display any clinical adverse event. The parameters of the clinical events measured were unclear.[120] Similarly,

Kvachadze *et al* reported no adverse events but the parameters were unclear.

In the animal subjects, inflammatory changes were observed in both studies. The mice model demonstrated a reduction of immune cell infiltration in infected mice undergoing BT. In the zebrafish models, a reduction in TNF alpha and IL 1 was observed in infected zebrafish embryos undergoing BT.

Discussion

Multidrug resistance to infectious agents is a significant healthcare crisis, with estimates predicting that 10 million people will be affected annually by 2050.[123] In response, research has explored alternatives or adjuvants to antibiotics, including bacteriophages. These highly bacterial specific viruses have gained significant interest in recent years, with an exponential increase in the number of in-vitro studies examining their characteristics and effectiveness against bacteria.

Our review found an increased number of publications on the use of bacteriophages in CF, particularly between 2020 and 2023. These studies provide valuable insight into the qualities of bacteriophages, testing and production methods. However, studies involving human participants remain limited, especially in the realms of cystic fibrosis, where MDR infections in the respiratory tract are a major concern.

In this systematic review, we evaluated the safety and efficacy of bacteriophages for the treatment of *P. aeruginosa* and *S. aureus* in CF. Our analysis of five studies provided important early information on the promise of this treatment. We included three case reports of this treatment in CF that showed demonstrable improvements with a reduction or eradication of bacteria, a reduction in exacerbations, and clinical improvement in terms of hospitalizations or medical support required. Although only one study evaluated *S. aureus* in CF, the results were encouraging. More importantly, none demonstrated any adverse outcomes following BT. As these cases were exclusively derived from patients requiring compassionate access to treatment, the results proved efficacious for these individuals. Furthermore, they underscore the importance of performing larger, controlled studies outside the realms of compassionate access.

Animal model studies involving CFTR deficient models have also demonstrated similar results. In these simulated CF models, studies have demonstrated positive outcomes, particularly in reducing bacterial load and inflammation. This provides further background evidence of the potential utility of this treatment in people with CF infected with *S. aureus* and *P. aeruginosa* in their airways.

In recent years, there has been renewed hope due to the introduction of CFTR modulators and correctors and their potential impact on improving life expectancy, lung function, growth, and reducing pulmonary

exacerbations. Consequently, there is a potential reduction in chronic lung infections such as *P. aeruginosa.*[124-126] With such significant advancements in the CF world, it is not surprising to anticipate a decrease in the need for BT in CF patients over time. However, in paediatric respiratory medicine, a substantial group of children with non-CF bronchiectasis,[127] those on long-term ventilation, and/or individuals who have undergone tracheostomy [128] will continue to require treatment options for managing these infections. Therefore, we believe that while CF patients will be the primary beneficiaries of BT, the implications and utility of clinical research within this patient cohort will extend beyond CF in terms of its reach.

Although our systematic review provides insights into current research on bacteriophages in cystic fibrosis, there are some limitations that should be considered. One limitation is that of publication bias, as we excluded unpublished studies, such as those presented with published abstracts at conferences. Our focus on English language papers also introduces bias, as eastern European countries have been using bacteriophages for almost 100 years. Thirdly, there is heterogeneity in these studies in terms of type of study, dosing, duration, and subjects that limit the generalizability of our findings. Finally, given the lack of randomization and the small size of these studies and differing outcomes and reporting measures, it is difficult to compare and synthesize results. Despite these limitations, our systematic review provides a thorough summary of the limited current

evidence regarding the safety and efficacy of bacteriophages for the treatment of staph or pseudomonal infection in CF.

In conclusion, this systematic review has shown that early evidence suggests that bacteriophage treatment has potential in the treatment of pseudomonas and staphylococcus aureus in CF with a promising safety profile. Given the data generated in the pre-clinical space in studying the efficacy of BT, the ability to move forward towards human trials and standardise the approach (dosing, duration, route and use of singular or combination of phages) to using this treatment in CF is on the horizon. Multiple studies are currently underway and are paving the way towards more information on the safety and efficacy of this treatment, this includes and is not limited to the Cystic Fibrosis Bacteriophage Study at Yale (CYPHY, NCT04684641) and the Children's Hospital at Westmead Phage Therapeutics and Application in CF (CHIP-CF) study.[129] This will provide the necessary body of evidence to help establish legislative frameworks globally.

AUTHOR	SUBJECT	BACTERIA	ROUTE AND DOSAGE	FREQUENCY OF ADMINISTRA	SUMMARY DATA	CONCLUSION BY AUTHORS
L.KVACHADZE 2011[118] CASE REPORT	7-year-old Female Outpatient	*S. aureus* *P. aeruginosa*	Nebulised Cocktail Pyo-bacteriophage 1×10^5 cfu/mL, Sb-1 1×10^7 pfu/mL	Pyo phage once every 4-6 weeks. Sb-1 added from doses 5 and above (36 to 54 weeks)	The concentration of *P. aeruginosa* decreased from 8×10^6 cfu/mL to 1×10^6 cfu/mL following treatment. While the concentration of *S. aureus* reduced from 1x107 cfu/mL to 1×10^3 cfu/mL. Both bacteria were no longer detected after 2 and 12 months for *S. aureus* and *P. aeruginosa* respectively.	No adverse events of phage application in a nebuliser and a promising clinical response were observed in this patient. Placebo-controlled double-blinded clinical trials are now warranted to test the value of Sb-1 phage against *S. aureus* infections in defined clinical situations.

26-year-old Female Inpatient	*P. aeruginosa* (non-mucoid and mucoid)	IV cocktail of 4 lytic phages AB-PA01 $4x10^9$ pfu/mL in 5 mL	6 hourly (8 weeks)	This patient had severe pneumonia as a result of multidrug-resistant *P. aeruginosa* and was successfully treated with bacteriophage therapy, along with antibiotics. The patient's pneumonia resolved, and no adverse events related to the therapy were reported. The patient's isolates remained mostly sensitive throughout the treatment. The patient did not have a recurrence of pneumonia or cystic fibrosis exacerbation within 100 days following the end of the therapy, and underwent successful lung transplantation 9 months later.	Safe and efficacious use of intravenous bacteriophage therapy in a CF patient awaiting a lung transplant was observed. Given the concern for MDR *P. aeruginosa* in CF patients, BT may offer a viable adjunct to antibiotic therapy in CF patients and further study in clinical trials is urgently needed.

E. ZALDASTANISHVILI 2021[117] CASE REPORT	43-year-old Male inpatient	*P. aeruginosa*	Oral and nebulized cocktail (pyo and intesti bacteriophage) 8 mL oral 2 mL nebulised	Daily and 3 monthly for 20 days) (3 years)	The patient received Pyo and Intesti bacteriophage inhalations, followed by a custom phage tailored for his specific strain as resistance emerged. After completing four courses of custom phages, the patient's P. aeruginosa culture responded to Pyo and Intesti bacteriophages again. The patient only resorted to antibiotics once due to a viral infection and a shortage of phages.	The bacterial strains before and after BT were indistinguishable according to their genetic profile but differed in their phage susceptibility properties. The authors raised the question of whether phage therapy should be tailored to the bacterial composition, diversity, and adaptation potential of individual patients.

5 CFKO	*P. aeruginosa*	Nebulised cocktail of 4 microparticle-engineered (MP) bacteriophages 2.6×10^6 pfu/mg	once	Phage-MPs treatment resulted in high phage titres and significantly reduced bacterial counts in the lungs of mice infected with *P. aeruginosa.* Compared to free phage treatment, phage-MPs treatment resulted in lower bacterial counts and no detectable bacteria in the liver. Additionally, histological analyses showed low levels of immune cell infiltration in infected mice treated with phage-MPs, indicating reduced infection-associated inflammation. Furthermore, the tested bacterial colonies recovered from phage-MP-treated mice remained sensitive to phage-MPs, indicating no development of phage resistance.	Phage-loaded MPs significantly reduced *P. aeruginosa* infections and associated inflammation in healthy and CF mice and rescued mice from pneumonia-associated death. Importantly, phage-MPs were effective against clinically derived strains, including antibiotic-resistant strains, as well as lung infections caused by two strains of bacteria.

M. CAFORA 2020[15] EXPERIMENTAL ANIMAL PROTOCOL	15 zebrafish CFTR-MOs	*P. aeruginosa*	Intra ductal cocktail of 4 phages 5 µL $5x10^8$ pfu/mL	Twice (30 minutes and 3 hours)	In CF zebrafish embryos infected with *P. aeruginosa* (PAO1), phage therapy reduced the bacterial burden and lethality. Live imaging also showed a reduction in the spread of fluorescent bacteria over the yolk sac. In addition, phage therapy reduced the inflammatory response generated by PAO1 infection in CF embryos. These results suggest that phage therapy could be an effective treatment for infections caused by PAO1 bacteria in CF patients.	The authors acknowledge that zebrafish is not a mammalian model but suggest that since it has a comparable innate immune system to humans, the data on the immune response could be reproducible. Zebrafish is well-suited for bacterial infection studies and can be used as a tester for phage therapy efficacy. Both systemic and localized infections in zebrafish embryos showed that phage therapy decreased both lethality, bacterial burden and inflammation.

Table 4: Summary of studies included. pfu/mL: plaque forming unit per millilitre, BT: bacteriophage therapy, CFKO: cystic fibrosis knock out, CFTR-MOs: cystic fibrosis transmembrane conductance regulator morpholinos.

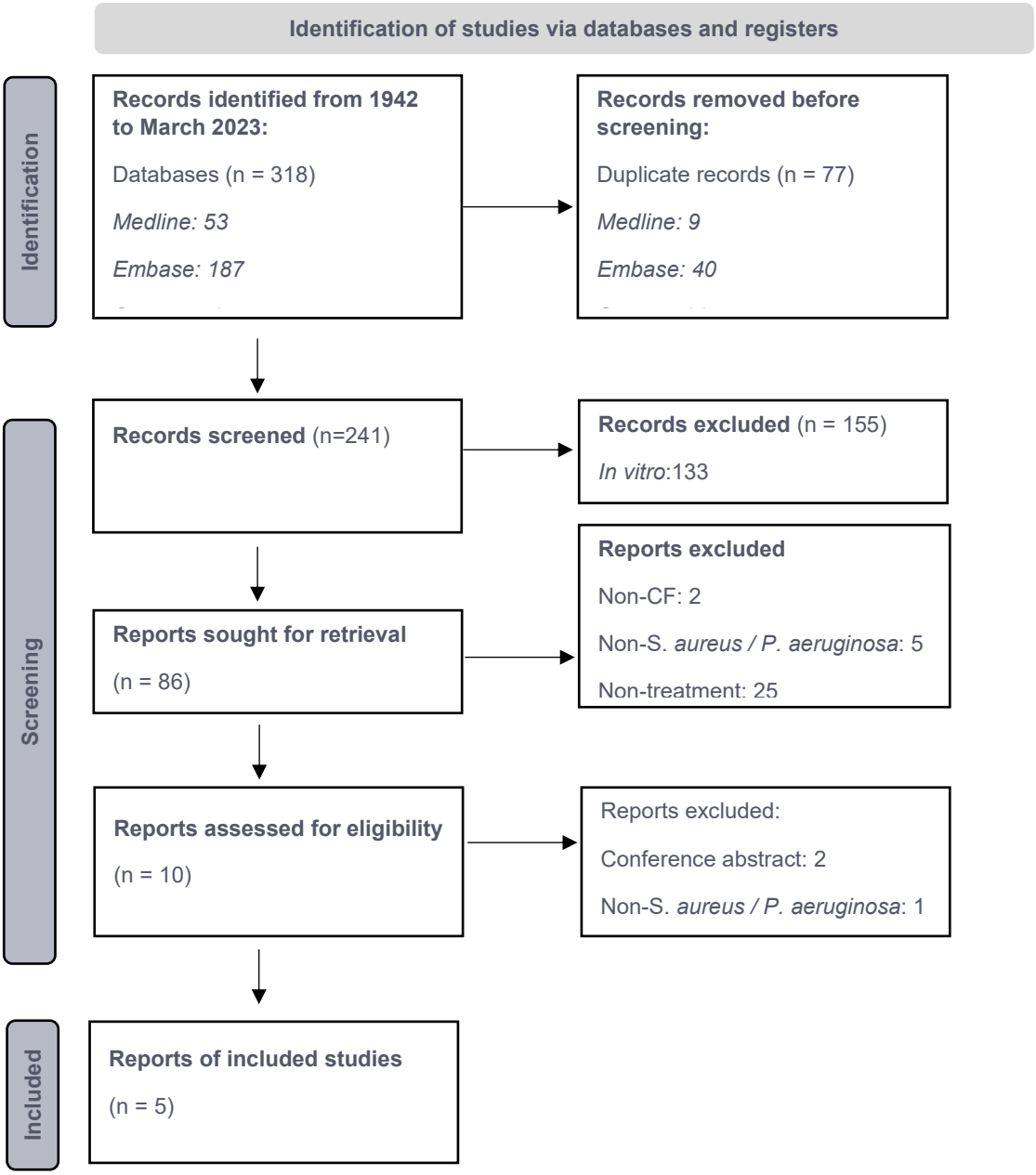

Figure 7: PRISM flow diagram

MEDLINE/EMBASE SEARCH

Phage OR Bacteriophage AND

Cystic fibrosis AND

Pseudomona* OR aeruginosa OR

Staphylococc* OR aureus OR MRSA OR Methicillin-resistant

staphylococcus aureus AND

Therap* OR treat* OR application*

SCOPUS SEARCH

TITLE-ABS-KEY (*phage) AND TITLE-ABS-KEY (bacteriophage*) AND TITLE-ABS-KEY ("cystic fibrosis") AND TITLE-ABS-KEY (pseudomona*) OR TITLE-ABS-KEY (aeruginosa) OR TITLE-ABS-KEY (staphylococc*) OR TITLE-ABS-KEY (aureus) OR TITLE-ABS-KEY (mrsa) AND TITLE-ABS-KEY (therap*) OR TITLE-ABS-KEY (treat*) OR TITLE-ABS-KEY (application*) AND NOT INDEX (medline) AND (LIMIT-TO (LANGUAGE , "English"))

Figure 8: String search

	L.KVACHADZE 2011[118]	***E. ZALDASTANISHVILI 2021[117]***	***N. LAW 2019[119]***
Were patient's demographic characteristics clearly described?	**Yes**	**No**	**No**
Was the patient's history clearly described and presented as a timeline?	**Yes**	**Yes**	**Yes**
Was the current clinical condition of the patient on presentation clearly described?	**Yes**	**No**	**Yes**
Were diagnostic tests or assessment methods and the results clearly described?	**No**	**No**	**Yes**
Was the intervention(s) or treatment procedure(s) clearly described?	**Yes**	**No**	**Yes**
Was the post-intervention clinical condition clearly described?	**Yes**	**Yes**	**Yes**
Were adverse events (harms) or unanticipated events identified and described?	**No**	**Yes**	**Yes**
Does the case report provide takeaway lessons?	**Yes**	**Yes**	**Yes**

Figure 9: Critical appraisal checklist for case reports selected.

		R. Agarwal 2018 [122]	M. Cafora 2020 [130]
Selection bias	*Sequence generation*	+	+
	Baseline characteristics	+	+
	Allocation concealment	-	-
Performance bias	*Random housing*	?	?
	Blinding	?	?
Detection bias	*Random outcome assessment*	-	-
	Blinding	-	-
Attrition bias	*Incomplete outcome data*	+	-
Reporting bias	*Selective outcome reporting*	+	+
Other	*Other sources of bias*	+	+

Figure 10: Risk of Bias in animal studies selected.

STUDY	REASON FOR EXCLUSION
S. ASLAM 2019 [120]	3 case studies were reported but case study involving CF subject was not *P.aeruginosa* or *S.aureus* related.
B. CHAN 2019 [48]	Conference abstract
CL. FURR 2018 [131]	Conference abstract
D. ALEMAYEHU 2012 [132]	Non- CF based animal model
L. DEBARBIEUX 2010 [133]	Non- CF based animal model

Table 5: Studies excluded following eligibility assessment

Chapter 5: Isolating and characterising phages against Pseudomonas aeruginosa and Staphylococcus aureus in children with cystic fibrosis

https://doi.org/10.1016/S1569-1993(20)30334-9

Introduction and objective

Phage therapy to treat chronic lung infections in patients with Cystic Fibrosis (CF) is attractive due to its safety, specificity, natural occurrence and ability to potentially be utilised as a targeted inhaled aerosol therapy. *In vitro* studies have also shown the ability of phages to penetrate bacterial biofilms and thus help eradicate them. This study was designed to evaluate the feasibility of isolating, propagating and purifying phages against bacterial isolates commonly seen in children with CF.

Methods

Nine clinical bacterial isolates of Staphylococcus aureus (n = 6), non-mucoid *Pseudomonas aeruginosa* (n = 2) and mucoid *P. aeruginosa* (n = 1) were obtained from sputa of patients followed up in The Children's Hospital at Westmead CF clinic. Phages for each strain were isolated from cocktails of environmental water samples. The lysates produced were extracted and subjected to phage propagation and purification using the Phage on Tap (PoT) protocol to produce high-titre homogenous phages.

Results

Fourteen morphologically distinct plaques were observed on the respective bacterial culture plates (S. aureus n = 8, P. aeruginosa n = 6). Five phages (S. aureus n = 3 and P. aeruginosa n = 2) were selected and amplified to high titres phages showed highly isolate-specific lytic activity. Both *P. aeruginosa* phages that were isolated exhibited a wider host range against all of the *P. aeruginosa* clinical isolates tested, which included both mucoid and non-mucoid strains. The bactericidal activity was further demonstrated on a time-kill curve.

Conclusion

We were able to successfully isolate and purify phages with lytic activity against both mucoid and non-mucoid *P. aeruginosa* as well as *S. aureus* clinical isolates. This pilot study demonstrates the feasibility of isolating phages that may potentially be useful in the treatment of CF patients with chronic respiratory infections


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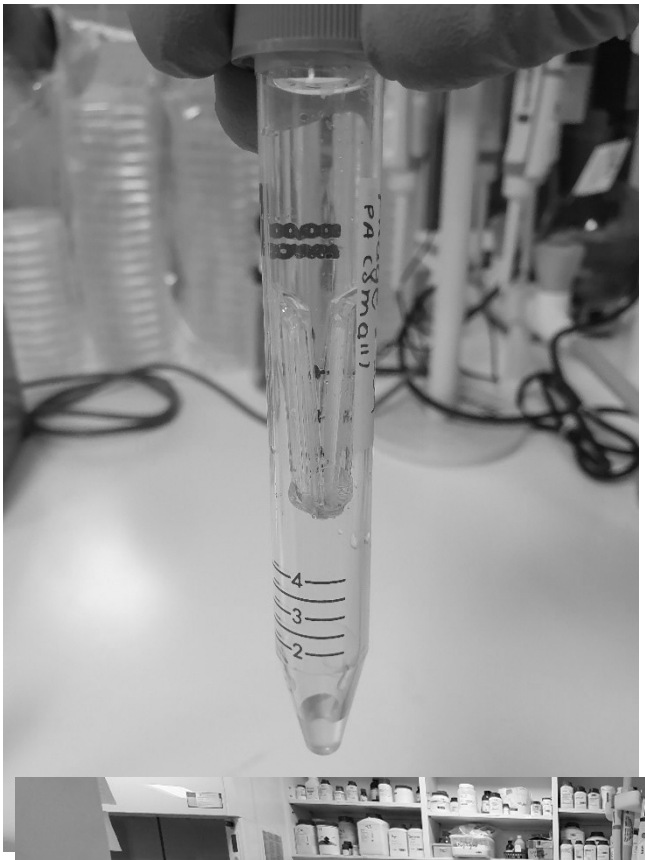
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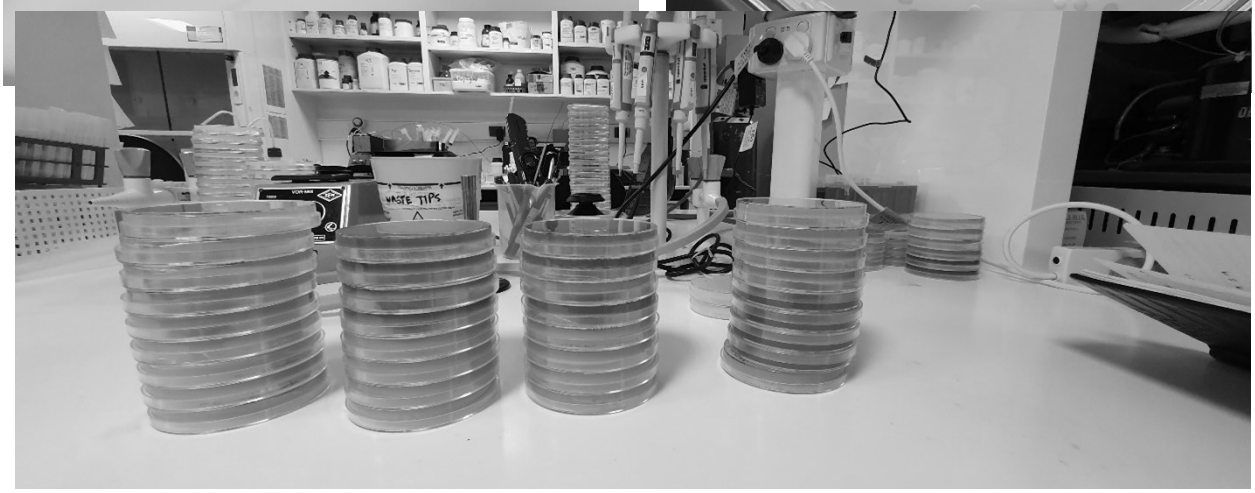
WASTE TIPS

Figure 11: Clockwise from top: Clear circles (plaque) produced on a *Pseudomonas aeruginosa* bacterial lawn by bacteriophage 509 PA L; Clear circles (plaque) produced during spot testing of bacteriophages (labelled L,S and C); Lysis demonstrated by Phage 01 on a *Staphylococcus aureus* bacterial lawn; Sets of bacterial lawn awaiting plaque formation; The final highly purified distillate of PA 509 S with under 5 EU/kg/hour of endotoxin unit (based on the weight of a child of 30kg).

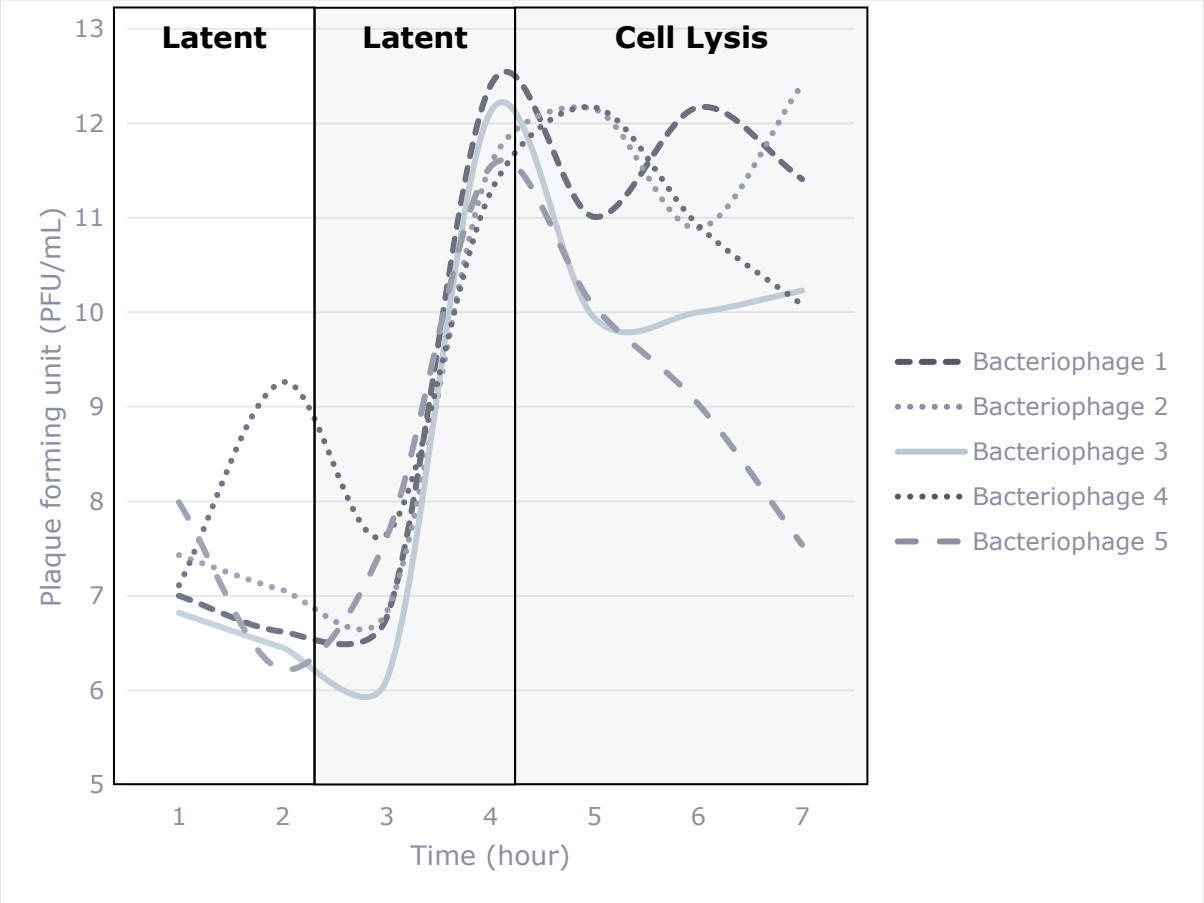

Figure 12: Demonstrating the growth of the bacteriophages in the latent, rise period and bacterial cell lysis phase of the five bacteriophages that were isolated. Bacteriophage 1 to 3 were isolated using *Pseudomonas aeruginosa* isolated while bacteriophage 4 and 5 were isolated using the *Staphylococcus aureus* isolates.

Chapter 6: Viability and infectivity of bacteriophage PBPA103 after nebulisation with a vibrating mesh nebuliser

https://doi.org/10.1164/ajrccm-conference.2024.209.1_MeetingAbstracts.A4125

Introduction

Pseudomonas aeruginosa, a pathogen categorized as a critical drug-resistant organism by the World Health Organization, remains a challenging organism to treat. This has sparked a race to uncover alternatives to antibiotics, with growing interest in bacteriophages. While various methods of administering bacteriophages have been explored, it is hypothesized that localised administration of these viruses can promote bacteriophage replication in the presence of highly concentrated pathogens, thereby enhancing the efficacy of phage therapy. However, nebulising bacteriophage solutions can subject them to mechanical stressors such as vibrations, heat stress, and shearing forces, potentially compromising phage viability and infectivity. The aim of this study was to assess the viability of a bacteriophage strain following nebulisation.

Methodology

PBPA103 was, sourced from Lyse N Tech (Heejoon Myung) South Korea, for personalized bacteriophage therapy for a child with CF with multidrug-resistant Pseudomonas aeruginosa. To aerosolize the solution, the Portable MicroAir® Battery-Operated Nebuliser by Omron Healthcare was

used. Before nebulisation, the bacteriophage solution was titered. The aerosolized solution was collected in a test tube which was connected to the nebuliser's mouthpiece via a closed circuit using an appropriately sized silicone tube. The bacteriophage solution remaining within the nebuliser chamber (retentate), aerosolized liquid collected in the collection chamber (flowthrough) and any leakage from the nebuliser's output valve (effluent) was collected and titered. The effluent was collected directly on agar plates dispersed around the area where the most visible effluent was produced. The trial was repeated twice, resulting in a triplicate experiment.

Results

Four mL of PBPA103 (titered at 4.0 $x10^8$ PFU/mL) was nebulised over five minutes through the nebuliser device. Two mL of flowthrough was collected, 1.5 mL remained as a retentate, and 0.5mL was lost as effluent. Flowthrough was titered as 1.3 $x10^8$ PFU/mL. A total loss of 2.7 $x10^8$ PFU/mL was observed following the nebulisation of PBPA103. Effluent collected resulted in complete clearance of the *P. aeruginosa* from the agar plates

Conclusion

PBPA103 maintains adequate titres and infectivity following nebulisation through a vibrating mesh nebuliser using this simple experimental method.

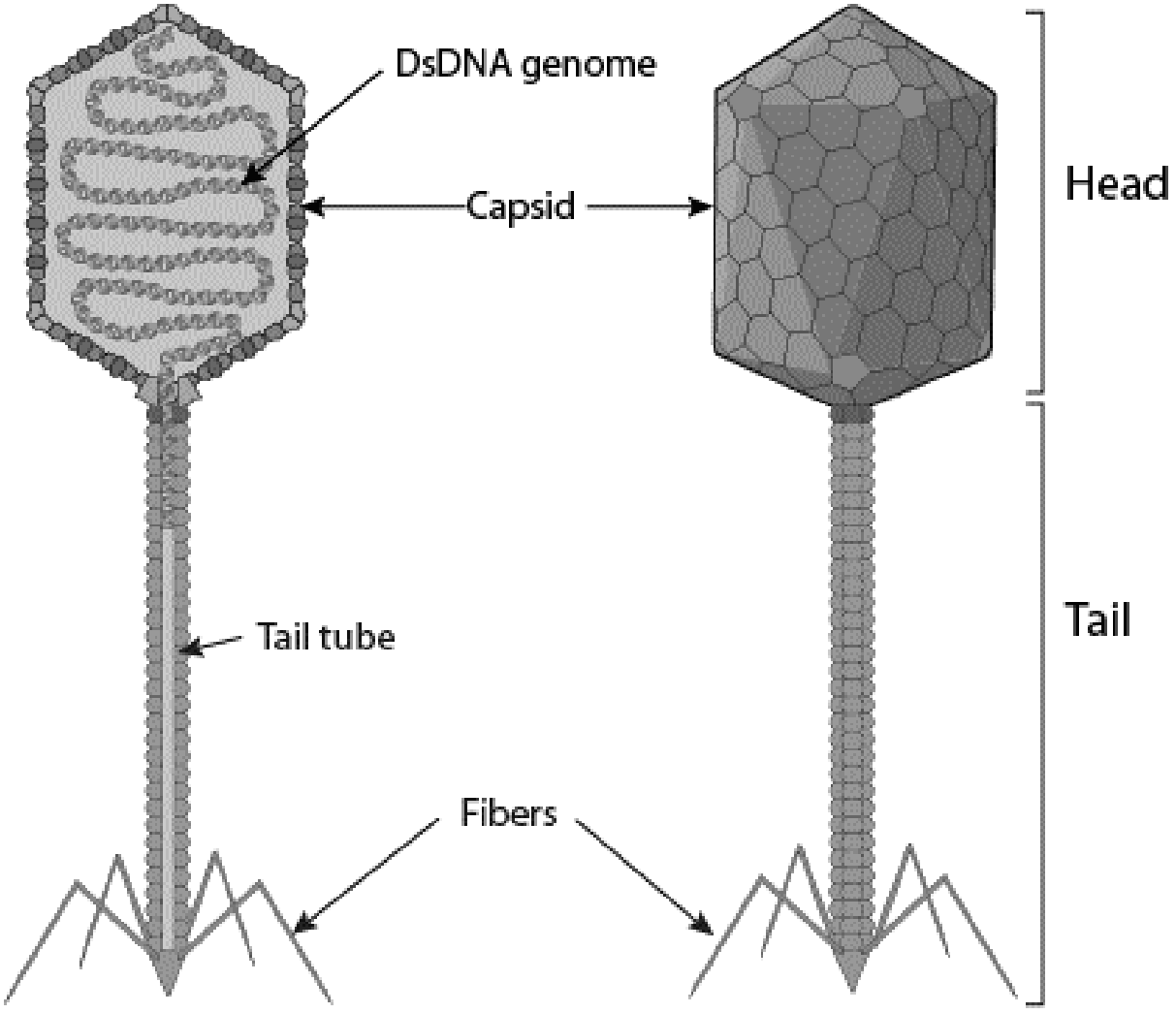

Figure 13: Structure of a PBPA103 (similar to *Pseudomonas* bacteriophage M6), under the genus of Yuavirus. *Source: Swiss Institute of Bioinformatics (ViralZone 2014*

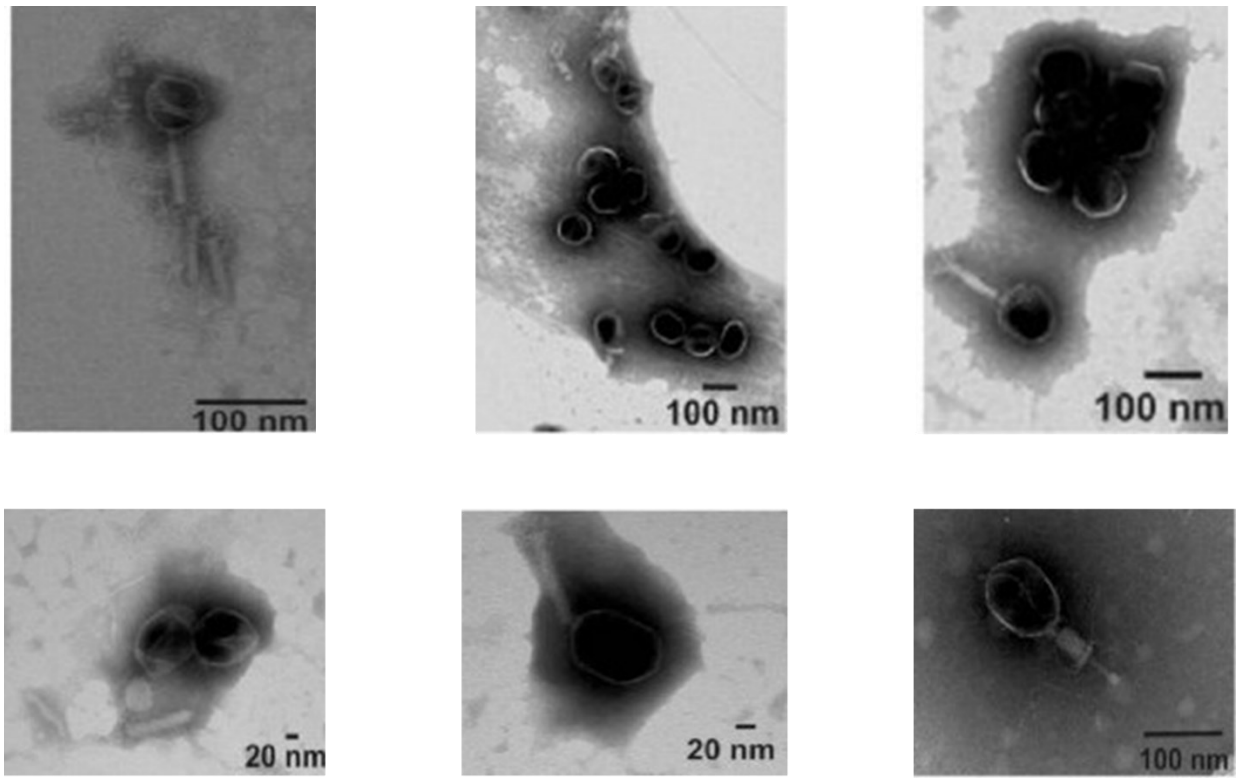

Figure 14: Transmission electron microscopy demonstrating the damage that can occur during aerosolization of bacteriophages. The bacteriophage shown here is PP01; (A) Aerosol containing one damaged phage with other body parts; (B) aerosol containing 13 phages' head; (C) aerosol containing 6 phages head and 1 full phage; (D) aerosol containing 2 damaged phages with other body parts; (E) aerosol that contains one full bacteriophage; and (F) biosampler control of an undamaged bacteriophage. *Adapted from: N. Groulx, et al 2016, Aerosol Science and Technolog*

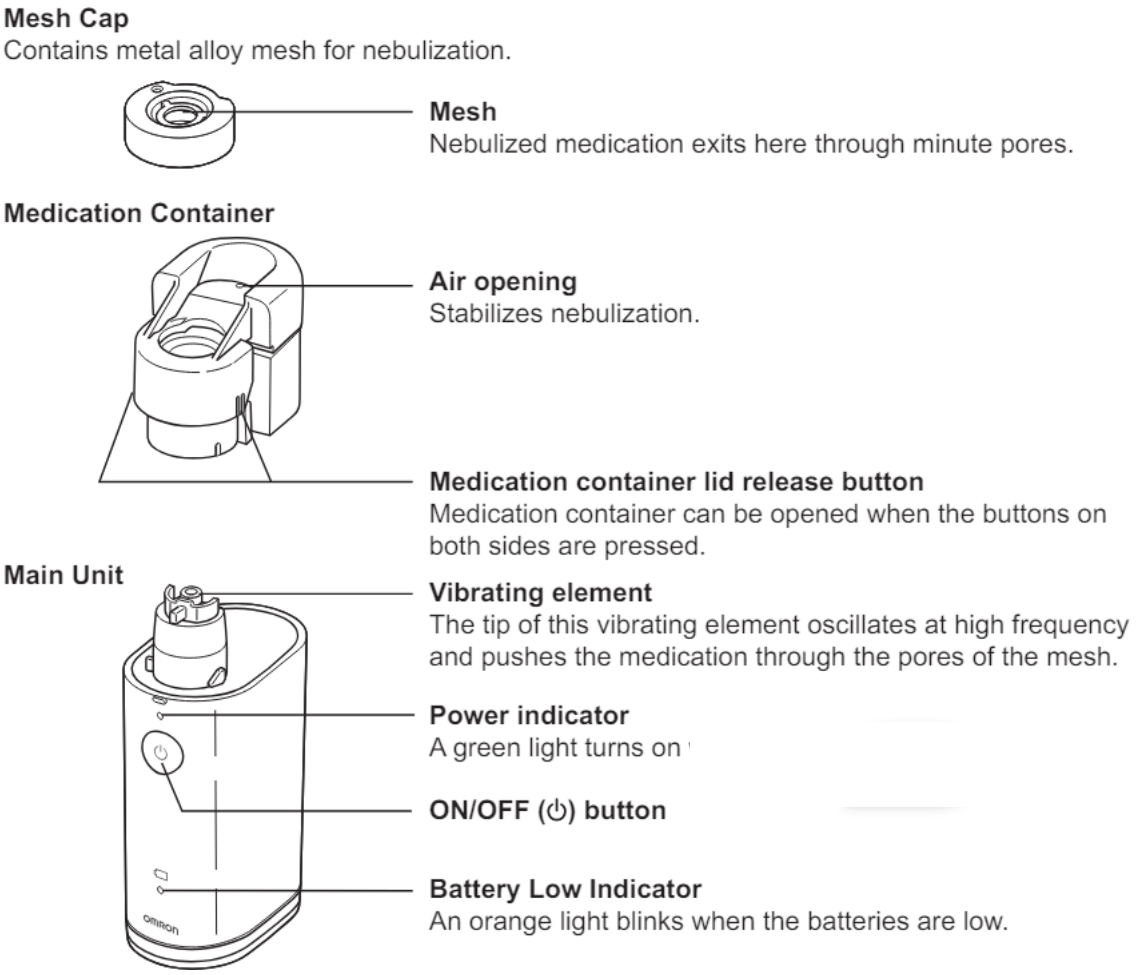

Figure 15: The nebuliser used during the experiment and for therapy. Vibrating mesh nebulisers use mesh deformation or vibration to push the liquid through the mesh. *Source: Product Manual, Omron Healthcare*

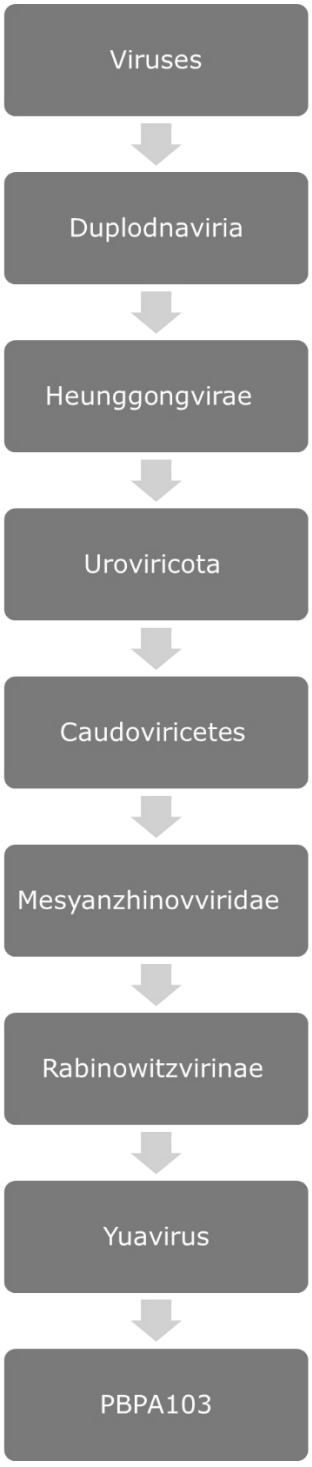

Figure 16: The NCBI taxonomy of PBPA103 (based on the 2022 taxonomy update of the ICTV bacterial viruses subcommittee) which closely resembles *Pseudomonas* bacteriophage M6, sourced from Lyse N Tech in South Korea, database: http://www.phagebank.or.kr/

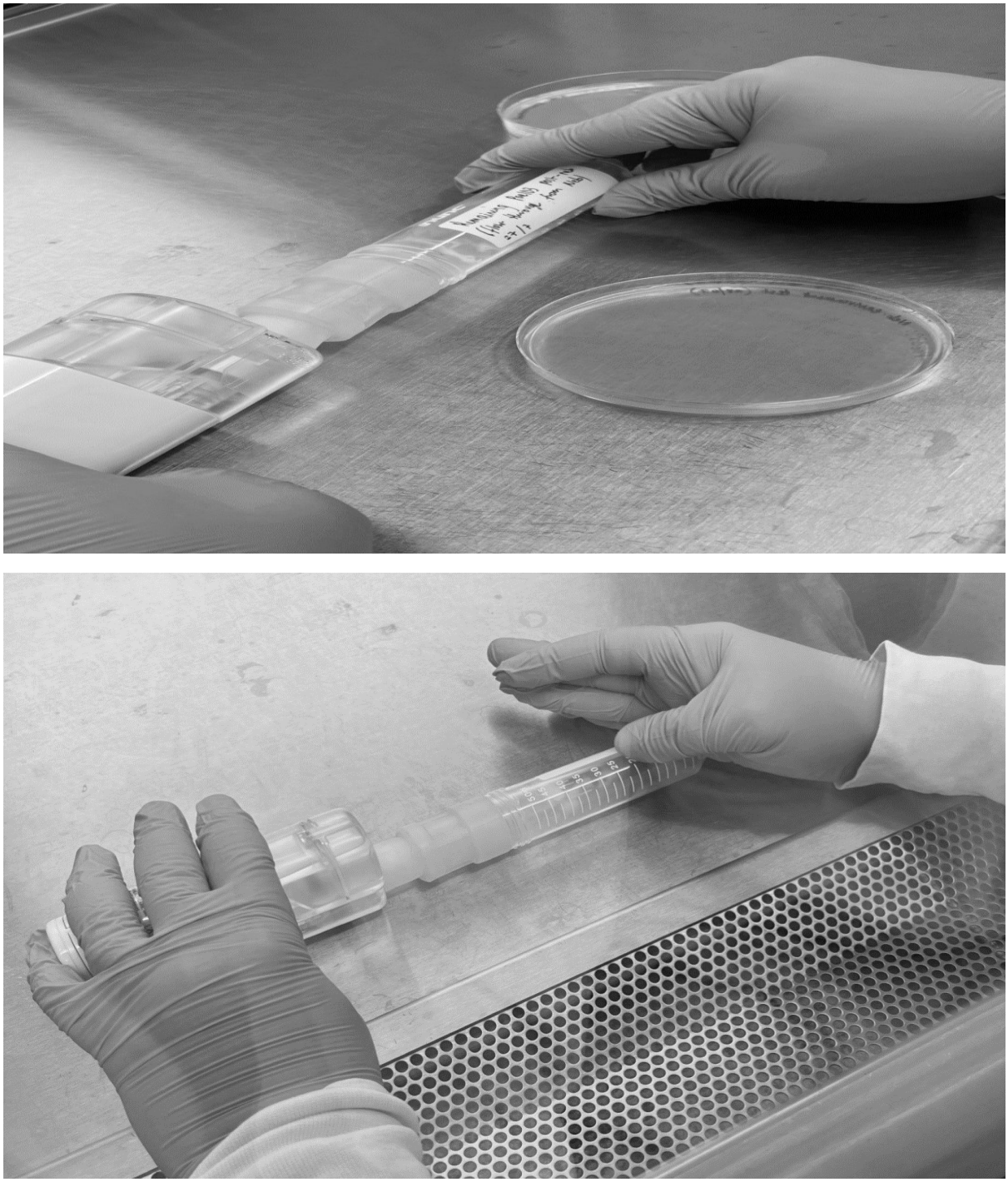

Figure 17: The bacteriophage undergoing aerosolization using a mesh nebuliser in a biocontainment cabinet. Flow through is collected with the test tube connected to the mouthpiece using a silicon tube. Bacterial lawn

plates were exposed to the aerosolized effluent and showed complete clearance of the bacteria.

Chapter 7: Single-Arm, Open-Labelled, Safety and Tolerability of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with Cystic Fibrosis and *Pseudomonas aeruginosa.*

https://doi.org/10.1136/bmjresp-2022-001360

Introduction

Cystic fibrosis (CF) is a life-limiting genetic condition of the lungs, exocrine organs and gastrointestinal tract that occurs in 1 in 2,500 births. This condition affects mucous, sweat and digestive enzyme production, and is characterised by recurrent pulmonary infections, each of which requires aggressive antibiotic treatment. One of the most common organisms that affect children with CF is *Pseudomonas aeruginosa* which may be acquired as early as infancy. An estimated 25% of children with CF will be infected by this organism which increases their morbidity and mortality.[83,134] A child with CF infected with *P. aeruginosa* within their airway has 2.6 times higher risk of death within 8 years when compared to a child with CF who has not been infected with the organism. These children also have a lower baseline weight and a higher number of CF-related hospitalisations.[83,135,136] Over time, 92% of children found to have *P. aeruginosa* within their airways develop resistance by 16 years of age. This further decreases traditionally available treatment options and increases the risk of morbidity and mortality.[134]

Treating respiratory tract infections caused by *P.aeruginosa* with prolonged and repeated antibiotic therapies have significant side effects including sensorineural hearing loss, hypersensitivity and impaired renal function.[137-139] Additionally, there is the added risk of a change in the airway microbiota allowing for opportunistic infections and a rise in resistant organisms.[140,141] An adjunct or alternative to antibiotics that may reduce treatment failure and medication side effects, is highly desirable

Bacteriophage (phage) therapy may offer a solution to this problem. Phages are viruses that replicate within bacteria, causing highly selective bacterial cell death and reducing the impact on the healthy microbiome when compared to antibiotic therapy. In the face of increasing antimicrobial resistance and dwindling prospects for the discovery of new antibiotics, there has been a resurgence of interest in phage therapy.[142,143] However, such research has generally excluded children who potentially stand to gain the most from this treatment, if it is shown to be safe and effective.[144]

Existing knowledge and evidence that influenced the design of this study

A summary of the existing evidence, which has informed the design of this study, is as follows:

Phages have been utilised in multiple n=1 studies in different countries.[145-153] Phages have low natural toxicity as they target specific bacteria which are largely strain-specific without disrupting the host's normal flora or human cells.[154] Although not persistently observed, phages have been shown to reverse antibiotic resistance and restore susceptibility to various classes of antibiotics in *P.aeruginosa.*[155] Phages have been used intravenously in the treatment of children with CF with no local infusion site reactions, anaphylaxis, seizures, abnormal vital signs or gastrointestinal disturbances.[156] Phages have been used in children in multiple situations with the youngest child being two years of age, treated with intravenous bacteriophage against *P. aeruginosa*.[50] Phages are a part of the human microbiome and the environment.[157,158] Lytic phages are specific to a particular bacterial strain while some are known to affect multiple strains of the bacteria.[159-161] The microbiota has been shown to be less affected by phages when compared to antibiotics;[162]this has been shown in both murine and human studies (adults and children).[163,164] A trial performed in children with diarrheal disease demonstrated that phages pass the alimentary tract without any change in the microbiome.[165] Phages when inhaled are the least systemically absorbed compared to other methods of delivery.[166,167] Although the anatomy of the human airway with its proximity to the alimentary tract may suggest contamination of the alimentary tract, studies performed on humans have shown that the contamination from tracheal instillation to the alimentary

tract is negligible.[168] Phages are able to stimulate an adaptive immune response. Dose,[52] duration,[51-53] and route of administration may influence this with topical, enteral and inhaled routes of administration considered less immunogenic compared to intravenous and intraperitoneal administrations.[169] As for the innate immune response, studies have shown that purified phages (as opposed to raw phage lysates) do not expose pathogen-associated molecular patterns [PAMPs] effectively and therefore are not able to induce an inflammatory response.[54,55,170] Phages are used in the food industry to reduce the bacterial load in food used for human consumption.[171,172] Phages that are non-genetically modified organisms are used as a surrogate for viruses in aerosolization studies to evaluate the efficacy of viral filters.[56]

Based on a review of 29 *in-vivo* animal model studies, no significant side effects were observed despite differing doses, routes of administration and infection models.[173] Phages have shown some potential to remain intact following nebulisation in a murine model.[59,144] Optimum dosing remains unclear, however, based on previous pre-clinical studies that include *in-vitro* and animal models, the PFU/mL to achieve treatment effect is commonly between 10^6-10^{10} PFU/mL.[147,174-176]

A review of human trials and the use of phage in children via respiratory routes are described in Tables 6 and 7.

Our study hypothesises that phage therapy is a safe and well-tolerated adjunct therapy in paediatric patients with CF with *P. aeruginosa* infection

in their lower airways. Our research questions are 1) Are endo-bronchially instilled and nebulised phages safe, easily administered and well-tolerated? 2) Are phages which are delivered directly into the bronchial tree via bronchoscopy followed by nebulised bacteriophage therapy effective in reducing the load of *P. aeruginosa* within the sputum?

The primary objective of this study is to demonstrate proof of the principle of tolerability and safety of endo-bronchially instilled and nebulised bacteriophages against *P. aeruginosa* in children with CF. As a secondary objective, we will measure the bacterial load of *P. aeruginosa* before and after treatment with phage.

Methods and analysis

Design

This trial is designed as a small, pilot, single-arm, open-labelled, non-randomized, safety, and tolerability study with a primary endpoint of tolerance towards endo-bronchially instilled initial phage dose followed by maintenance treatment via nebulisation. This study is designed to provide pilot data pertaining to safety and tolerability in children and thus allow for larger, dosing or placebo trial studies that will fulfil Australian legislation. This study has undergone scientific review and has obtained approval from the Sydney Children's Hospital Network Ethics committee (ethics approval number 2022/ETH00241, version 3). This trial has been registered on the Australia and New Zealand Clinical trial registry

(ANZCTR) (trial registration number ACTRN12622000767707, registered on 22 May 2022,). Amendments will be notified to the approving ethics committee and is subject to review and approval. Approved amendments will be updated with the clinical trials registry. The trial result will be disseminated through publication (s) obtained from the data of this clinical trial. Cleaned data and complete protocol may be submitted for review to assist with publication. Authorship will be based on the Australian Code for the Responsible Conduct of Research.

Patient and Public Involvement

None.

Study population

Inclusion criteria

1. Adolescents (≥12 years and <18 years of age) with CF
2. Positive sputum or bronchoalveolar lavage culture (*P. aeruginosa*) in more than 50% of sputum samples over the past year.[177]
3. Continues to shed *P. aeruginosa* in sputum despite undergoing eradication therapy using two anti-pseudomonal antibiotics and/or is currently on suppressive nebulised antibiotic treatment
4. The latest clinical isolates of *P. aeruginosa* taken within 3 months of enrolment are susceptible to (demonstrates lytic activity) available anti- *P. aeruginosa* phages.

5. Ability to perform reliable spirometry.[90]

Exclusion criteria

1. Children who have received more than 1mg/kg of prednisolone continuously for more than 7 days before study enrolment or have a diagnosed immunosuppressive condition.
2. Children that require ≥18 hours/day of non-invasive ventilatory (NIV) support during admission.
3. A current diagnosis of allergic bronchopulmonary aspergillosis (ABPA).
4. History of haemoptysis in the past 12 months before study enrolment.
5. Prior known inability to expectorate sputum.
6. Has undergone solid organ transplantation
7. Positive sputum isolation of *Burkholderia cepacia* or non-tuberculous *mycobacterium* within the past 1 year.
8. A positive COVID-19 PCR nasal swab with a confirmed COVID-19 infection (true positive) in the past 6 months before enrolment.[178]
9. Within 3 months of having received a booster COVID-19 vaccine or within 6 months of receiving the first dose of vaccine.[179]

Study site

This study will be performed at the Children's Hospital at Westmead and The Sydney Children's Hospital, Sydney, Australia (under the Sydney Children's Hospital Network). The CF multidisciplinary team currently treats 400 children with CF. The study will be conducted initially in an in-

patient setting for a duration of 7 inpatient days of bacteriophage treatment in addition to standard CF treatment (IV antibiotics, physiotherapy to be completed inpatient for 10 to 14 days). Clinical reviews will be performed at 3-, 6-, 9- and 12 months following phage treatment.

Recruitment

The estimated sample size in this study is up to 10 participants. Recruitment into this study will be extended to all children with CF under the care of the Children's Hospital at Westmead and Sydney Children's Hospital, Randwick (SCHN) who fulfil the criteria for this study. To ensure adequate power to detect any adverse events, we calculated the sample size based on practical considerations. We assumed an expected probability of adverse events of 30%, a desired precision of the estimate of 0.2, and a statistical significance level of 0.05, with a power level of 80%.

Potential study participants who meet the inclusion and exclusion criteria will be selected by the study investigators. These potential participants will be discussed for suitability in the weekly multidisciplinary CF meetings. This is to ensure that clinicians (respiratory physicians, CF clinical nurses, physiotherapists and dieticians) involved in the care of the participant will be able to provide input about the suitability of the potential participant in the trial. If the participant is deemed suitable based on the criteria, the study investigator will arrange for a discussion

with the participant and their guardian. Consent for enrolment into this trial will be obtained by the principal investigator to ensure a standardised approach. A parent information sheet will be provided to the family. A young person and child information sheet has also been provided in age-appropriate language. The consent will include consent for ancillary studies as well.
Copy of the signed page of the consent form will be uploaded into the patient's electronic medical record.

The enrolment period will be 36 months or until the sample size is achieved, whichever comes first. As part of the trial, participants will be staggered on a 6-weekly interval. For example; participant 1 will undergo 2 weeks of treatment (7 days of phage treatment with a total of 10 to 14 days of inpatient care) and will continue to progress following the scheduled study review. A data safety monitoring board (DSMB) and trial steering committee (TSC) will review the outcome of each participant before progressing to the next participant. Participant 2 will commence trial after participant 1 has completed 2 weeks of treatment and the DSMB and TSC are satisfied with the safety outcomes and conduct of the trial of the preceding participant.

Study protocol and intervention

This is an open-label, single-arm study using phages that have demonstrated obligate lytic activity against each of the study participants'

P. aeruginosa sputum isolates. The selected phage will be prepared according to established protocols to achieve close to good manufacturing practice (GMP). The phages will be obtained through the phage bank in the Westmead Institute of Medical Research.

The preparation and testing of the phages will be performed at the Westmead Institute of Medical Research. Each selected phage will be amplified using the participants' bacterial strain to avoid any contamination. Bacterial isolates obtained from the study participant will be tested using spot plaque and top agar testing. Phages selected must not only have lytic activity as demonstrated by the plaque formation but will need to be $\geq 10^8$ PFU/mL when tested against the participants' bacterial strain to be considered for therapy.

The phage solution will undergo regular monitoring throughout the entire study following processes that may reduce the phage titres (Figure 11). To test these bespoke phage solutions, any nebulised medication that the participant is on during the trial will be tested against the selected phage before commencing treatment to ensure stability and maintenance of potency (synergy testing). A time-kill curve will be performed before and after *in-vitro* exposure of the medications and phage solution. Additionally, phage titre will be assessed following the addition of these medications to ensure titres remain high ($\geq 10^8$ PFU/mL).

Nebulisers used in this study will be TGA-approved mesh nebulisers. To confirm the viability of the phage-nebuliser pairing, all candidate phages

will undergo testing to ensure titres remain ≥10^8 PFU/mL before administration. Candidate phage will be nebulised and the nebulised aerosol will be collected and phage titration (spot plaque testing will be performed).

Bacterial cell debris such as endotoxins [i.e., lipopolysaccharides (LPS)], peptidoglycan, exotoxins, flagella, nucleic acids and other compounds will be separated from the phage solution. A combination of protocols have been adapted to address this including;

1. Standardised bacteriophage purification for personalised phage therapy, Luong et al 2020.[180]
2. The test for sterility has been adapted from the Forty-sixth WHO Expert Committee on Specification for Pharmaceutical Preparations in October 2011 and the 4th Edition of the International Pharmacopoeia. [181]
3. Test for sterility guidelines published by the Therapeutic Goods Administration (TGA) as part of the TGA guidelines for sterility testing of the therapeutic group has been included. [182]
4. Packaging selection is based on Guidance for industry, Container Closure Systems for packaging human drugs and biologics by Food and Drug Administration (FDA). [183]
5. Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products--Chemistry, Manufacturing, and Controls Documentation; guidance for industry. [184]

The concentration of phage administered will be 10^8 PFU/4 mL of a single, bespoke phage. Primary packaging of phage solution that is sterile will be in sealed sterile glass flasks. This flask will then be transported to a sterile medication preparation room (in the nearby pharmacy of the Children's Hospital at Westmead). The solution (1mL of $1x10^8$ PFU/mL of phage and added with 3ml of sterile normal saline) will be decanted into non-pyrogenic sterile Type 1 glass vials and sealed. These vials will be stored in a 4°C fridge and labelled. Sterile transfer of the final lysate into sterile vials will be conducted in the sterile medication preparation room in the pharmacy of the Children's Hospital at Westmead.

Bronchoscopy will be performed using 1 mL/kg of lavage fluid of normal saline 0.9% will be prepared in total to obtain lavage fluid for microbiology and to aid the bronchoscopy. The remaining 1 mL/kg will be made up of 0.9% normal saline with 8 mL of phage solution (equivalent to $2x10^8$ PFU/ 8mL in the total solution or 2 sterile vials of phage solution that contain $1x10^8$ PFU/4mL). Therefore, 2mL/kg of fluid will be administered during the bronchoscopy[62]. The final solution of 1 mL/kg of phage and saline solution will be instilled in equal aliquots in all five lobes of the lung. This translates to 0.2mL/kg in each lobe.

The subsequent phage dose will be administered via nebulisation. The solution from the vial will be transferred into the nebuliser chamber using a sterile syringe and diluted with sterile saline.

Phage nebulisation will occur after physiotherapy sessions and/or nebulised mannitol and/or nebulised hypertonic saline and/or nebulised dornase alfa and/or nebulised antibiotics.

The nebulised solution will contain 1 $x10^8$ PFU/4mL and be administered twice daily. The ideal timing of phage dosing should be 12 hours apart (range 10 to 14 hours).

Concomitant therapy for intravenous anti-pseudomonal antibiotics will be based on the sensitivity of the most recent *P.aeruginosa* isolate and current dosing and guideline as per the Children's Hospital Westmead CF treatment protocol. This concomitant treatment will continue for 10 to 14 days in an inpatient setting.

Data collection, monitoring and follow-up details

Data will be collected based on the tests, examinations, follow-up schedules, and procedures outlined in Figures 11 and 12. Results will be transcribed onto a data collection sheet that will be de-identified and stored under a password-protected file. The data will be available for review by the TSC and DSMB at the stipulated time points as per the individual charters. Data points collected will be standardised using;

1. Phage identification, isolation, purification, viability testing and sterility data template.
2. Bronchoscopy report.

3. Lung function indices and anthropometric data (weight and height) will be obtained from standardised measurements and report available on the study participants' electronic medical records.
4. Laboratory results will be obtained from standardised measurements and reports are available on the study participants' electronic medical records.

Data forms for points 1 and 2 can be made available upon reasonable request to the author.

Biological samples collected will include; Bronchoalveolar lavage and sputum sample [culture and sensitivity (reported as scant 1+, light growth 2+, moderate growth 3+ and heavy growth 4+) , strain analysis, quantitative polymerase chain reaction (qPCR, with limit of detection to be determined during the experimentation), Interleukin-6 and tumour necrosis factor-alpha (TNFα), NanoString RNA sequencing, bacterial population diversity (genomic sequencing, 16S rRNA transcriptomic analysis), cytology (for bronchoalveolar only), Immunoglobulin G, A, M, phage neutralizing antibodies]; Blood sample (full blood count, renal and liver profile, C-reactive protein, blood culture, interleukin-6, TNFα, Immunoglobulin G, A, M, NanoString RNA sequencing, phage neutralizing antibodies); Stool sample [Bacterial population diversity (16S rRNA transcriptomic analysis) and genomic sequencing before first administration and after last administration].

To assess the pharmacokinetics and pharmacodynamics, we plan to measure the levels of phage using the qPCR and Nanostring RNA sequencing methods at different time points (sputum, blood and stool samples). Additionally, cytokine levels as well as bacterial diversity will be evaluated. (Figure 12). Bacterial diversity (16s RNA and, pre and post-treatment genomic sequencing of the bacterial host), load and change in antibiotic sensitivities will be assessed over subsequent sputum samples. Monitoring the isolates and their changes in the efficiency of plating (EOP) to determine the emergence of phage resistance will also be conducted on each bacterial sample during the treatment phase.

Outcomes and endpoints

Primary outcomes (safety and tolerance)

Treatment success will be defined as the **absence** of the following adverse events:

1. Fever > 38.5°C over 3 consecutive temporally related administration or 3 episodes of temporally related fever above 38.5°C over 48 hours following administration of treatment. Temporally related fever is defined as fever above 38.5°C occurring within 1 hour of the administration of the nebulised bacteriophage.
2. Bronchospasm defined as a 10% decline in FEV_1% measured pre and post-administration of the first dose of nebulised bacteriophage

despite a reduction of dosing and pre-treatment with inhaled salbutamol.[185]

Secondary outcomes

A reduction in sputum bacterial culture titres of *Pseudomonas aeruginosa* from pre-treatment to day 7 of treatment.

Indications to stop treatment on the participant

1. Report of fevers > 38.5°C that fit the adverse event criteria stated above.
2. Severe allergy within 1 hour of the administration towards the nebulised liquid that requires inhaled salbutamol (x 3, 20 minutely) or intramuscular adrenaline.
3. Severe bronchospasm despite a reduction in the dosing of the nebulised bacteriophage and pre-treatment inhaled salbutamol is administered.
4. Any unexpected side effect that is deemed to be significant by the TSC/ DMSB or Principal Investigator. A severe unexpected side effect report will be submitted to the Sydney Children's Hospital Network Ethics committee.

Ancillary and post-trial care will be provided by the Sydney Children Hospital Network.

Data collection, monitoring and follow-up details

Participant demographic and clinical characteristics and study outcomes will be presented using standard descriptive statistics: mean/median and range for continuous variables and frequency and percentages for categorical variables.

The primary outcome of the proportion of participants who achieve a treatment response will be presented with an exact 95% confidence interval. The secondary outcome of change in sputum bacterial titres will be described by mean/median and range. For the secondary end-point, continuous data will be reported using the mean/median range. Changes of bacterial load, phage pharmacokinetics and pharmacodynamics will be compared using one-way ANOVA and/or t-test.

Additional statistical methods may be employed and will be mentioned separately when used during publication. This includes methods such elimination rate constant obtained from the log transformed concentration-time curve. Data of study participants that drop out of the study will be used up to the last available data point before the drop-out date.

Trial oversight

There will be 2 committees that will be convened for this trial which include The Trial Steering Committee (TSC), members of whom are involved in the trial and The Data and Safety Management Board (DSMB), members of whom are from an independent institution and are not involved in the trial.

These committees will be convened during different stages of the study to review technical data, titres, sterility and adherence to protocol. Safety data will be monitored regularly by both the TSC and DSMB as outlined within their respective charters. This will be based on the enrolment of each study participant (before administering the bacteriophage and at the end of admission). Auditing of record keeping and adherence to protocol will be based on the stipulation by the Sydney Children's Hospital Network Ethics Committee. Safety data, record keeping and adherence to the protocol will be monitored regularly by both the TSC and DSMB as outlined within their respective charters

Discussion

This pilot trial is designed specifically to examine the safety and tolerability of bacteriophages in children with cystic fibrosis. By design, we deliberately chose to directly instil phages via bronchoscopy into the airways to allow for a targeted delivery. This is followed by nebulisation as a delivery method to demonstrate that phages can be delivered safely and relatively without encumbrances of long hospital stays as is required through the delivery of long-term IV bacteriophage treatments. This is important as a patient-centred and cost-effective clinical strategy as we are trying established techniques that are familiar and easily adaptable to the daily routines of children and families with CF.

We designed this trial to allow a close to GMP grade of phages that can be safely delivered to children. This not only involves bespoke production of

phages but also genomics, purification and specific testing of individual phages against the participants' medications, making it a personalised medicine endeavour. We appreciate that this may be the main limitation of the study given the additional level of complexity, stringent approval requirements, costs and labour intensiveness and may subject the trial to delays. However, we strongly believe that this is an important step in bringing phage therapy into mainstream CF treatment.

Summary

In this protocol paper, we described our single-arm, open-labelled, non-randomised trial examining the safety and tolerability of the use of close to GMP-produced phage treatment in children with CF and *P.aeruginosa* which will be delivered via the endo-bronchial and inhalational routes. This study will run for 36 months or up to 10 participants, whichever is achieved first

Reference	**Year**	**Indication**	**Etiology**	**Delivery method**	**Efficacy**
Delacoste *el al*[186]	1959	Refractory coughs	N.A.	Inhalation	100%
Hoeflmayr *et al*[187]	1962	Bronchitis	Streptococci (2/3); Staphylococci (1/3)	Inhalation	90%
Garsevanishvili *et al*[188]	1974	Pneumonia	Staphylococci, streptococci, and	Inhalation	N/A

			enterics were targeted		
Ioseliani *et al*[189]	1980	Lung infections	Staphylococci and/or others	Inhalation, Bronchoscopy	>90%
Meladze *et al*[190]	1982	Parenchyma and pleura infections	*Staphylococcus*	Inhalation, topical, parenteral	>90% (223)
Kvachadze *et al*[191]	2011	Cystic fibrosis-associated infections	*Staphylococcus* and *Pseudomonas*	Inhalation	Improvement

Table 6*:* Existing human trials previously published (only relevant inhaled route is shown). *This table was adapted from Abedon et al 2015. These studies that have been cited may be in languages other than English and therefore are obtained from a summarised review article that has been published in English.*[192]

Reference	Route of administration	Main safety outcomes
Lebeaux et al 2021[193]	Inhaled phage against *A. xylosoxidans*	Well tolerated
Gainey et al 2020	IV against *achromobacter*	No adverse events
Dedrick RM et al 2019[194]	IV and topical	Weak phage-neutralisation antibody and cytokines. Diaphoresis and flushing without fever. No adverse reaction
Hoyle et al 2018[195]	Inhaled and oral against *Achromobacter xylosoxidans*	No safety data, patient improved
Kvachadze *et al* 2011[191]	Inhaled against *Pseudomonas aeruginosa*	No adverse events

Table 7: Recent case reports of children with CF treated with bacteriophage[169]

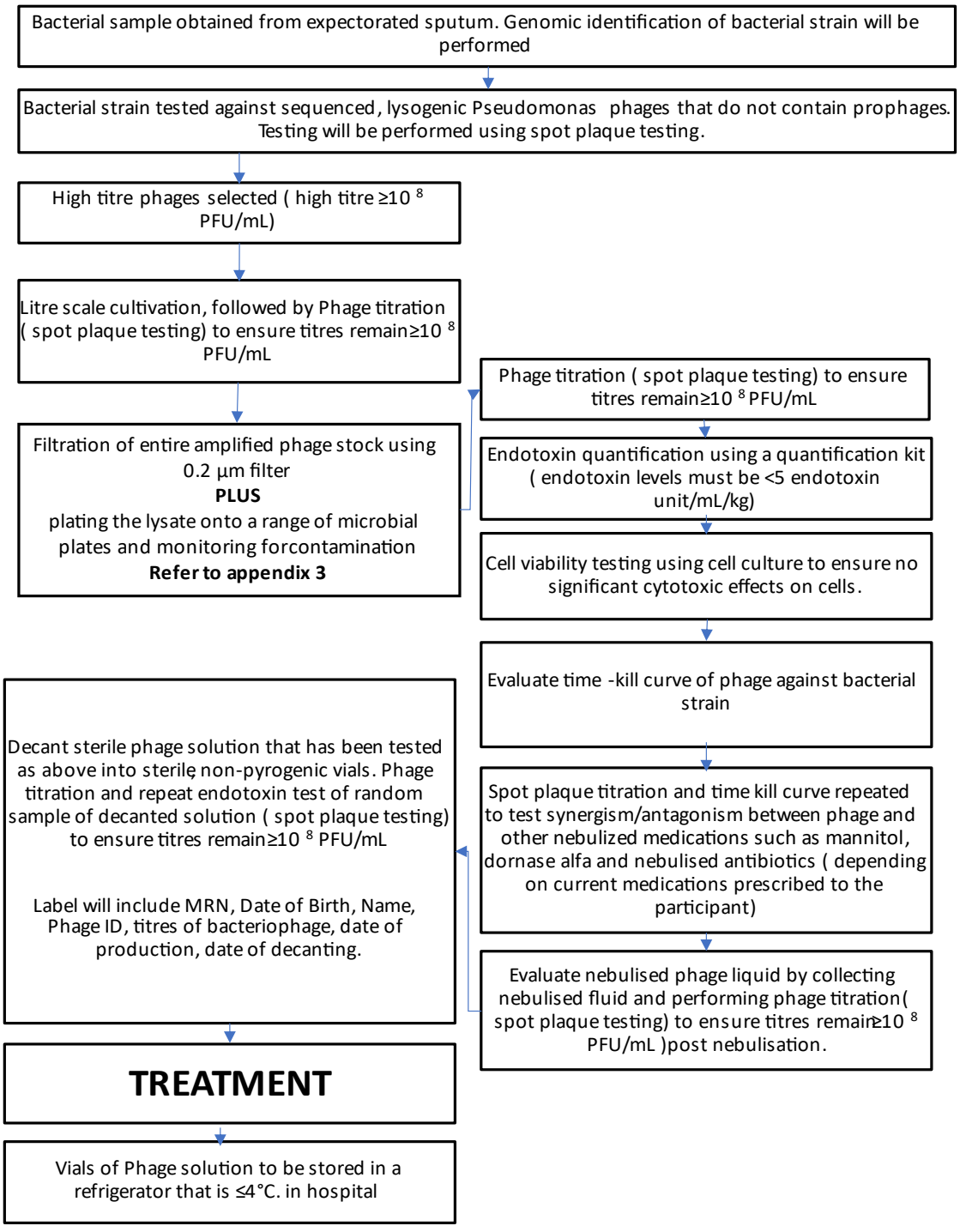

Figure 18: Phage production and quality control

TIMEPOINT	Pre-admission	Day 0	Day 1	Day 2	Day 3-6	Day 7	Day 8 to 9 * or up to 13	Discharge (day 10-14)	Week 3	Month 3	Month 6	Month 9	Month 12
Eligibility screen	X												
Informed consent	X												
Bronchoscopy and instillation of bacteriophage		X											
Nebulization of bacteriophage			X	X	X	X							
Standard IV		X	X	X	X	X	X	X					
Physiotherapy		X	X	X	X	X	X	X					
Sputum collection	X		X[@]			X		X		X	X	X	X
Anthropometry	X		X			X		X			X	X	X
Spirometry	X		X*			X		X		X		X	X

Blood investigation**	X			X				X					
Clinical review	X	X[a]	X	X	X	X	X	X			X	X	X
Discharge review (telephone)									X				

Figure 19: Timetable of schedule, footnote: [@]= sputum on day 1 to be performed before the first dose of nebulised bacteriophage, Blood investigations are timed to coincide with standard blood taking practices; admission, day 2 to review tobramycin level and day 14 during removal of the central line. [a]= This clinical review will be conducted prior to performing the bronchoscopy.

Chapter 8: Safety and Tolerability of Bronchoscopic and Nebulised Administration of Bacteriophage.

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Introduction

Pseudomonas aeruginosa is well known for causing significant morbidity and mortality in people living with chronic lung conditions such as cystic fibrosis.[27,196] Paired with an increase in antimicrobial resistance of this organism, treating *P. aeruginosa* continues to pose a challenge to clinicians and exposes patients to recurrent, prolonged antibiotic treatment that can lead to significant side effects.[197-199]

While significant and promising short-term benefits have been demonstrated following the introduction of CF transmembrane conductance regulator (CFTR) modulators, concerns persist regarding the potential resurgence of pathogens, particularly in those with pre-existing lung damage.[16,200]

Novel therapies, such as bacteriophages, have shown promise but face regulatory challenges due to their high specificity and the variable nature of these viruses.[201] Here, we report on the two adolescents receiving bacteriophage therapy (BT) through bronchoscopy and nebulisation, focusing on the tolerability, safety and clinical outcomes of this administration modality.[129,202]

Clinical Characteristics

A 12-year-old female (F12) and a 17-year-old male (M17) were both diagnosed with cystic fibrosis (CF) featuring homozygous F508*del* mutation and pancreatic insufficiency since birth.

In the case of F12, non-mucoid *P. aeruginosa* was initially identified in her sputum at the age of six, and three years later, a mucoid strain of *P. aeruginosa* was first isolated. Subsequently, mucoid *P. aeruginosa* consistently appeared in all sputum samples collected every 3-4 months (n=17 samples). For M17, non-mucoid *P. aeruginosa* was initially detected in his sputum at the age of 11, and consistently appeared in all subsequent sputum samples collected every 3-4 months (n=26 samples).

Both have been subjected to multiple courses of intravenous antibiotics (mainly courses of Piperacillin/Tazobactam and Tobramycin) following the first isolation of *P. aeruginosa* within their sputum (F12; 11 admissions, M17; 13 admissions). F12 commenced Elexacaftor-Tezacaftor-Ivacaftor (ETI) for 3 months, and M17 for 18 months before BT. Both participants have evidence of bronchiectasis on their chest computed tomography scan.

Both met the inclusion criteria which included; age ≥12 years and <18 years of age) with CF, positive sputum or bronchoalveolar lavage culture (*P. aeruginosa*) in more than 50% of sputum samples over the past year, continue to isolate *P. aeruginosa* in sputum despite undergoing

eradication therapy using two antipseudomonal antibiotics, the latest clinical isolates of *P. aeruginosa* taken within 3 months of enrolment are susceptible to (demonstrates lytic activity) available anti-*P. aeruginosa* bacteriophage, and the ability to perform reliable spirometry.[129] F12 had a baseline FEV_1% (determined by the average of FEV_1% performed in the past 12 months before BT) of 92% over the past 12 months and the best FEV_1% of 100% over the past three years while M17 had a baseline FEV_1% of 75% over the past 12 months and best FEV_1% of 79% over the past three years (Figure 20).

Phage Administration

The methodology of the BT protocol (Sydney Children's Hospitals Network Human Research Ethics Committee ethics approval; 2022/ETH00241) has been previously published.[129] Bacteriophage PBPA103 (Lyse N Tech, Heejoon Myung, South Korea) was selected for both participants as it provided the best lytic reaction for mucoid (F12) and non-mucoid (M17) strains of *P. aeruginosa*. [203]

PBPA103 was instilled in all lobes of the lungs in equal aliquots. Intravenous Piperacillin/Tazobactam and Tobramycin were administered concurrently. Both had received this antibiotic combination in their previous admissions.

Both participants received nebulised bacteriophage on the following day, delivered through a non-vibrating mesh nebuliser over five minutes. PBPA103 was nebulised twice a day for the remaining seven days of BT.

Clinical and Microbiological Outcomes

Following bronchoscopic and nebulised administration, vital signs taken every 15 minutes for one hour post-administration remained normal. No wheeze or respiratory distress was observed at one hour post-administration. Four-hourly vital signs remained within the normal range throughout BT. Spirometry performed before and after nebulisation of PBPA103 did not show any bronchospasm.

In terms of clinical outcomes, decreased cough and sputum expectoration were reported by both participants and the CF physiotherapist (performing twice-daily supervised physiotherapy). At the end of the BT, F12 demonstrated an increase of FEV_1% by 4%, while M17 demonstrated an increase of FEV_1% by 5% from their best FEV_1% in the past three years. F12 demonstrated an increase of 12% and M17 demonstrated an improvement of 8% in terms of improvement from baseline FEV_1%. An increase in markers such as white blood cells, liver transaminases and interleukin-6 were observed without any discernible clinical effects (Table 8). The serial chest x-ray of M17 before and after BT is shown in Figure 21.

For F12, no *P. aeruginosa* species was isolated from induced sputum samples taken on days three, seven and 14, three, six- and nine-months post-BT. M17 continued to isolate non-mucoid *P. aeruginosa* from samples taken during the same intervals as was performed on F12 (Figure 22). PBPA103 continued to cause lysis when tested on the bacterial lawn of his *P. aeruginosa* species isolated during and after BT.

Discussion

The absence of fever, bronchospasm (no wheezing or cough and, stable spirometry), and localised reactions during and after BT in these two adolescents have been promising. There was an improvement in cough and sputum production. Furthermore, we found that both demonstrated a rise of FEV_1% above their best FEV_1% in the past three years and the improvement exceeded what was achieved in previous admissions without BT. The improvement in lung function demonstrates a higher average of increase attributable to ETI.[204] In terms of the effect of ETI on FEV_1%, studies have demonstrated that improvements of ppFEV_1% of about 10% in lung function are seen in the first few weeks of commencing ETI, which plateaus thereafter. [204,205] In our cohort, a similar pattern was observed following the initial initiation of ETI which then plateaued. A further increase in FEV_1% was then observed following BT, which is outside of the typical time and magnitude observed from ETI alone.

A temporary rise in white blood cell count, tumour necrosis factor α (TNF-α), and interleukin-6 (IL-6) levels was observed. While TNF-α and IL-6 increased in both F12 and M17, an increase in white blood cells was only observed in F12. A review of her blood results from previous admissions showed a similar trend, possibly related to inherent factors. An alternative explanation that favours BT could be that the administration of BT may improve phagocytic activity, thereby enhancing the intracellular killing of chronic *P. aeruginosa* which will show a neutrophilic reaction. [206,207] Given the successful eradication of the organism from her sputum, this rise in neutrophils could be a hallmark of neutrophil-phage synergy, signalling effective phage therapy.[206]. The changes observed in the TNF-α and IL-6 is in contrast to several studies performed *in-vitro* and in animal models that showed either no change or a reduction in the expression of these cytokines.[208] [209] However, our findings align with Van Belleghem *et al,* who described an increase in these cytokines that were clear, reproducible and, independent of the level of endotoxins within the bacteriophage preparation.[210] From this, we postulate that the expression of these cytokines in our cohort is; 1) due to the interaction between the bacteriophage and bacterial host and not due to the bacteriophage alone; 2) while bacteriophages share similar properties (e.g., capsid protein folds)[211], a single-point mutation can alter the serotype and the subsequent immune response towards the bacteriophage and therefore is strain specific;[210] 3) the modality of administration of our bacteriophage,

particularly through bronchoscopy, may induce a more significant innate immune response that commences at the level of the respiratory epithelium. This transient upregulation in cytokines following BT will need to be further studied as it will add to our understanding of the effects of bacteriophages in the immune system and cannot be concluded from our findings alone. A two-fold rise in their liver transaminases could also be attributed to factors such as β-lactams administration will require further monitoring.

This is the first time *P. aeruginosa* was not found in the sputum sample of F12 over six years, despite repeated hospitalisations requiring intravenous antibiotics and the introduction of ETI. *P. aeruginosa* has not been detected within her sputum samples three months after BT. An important aspect of this report that needs to be considered is the confounding role that ETI may have in our findings. Studies have demonstrated that the introduction of ETI has been shown to cause a decline in density of *P. aeruginosa.* These studies have shown that while ETI results in a decline in *P. aeruginosa*, the decline in bacterial density usually stabilises within the first three months following initiation of ETI, after which BT was commenced in both our participants. [212,213]

This report outlines the innovative application of bacteriophages delivered bronchoscopically and through nebulisation in treating challenging infections in children. While presenting two cases, it is important to acknowledge a significant limitation in the form of a small participant pool

undergoing this treatment. The success in eradicating chronic infection warrants further microbiological and clinical surveillance over six and 12 months for a more conclusive assessment. However, the success in achieving eradication, following repeated courses of intravenous antibiotics over the past six years for chronic mucoid *P. aeruginosa*, cannot be understated.

Commencing BT poses multiple challenges, notwithstanding a decade of compassionate access treatments. The establishment of robust clinical trials, particularly in the context of our experience in conducting the first personalised BT as part of a clinical trial in children through bronchoscopy and nebulisation, faced formidable yet conquerable hurdles in ethics, governance, regulation, and production. This inaugural report emphatically underscores the feasibility of conducting clinical trials beyond the conventional bounds of compassionate access. Consequently, it serves as a compelling call to action for clinicians and researchers to actively explore and embrace this innovative approach.

	Baseline before BT	Day 2 following BT	Day 14 following BT
Haemoglobin (115-160 g/L)			
F12	103	122	113
M17	121	130	139
White blood cell count (4.5-13.5x10^9/L)			
F12	6.6	12.6	6.2
M17	5.1	5.9	4.2
Neutrophils (1.5-8x10^9/L)			
F12	2.9	10.3	4
M17	2.8	4.5	2.6
Lymphocytes (1.5-7x10^9/L)			
F12	3.1	1.5	1.6
M17	1.5	0.6	1.1
Eosinophil (0-1.1x10^9/L)			
F12	0.1	0	0.1
M17	0.1	0.1	0.1
Monocytes (0.2-1x10^9/L)			
F12	0.5	0.7	0.5
M17	0.6	0.6	0.4
Basophils (0-0.2x10^9/L)			
F12	0.1	0	0
M17	0	0	0
C- Reactive protein (0-10 mg/L)			
F12	<0.3	<0.3	<0.3
M17	0.9	4.5	0.5
Aspartate aminotransferase (U/L)			
F12 (0-41 U/L)	32	91	81
M17 (0-49 U/L)	27	83	
Alanine transaminase (U/L)			
F12 (0-36 U/L)	25	34	86
M17 (0-39 U/L)	31	103	**
Bilirubin (0-10 umol/L)			
F12	<2	11	6
M17	<2	2	
Urea (2-6.8 mmol/L)			
F12	1.8	3.9	2.8
M17	4.4	5	
Creatinine (35-74 umol/L)			
F12	26	35	38
M17	57	64	

IgG (6.24-14.4 g/L)			
F12	6.99	9.18	9.87
M17	9.17	10.2	12.2
IgA (0.74-2.28 g/L)			
F12	0.6	0.84	0.83
M17	1.25	1.44	1.7
IgM (0.48-2.57 g/L)			
F12	1.25	1.53	2.03
M17	1.33	1.48	1.9
Tumour necrosis factor-α (<0.067 pg/mL)			
F12	<0.067	<0.067	0.26 *
M17	0.39	0.67	1.23
Interleukin-6 (< 5.22 pg/mL)			
F12	3.84	5.72	9.77 *
M17	0.72	13.85	1.78

Table 8: Blood investigations performed to monitor the effects of bacteriophage therapy. BT; bacteriophage therapy, Ig; immunoglobulin. * A repeat blood test performed 6 weeks post-BT showed a decline of tumour necrosis factor α to <0.067 pg/mL and Interleukin-6 to 6.77 pg/mL. ** Results not available due to sample haemolysis.

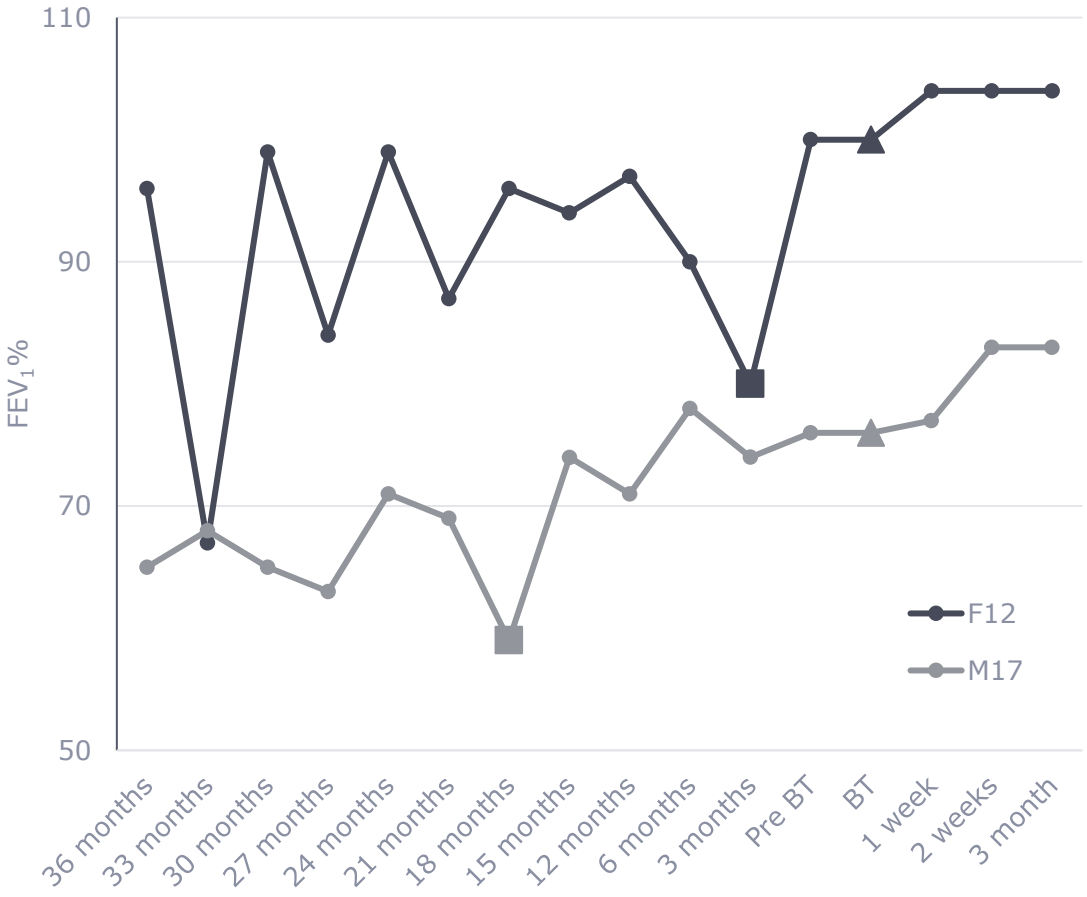


Figure 20: Spirometry trend over three years leading up to bacteriophage therapy. ▲ ; commencement of bacteriophage therapy, ■ ; commencement of Elexacaftor-Tezacaftor-Ivacaftor. BT; bacteriophage therapy.

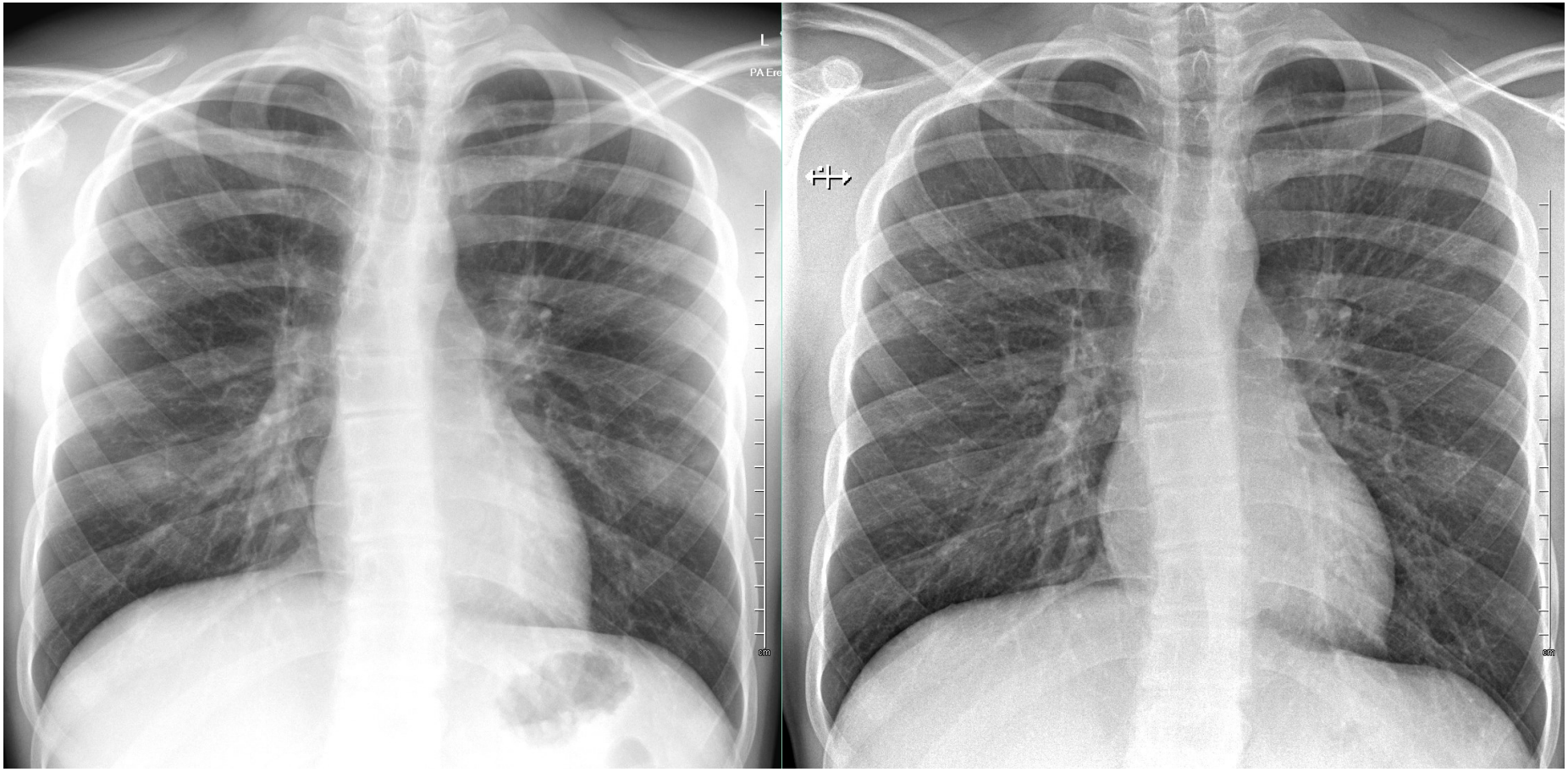


Figure 21: Chest X-rays of M17 taken 6 months before BT (Before) and 6 months after completion of bacteriophage therapy (After).

Antibiotic	**F12 (mucoid *P.aeruginosa*)**	**M17 (non-mucoid *P.aeruginosa*)**	
		Before BT	After BT
Amikacin	S	R	S
Aztreonam	S-IE	R	S-IE
Cefepime	S-IE	R	S-IE
Ceftazidime	S-IE	R	S-IE
Ceftolazane/Tazobactam	S	R	S
Ciprofloxacin	S-IE	R	S-IE
Gentamicin	INS	R	INS
Imipenem	S-IE	R	S-IE
Meropenem	S-IE	R	S-IE
Piperacillin/Tazobactam	S-IE	R	S-IE
Tobramycin	S	R	R

Figure 22: The antibiotic susceptibility profile of *P.aeruginosa* of F12 and M17. S: sensitive; R: Resistant; S-IE: susceptible with increased exposure; INS: insufficient evidence

Chapter 9: Summary and Conclusion

This thesis has established significant insights, experience, and expertise in bacteriophage therapy for chronic lung infections. The studies that comprise it have addressed several questions that will facilitate translational work in this field.

What is the incidence and prevalence of *Pseudomonas aeruginosa* within one of the largest paediatric CF centres in the country?

The 18-year longitudinal study provides essential and compelling data on the evolving epidemiology of respiratory pathogens in children and adolescents with cystic fibrosis (CF), marking it as a crucial component of this thesis. The analysis highlights a steady decline in the incidence and prevalence of key CF pathogens, including *P.aeruginosa*, *S. aureus*, and *H. influenzae*, while also noting an increase in the mean age at which these pathogens are first isolated. Despite these positive trends in infection control and management, *P. aeruginosa* remains a predominant organism affecting adolescents, raising ongoing concerns about persistent infection and the potential for antibiotic resistance.

Furthermore, the study reveals a significant rise in the use of second-line antipseudomonal antibiotics, such as nebulised amikacin and colistin,

which suggests a developing trend of resistant *P. aeruginosa* strains. This aligns with concerns about the impact of antibiotic resistance on treatment outcomes and highlights the growing urgency for alternative, more targeted therapeutic strategies. In addition, the study reports a numerical rise in the incidence of atypical infections, particularly non-tuberculous mycobacteria (NTM), which further complicates clinical management and underscores the need for novel treatments to address these emerging pathogens.

While the study predates the widespread adoption of CFTR modulator therapy, which has revolutionised CF treatment, the findings remain highly relevant. The use of modulator therapies, which have shown significant improvements in lung function and overall clinical outcomes for many CF patients, is expected to have an impact on the prevalence and management of respiratory pathogens. The data from this study will serve as a valuable comparator, offering insights into pathogen trends and treatment outcomes in the pre-modulator era. It provides a baseline for future investigations into how modulator therapy influences the incidence and prevalence of *P. aeruginosa* and other pathogens, enabling a more comprehensive understanding of the evolving landscape of CF treatment and infection control.

The findings from this study are directly relevant to this thesis on bacteriophage therapy, as they underscore the limitations of current antimicrobial therapies in managing resistant *P. aeruginosa* strains. The steady decline in the incidence of common respiratory pathogens is a promising trend, but the persistent prevalence of *P. aeruginosa*, particularly in adolescents, and the rising incidence of atypical pathogens like NTM, highlight the need for innovative therapeutic approaches. Bacteriophage therapy, which offers the potential to target specific bacterial pathogens without disrupting the overall microbiota, presents an exciting avenue for overcoming the challenges posed by resistant infections

What are the baseline characteristics of children with CF presenting with a pulmonary exacerbation, and what impact does *P. aeruginosa* have?

This study provides critical insights into the impact of *Pseudomonas aeruginosa* infections on lung function in children with cystic fibrosis (CF), particularly during pulmonary exacerbations. As one in three children admitted for a pulmonary exacerbation had *P. aeruginosa* isolated from their airway sample, it reinforces the significant role this pathogen plays in driving morbidity and the subsequent decline in lung function, with an average 13% reduction in FEV_1% observed. Despite treatment, fewer than half of these children returned to their baseline lung function, and most experienced a decline within six weeks post-discharge, leading to the need for readmission.

These findings underscore the critical importance of managing *P. aeruginosa* infections, particularly chronic colonisation, which has long been a major contributor to the progressive deterioration in lung function in CF. Given the challenges in sustaining lung function after exacerbation treatment, particularly with standard antibiotic therapies, the need for alternative and potentially more effective treatment strategies is apparent.

This is where bacteriophage therapy emerges as a promising approach. With the increasing concern around antibiotic resistance and the limitations of current treatments, bacteriophage therapy offers a targeted, pathogen-specific option for addressing *P. aeruginosa* infections. As this study demonstrates that *P. aeruginosa* continues to be a significant threat to lung function, bacteriophage therapy could complement existing treatment strategies by providing an alternative method of infection control. The fact that children with CF often struggle to maintain gains in lung function post-treatment presents a strong case for exploring bacteriophage therapy as a way to break the cycle of exacerbations and hospital readmissions. By precisely targeting *P. aeruginosa*, bacteriophage therapy could reduce the frequency of these infections and potentially offer longer-lasting improvements in lung health.

Furthermore, the study's findings align with previous research, including a larger US study by Sanders *et al*., which found that a significant proportion of children with CF returned to baseline lung function after

treatment for a pulmonary exacerbation. However, the present study adds depth by showing that gains made during treatment are not always sustained, which is particularly concerning as it links this decline in lung function to an increased risk of readmission and potential treatment failure.

Moreover, as this study was conducted in the era prior to the widespread use of CFTR modulators, it presents a valuable comparison point for future studies evaluating the impact of modulator therapies alongside newer treatments like bacteriophage therapy. As we move into an era where modulator therapies may reduce exacerbation frequency and hospital admissions, the addition of bacteriophage therapy could provide a potent tool for addressing persistent *P. aeruginosa* infections, improving outcomes, and reducing long-term lung function decline.

To what extent have bacteriophages been used in treating *P. aeruginosa* infections, particularly in CF?

The systematic review, contained within this thesis, highlights the growing interest in bacteriophage therapy as a potential treatment for multidrug-resistant infections, particularly in CF. While there has been a notable increase in *in-vitro* studies and compassionate use cases of bacteriophages, the clinical application of bacteriophage therapy in CF remains underdeveloped. The review revealed a significant gap in clinical trials, particularly in paediatric populations, with no phase 1 trials, no

studies exploring bronchoscopic delivery, and no trials targeting bacteriophage therapy specifically in children with CF.

Despite these gaps, the studies reviewed demonstrate promising early results, including reductions in bacterial load, clinical improvement, and a favourable safety profile for bacteriophage treatment in CF. Animal model studies further support the potential of bacteriophage therapy, showing positive effects on reducing bacterial load and inflammation in CFTR-deficient models. However, the lack of robust, randomised controlled trials and the heterogeneity in study designs limit the ability to draw definitive conclusions.

The lack of clinical trials and standardised approaches in bacteriophage therapy presents a significant challenge for its future implementation. However, studies such as the CHIP-CF study and others (e.g., CYPHY study at Yale) are paving the way for future human trials and regulatory frameworks. These studies are crucial to address the existing gaps, including the lack of phase 1 trials and bronchoscopic use, and to establish the evidence needed to move forward with more widespread adoption of bacteriophage therapy in clinical practice.

With recent advancements in CF treatment, including CFTR modulators, the need for bacteriophage therapy in CF may decrease over time. However, other patient groups, such as those with non-CF bronchiectasis,

long-term ventilation, or tracheostomies, could continue to benefit from bacteriophage therapy. The findings of this review underscore the importance of advancing research in this area, not only for CF patients but also for broader applications in paediatric respiratory medicine.

Can bacteriophages be isolated and purified for therapeutic use by the candidate/clinician to better understand the process of bacteriophage isolation and purification?

The candidate of this thesis, a clinician, was specifically trained to isolate and purify bacteriophages using established protocols. This hands-on experience provided a solid foundation for the candidate, enhancing their understanding of bacteriophage biology and production. By actively engaging in the isolation and purification process, the candidate was able to gain crucial insights into the complexities of bacteriophage-host interactions, the specificity of bacteriophages, and the various steps involved in their preparation for therapeutic use.

This invaluable training allowed the candidate to comprehend the intricacies of bacteriophage biology—such as their life cycle, host range, and potential for therapeutic application. With a deepened understanding of bacteriophage dynamics, the candidate was equipped with the knowledge necessary to contribute to the development of clinical protocols for bacteriophage therapy. The experience also fostered a more nuanced approach to addressing challenges related to bacteriophage production, including issues of quality control, batch consistency, and optimal therapeutic delivery. Ultimately, this practical expertise

has provided the candidate with the ability to apply these learnings directly to clinical settings, advancing the implementation of bacteriophage therapy.

How can the integrity and infectivity of bacteriophages be assessed following nebulisation for therapeutic use?

Ensuring the integrity and infectivity of bacteriophages following nebulisation is crucial for their effective therapeutic application, as their inherent fragility can be compromised by mechanical stressors during the nebulisation process. Nebulisation subjects bacteriophage solutions to vibrations, shear forces, and temperature changes that could negatively affect phage viability and infectivity. This is particularly important for personalised bacteriophage therapy, where each patient may receive a bespoke bacteriophage preparation tailored to their unique infection profile.

Through this thesis, a protocol using a mesh nebuliser—selected based on previously published studies that highlighted its ability to preserve bacteriophage integrity—was implemented to nebulise the phage solution. This decision builds upon existing knowledge, ensuring a robust and well-established standard for nebulisation while accounting for the delicate nature of bacteriophages. Detailed measurements of the retentate (remaining solution in the nebuliser) and flow-through (aerosolised solution) were taken. The flow-through was titrated to evaluate the

concentration of viable bacteriophages, ensuring that adequate plaque-forming units (PFU/mL) remained for therapeutic use.

This protocol is not only essential for verifying the integrity of bacteriophages, but also for ensuring that each individual bacteriophage preparation can be easily tested and replicated. Personalised therapy—the hallmark of this thesis—relies on the ability to tailor treatments to individual patients, and this method facilitates that process. The ability to rapidly and accurately assess the viability of bacteriophages following nebulisation is critical for ensuring that patients receive a potent and effective dose.

Can a clinical trial be implemented outside the confines of compassionate access schemes?

This thesis demonstrates that the field of bacteriophage therapy needs advancement into more robust and standardised clinical trials. While compassionate access schemes offer a route for treatment in critically ill patients, they are inherently limited to those in extreme conditions, typically at the end of the therapeutic spectrum. These schemes are important, but they do not address the broader need for preventive and therapeutic options in managing chronic infections caused by antimicrobial-resistant organisms, nor do these schemes enable access to bacteriophage therapeutic trials to the broader cohort of patients that are

not as ill as those who would typically be considered for compassionate access schemes.

The clinical trial outlined in this thesis seeks to move beyond the confines of compassionate access by implementing bacteriophage therapy in children with CF who are not in *extremis* or undergoing exacerbation, and doing so in a controlled and regulated environment. The focus on children, rather than adults, stemmed from a desire to intervene earlier in the progression of chronic infection, with the aim of preventing the long-term lung damage that often accompanies persistent infections. Despite the challenges in obtaining necessary approvals, particularly for paediatric patients, the decision to initiate the trial in children was intentional, driven by the need to address chronic infections before they result in irreversible damage.

This approach represents a shift towards considering bacteriophage therapy not as a last resort, but as a proactive treatment option for managing chronic infections, particularly in vulnerable populations such as children with CF. It is through trials like this that we can build a foundation for more widespread and evidence-based use of bacteriophages in clinical settings.

What is the outcome of this therapy in terms of tolerability and safety?

This novel clinical trial is the first to implement personalised bacteriophage therapy in children, in general, and those with CF, in

specific, via bronchoscopy and nebulisation. Both participants demonstrated excellent tolerability, with no adverse effects observed during treatment or up to ten months post-therapy. A lower bacteriophage dose was used compared to previous reports, yet it remained effective. One participant achieved complete eradication of *P.aeruginosa* for the first time in six years, despite multiple prior courses of intravenous antibiotics and the introduction of elexacaftor-tezacaftor-ivacaftor (ETI). One patient received bacteriophage therapy three months after commencing ETI but continued to have P. aeruginosa in her sputum samples. Another patient remained positive for P. aeruginosa despite being on ETI for more than 18 months. The timing of bacteriophage therapy in relation to ETI initiation is an important consideration. Anecdotally, P. aeruginosa persistence three months after starting ETI may represent the minimum duration before considering bacteriophage therapy in a CFTR modulator corrected airway. Both participants also showed significant improvements in lung function beyond what was previously observed with ETI alone. A third participant has completed treatment and also similarly shown no adverse event but more importantly, complete eradication of the organism eight months post treatment.

While, a transient rise without (any clinical events) in inflammatory markers, including TNF-α and IL-6, was detected, aligning with previous findings that suggest an interaction between bacteriophages and host

immune responses rather than endotoxin contamination. The results indicate potential neutrophil-phage synergy, which may enhance bacterial clearance. While further studies are needed to characterise this immune response, the findings provide crucial insights into the interplay between bacteriophage therapy and the immune system.

This trial represents a critical step towards transitioning bacteriophage therapy from a last-resort option under heterogeneous compassionate access schemes to a more structured and proactive treatment approach. By demonstrating safety, feasibility, and efficacy in a controlled setting, this work lays the foundation for expanding bacteriophage therapy beyond emergency use, ultimately moving it towards routine clinical application. The challenges in ethics, governance, and regulatory approvals were significant but surmountable, establishing a clear precedent for future clinical trials. More importantly, this study confirms that bacteriophage therapy can be safely and effectively delivered to children with CF, marking a pivotal step in integrating this treatment into standard care pathways for chronic infections.

The future direction of the CHIP-CF study

This study was designed to lay the foundation for establishing a bacteriophage therapy service for children and adolescents with difficult-to-treat lung infections. With the successful implementation of this clinical trial, ethics approval has now been obtained to expand the inclusion criteria, allowing children as young as six years old to receive bacteriophage therapy, regardless of their underlying lung condition. The revised protocol will encompass children with chronic *Pseudomonas aeruginosa* infection and any form of suppurative lung disease, broadening the reach of this personalised therapy while maintaining a rigorous and controlled treatment framework.

Looking ahead, the next critical step is advancing to a phase 2 clinical trial to evaluate efficacy on a larger scale. Expanding collaborative efforts will be key, both within Australia and internationally. Strengthening partnerships with leading bacteriophage research centres overseas will facilitate access to expertise, enhance protocol development, and accelerate the integration of bacteriophage therapy into routine clinical practice. These collaborations will also support the establishment of standardised production, regulatory pathways, and training programs to ensure safe and effective implementation.

The recent conferment of the Churchill Fellowship presents a unique opportunity to gain firsthand experience in developing a dedicated phage

therapy centre in Australia. This initiative will be instrumental in scaling up access to bacteriophage therapy beyond CF, extending its application to other chronic lung infections and difficult-to-treat bacterial diseases. By translating the findings from CHIP-CF into broader clinical practice, this work will help shape the future of bacteriophage therapy in paediatrics and beyond, paving the way for a structured and sustainable model of care.

Appendix 1: Protocol

Single-Arm, Open-Labelled, Safety and Tolerability of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with *Pseudomonas aeruginosa*.

Version: 6.1

Date: 4th March 2024

CONFIDENTIAL

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

TABLE OF CONTENTS

ADMINISTRATIVE INFORMATION

1. TITLE

A Single-Arm, Open-Labelled, Safety and Tolerability of Intrabronchial and Nebulised Bacteriophage Treatment in Children with *Pseudomonas aeruginosa*.

2. TRIAL REGISTRATION

2A REGISTRY

This study will be registered in the following registries;

1. Clinicaltrials.gov
2. Australianclinicaltrials.gov.au

2B DATA SET

Data Category	**Information**
Primary registry and study identifying number	Registration in the above registries is intended.
Date of registration in primary registry	Pending.

Data Category	**Information**
Secondary identifying numbers	Pending.
Source(s) of monetary or material support	Respiratory Department, The Children's Hospital at Westmead.
Primary sponsor	Respiratory Department, The Children's Hospital at Westmead.
Secondary sponsor(s)	N/A.
Contact for public queries	Dr Jagdev Singh.
Contact for scientific queries	Dr Jagdev Singh.
Public title	The evaluation of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with *Pseudomonas*. (CHIP-CF)

Data Category	**Information**
Scientific title	A Single-Arm, Open-Labelled, Safety and Tolerability of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with *Pseudomonas aeruginosa*.
Country of recruitment	Australia
Site (s) of recruitment	The Sydney Children's Hospital Network • The Children's Hospital at Westmead • Sydney Children's Hospital Randwick
Health condition(s) or problem(s) studied	Presence of *Pseudomonas aeruginosa* in the airways of children.
Intervention(s)	Personalised intra-bronchial and nebulised bacteriophage in children that continue to isolate *Pseudomonas aeruginosa* despite undergoing eradication and suppressive treatment(s).

Key inclusion and exclusion criteria	**Inclusion criteria** 1. Patients (≥6 years and) *2.* Positive sputum or bronchoalveolar lavage culture (*Pseudomonas aeruginosa)* in more than 50% of sputum samples over the past 1 year[177]. *3.* Continues to isolate *Pseudomonas aeruginosa* in sputum despite undergoing *Pseudomonas aeruginosa* eradication therapy using two anti-pseudomonal antibiotics and/or is currently on suppressive treatment 4. The latest clinical isolates of Pseudomonas aeruginosa taken within 3 months of enrolment are susceptible (demonstrates lytic activity) to available anti-*Pseudomonas aeruginosa* bacteriophages. 5. Ability to perform spirometry **Exclusion criteria:** 1. Children who have received more than 1mg/kg of Prednisolone continuously for more than 7 days before study enrolment.

	2. Children that require ≥18 hours/day of non-invasive ventilatory (NIV) support during admission. 3. A current diagnosis of Allergic bronchopulmonary aspergillosis (ABPA). 4. History of hemoptysis in the past 12 months prior to study enrolment. 5. Prior known inability to expectorate sputum. 6. Has undergone solid organ transplantation 7. Positive sputum isolation of *Burkholderia cepacia* within the past year 8. Treatment of non-tuberculous mycobacterium within the past 1 year. 9. A positive COVID-19 PCR nasal swab or rapid antigen test with a confirmed COVID-19 infection in the past 6 months prior to enrolment[178]. 10. Within 3 months of having received a booster COVID-19 vaccine or within 6 months of receiving the first dose of vaccine[179].

Data Category	Information
Study type	A Single-Arm, Open-Labelled, Non-Randomized, Safety and Tolerability Study.
Date of the first enrolment	Expected enrolment in May 2022 or as soon as ethics is approved.
Target sample size	Up to 10 participants
Primary outcome(s)	Tolerability and safety of intra-bronchial and nebulised bacteriophages against *Pseudomonas aeruginosa.*
Key secondary outcomes	A reduction in colony-forming units (CFU/mL) of *Pseudomonas aeruginosa* following treatment.

3. PROTOCOL VERSION

Issue Date:	**4th March 2024**
Protocol amendment number:	**Version 6.1**
Author(s):	**Dr Jagdev Singh**

REVISION CHRONOLOGY:

Date of change	Summary of changes
8th February 2022	Addition of table for SUSAR and AE notifications Addition of checklist and data collection sheet Addition of blood volume Change in schedule of events to include day 3 to 6 and 8 to 9 Removed section 10 (c)
8th April 2022	Addition of third party to assess sterility of final product
3rd March 2024	Inclusion criteria age range to be extended to ≥6 years with no age limit

4. FUNDING

Current funding will be obtained from The Respiratory Department, The Children's Hospital at Westmead.

5. ROLES AND RESPONSIBILITIES

5A CONTRIBUTORSHIP

Author Name	**Summary of contribution**
Dr Jagdev Singh Respiratory Department, The Children's Hospital at Westmead	Main Author and Principal Investigator
Professor Hiran Selvadurai Head of Department, Respiratory Department, The Children's Hospital at Westmead	Primary Supervisor, Associate investigator, and reviewer
Professor Dominic Fitzgerald CF Clinical Lead, Senior Clinician Respiratory Department, The Children's Hospital at Westmead	Senior Clinical reviewer, Associate investigator, and reviewer
Professor Adam Jaffe Senior Clinician and Research Lead Respiratory Department Sydney Children's Hospital	Senior Clinical reviewer, Associate investigator, and reviewer

Author Name	Summary of contribution
Professor Jon Iredell Head of Infectious Diseases and WIMR phage research laboratory Westmead Hospital	Supervisor and reviewer
Dr Jeremy Barr Head of Barr Laboratory Monash University	Supervisor and reviewer
Ms Sharon Hunt CF Clinical Lead, Senior Clinical Nurse Consultant Respiratory Department, The Children's Hospital at Westmead	Clinical reviewer

5B SPONSOR CONTACT INFORMATION

Study Sponsor	Respiratory Department, The Children's Hospital at Westmead.
Contact name	Professor Hiran Selvadurai
Address	Respiratory Department, The Children's Hospital at Westmead.
Telephone	98453395
Email	Hiran.Selvadurai@health.nsw.gov.au

5C COMMITTEES

Principal Investigator and Research Staff

Dr Jagdev Singh

Steering Committee

Professor Hiran Selvadurai

Professor Jonathan Iredell

Dr Jeremy Barr

Professor Adam Jaffe

Professor Dominic Fitzgerald

Ms Sharon Hunt

INTRODUCTION

6. BACKGROUND AND RATIONALE

Cystic fibrosis (CF) is a life-limiting genetic multisystem condition causing chronic lung disease that occurs in 1 in 2,500 births. It is complicated by recurrent pulmonary exacerbations requiring aggressive antibiotic treatment for 10 to 14 days. In Australia, almost 30% of adolescents with CF are hospitalised for intravenous (IV) antibiotic treatment each year. An estimated 25% of children with CF will be infected by *Pseudomonas aeruginosa* which increases their morbidity and mortality[83,134].

A child with CF who has an infection with *Pseudomonas aeruginosa* within his/her airway has a 2.6 times higher risk of death in an 8-year period than children that have not been infected with the organism. These children will also have a poorer baseline weight and a higher number of CF-related hospitalisations[83,135].

A child with CF may acquire *Pseudomonas aeruginosa* as early as 1 year of age (non-mucoid). This initial infection can be possibly eradicated by aggressive anti-*pseudomonal* treatments[214,215]. Over time, 92% of children that have isolated non-mucoid pseudomonas develop mucoid strains (or resistant strains) by the age of 16 years which makes treatment difficult [136,216] and a life-threatening situation ensues[134].

A window of opportunity exists between first acquisition and when treatment becomes difficult (median of 10.9 years) to intervene by eradicating or suppressing this organism[217].

In addition to CF, suppurative lung diseases such as non-CF bronchiectasis and primary ciliary dyskinesia also have higher rates of recurrent exacerbations and higher mortality. [218-220]. The impact of chronic *P.aeruginosa* infection within the lung remains similar irrespective of the underlying diseases. [221]

Thus, the prospect of an adjunct or alternative to antibiotics to target this organism that may prevent treatment failure is essential and urgently required in paediatrics.

6A THE PROBLEM

Treating respiratory tract infection caused by *Pseudomonas aeruginosa* may require prolonged and repeated antibiotic therapy, which may have significant side effects such as sensorineural hearing loss and impaired renal function[137-139]. Additionally, there is the added risk of a change in the microbiota thus possibly allowing for opportunistic infections and a rise in resistant organisms[140,141].

An adjunct or alternative to antibiotics must be found to address these issues. Phage therapy offers a solution to this problem. At present, the only option of treatment lies within aggressive antibiotic use that may not

eradicate the organisms but may lead to antibiotic resistance or systemic complications.

6B THE PROPOSED SOLUTION

Bacteriophages (phages) are viruses that replicate within bacteria, causing highly selective bacterial cell death and reduced impact on our healthy microbiome compared with antibiotic therapy. In the face of increasing antimicrobial resistance and dwindling prospects for new antibiotics to be discovered, there has been a resurgence of interest in phage therapy[142,143]. However, such research has so far excluded children who potentially stand to gain the most from this treatment, if it is shown to be effective[144].

For patients with CF, phage therapy is highly attractive due to its relatively safe profile and its ability to potentially be utilised as a targeted inhaled aerosol therapy. Laboratory studies have shown the ability of phages to penetrate the biofilm layer of bacteria like *P. aeruginosa* which would be key in the treatment of patients with CF or other suppurative lung diseases.

6C EXISTING KNOWLEDGE AND EVIDENCE THAT INFLUENCED THE DESIGN OF THIS STUDY

1. Obligatory lytic bacteriophages have shown to be effective against different organisms causing life-threatening infections. Phages have been utilized in multiple n=1 studies under the magisterial[145-147] use in

multiple different countries.[148-151]. Multiple trials have also been conducted in Australia[151-153].

2. Bacteriophages have low natural toxicity as they target specific bacteria and is largely strain specific without disrupting the host normal flora or human cells[154]
3. Bacteriophages have been shown to reverse antibiotics resistance and restore susceptibility to various classes of antibiotics in Pseudomonas aeruginosa[155]
4. Bacteriophage has been used intravenously in the treatment of children with CF with no local infusion site reactions, anaphylaxis, seizures, abnormal vitals or gastrointestinal disturbances.[49]
5. Bacteriophages have been used in children in multiple situations with the youngest child being 2 years of age, treated with intravenous bacteriophage against Pseudomonas aeruginosa.[50]
6. Bacteriophages are a part of the human microbiome[157] and the environment[158], lytic bacteriophages are specific to a particular bacterial strain while some are known to affect multiple strains of the bacteria[159-161].
7. The microbiota has been shown to be less affected by bacteriophages when compared to antibiotics[162]. This has been shown in both murine and human studies (adults and children)[163,164]. A trial performed in children with diarrheal disease demonstrated that bacteriophages pass the alimentary tract without any change in the microbiome.[165]

8. Inhaled bacteriophages are the least effective in systemic absorption when compared to other methods of delivery [166,167].
9. Although the anatomy of the human airway and its' proximity to the alimentary tract may suggest contamination of the alimentary tract, studies performed in humans have shown that the contamination from tracheal instillation to the alimentary tract is negligible[168]
10. Phage are able to stimulate the adaptive immune response. These responses have been demonstrated to be either dose[52], duration[51-53] or route of administration dependent with topical, enteral and inhaled considered lower immunogenic compared to intravenous and intraperitoneal administrations.[169] In terms of the adaptive immune response, studies have shown that the main antibodies are IgG and IgM and their subtypes with IgE being understudied.[222,223]
11. As for the innate immune response, studies have shown that purified phages (as opposed to raw phage lysates) do not expose pathogen-associated molecular patterns [PAMPs] effectively and therefore are not able to induce an inflammatory response.[54,55,170]
12. Bacteriophages are used in the food industry to reduce bacterial load in food used for human consumption[171,172].
13. Bacteriophages that are non-GMO modified are used as a surrogate for viruses in FDA-approved aerosolization studies to evaluate the efficacy of viral filters[56].

14. Based on a review of 29 in-vivo animal model studies, no significant side effects were observed despite differing doses, route of administration and infection models.[173]
15. Bacteriophages have been shown to remain intact following nebulization in murine model[224-227].
16. Optimum dosing remains unclear, however, based on previous pre-clinical studies that include in-vitro and animal models have shown that the PFU/mL to achieve treatment effect is commonly between 10^6-10^{10}. [147,174-176]

6D EXISTING HUMAN TRIALS PREVIOUSLY PUBLISHED (ONLY RELEVANT INHALED ROUTE IS SHOWN)

Reference	Year	Indication	Etiology	Delivery method	Efficacy
Delacoste[186]	1959	Refractory coughs	N.A.	Inhalation	100%
Hoeflmayr[187]	1962	Bronchitis	Streptococci (2/3); Staphylococci (1/3)	Inhalation	90%
Garsevanishvili[188]	1974	Pneumonia	Staphylococci, streptococci, and enterics were targeted	Inhalation	N/A
Ioseliani *et al.* [189]	1980	Lung infections	Staphylococci and/or others	Inhalation, Bronchoscopy	>90%
Meladze *et al.*[190]	1982	Parenchyma and pleura infections	*Staphylococcus*	Inhalation, topical, parenteral	>90% (223)

Kvachadze *et al*.[191]	2011	Cystic fibrosis-associated infections	*Staphylococcus* and *Pseudomonas*	Inhalation	improvement

This table was adapted from Abedon et al 2015. These studies that have been cited may be in languages other than English and therefore are obtained from a summarised review article that has been published and has been cited 92 times [192].

6E RECENT CASE REPORTS OF CHILDREN WITH CF TREATED WITH BACTERIOPHAGE [169]

Reference	Case	Route of administration	Main safety outcomes
Lebeaux et al 2021[193]	12-year-old post lung transplant	Inhaled phage against *A. xylosoxidans*	Well tolerated
Gainey et al 2020[49]	10-year-old female	IV against *achromobacter*	No adverse events
Dedrick RM et al 2019[194]	15 year old	IV and topical	Weak phage-neutralisation antibody and cytokines. Diaphoresis and flushing without fever. No adverse reaction
Hoyle et al 2018[195]	17 year old	Inhaled and oral against *Achromobacter xylosoxidans*	No safety data, patient improved

Kvachadze *et al*.[191]	7 year old	Inhaled against *Pseudomonas aeruginosa*	No adverse events

6F EXPLANATION FOR CHOICE OF COMPARATORS

This is an open, single-arm, /II trial with no comparators. The participants act as their own controls as they have previously undergone *Pseudomonas aeruginosa* eradication therapy but remain chronically infected (as defined by the Leeds criteria)[177].

7. OBJECTIVES

HYPOTHESIS:

Bacteriophage (phage) therapy is a safe and well-tolerated adjunct in paediatric patients with with *Pseudomonas aeruginosa*.

RESEARCH QUESTIONS:

1. Are bronchoscopically instilled and nebulised bacteriophages safe, tolerable, easily administered and well-tolerated as part of a twice a day treatment regimen for 7 days?
2. Are bacteriophages that are delivered directly into the bronchial tree via bronchoscopy followed by nebulised bacteriophage therapy effective in reducing the colony forming units (CFU/mL) of *Pseudomonas aeruginosa* within the sputum*?*

STUDY OBJECTIVE

PRIMARY

To demonstrate tolerability and safety towards endo-bronchially instilled and nebulised bacteriophages against Pseudomonas aeruginosa in children..

SECONDARY

To assess the number of colony-forming units (CFU/mL) of *Pseudomonas aeruginosa* following treatment.

8. STUDY DESIGN

This trial is designed as an a Single-Arm, Open-Labelled, Non-Randomized, Safety, and Tolerability Study with a primary end point of tolerance towards endo-bronchially instilled initial bacteriophage dose followed by maintenance treatment via nebulisation.

METHODS: PARTICIPANTS, INTERVENTIONS, OUTCOMES

9. STUDY SETTING

This study will be performed in the Children's Hospital at Westmead and The Sydney Children's Hospital, Sydney, Australia (under the Sydney Children's Hospital Network). The Respiratory team currently treats 400 children with Cystic Fibrosis and provides support to a similar number of children with suppurative lung diseases. The study will be conducted initially in an in-patient setting for a duration of 7 inpatient days of bacteriophage treatment in addition to standard treatment (IV antibiotics, physiotherapy to be completed as inpatient for 10 to 14 days). Clinical reviews will be performed at 3, 6, 9 and 12 months following bacteriophage treatment.

10. ELIGIBILITY CRITERIA

10A INCLUSION CRITERIA:

6. Patients (≥6 years).
7. Positive sputum or bronchoalveolar lavage culture (Pseudomonas aeruginosa) in more than 50% of sputum samples over the past 1 year[177].
8. Continues to isolate Pseudomonas aeruginosa in sputum despite undergoing Pseudomonas aeruginosa eradication therapy using two

anti-pseudomonal antibiotics and/or is currently on suppressive treatment

9. The latest clinical isolates of Pseudomonas aeruginosa taken within 3 months of enrolment are susceptible to (demonstrates lytic activity) available anti-Pseudomonas aeruginosa bacteriophages.

10. Ability to perform spirometry[90].

10B EXCLUSION CRITERIA:

10. Patients who have received more than 1mg/kg of Prednisolone continuously for more than 7 days before study enrolment.
11. Patients that require ≥18 hours/day of non-invasive ventilatory (NIV) support during admission.
12. A current diagnosis of Allergic bronchopulmonary aspergillosis (ABPA).
13. History of hemoptysis in the past 12 months prior to study enrolment.
14. Prior known inability to expectorate sputum.
15. Has undergone solid organ transplantation
16. Positive sputum isolation of *Burkholderia cepacia* within the past 1 year.
17. Treatment of non-tuberculous *mycobacterium* within the past 1 year.
18. A positive COVID-19 PCR nasal swab with a confirmed COVID-19 infection (true positive) in the past 6 months prior to enrolment[178].

19. Within 3 months of having received a booster COVID-19 vaccine or within 6 months of receiving the first dose of vaccine[179].

11. INTERVENTIONS

11A INTERVENTION WILL BE PERFORMED BY:

1. Bronchoscopy will be performed by Dr Jagdev Singh and/or Professor Hiran Selvadurai
2. Sputum collection will be performed by the nursing members of the Respiratory multidisciplinary team.
3. Lung function (Spirometry) will be performed by the Respiratory Scientist in the Respiratory Laboratory, The Children's Hospital with Dr Jagdev Singh and/or Prof Selvadurai in attendance)
4. Administration of nebulised solution in the ward will be performed by the nurse that is looking after the study participant during admission. An in-service will be conducted prior to the commencement of this trial.
5. A pharmacist will be appointed to ensure labelling, supply and the correct volume of phage is provided.

11B INTERVENTIONS

This is an open-label, single-arm study of bacteriophage that has demonstrated obligate lytic activity against each of the study participants' *Pseudomonas aeruginosa*. This selected bacteriophage will be prepared according to established protocols (refer to appendix 3) and instilled

endo-bronchially using an appropriately sized bronchoscope (refer to appendix 4).

The bacteriophages will be obtained through Professor Jonathan Iredell's Phage Bank in the Westmead Institute of Medical Research. Any master stock phage that is used for this study (particularly if obtained from outside of the above-mentioned phage bank will still require to undergo the same requirement and process as stipulated in appendix 3).

The preparation and testing of the phages will be performed in the Iredell's Phage Laboratory in the Westmead Institute of Medical Research. Sterile transfer of the final lysate into sterile vials will be conducted in the sterile medication preparation room in the pharmacy of the Children's Hospital at Westmead.

Phages that exceed titres of $\geq 10^8$ PFU/mL when tested using the participant's own bacterial isolate will be selected. Bacterial isolate obtained from participants will be tested using spot plaque testing. Phages selected must not only have lytic activity as demonstrated by the plaque formation but will need to be $\geq 10^8$ PFU/mL to be considered for therapy.

As for the participants' bacterial strain, genomics will be performed on the initial bacterial isolate used to perform spot testing on available phages, BAL isolates at the start and at the end of treatment (if pseudomonas continues to be isolated). Each participant will be treated with a bespoke bacteriophage. This trial has an element of compassionate/magisterial[146]

use as these participants have previously been treated with standard treatment available to treat the infection and continue to isolate *Pseudomonas aeruginosa*.

All sputum cultures that continue to isolate *Pseudomonas* (as per the intervention schedule in figure 3) will undergo titration study (spot plaque testing). The phage solution will undergo regular monitoring throughout the entire study following processes that may reduce the phage titres. Examples of steps that may cause a reduction in phage titres include litre scale cultivation, filtration of phage solution, following exposure to medications (mannitol, dornase alfa and nebulised antibiotics), nebulisation and storage process. Please refer algorithm for phage preparation, sterilisation and stock monitoring. (Appendix 1)

A step by step protocol for isolation, purification and packaging is attached in appendix 3

In order to test these bespoke phage solutions, any nebulised medication that the participant is on during the trial will be tested against the selected phage prior to commencing treatment to ensure stability and maintenance of potency. A time-kill curve will be performed before and after in vitro exposure of the medications and phage solution. Additionally, phage titre will be assessed following the addition of these medications to ensure titres remain high ($\geq 10^8$ PFU/mL).

Nebuliser used in this study will be a TGA-approved mesh nebuliser. In order to confirm the viability of the phage-nebuliser pairing, all candidate

phage will undergo testing to ensure titres remain ≥10^8 PFU/mL prior to administration. Candidate phage will be nebulised and the nebulised aerosol will be collected and phage titration (spot plaque testing will be performed). (as per the viability testing of bacteriophage in appendix 1).

The dose of phage administered will be 10^8 PFU/mL of a single individually tailored phage (bespoke), diluted as appropriate in Pharmacy. Primary packaging of phage solution that is sterile will be in sealed sterile glass flasks. This flask will then be transported to a sterile medication preparation room (in the pharmacy of the Children's Hospital at Westmead). The solution (1mL of 1x10^8 PFU/mL of phage and added with 3ml of sterile normal saline) will be decanted into non-pyrogenic sterile Type 1 glass vials and sealed. These vials will be stored in a 4°C fridge and labelled. Solution from the vial will be transferred into the nebuliser chamber using a sterile syringe and diluted with sterile saline. (phage packaging details is attached in appendix 3)

Bronchoscopy will be performed using 1 mL/kg of lavage fluid of normal saline 0.9% will be prepared in total to obtain lavage fluid for microbiology and to aid the bronchoscopy. The remaining 1 mL/kg will be made up of 0.9% normal saline with 8 mL of phage solution (equivalent to 2x10^8 PFU in the total solution or 2 sterile vials of phage solution that contain 1x10^8 PFU/4mL), therefore 2mL/kg of fluid used during the bronchoscopy[228]. The final solution of 1 mL/kg of phage and saline

solution will be instilled in equal aliquots in all five lobes of the lung. This translates to 0.2mL/kg in each lobe.

A step by step protocol for bronchoscopic instillation is attached in appendix 4

The subsequent bacteriophage dose will be administered via nebulisation. Phage nebulisation must occur after physiotherapy sessions and/or nebulised mannitol and/or nebulised hypertonic saline and/or nebulised dornase alfa and/or nebulised antibiotics

The nebulised solution will contain 1 $x10^8$ PFU/4mL and be administered twice daily. The ideal timing of phage dosing should be 12 hours apart (range 10 to 14 hours)

A step by step protocol for ward nebulisation is attached in appendix 5

Therapy for intravenous anti pseudomonal antibiotics will be based on the sensitivity of the most recent *Pseudomonas aeruginosa* isolate and current dosing and guideline as per the Children's Hospital Westmead treatment protocol and is as follows:

Drug	Dosage	Route	Frequency
Tobramycin	10mg/kg/dose (for CF patients) and 7.5 mg/kg/dose (for non CF patients)	IV	Daily

	Then adjusted as per AUC taken following dose 3.		
Plus the following; *Note:* *Tobramycin is given in combination with one of those listed below. Used only after discussion with the consultant when the patient has multi-resistant Pseudomonas or poor clinical response to the 1st line therapy.* *Two non-aminoglycosides would be considered from those listed below if therapies are ineffective.*			
Ceftazidime *preferred	50mg/kg/dose (max 2g)	IV	TDS
Tazocin (piperacillin and tazobactam)	100-150 mg/kg (Max 4g)	IV	QID
Meropenem	40mg/kg/dose (max 2g)	IV	TDS
Cefepime	50 mg/kg (max 2g)	IV	TDS

Imipenem and cilastatin	25mg/kg/dose (max 1g)	IV	QID
Aztreonam	50mg/kg/dose (max 2g)	IV	TDS
Ciprofloxacin	10-15mg/kg (max 400mg)	IV	TDS

11C POTENTIAL ADVERSE EFFECTS AND MODIFICATION

TEMPORALLY RELATED FEVER EVENTS > 38.5C

It is expected that 20% of children undergoing bronchoscopy will experience fever (≥38.5°C) within the first 6-8 hours following the procedure[229]. If the study participant develops fever following administration of either endobronchial or nebulised phage, routine care should be given based on the clinical condition of the study participant which includes a clinical review based on the *between the flags* [230] guidelines.

Blood culture must be performed with interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), full blood count (FBC), liver function test (LFT) and electrolyte, immunoglobulin G, A and M, urea and creatinine (EUC) and C-reactive protein (CRP) if fever develops within 1 hour of administration of bacteriophage.

If fever ≥38.5°C with no other clear source of infection occurs 3 consecutive times within 1 hour of each administration of nebulised phage or 3 episodes of fever over 48 hours within 1 hour of each administration of nebulised phage, (temporally related fever events) a repeat sputum sample for microbiology culture, TNF-α and IL-6, Immunoglobulin G, A, M, FBC, EUC, LFT, CRP and blood culture will be performed.

The persistent fever will be reported as an adverse event and a Suspected Unexpected Serious Adverse Event (SUSAR) will be completed by Dr Singh with notifications to the (1) Chairman of the Study Steering Committee (SSC), (2) Chairman of the Data Safety Monitoring Board DSMB, (3) Representative of the Research Governance Office (RGO) within 72 hours using the SCHN SUSAR form. Further administration of bacteriophage will be ceased.

A step by step protocol in the management of fever during the trial is described in appendix 6

ALLERGY REACTION TOWARDS ADMINISTERED BACTERIOPHAGE SOLUTION

An allergic reaction may occur within 1 hour of administration of bacteriophage. A mild to moderate allergy reaction (as per ASCIA guideline) is defined as swelling of lips, face, eyes; hives or welts; or tingling mouth.

A severe reaction (or anaphylaxis) is defined as difficult or noisy breathing; swelling of tongue; swelling or tightness in throat; difficulty talking or hoarse voice; wheeze or persistent cough - unlike the cough in asthma, the onset of coughing during anaphylaxis is usually sudden; persistent dizziness or collapse; pale and floppy (young children); abdominal pain, vomiting.

If any of the severe symptoms occur during nebulisation, the nebulisation must be stopped immediately.

A severe allergy (anaphylaxis) will be reported as an adverse event and a Suspected Unexpected Serious Adverse Event (SUSAR) will be completed by Dr Singh with notifications to the (1) Chairman of the Study Steering Committee (SSC), (2) Chairman of the Data Safety Monitoring Board DSMB, (3) Representative of the Research Governance Office (RGO) within 72 hours using the SCHN SUSAR form. Further administration of bacteriophage will be ceased.

A step by step protocol in the management of allergic reactions during the trial is described in appendix 6

BRONCHOSPASM OBSERVED DURING FIRST ADMINISTRATION OF THE NEBULISED BACTERIOPHAGE BY MEANS OF SPIROMETRY

Each participant will undergo spirometry to assess for any evidence of bronchospasm following the first nebulised administration of

bacteriophage. This will be performed in the presence of Dr Singh or Prof Selvadurai.

The results will be interpreted as follows;

a. If lung function (FEV_1%) decline is less than 10% from baseline, the study participant is considered to not have bronchospasm and can have subsequent doses of nebulised bacteriophage.

b. If lung function decline is between 10 to < 15%, 6 puff of MDI Ventolin through a spacer will be delivered and lung function repeated, if change from baseline is now less than 10%, the study participant will be prescribed 6 puffs of Ventolin pre each nebulisation of phage solution over the course of the study (remaining 11 doses)

c. If lung function decline is >15%, the study participant will be given 6 puffs of Ventolin and lung function repeated, if change from baseline is now less than 10%, the study participant will be prescribed 6 puffs of Ventolin pre each nebulisation of phage solution over the course of the study (remaining 11 doses)

d. If lung function in (b) and (c) does not improve to <10% from baseline, the study participant will not receive the second dose on the same day.

e. A repeat medication tolerance test will be repeated on the following day using a diluted phage solution (dilute 2mL of the

prepared phage solution with 2mL of sterile normal saline, and the test is repeated. The decision will be made as per (a), (b) and (c) using this diluted phage sample. (remaining 10 doses).

f. If dose dilution is required, this will be updated in the medication chart and each vial containing 4mL of phage solution will need to be diluted with 2mL of sterile normal saline prior to administration as per appendix 5

g. If (d) occurs again despite the reduction of dose, further administration will be discontinued.

Severe bronchospasm (a decline of FEV_1% of >10%) occurring despite a reduction of dosing will be reported as an adverse event and a Suspected Unexpected Serious Adverse Event (SUSAR) will be completed by Dr Singh with notifications to the (1) Chairman of the Study Steering Committee (SSC), (2) Chairman of the Data Safety Monitoring Board DSMB, (3) Representative of the Research Governance office (RGO) within 72 hours using the SCHN SUSAR form. Further administration of bacteriophage will be ceased.

A detailed protocol of first administration of nebulised bacteriophage is detailed in appendix 7

Adverse event type and notification steps		
Events	**Who reports**	**How and When**

Adverse event (as enumerated above) *Any untoward medical occurrence in the participant administered a medicinal product and that does not necessarily have a causal relationship with this treatment.*	PI	As soon as possible and no later than 72 hours of the PI becoming aware of the adverse event. Report to Research Governance Office Email notification to Chair of TSC and DSMB
Suspected Unexpected Serious Adverse Reaction (SUSAR) *An adverse reaction that is both serious and unexpected.*	PI	As soon as possible and no later than 72 hours of the PI becoming aware of the adverse event. Report to Research Governance Office using SUSAR notification form

		Email notification to Chair of TSC and DSMB
Unexpected & Related SAEs (URSAE) *An adverse event that is:* ***Serious*** *– meets the definition of a serious adverse event* ***Related*** *– resulted from administration of the trial intervention* ***Unexpected*** *– the event is not described in the protocol as an expected occurrence.*	PI	As soon as possible and no later than 72 hours of the PI becoming aware of the adverse event. Report to Research Governance Office using URSAE notification form Email notification to Chair of TSC and DSMB

**Adapted from Sydney Local Health District Research and Ethics Governance Office Safety Reporting guideline.*

DEVELOPMENT OF BACTERIAL RESISTANCE AGAINST BACTERIOPHAGE

Sputum collected from study participants who continue to have growth of *Pseudomonas aeruginosa* will undergo phage-bacterial isolate testing to evaluate the presence of a lytic reaction. If no lytic reaction is observed, a new phage that is "obligately lytic" to the bacterial isolate will be prescribed to replace the initial phage used.

11D INDICATIONS TO STOP TREATMENT ON THE PARTICIPANT

5. Report of fevers > 38.5°C that fit the adverse event criteria stated above
6. Severe allergy within 1 hour of the administration towards the nebulised liquid that requires nebulised ventolin (x 3, 20 minutely) or intramuscular adrenaline.
7. Severe bronchospasm despite a reduction in the dosing of the nebulised bacteriophage and pre-treatment inhaled salbutamol is administered
8. Any unexpected side effect that is deemed to be significant by the TSC/ DMSB or Principal Investigator.

11E CONCOMITANT CARE

There are no barriers to concomitant care throughout the entire study. The participant is allowed to have any CF or other medically related treatment or antibiotics.

There are no restrictions towards the administration of any other medications in the event of an expected or unexpected side effect, which

includes but is not limited to resuscitation medications, antihistaminics, laxatives, antipyretics or antibiotics.

If the study participant is prescribed more than 1mg/kg of prednisolone or any parenteral/enteral corticosteroids for more than 7 days a sputum culture, TNF-α, IL-6 must be repeated on day 7 of the corticosteroid treatment.

12. OUTCOMES

PRIMARY OUTCOMES (SAFETY AND TOLERANCE)

Treatment success will be defined as the **absence** of the following adverse events:

3. Fever > 38.5°C over 3 consecutive temporally related administration or 3 episodes of temporally related fever above 38.5°C over 48 hours following administration of treatment. Temporally related fever is defined as fever above 38.5°C occurring within 1 hour of the administration of the nebulised bacteriophage.
4. Bronchospasm defined at 15% decline in FEV1% measure pre and post administration of first dose of nebulised bacteriophage despite a reduction of dosing and pre-treatment with inhaled salbutamol[185].

SECONDARY OUTCOMES

A reduction in sputum bacterial culture titres of *Pseudomonas aeruginosa* (CFU/mL) from pre-treatment to day 7 of treatment.

13. PARTICIPANT TIMELINE

The timeline of this study and the intervention schedule is enumerated in Appendix 2

14. RECRUITMENT

The estimated sample size in this study is up to 10 participants. The rationale for this number has been decided primarily on practical considerations. If all 10 participants achieve a treatment response (as defined in section 11) then the 95% confidence interval for response rate will be 72-100%.

Recruitment into this study will be extended to all patients with cystic fibrosis under the care of the Children Hospital at Westmead and Sydney Children's Hospital, Randwick (SCHN) who fulfill the criteria for this study.

Potential study participants that meet the inclusion and exclusion criteria will be selected by Dr Singh. These potential participants will be discussed for suitability in the weekly departmental CF meeting. This is to ensure that clinicians (respiratory physicians, CF clinical nurses, physiotherapists and dieticians) involved in the care of the participant will be able to provide input about the suitability of the potential participant in the trial. If the participants are deemed suitable based on the criteria, Dr Singh will arrange for a discussion with the participant and their guardian or

participant (if ≥18). (This request was made in view of some of our CF patients have not been transitioned to the adult services but were admitted for treatment and missed the opportunity of obtaining this treatment as they were over 18 years of age). For participants who are over 18 years of age and have been transitioned, a joint discussion will be made with the patients current respiratory (adult) physician about the feasibility of being part of this trial. A separate ethics approval will need to be organised as this would need to consider the site, the need for anaesthesia and the clinicians involved.

The enrolment period will be 36 months or until the sample size is achieved, whichever comes first. As part of the trial, participants will be staggered on a 6 weekly duration. For example; participant 1 will undergo 2 weeks of treatment (7 days bacteriophage treatment with a total of 10 to 14 days of inpatient care) and will continue to progress following the scheduled study review. A DSMB and TSC meeting will review the outcome of each participant before progressing to the next participant.

Participant 2 will commence trial after participant 1 has completed 2 weeks of treatment and the DSMB and TSC are satisfied with the safety outcomes and conduct of the trial of the preceding participant.

15. DATA COLLECTION METHODS

15A DATA COLLECTION METHODS

Results will be transcribed onto a Data collection sheet that will be de-identified and stored under a password-protected file. The data will be available for review by the TSC and DSMB at the stipulated time points as per the individual charters. Data points collected will be standardized using;

5. Phage identification, isolation, purification, viability testing and sterility data template (refer to appendix)
6. Bronchoscopy report
7. Lung function indices and anthropometric data (weight and height) will be obtained from standardized measurements and report available on the study participants' electronic medical records.
8. Blood test results will be obtained from standardized measurements and report available on the study participants' electronic medical records.

A checklist and data sheet will be provided in the participants file during the treatment.

15B RETENTION

No monetary remuneration will be provided for this study. Research data of participants that withdraw from the study will be used up to the date of withdrawal, following which only routine clinical sputum collection data will be reviewed.

15C DATA MANAGEMENT

All data will be entered electronically into the study participants' electronic medical notes. Data entry will be performed by Dr Singh into a data collection sheet. A data collection form that contains non-identifiable data will be used.

The data will be stored as per the SCHN data retention policy for clinical trials which requires for the data to be stored for 15 years or until the youngest participant turns 25 years of age.

16. STATISTICAL METHODS

16A OUTCOME

Participant demographic and clinical characteristics and study outcomes will be presented using standard descriptive statistics: mean/median and range for continuous variables and frequency and percentages for categorical variables.

The primary outcome of the proportion of participants who achieve a treatment response will be presented with an exact 95% confidence interval. The secondary outcome of change in sputum bacterial titres will be described by mean/median and range.

For secondary end-point continuous data will be reported using the mean/median range.

16B ADDITIONAL ANALYSES

Additional statistical methods may be employed and will be mentioned separately when used during publication.

16C ANALYSIS POPULATION AND MISSING DATA

Data of study participants that drop out of the study will be used up to the last available data point before the drop-out date.

DATA MONITORING

17A FORMAL COMMITTEE

There will be 2 committees that will be convened for the purpose of this trial

1. The Trial Steering Committee (TSC)
2. The Data and Safety Management Board (DSMB)

Each committee has its' own charter as per appendix 6 and appendix 7 respectively.

17B INTERIM ANALYSIS

Safety data will be monitored regularly by both the TSC and DSMB as outlined within their respective charters. This will be based on the

enrolment of each study participant (before administering the bacteriophage and a the end of admission) (appendix 6 and 7)

18. HARMS

All adverse events occurring after the informed consent document is signed (entry) but the study participant has not started to receive study intervention will not be recorded. All adverse events occurring after receiving the first dose of phage via endobronchial instillation and until 72 hours following the cessation of the last nebulised phage will be recorded.

Suspected Unexpected Serious Adverse Event (SUSAR) will be completed by Dr Singh with notifications to the (1) Chairman of the Study Steering Committee (SSC), (2) Chairman of the Data Safety Monitoring Board DSMB, (3) Representative of the Research Governance Office (RGO) within 72 hours using the SCHN SUSAR form.

19. AUDITING

Auditing of record keeping and adherence to protocol will be based on the stipulation by the Sydney Children's Hospital Network Ethics Committee. Safety data, record keeping and adherence to the protocol will be monitored regularly by both the TSC and DSMB as outlined within their respective charters (appendix 6 and 7)

ETHICS AND DISSEMINATION

20. RESEARCH ETHICS APPROVAL

This protocol, informed consent form, participant information sheet(s), and data record template(s) contained in the appendix of this document will be reviewed and will require approval by the Sydney Children Hospital Network Ethics Committee with respect to scientific content and compliance with applicable research and human study participants regulations before commencement.

21. PROTOCOL AMENDMENTS

Protocol amendments can be suggested by the TSC, DSMB as per their respective charters. Protocol amendment(s) will be subject to a review and approval by the Sydney Children Hospital Network Ethics Committee with respect to scientific content and compliance with applicable research and human study participants regulations.

22. CONSENT

22A CONSENT

Consent for enrolment into this trial will be obtained by Dr Jagdev Singh as a principal investigator to ensure a standardised approach. If a participant is <18, a parent information sheet will be provided to the family. A young person and child information sheet has also been provided in age-appropriate language. The consent will include consent for ancillary studies as well. If a participant is ≥18, they will be provided with a participant information sheet.

Copy of the signed page of the consent form will be uploaded into the patient's electronic medical record.

In the unlikely event that a participant/participant's family do not understand English, a professional medical interpreter will be used to translate the consent document into their preferred language and the consent will be taken in the presence of the interpreter. The interpreter service that will be used is the service that is linked to SCHN.

22B ANCILLARY STUDIES

Ancillary studies of samples collected and biological specimens for future studies will be undertaken and will be stipulated in the consent and information sheet.

23. CONFIDENTIALITY

Confidentiality will be ensured using data format and storage arrangements that are de-identified, appropriate and protect the participant's privacy.

24. DECLARATION OF INTERESTS

The principal investigators for the overall trial have no conflict of interests to declare.

25. ACCESS TO DATA

As this is a single-centered (SCHN) study, data will be made available when requested for auditing by The Sydney Children Hospital Network Ethics Committee.

26. ANCILLARY AND POST-TRAIL CARE

Ancillary and post-trial care will be provided by the Sydney Children Hospital Network.

27. DISSEMINATION POLICY

27A TRIAL RESULTS

The trial result will be disseminated through publication (s) obtained from the data of this clinical trial. Cleaned data, complete protocol may be submitted for review to assist with publication.

27B AUTHORSHIP

The PI [Dr Jagdev Singh] will be the lead author of this trial. Ancillary studies will include Dr Singh but will be subject to authorship guidelines (International Committee of Medical Journal Editors, ICMJE).

27C REPRODUCIBLE RESEARCH

The trial protocol can be provided (if requested) to journals for purpose of publication of the trial results.

28. BIOLOGICAL SPECIMENS

BRONCHOALVEOLAR LAVAGE AND SPUTUM SAMPLE

The tests that will be conducted on the samples will include; Culture and sensitivity, strain analysis IL-6 and TNFA, RNA nanostring study, Bacterial population diversity (16S rRNA transcriptomic analysis), cytology (for bronchoalveolar only), Immunoglobulin G,A,M, phage neutralizing antibodies,

BLOOD SAMPLE

The tests that will be conducted on the samples will include; FBC,EUC,LFT,CRP, Blood culture, IL-6, TNFα, Immunoglobulin G,A,M, RNA nanonstring study, phage neutralizing antibodies.

Lavage, sputum and blood samples will be stored following the finalisation of the respective test results. Results from these samples (de-identified) will be used for ancillary studies. Study participant DNA studies will not be evaluated in this trial or any other ancillary studies.

STOOL SAMPLE

Bacterial population diversity (16S rRNA transcriptomic analysis) before first administration and after last administration.

All biological samples derived from this study will be stored for potential future ancillary studies in the NSW biobanking facility under the care of

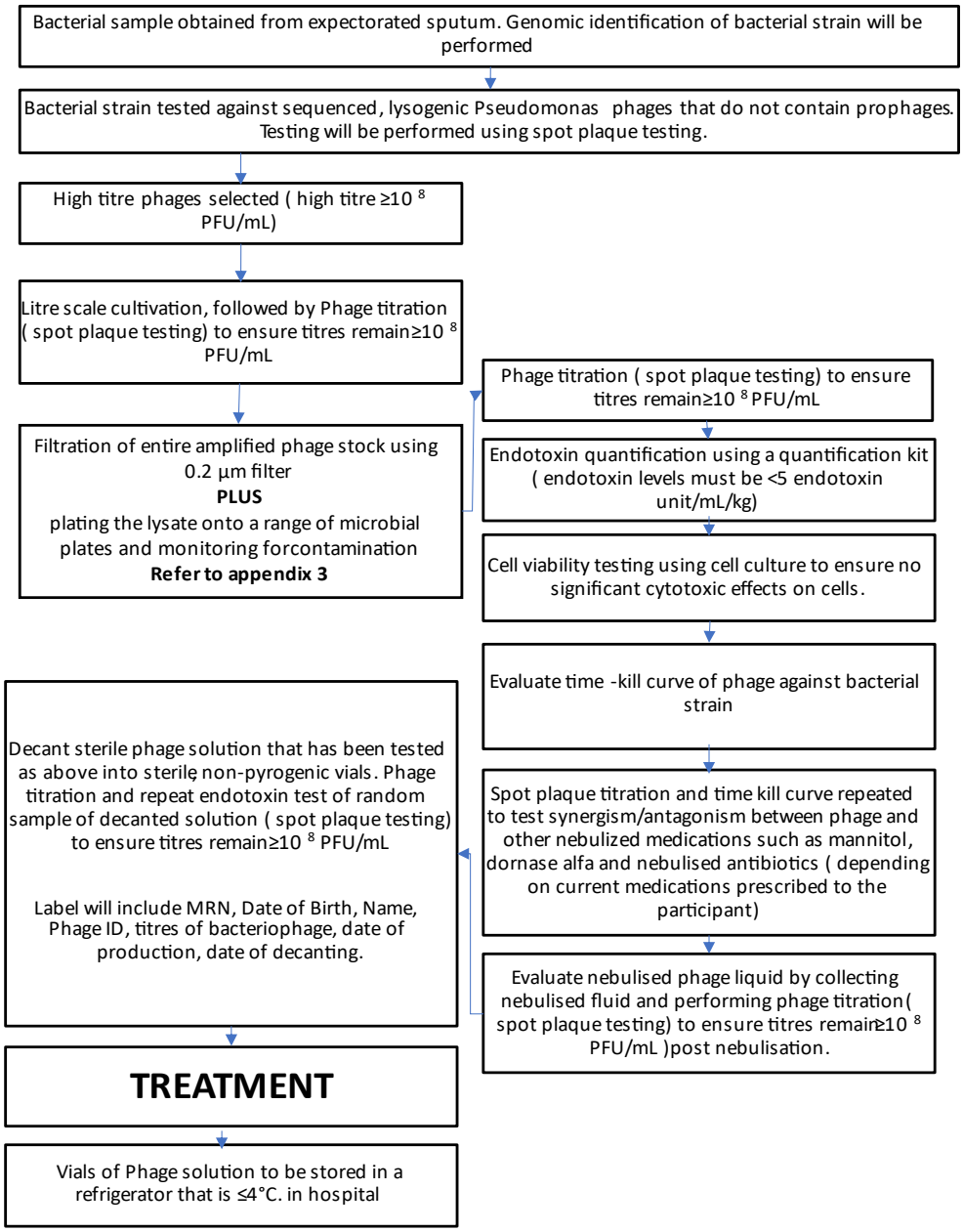
Prof Hiran Selvadurai. (the use of these samples will be subject to the consent as per the consent form)

29. SERIOUS BREACHES

Serious Breaches will be reported to the Research Governance Officer within 72 hours of any serious breach occurring at the site. An email will be sent to the Chair of the TSC and the DSMB and decisions will be subject to the discussions as per stipulate in their respective charters.

APPENDICES

1 SUMMARY OF BACTERIOPHAGE PROCESSING


Bacterial sample obtained from expectorated sputum. Genomic identification of bacterial strain will be performed
Bacterial strain tested against sequenced, lysogenic Pseudomonas phages that do not contain prophages. Testing will be performed using spot plaque testing.
High titre phages selected (high titre ≥10 8 PFU/mL)
Litre scale cultivation, followed by Phage titration (spot plaque testing) to ensure titres remain≥10 8 PFU/mL
Filtration of entire amplified phage stock using 0.2 µm filter
PLUS
plating the lysate onto a range of microbial plates and monitoring forcontamination
Refer to appendix 3
Phage titration (spot plaque testing) to ensure titres remain≥10 8 PFU/mL
Endotoxin quantification using a quantification kit (endotoxin levels must be <5 endotoxin unit/mL/kg)
Cell viability testing using cell culture to ensure no significant cytotoxic effects on cells.
Evaluate time -kill curve of phage against bacterial strain
Spot plaque titration and time kill curve repeated to test synergism/antagonism between phage and other nebulized medications such as mannitol, dornase alfa and nebulised antibiotics (depending on current medications prescribed to the participant)
Evaluate nebulised phage liquid by collecting nebulised fluid and performing phage titration(spot plaque testing) to ensure titres remain≥10 8 PFU/mL)post nebulisation.
Decant sterile phage solution that has been tested as above into sterile non-pyrogenic vials. Phage titration and repeat endotoxin test of random sample of decanted solution (spot plaque testing) to ensure titres remain≥10 8 PFU/mL
Label will include MRN, Date of Birth, Name, Phage ID, titres of bacteriophage, date of production, date of decanting.
TREATMENT
Vials of Phage solution to be stored in a refrigerator that is ≤4°C. in hospital

2 STUDY TIMELINE AND INTERVENTION SCHEDULE

TIMEPOINT	Pre-admission	Day 0	Day 1	Day 2	Day 3-6	Day 7	Day 8 to 9 * or up to 13	Discharge (day 10-14)	Week 3	Month 3	Month 6	Month 9	Month 12
Eligibility screen	X												
Informed consent	X												
Bronchoscopy and instillation		X											

of bacteriophage													
Nebulization of bacteriophage			X	X	X	X							
Standard IV		X	X	X	X	X	X	X					
Physiotherapy		X	X	X	X	X	X	X					
Sputum collection	X		X@			X		X		X	X	X	X
Anthropometry	X		X			X		X			X	X	X
Spirometry	X		X*			X		X		X		X	X
Blood investigation**	X			X				X					
Clinical review	X	Xa	X	X	X	X	X	X			X	X	X

Discharge review (telephone)									X				

Timetable of schedule, footnote: [@]= sputum on day 1 to be performed before first dose of nebulised bacteriophage, *= first spirometry will be based on procedure outlined in appendix 7, **= FBC,EUC,LFT,CRP, Blood culture, IL-6, TNFα, Immunoglobulin G,A,M,RNA nanonstring study, neutralizing antibodies. Blood investigations are timed to coincide with standard blood taking practices; admission, day 2 to review tobramycin level and day 14 during removal of the central line. [a]= This clinical review will be conducted before performing the bronchoscopy

3 BACTERIOPHAGE PURIFICATION PROTOCOL

1 OVERVIEW

As a type of virus, phages can reproduce only within a bacterial cell[231]. Therefore, a major hurdle to creating medicinal phages is the separation of phages from bacterial cell debris such as

1. endotoxins (i.e., lipopolysaccharides (LPS))
2. peptidoglycan
3. exotoxins
4. flagella
5. nucleic acids
6. other compounds.

If not adequately removed, these gross impurities could trigger potentially life-threatening inflammation, sepsis, and septic shock in humans [232-234]. To date, the major safety concern with phage products for human use has been endotoxin content[235-237]. Indeed, phage preparations can be safe in animal models [206,238-240].

This protocol has been adapted from;

1 Standardised bacteriophage purification for personalised phage therapy, Luong et al 2020[180], published in nature protocols (impact factor 14).

2 The test for sterility has been adapted from the Forty-sixth WHO Expert Committee on Specification for Pharmaceutical Preparations in October 2011 and the 4th Edition of the International Pharmacopoeia. [181]

3 A further test for sterility guidelines published by the Therapeutic Goods Administration (TGA) as part of the TGA guidelines for sterility testing of the therapeutic group has been included [182]

4 Packaging selection is based on Guidance for industry, Container Closure Systems for packaging human drugs and biologics by Food and Drug Administration (FDA). [183]

5 Nasal Spray and Inhalation Solution, Suspension, and Spray Drug Products--Chemistry, Manufacturing, and Controls Documentation; guidance for industry [184]

2 REAGENT PREPARATION

All solutions will be prepared with sterile double-distilled water (ddH_2O) and stored at room temperature (RT; 20–25 °C) for up to 3 months.

Liquid growth medium

A mixture of 10 g of tryptone, 5 g of yeast extract and 5 g of NaCl in 1 L of ultrapure ddH_2O. Autoclave.

Solid growth medium

Add 14 g of agar to the growth medium and autoclave. Then, cool to 55 °C in a water bath for 30 min–1 h. Sterilely pour solid growth medium

into sterile Petri plates. The thickness should be 5–10 mm. Let cool at RT and store at 4 °C for up to 2 weeks.

Molten soft agar

A mixture of 7 g of agar to growth medium and autoclave. Then, cool to 55 °C in a water bath to prevent agar solidification before plating. Make fresh.

Tris-sodium chloride (TN) buffer

Mix 10 mM Tris (pH 7.0) and 150 mM NaCl. Adjust to pH 7.0. Autoclave and store at 4 °C for up to 3 months.

Tris-EDTA buffer

Mix 10 mM Tris (pH 7.0) and 1 mM EDTA (pH 8.0). Adjust to pH 7.5.

10× Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) running buffer Mix 30.0 g of Tris base, 144.0 g of glycine and 10.0 g of SDS in 1 L of ddH_2O. Make fresh.

Nuclease solution

Mix 20 mg·mL−1 of DNase I and 20 mg·mL−1 of RNase A. Store at −20 °C for up to 30 months.

Precipitant solution (30% (wt/vol) PEG-8000 and 3 M NaCl)

In a sterile bottle, add 110 ml of ddH2O and 35 g of NaCl and dissolve completely. Add 60 g of PEG-8000, cap bottle and shake. Incubate bottle

in a 50–60 °C water bath for ~2 h, shaking occasionally (at this point, it is normal for the solution to be turbid and separated into two phases). Remove and let cool to RT, shaking occasionally. The solution should be clear or slightly turbid. Dilute to 200 mL with ddH2O.

Resuspension buffer (5 mM MgSO4)

Add 0.0123 g of MgSO4 heptahydrate per 10 mL of ddH_2O

STEP WISE PROCEDURE

SELECTION OF BACTERIOPHAGE FROM PRE-EXISTING LIBRARY

1. Phage samples will be obtained through Iredell Laboratory, Westmead Institute of Medical Research (WIMR).
2. The origin of the bacteriophage sample, PFU/mL, date of isolation, medium of isolation will be recorded. Only bacteriophages that have undergone genomic identification will be considered. The bioinformatics data will be reviewed independently.
3. An independent review of the FASTA file will be repeated by uploading the genome to PHACTS to predict whether it is a virulent or temperate virus. Temperate bacteriophages will be excluded.
4. Genome will be uploaded to PATRIC (https://patricbrc.org/) to annotate other genes in the genome.

5. Bacteriophages selected for large-scale production based on the lack of predicted antibiotic and virulence genes and the likelihood that the isolated phage is virulent (i.e., strictly lytic lifecycle).
6. In the event that no bacteriophage is suitable, environmental phage will be isolated based on the attached isolation protocol.

OBTAINING LYSIS

7. Growth agar Petri plate will be dried for 30 min in a biosafety cabinet. These petri plates will be labeled with the date of preparation, participants' unique study ID, and the phage ID.
8. To seed the lawn, 3 mL of the participants' *Pseudomonas aeruginosa* grown to OD600 0.2 will be poured onto the dry growth agar Petri plate.
9. Petri plate will be dried in a biosafety cabinet for 15 min.
10. While drying, 90 µL of PBS will be added to eight wells row-by-row in a 96-well microtiter plate.
11. 10 µL of phage sample selected (steps 1 to 6) will be added to the first well and mixed well.
12. 10 µL will be pipetted out from the first well into the second well and mixed well.
13. Step (12) will be repeated for the remaining wells to create a dilution series (10^{-1} to 10^{-8}).
14. 4 µL from each well will be spotted using an 8-channel pipette onto a dried seeded lawn of bacteria prepared in Steps 7 to 9.

15. The plate(s) will be incubated at 37°C and atmospheric growth conditions 12–18 h or until plaques form on a confluent lawn of bacteria.
16. If plaques are formed, bacteriophage stock concentration will be determined as PFUs·mL−1 (PFU per ml = plaques per plate × volume plated in ml × dilution factor)
17. If no plaques are found, steps 7 to 15 will be repeated with a different bacteriophage sample as per steps 1 to 6.

SMALL SCALE CULTIVATION

18. 1 PFU will be selected using a Pasteur pipette, selected and re-suspended in 100 µL of PBS in a microcentrifuge tube.
19. 50 mL of growth medium will be warmed in a sterile 250-mL GL45 screw-top flask with a GL45 0.22-µm PTFE membrane vented cap.
20. 500 µL of bacteria grown to OD600 0.2 will be added and incubated at 37°C and
atmospheric growth conditions for 20 min.
21. 50 µL of phage obtained in Step 20 will be added and incubated under appropriate bacterial growth conditions for 12–18 h.
22. Lysates will be transferred to 50-mL conical centrifuge tubes and centrifuge at 6,000g for 30 min at 4 °C to remove bulk bacterial debris.

23. The supernatant will be filter sterilized with a 0.2-µm syringe filter into a sterile 50-mL conical tube.
24. The sample obtained will be titred as described in Steps 7 to 15.

LITER-SCALE SHAKE FLASK CULTIVATION

25. In a sterile 2-L GL45 screw-top flask with a GL45 0.22-µm PTFE membrane vented cap, 1 L of complex growth medium will be warmed.
26. 5 mL of bacteria grown to OD600 0.2 will be added and incubated under 37°C temperature and atmospheric growth conditions for 20 min.
27. Phage from Step 23 at an MOI of 0.1 will be added and incubated under 37°C temperature and atmospheric growth conditions for 12–18 h.
28. Lysates will be transferred to 1-L centrifuge bottles and centrifuge at 8,000g for 45 min at 4 °C to remove bulk bacterial debris.
29. Supernatants will be decanted into fresh, sterile 1-L centrifuge bottles without disturbing the bacterial pellet.
30. This sample will be centrifuged again at 8,000g for 45 min at 4 °C.
31. The supernatant will be decanted into a sterile 1-L glass bottle.

DEAD END FILTRATION

32. 0.8/0.45-µm and 0.45/0.2-µm capsule filters will be assembled inline. The peristaltic pump will be used to filter the sterilized supernatant into sterile glass bottles.
33. Titer the sample as described in Steps 7 to 15.

ULTRAFILTRATION, DIAFILTRATION AND CONCENTRATION

34. Peristaltic pump and cross-flow ultrafiltration cassette to be assembled.
35. 500 mL of sterile ddH_2O will be circulated through the cassette and discarded.
36. The intake and retentate hoses is placed into the 0.2 µm sterile phage supernatant solution from Step 33.
37. The filtrate hose is placed into a sink or waste container.
38. Up to 1.5 L per batch of the supernatant is recirculated until ~200 mL remains.
39. 400 mL of sterile ddH2O is added.
40. The supernatant is recirculated until ~200 mL remains.
41. 400 mL of sterile TN buffer is added.
42. The supernatant is recirculated until ~200 mL remains.
43. Steps 41 and 42 are to be repeated until the supernatant becomes clear and colorless.
44. Pump paused, intake and retentate hoses placed in a sterile 50-mL conical tube and recirculate while the remaining supernatant

is continually added until 40 mL of concentrate remains (first fraction).

45. Pump paused and intake hose with retentate hose placed into a sterile 50-mL conical tube with 30 mL of TN buffer. Circulate briefly and remove the intake hose. 30 mL of concentrate is collected (second fraction).
46. Titer the sample as described in Steps 7 to 15.

LPS-AFFINITY CHROMATOGRAPHY

47. The spin column is equilibrated and regenerated as per the manufacturer's instructions (Pierce High-Capacity Endotoxin Removal Spin Column).
48. The spin column is placed into a collection tube and centrifuge at 500g for 1 min at RT to discard the regeneration solution.
49. Cap removed and the bottom plug inserted. 8 mL of 2 M NaCl is added, the cap replaced, and the column is inverted several times.
50. The cap is loosened, and the bottom plug is removed. The column is placed in a collection tube and centrifuged at 500g for 1 min at room temperature (RT) to discard the solution.
51. The cap is removed, and the bottom plug inserted. 8 mL of supplied endotoxin-free water added. The cap removed and the column inverted several times.

52. The cap loosened and the bottom plug removed. The column is placed in a collection tube and centrifuged at 500g for 1 min at RT to discard the water.
53. The cap is removed, and the bottom plug inserted. 8 mL of endotoxin-free PBS added, the cap is replaced, and the column inverted several times.
54. The cap is loosened, and the bottom plug removed. The column is placed in a collection tube and centrifuged at 500g for 1 min at RT to discard the PBS.
55. Steps 53 and 54 is to be repeated two additional times.
56. The cap is removed, and the bottom plug inserted. 10 mL of dialyzed phage concentrate added from Step 45 to the resin, the cap replaced, and the column inverted several times.
57. The column is incubated with gentle end-over-end mixing at 4 °C for 45 min.
58. The cap is loosed, and the bottom plug removed. The column is placed in a sterile collection tube and centrifuged at 500g for 1 min at RT to collect the sample.
59. Repeat Steps 47 to 58 is repeated until all phage concentrate is processed.
60. The sample will be titred as described in Steps 7 to 15.

PHAGE PREPARATION ENDOTOXIN QUANTIFICATION ● TIMING 2 H

61. Solutions to be equilibrated to RT, as per the manufacturer recommendation (Pierce LAL Chromogenic Endotoxin Quantitation Kit)
62. LPS standards provided in the kit is prepared using the 'High Standards' option.
63. Prepared standards added, the blank and samples from Step 59 to a 96-well microtiter plate.
64. The plate is warmed to 37 °C and 50 µL of LAL is added to each well. The plate is tapped lightly ten times to mix.
65. The chromogenic substrate solution is reconstituted and warmed to 37 °C for 5 min before use.
66. 100 µL of the chromogenic solution is added to each well.
67. At 6 min, 50 µL of 25% (vol/vol) acetic acid is added to stop the reaction.
68. OD405nm in a microplate reader is performed immediately.
69. The endotoxin level from the standard curve is extrapolated.

PHAGE PREPARATION PROTEIN ANALYSIS

70. The absorbance of phage preparations from Step 59 at 280 nm is determined to measure protein level. (This will vary between phage strains but should be between 1 and 3 $mg \cdot mL^{-1}$)
71. Phages diluted to 15 µg in 20 µL of 1× Laemmli sample buffer.
72. The sample is to be incubated at 90 °C for 5 min.
73. Samples to be centrifuged at 13,000g for 60 s at RT.

74. A 10% (wt/vol) acrylamide gel is prepared for SDS–PAGE electrophoresis.
75. The gel is mounted into the tank, combs removed and the inner chamber of the tank and three-fourths of the outer chamber is completely filled with 1× SDS–PAGE running buffer.
76. 3 µl of the standard is pipetted and 20 µL of the sample into the subsequent wells.
77. Electrophoresis is to be run at 100 V for ~60 min.
78. The gel is washed with ddH_2O three times for 15 min each.
79. The gel is incubated with 50 mL of Coomassie blue staining solution at RT for 1 h.
80. The solution is decanted and 50 mL of ddH_2O added. This solution is incubated at RT for 30 min on a rocker.
81. Decant and repeat Step 80 three times.
82. The gel is imaged using a conventional camera.

PHAGE SOLUTION TESTING FOR STERILITY [182] [181]

83. Phage solutions are tested routinely by Prof Iredell's Laboratory in WIMR through an external laboratory (Institute of Clinical Pathology and Medical Research), *please refer to section on packaging*

VIABILITY TESTING

84. 10 uL of each nebulised medication (e.g dornase alfa, tobramycin, colistin, hypertonic saline) will be added to 10 uL of phage solution, spot plaque testing will be repeated to determine the stability of phage concentration.
85. All bacterial-phage-medication combinations will be done in triplicates.
86. A drop of concentration from 10^8 to 10^6 will be tolerated as a minimum dose required for treatment. A drop of phage concentration to under 10^6 will invalidate the phage sample for further treatment use

PHAGE NEBULIZATION [227]

87. A volume of 4 mL phage stock (that will contain $1x10^8$ PFU) suspension will be nebulised via a vibrating mesh nebuliser (Philips Respironics innospire GO)
88. Nebulization will be performed in a fume hood
89. the nebulized aerosol was drawn into an ice-cooled test tube placed inside an aspiration flask through a Tygon tubing connected with a 2.0 mL plastic pipette at 8 L/min.
90. The sample collected in the test tube was used for plaque assay.
91. All nebulizations were done in triplicate. Nebuliser will be sterilized using Korsolex® disinfectant solution followed by boiling prior to each use.

92. A drop of concentration from 10^8 to 10^6 will be tolerated as a minimum dose required for treatment. A drop of phage concentration to under 10^6 will invalidate the phage sample for further treatment use

PACKAGING [183]

93. Litre scale solution (Step 59 of which a representative portion that has successfully reviewed until Step 101) will be decanted individually into sterile, clear type 1 container (tubular glass vials with crimp neck finishes), size 4mL (brimful 6mL), 14 vials will be prepared in a labelled box for treatment
94. 7 vials for phage PFU/mL quantification- Day 3, Day 7 for phage concentration via spot testing.
95. The vial selected will meet ISO 9001 and ISO 15378 and be compliant with EP, JP and USP.
96. As this will be the last step in assuring sterility, 1 vial containing 4mL of phage solution (within the 5% of the total volume of bacteriophage solution) stipulation will be submitted to an independent third party for a repeat sterility testing. Prof Iredell's laboratory submits these samples to the Institute of Clinical Pathology and Medical Research and a sterility report is obtained after 5 days.

LABELLING [184]

97. The following details will be listed on each vial

    a. Name of phage used (based on nomenclature provided from master bank stock)

    b. Manufacturing and expiration date

    c. Serial number of each vial (phage name/participants ID/date of manufacturing/order in which the phage solution is decanted into the container) e.g ØPA297/01/24112021/1

    d. Amount of the phage (mL) per container and concentration (PFU/mL)

    e. Participants' name (this is required as part of medication delivery) and study ID

    f. Dosing and route of administration

4 BRONCHOSCOPIC ADMINISTRATION OF BACTERIOPHAGE

1. Phage solution vials prepared will be checked against patients' details
2. Consent for bronchoscopy to be checked by Dr Singh/ Prof Selvadurai with theatre staff present in the procedure room prior to the procedure. The results of a COVID test PCR performed in the past 72 hours is checked and must be negative.
3. Study participants will be reviewed in anaesthetic bay and procedure will be explained again to the accompanying parent/guardian. An

examination will be recorded which includes- the presence of crackles, site, wheeze, site.

4. Discussion with anaesthetic colleague about age and size appropriate airway- this could be either a laryngeal mask airway (LMA) or an endotracheal tube (ETT). Bronchoscope will be selected based on the size of the suggested airway to allow.
5. Bronchoscopic setup will be cleaned using disinfectant wipes
6. All staff and operators (Dr Singh/ Prof Selvadurai) will be in personal protective equipment which includes goggles, fitted N95 mask, gown, and glove.
7. The collection jar will be set up in-line with the bronchoscopic unit
8. Participants' weight confirmed and 1 ml/kg of sterile normal saline will be prepared in sterile syringes. This will be divided into 10mL for each aliquot. The number of syringes and saline will be recorded. These syringes will be individually labelled as Normal Saline.
9. Another 1 mL/kg will be made up of 0.9% normal saline with 8 mL of phage solution (equivalent to $2x10^8$ PFU in the total solution), this solution will be placed within 5mL syringes and labelled to discern from the normal saline solution. Additionally, these syringes will be individually labelled as phage solution.
10. A bronchoscope will be placed on a sterile surface (on the bronchoscopic trolley)
11. Routine patient check as per theatre protocol to be performed.

12. Bronchoscopy will be commenced after confirmation with anaesthetic colleagues
13. A review of the anatomy and airways of the lungs will be performed first, areas of increased secretion will be recorded, suction and lavage will be performed in tandem with the review.
14. Lavage sample will be drained into the inline suction collection jar (previously prepared)
15. Following saline bronchoalveolar lavage the 1 mL/kg of phage and saline solution (phage solution) will be instilled in equal aliquots in all five lobes of the lung (0.2mL/kg in each lobe).
16. 1 minute of ventilation will be continued following administration of the phage solution and if deemed appropriate by the anaesthetic colleagues, the reversal of anaesthesia can then be commenced, and the participant extubated.
17. Lavage sample will be labelled and sent to the laboratory.
18. The participant will be reviewed in the recovery bay by Dr Singh/ Prof Selvadurai to update about the progress of the procedure. Another clinical examination will be performed, and the findings will be recorded.
19. Observations will be 15 minutely for 1 hour (4 observations) which will include temperature, heart rate, blood pressure and saturation. A repeat clinical examination will be repeated by Dr Singh/Prof Selvadurai after 1 hour.

5 NEBULISATION PROTOCOL

1. Phage samples prepared will be checked against participants' details. Dose prescribed will be confirmed. There are 2 possible doses 4mL of phage solution (containing 1 x10^8 PFU in 4mL) or a half-strength solution that is diluted with 2 mL of normal saline. (refer to appendix 7 for the rationale behind the dilution of administration dose). The frequency of administration will be twice a day for 6 days.
2. The nurse administering the solution will be in personal protective equipment which includes goggles, fitted N95 mask, gown, and glove. A nurse who is pregnant or considered to have immunocompromised status will not be allowed to deliver the medication.
3. A portable HEPA air filter will be turned on. Ward access door into the room will be shut. Participants of this study must be placed in a single room (as per usual CF admission guidelines). If the room has a door towards the outside of the ward (private balcony space), the door can be opened. This will be notified to the bed manager during the placement of the eRFA.
4. 4 mL of phage solution will be added into the nebuliser. This solution will be removed from the medication vial using a sterile syringe. If a half-strength dose is required, this must be done on a

sterile surface in the patient room (a sterile pack on top of a flat stable surface that has been cleaned with sterile wipes, 2mLs of phage solution from the prepared vial will be added to 2mLs of 0.9% saline)

5. 4mL of phage solution will be nebulised over 5 minutes
6. Observation in the ward at 15-minute intervals- this includes saturation, heart rate, temperature, blood pressure. Subsequently, observation at 1 hour and subsequently 4 hourly until next administration of nebulization.
7. The HEPA filter can be turned off after 1 hour following the completion of nebulisation.

6 ADVERSE EVENT PROTOCOL

FEVER > 38.5

1. If the temperature during the first hour of observation following nebulisation is above 38.5C
2. Paracetamol 15 mg/kg to be given orally (maximum 4 times/day
3. Temperature repeated after 1 hour
4. If the temperature is reducing, 4 hourly observation is continued
5. If continues to have temperature >38.5C at repeat observation post fever and administration of paracetamol (observation after 1 hour) or

during subsequent 4 hourly observations, oral ibuprofen 10 mg/ kg can be prescribed (maximum 3 times a day, in addition to paracetamol) if last paracetamol dose was provided within the past 6 hours. If > 6 hours has passed between the last paracetamol administration, paracetamol can be administered again

6. A clinical review must be initiated as per the between the flag guidelines if indicated. If clinical review is requested the following must be recorded;
    a. Reason for clinical review
    b. Observation- vitals, general condition
    c. Respiratory examination- wheeze, crackles
    d. midline or PICC site- erythema
    e. ENT examination to include erythema of tympanic membrane, throat or swelling of tongue, or increase in redness.
    f. Clinician reviewing the participant must call Dr Jagdev Singh via switch
7. If the attending clinician decide to take blood culture (as per their clinical impression) then the following bloods should be performed at the same time

    Blood culture must be performed with interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), full blood count (FBC), liver function test (LFT) and electrolyte, immunoglobulin G,A and M, urea and creatinine (EUC).

8. Please refer to section 11C for criteria of termination of the study.
9. SUSAR will be submitted iffever ≥38.5°C with no other clear source of infection occurs 3 consecutive times within 1 hour of each administration of nebulised phage or 3 episodes of fever over 48 hours within 1 hour of each administration of nebulised phage, (temporally related fever events)

RASH, OR SWELLING OF THE ORAL MUCOSA

1. If rash or swelling of oral mucosa is noted a clinical review must be requested
2. If clinical review is requested the following must be recorded;
    a. Reason for clinical review
    b. Observation- vitals, general condition
    c. Rash – extent, distribution, site, nature (macule, maculopapular, papule)
    d. midline or PICC site- erythema
    e. ENT examination to include erythema of tympanic membrane, throat or swelling of tongue, or redness.
3. The reaction will be classified as per the ASCIA [241] guideline which includes:
    a. Mild or moderate reactions
        i. Swelling of lips, face, eyes
        ii. Hives or welts

iii. Tingling mouth

b. Anaphylaxis

i. Difficult or noisy breathing
ii. Swelling of tongue
iii. Swelling or tightness in the throat
iv. Difficulty talking or hoarse voice
v. Wheeze or persistent cough - unlike the cough in asthma, the onset of coughing during anaphylaxis is usually sudden
vi. Persistent dizziness or collapse
vii. Pale and floppy (young children)
viii. Abdominal pain, vomiting

1. If any of these symptoms occur while nebulization is ongoing. To stop nebulization immediately.
2. Oral loratadine 10 mg/kg 24 hourly (other alternatives anti-histaminic medication available can be also be considered) can be prescribed for symptoms that are limited to (a). Close monitoring and observation will need to be performed 15 minutely for the first hour following the reaction and then hourly for 4 hours.
3. If the participant develops airway compromise or the symptoms enumerated in (b) eg swelling of the airway, difficulty in breathing (anaphylaxis) then 10 micrograms/kg (0.01 mL/kg of

adrenaline 1:1000) up to a maximum of 500 micrograms (0.5 mL).

   i. Repeat dose every 5 minutes if required. Use IV infusion only if the response to repeated IM doses and volume expansion is inadequate. Continuous monitoring of ECG, pulse oximetry and BP is essential. If a repeat dose is required PICU outreach team will need to be informed.
   ii. Close monitoring and observation will need to be performed 15 minutely for the first hour following the administration of IM adrenaline and then hourly for 4 hours.

4. The clinician reviewing the participant must call Dr Jagdev Singh via switch for any reaction that requires medication.
5. Adrenaline infusion if required following the failure of IM medication, given by staff trained in its use or in liaison with an intensivist. IV adrenaline infusions should be used with a dedicated line, infusion pump and anti-reflux valves wherever possible
6. The suggested dosing of emergency medications is provided as below. Please refer to local administration guidelines for further information. ASCIA [241]

For upper airway obstruction	i. Nebulised adrenaline (5mL e.g. 5 ampoules of 1:1,000).

	ii. Consider the need for advanced airway management if skills and equipment are available.
For persistent hypotension/ shock	i. Give normal saline (maximum of 50mL/kg in first 30 minutes).
For persistent wheeze	i. Bronchodilators: Salbutamol 12 puffs of 100µg (spacer) or 5mg (nebuliser). ***Note: Bronchodilators must not be used as first-line medication for anaphylaxis as they do not prevent or relieve upper airway obstruction, hypotension or shock.*** ii. Corticosteroids: Oral prednisolone 1 mg/kg (maximum of 50 mg) or intravenous hydrocortisone 5 mg/kg (maximum of 200 mg).

7. A severe reaction (or anaphylaxis) as defined above (point 3b) will be reported as a SUSAR.

7 FIRST NEBULIZATION PROTOCOL

1. Phage samples prepared will be checked against participants' details
2. Room 2 or the exercise room in the lung function lab will be set up with a portable HEPA filter (between 8 to 12 exchanges/hour). These rooms have 12 exchanges per hour.
3. Study participant will be placed in either one of these rooms.
4. An examination will be recorded which includes- the presence of crackles, site, wheeze, site.

5. Lung function laboratory scientist and Dr Singh/ Prof Selvadurai will be in personal protective equipment which includes goggles, fitted N95 mask, gown and glove.
6. Study participant will be reviewed in the designated room and the procedure will be explained again to the accompanying parent/guardian. An examination will be recorded which includes- presence of crackles, site, wheeze, site.
7. Lung function pre will be performed and the results recorded
8. 4mL of phage solution will be nebulised over 5 minutes
9. A repeat lung function will be performed to assess for changes post-delivery of the nebuliser solution
10. Results will be interpreted as follows
    a. If lung function decline is less than 10% from baseline, the study participant is considered to not have a bronchospasm
    b. If lung function decline is >10%, 6 puff of MDI Ventolin through a spacer will be delivered and lung function repeated, if change from baseline is now less than 10%, the study participant will be prescribed 6 puffs of Ventolin pre each nebulisation of phage solution over the course of the study (remaining 11 doses)
    c. If lung function in (b) does not improve to <10% from baseline, the study participant will not receive the second dose on the same day.

d. A repeat medication tolerance test will be repeated on the following day using a diluted phage solution (dilute 2mL of phage solution with 2mL of sterile Normal Saline, and the test is repeated. Dilution will be performed by Dr Singh as per appendix 5 (point 5). The decision will be made as per (a), (b) and (c) using this diluted phage sample.
e. If (d) occurs again despite the reduction of dose, further administration will be discontinued. This will be reported as a SUSAR.

11. Surfaces will be cleaned with 70% alcohol following testing
12. Study participants that exhibited results (a), (b) and (c) will require observation in the ward at 15-minute intervals- this includes saturation, heart rate, temperature, blood pressure. Subsequently, observation at 1 hour and subsequently 4 hourly until next administration of nebulization.
13. A clinical review will be performed at 1 hour to review for increased crackles, wheeze or tachypnea.

8 TRIAL STEERING COMMITTEE (TSC) CHARTER

Protocol Title:	A Single-Arm, Open-Labelled, Safety and Tolerability of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with Cystic Fibrosis and Pseudomonas aeruginosa.

Protocol #:	
Protocol Version & Date this Charter is based on:	Version 3, 8th April 2022

REVISION HISTORY

Version No.	Date	Summary of Changes

AGREEMENT

Name and Title	Role	Signature	Date
Professor Hiran	Committee Chair		
Professor Jonathan	Member		
Dr Jeremy Barr	Member		
Professor Adam Jaffe	Member		

Professor Dominic	Member		
Ms Sharon Hunt	Member		
Dr Jagdev Singh	Principal		

CORE MEMBERSHIP

The Study/Trial Steering Committee membership will consist of:

Name and Title	Affiliation / Institution	Email
Professor Hiran Selvadurai	Committee Chair	Hiran.selvadurai@health.nsw.gov.au
Professor Jonathan Iredell	Member	Jon.Iridell@health.nsw.gov.au
Dr Jeremy Barr	Member	Jeremy.barr@monash.edu
Professor Adam Jaffe	Member	A.jaffe@unsw.edu.au
Professor Dominic Fitzgerald	Member	Dominic.Fitzgerald1@health.nsw.gov.au

Ms Sharon Hunt	Member	Sharon.Hunt@health.nsw.gov.au
Dr Jagdev Singh	Principal Investigator	Jagdev.Singh@health.nsw.gov.au

CHAIR OF THE TSC

The Chair of the Committee is Professor Hiran Selvadurai

RESPONSIBILITIES OF THE COMMITTEE

The Committee provides oversight and ensures that the study is conducted to the required standards. The Committee, through the Committee Chairperson, provides advice to the PI; the primary responsibility for the study/trial and its day-to-day management remains the responsibility of the PI.

This Committee will have responsibility for monitoring study progress with regards to:

1. Overseeing progress towards study milestones (i.e. recruitment accruals, timelines etc).
2. Reviewing adherence/compliance to the protocol and adherence/compliance to good clinical research practices.

3. Reviewing reports on participant safety and data (and acting on recommendations from the Data Safety Monitoring Board [DSMB].
4. Considering any new external information relevant to the study.
5. Advising on discontinuation or extension of recruitment.
6. Approving publication and authorship plans suggested by the principal investigators and settling of any associated disputes raised by any of the study team.
7. Review manuscripts, manuscript author lists, and/or order of authors, and providing final approval of any publication plans.

DATA

At each scheduled meeting, the TSC will be provided with a report for review, containing the following data elements:

- Summary of progress to date, including site activation status, recruitment update, timeline update.
- Summary of protocol compliance i.e. protocol deviations, serious breaches etc.
- Summary and any outcome/s and recommendation/s provided by the DSMB
- Summary of any upcoming manuscripts/abstracts

FREQUENCY AND FORMAT OF MEETINGS

MEETING FREQUENCY

The TSC will meet prior to commencement of the trial to discuss the protocol, the trial, any analysis plan, future meetings, and to have the opportunity to clarify any aspects with the Investigator.

The TSC will meet via videoconference prior to each admission (intake meeting) of a participant to allow for a review of phage purification data prior to administration.

Subsequently, TSC will convene 1 month (post-discharge meeting) after each study participant is recruited via videoconference to review the clinical progress and adverse events that may have arisen. This meeting may be done to coincide with the review of the next phage purification data for the next study participant. This meeting will be performed after the DSMB meeting to review and discuss any suggestions from the DSMB

During the follow-up phase, 3 monthly meetings will be convened via telehealth to discuss the clinical progress of the study participants. This can coincide with the intake meeting and or post-discharge meeting of previous study participants. The follow up meeting will continue until the last participant has completed their follow up review (at 12 months after bacteriophage therapy)

Additional ad hoc meetings of the TSC may be scheduled if requested by either the PI or the DSMB

MEETING ATTENDANCE AND QUORUM

The minimum number of members in attendance for the TSC to be quorate for decision-making is 50% of the membership, being 3 members. If at any time the number of members is less than a quorum, the Committee may meet only for discussion purposes.

If the report is circulated before the meeting, those TSC members unable to attend the meeting may pass comments to the TSC Chair for consideration during the discussions.

MEETING DELIBERATIONS

The Chair will facilitate and summarise discussions and will encourage decision-making via consensus. Meetings will be minuted and any decisions made electronically will be recorded in the form of meeting minutes and distributed to Committee members within 14 days of the meeting. All meeting agendas, data reports submitted to the Committee for review, meeting minutes generated, and other relevant documentation will be filed in the Study Master File.

The discussions of the Committee are confidential to its' members.

CHARTER REVIEW

This Charter will be reviewed and updated annually, as required.

COMMUNICATIONS

At any time during the study/trial, regulatory authorities, the Human Research Ethics Committee, the DSMB or any other body or individual involved with the conduct of the study/trial may seek the advice of the TSC about any concern that they may have about the conduct, outcome, or continuation of the study/trial. Any such requests should be forwarded in writing to the Committee Chairperson at the email address provided above.

9 DATA AND SAFETY MONITORING BOARD (DSMB) CHARTER

Trial title:	A Single-Arm, Open-Labelled, Safety and Tolerability of Intra-bronchial and Nebulised

	Bacteriophage Treatment in Children with Pseudomonas aeruginosa.
Trial registration number:	
Study protocol version this charter is based on:	Version 1, 8th February 2022
Investigator: & contact information:	Dr Jagdev Singh

AGREEMENT

Name and title	**DSMB Role**	**Signature**	**Date**
Prof Craig Mellis	Clinical Expert, Chair		
Prof Anne Cheng	Clinical expert		

INTRODUCTION

This charter is for the Data and Safety Monitoring Board (DSMB) for the clinical trial titled A , Single-Arm, Open-Labelled, Safety and Tolerability of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with Cystic Fibrosis and Pseudomonas aeruginosa.

This charter defines the responsibilities of the DSMB, its membership, and the purpose and timing of its meetings. The Charter also provides the procedures for ensuring confidentiality and proper communication, the statistical monitoring procedures to be implemented by the DSMB, and an outline of the content of the reports that will be provided to the DSMB.

DSMB MEMBERSHIP

The DSMB consists of the following members who collectively have experience in the clinical area of interest and randomised clinical trials. A quorum will require at least two members. The DSMB will consist of:

DSMB Role	**Name and title**	**Affiliation / Institution**	**Contact details**	**Summary of expertise**
DSMB Chair	Prof Craig Mellis	The University of Sydney		Professor Mellis conducted the first clinical trial in Australian

				children, of an inhaled corticosteroid for asthma, and was a co-investigator of the first paediatric trial demonstrating the efficacy of a leukotriene receptor antagonist in viral-induced wheezing.
DSMB Co-Chair	Prof Anne Chang	Queensland Children's Hospital		Professor Anne Chang AM is an established leading researcher with international recognition in cough, bronchiectasis and evidence-based medicine (EBM) related to paediatric respiratory medicine.

RESPONSIBILITIES OF THE DSMB

STEWARDSHIP OF THE TRIAL

The DSMB is responsible for the stewardship of the trial overall participating sites. The stewardship includes a continuous review of participant recruitment, accrual, retention, and withdrawal. It further involves oversight of participant management, adherence to protocol-specified regimens, and procedures for data management and quality control.

The DSMB will be responsible for safeguarding the interests of trial participants by assessing the safety of the interventions during the trial, and the general progress of the trial.

Specifically, the role of the DSMB will be to:

- Monitor and review participant safety in the trial (including evidence for treatment harm [e.g. toxicity data, safety events)
- Review participant recruitment, accrual, retention, treatment discontinuation, trial withdrawal, serious breaches, and protocol deviations that require exclusion of the participant from the per-protocol analysis,
- Monitor efficacy based on pre-planned interim data analyses
- Advice on protocol modifications suggested by investigator or sponsor (e.g. to inclusion criteria, trial outcomes or sample size)

- Suggest additional data analyses
- Assess data quality, including completeness (and by doing so encourage the collection of high-quality data)
- Monitor compliance with the protocol by participants and investigators
- Evaluate emerging literature that may have an impact on the scientific plausibility or need for the trial / assess the impact and relevance of external evidence
- Monitor continuing appropriateness of participant information
- Monitor compliance with previous DSMB recommendations

This responsibility will be exercised by providing recommendations about continuing, modifying or stopping the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection/recruitment/retention of participants, participant management, improving adherence to protocol-specified regimens, and the procedures for data management and quality control.

The DSMB will be advisory to the Investigator and through him to the Trial Steering Committee (TSC)

The Sponsor-Investigator holds ultimate responsibility for decisions regarding the trial.

SAFETY MONITORING

The DSMB is responsible for safeguarding the interests of trial participants by assessing the safety of the interventions during the trial. The DSMB members should review the plan for the collection of safety data prior to commencement of this trial in addition to the bacteriophage isolation and administration protocols. If important safety items are not considered in the reporting of safety data, the DSMB may request to change or add items to be included.

The DSMB will use the accumulating clinical data to differentiate reported safety events (i.e. [169] adverse events, suspected unexpected serious adverse reactions, urgent safety measures) associated with the trial interventions from those with other aetiologies. It is therefore important that, where possible, reported safety data indicate the likelihood of the relationship to the study interventions (i.e. causality).

MONITORING OF SAFETY DATA - INTERIM ANALYSES

The DSMB is also responsible for the assessment of the safety of the interventions during the course of the trial i.e. interim analyses of safety parameters. The current approved study protocol pre-specifies the following interim analyses and interim analysis of the safety will be applied for each individual participant.

- A review of the selected bacteriophage solution prior to administration, the review will include adherence to the approved protocol in purification, maintenance of the PFU/mL of the phage solution, sterility of the bacterial solution throughout the process and the desired concentration is achieved prior to the administration of the bacteriophage solution.
- A second analysis will be performed following the completion of the therapeutic administration to review the adherence to protocol and side-effects that may have arisen.

FREQUENCY AND FORMAT OF MEETINGS

INITIAL MEETING

The DSMB will meet before the trial starts to discuss the approved protocol, the trial, any analysis plan, future meetings, and to have the opportunity to clarify any aspects with the Investigator.

An initial meeting of the DSMB will review the role and functioning of the DSMB, discuss the format and content of the DSMB reports and review scientific and ethical issues relating to the design and conduct of the trial.

REVIEW MEETINGS

Subsequent DSMB meetings will occur as per the frequency stated above for individual participants. At the meetings, the following will be reviewed:

- Data related to safety
- Data related to efficacy
- Data relating to trial conduct.

AD HOC MEETINGS

Additional ad hoc meetings of the DSMB may be scheduled if requested by either the Investigator or TSC or the DSMB.

CONDUCT OF MEETINGS

Meetings will consist of an open session and a closed session. These meetings will be held via videoconferencing.

OPEN SESSION

Members of the TSC, which includes the Investigator, will meet with the DSMB who prepared the DSMB report at the commencement of each meeting. This "*Open Session"* provides the DSMB an opportunity to query the members about issues that have arisen during the review of the data. Once the DSMB members are satisfied that all their queries have been addressed, the TSC members will then leave the meeting to enable the

confidential *Closed Session* of the DSMB to commence. The Sponsor-Investigator will remain available to return, if required, to assist with any questions.

CLOSED SESSION

The remainder of the closed session will involve only DSMB members to allow discussion of confidential data from the clinical trial.

MEETING ATTENDANCE AND QUORUM

The minimum number of members in attendance for the DSMB to be quorate for decision-making is *two persons.*

If a member does not attend a meeting, it should be ensured that the member is available for the next meeting. If a member does not attend a second meeting, they should be asked if they wish to remain part of the DSMB. If a member does not attend the third meeting, they may be replaced.

MEETING DELIBERATIONS

The Chair will facilitate and summarise discussions and will encourage consensus.

Following its review of the data, the DSMB will reach a consensus on its list of recommendations. Consensus will be determined through informal or, where required, formal voting

DSMB RECOMMENDATIONS

The recommendations provided by the DSMB may include: continuing the trial unchanged; continuing the trial with modifications, or terminating the trial. The DSMB may also make recommendations about other aspects of the trial such as the recruitment of participants and the conduct of the trial. All recommendations will be sent to the Sponsor-Investigator promptly, within *2 weeks,* and through the Sponsor-Investigator to the *TSC.*

The TSC will advise on whether to continue or terminate the trial, and whether amendments to the protocol or changes in trial conduct are required based on the DSMB recommendations. The subsequent enrolment of a new participant will only be commenced after addressing the concerns, if any, raised by the DSMB has been satisfied.

In the event that the DSMB recommends the early termination of the trial, the final decision to stop the trial early or modify the trial protocol will be made by the Investigator following advice from the TSC. If this situation arises at any time, the decision of the Investigator will be discussed with the DSMB immediately.

The DSMB will be advisory to the Investigator and through him to the TSC. The Investigator holds ultimate responsibility for decisions regarding the trial.

The Investigator will be responsible for promptly presenting the recommendations of the DSMB to the TSC for ready review. The TSC will advise on whether to continue or terminate the trial, and whether amendments to the protocol or changes in trial conduct are required based on the DSMB recommendations.

Response to the DSMB's recommendations:

- If the Investigator/ TSC does not agree with the DSMB recommendations, a memo justifying the reasons for not complying with the recommendations of the DSMB will be promptlyforwarded to the DSMB and to the Sponsor (14 days).
- If the DSMB is not satisfied with the Investigator/TSC's response to their recommendations, the DSMB will promptly notify the Sponsor (14 days).

Note that in the event that the Investigator or TSC wishes to remove one or more DSMB members, a memo justifying the reasons for this will be promptly forwarded to the DSMB and to the HREC. (14 days)

MEETING MINUTES

The DSMB will have minutes taken for both the Open and Closed meetings. Meeting minutes should be signed by the DSMB Chair and distributed as soon as possible after the DSMB meeting.

TRIAL PUBLICATIONS

The DSMB may be sent copies of accepted papers for their information.

DSMB members will be named, and their affiliations listed in the main report/publication. A summary of the timings and conclusions of the DSMB meetings should be included in the body of the main trial paper.

STATISTICAL MONITORING AND REPORTS

OPEN AND CLOSED REPORTS

The open report will also be circulated to the TSC via email which will meet shortly after the DSMB to discuss any recommendations made by the DSMB along with any other trial-related issues.

OPEN REPORTS

Open reports will contain the following information:

- Trial title.
- Summary of the trial design and progress.
- Details of any protocol amendments since the previous report
- Status of accrual (actual vs target recruitment)
- Summary of baseline characteristics
- Summary of adverse events*
- Summary of serious adverse events*

- Summary of suspected unexpected serious adverse reactions*
- Summary of urgent safety measures
- Summary of significant safety issues
- Details of serious breaches
- Number of protocol deviations requiring exclusion from the per-protocol analysis (such as study treatment discontinuations and, where applicable, withdrawals from study procedures and follow up)

CLOSED REPORTS

Closed reports will contain the following information:

- Trial number and title.
- Summary of the trial design and progress.
- Details of any protocol amendments
- Status of accrual (actual vs target recruitment)
- Summary of baseline characteristics
- Summary of adverse events*
- Summary of serious adverse events*
- Summary of suspected unexpected serious adverse reactions*

- Summary of urgent safety measures
- Summary of significant safety issues
- Details of any serious breaches
- Number of protocol deviations requiring exclusion from the per-protocol analysis (such as study treatment discontinuations and, where applicable, withdrawals from study procedures and follow up)

Additional information may be presented in subsequent reports if specifically requested by the DSMB.

CONFIDENTIALITY

All DSMB reports will remain confidential until the end of the trial. Details of DSMB discussions and draft reports will remain confidential until formally delivered to the Investigator and through him to the TSC.

COMMUNICATIONS

At any time during the trial, regulatory authorities, the Human Research Ethics Committee, the TSC or any other body or individual involved with the conduct of the trial may seek the advice of the DSMB about any concern that they may have about the conduct, outcome or continuation of the trial. Any such requests should be forwarded in writing to the DSMB Chairperson at the address provided above.

If any suspected unexpected serious adverse events occur, which are thought to relate to the experimental treatment, the Chair of the DSMB will be notified within 72 hours. The Chair will then decide whether an additional meeting of the DSMB should be held.

LIABILITY STATUS OF THE DSMB

As the DSMB is an advisory body alone, its members are not liable for damage, harm, morbidity or mortality to recruited patients.

Appendix 2: Parent information sheet

PARTICIPANT INFORMATION SHEET/CONSENT FORM – PARENT/GUARDIAN

Interventional Study - *Parent/Guardian consenting on behalf of participant*

Title	Single-Arm, Open-Labelled, Safety and Tolerability of Intrabronchial and Nebulised Bacteriophage Treatment in Children with Chronic Pseudomonas aeruginosa Infection.

Short Title	The evaluation of Intrabronchial and nebulised bacteriophage treatment in children with chronic Pseudomonas infection. (CHIP-CF)
Protocol Number	2022/ETH00241, version 3, 8th April 2022
Project Sponsor	The Sydney Children's Hospital Network
Coordinating Principal Investigator/ Principal Investigator	Dr Jagdev Singh
Associate Investigator(s)	Professor Hiran Selvadurai Professor Jon Iredell (Iredell Laboratory, Westmead Institute of Medical Research) Professor Adam Jaffe Dr Jeremy Barr (Barr Laboratory, University of Monash) Professor Dominic Fitzgerald A/Prof Paul Robinson Dr Chetan Pandit

	Ms Sharon Hunt Ms Sharon Simonds Ms Christie Boyton Dr Anna Middleton Mr Brendan Kennedy Ms Beth Weldon Ms Merilyn John Ms Brooke Bailey
Location	The Children's Hospital at Westmead or The Sydney Children Hospital, Randwick.

Clinical contact person (in-hours)

Name	*Dr Jagdev Singh*
Position	*Paediatric Respiratory Physician*

Telephone	*98453395*
Email	*Jagdev.Singh@health.nsw.gov.au*

Clinical contact person (out of-hours)

Name	*Respiratory On-Call*
Position	*Paediatric Respiratory Physician/ Fellow*
Telephone	*98450000 (switch then respiratory On-Call)*

PART 1 WHAT DOES THE CHILD'S PARTICIPATION INVOLVE?

1 INTRODUCTION

Your child is invited to take part in this research project because they have a bacteria known as *Pseudomonas aeruginosa* in his/her sputum (phlegm) despite previous or ongoing standard antibiotic treatment. This research project is testing a new treatment for chronic *Pseudomonas aeruginosa* infection within the lungs. The new treatment is called personalised Bacteriophage therapy.

This **Participant Information Sheet/Consent Form** tells you about the research project. We have also prepared an age-appropriate information sheet for your child to read. It explains the tests and treatments involved.

Knowing what is involved will help you decide if you want your child to take part in the research.

Please read this information carefully. Ask questions about anything that you do not understand or want to know more about. Before deciding whether or not your child can take part, you might want to talk about it with a relative, friend or your child's local doctor.

Participation in this research is voluntary. If you do not wish your child to take part, they do not have to. Your child will receive the best possible care whether or not they take part.

If you decide you want your child to take part in the research project, you will be asked to sign the consent section. By signing this consent you are telling us that you:

• Understand what you have read

• Consent to your child taking part in the research project

• Consent for your child to have the tests and treatments that are described

• Consent to the use of your child's personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

2 WHAT IS THE PURPOSE OF THIS RESEARCH?

Bacteriophage is an experimental treatment. This means that it is not an approved treatment for *Pseudomonas* infection in Australia.

A bacteriophage is a small virus that is unique in that it only attacks bacteria. There are billions of bacteriophages in the environment.

In this study, we are selecting very specific bacteriophages that attack the specific bacteria your child has in his/her lungs.

This study aims to provide information for the safe and routine use of bacteriophages in the future. We hope that our study will help increase the evidence that bacteriophages can be used in the future to treat lung infections in CF.

3 WHAT DOES PARTICIPATION IN THIS RESEARCH INVOLVE?

This is a personalised treatment. The strain of *Pseudomonas aeruginosa* may be unique to your child and will be tested against the available bacteriophages we have discovered. If the bacteriophage is found to be effective against the *Pseudomonas* strain, a treatment dose will be prepared to be administered at first through bronchoscopy (placing a small camera into your child's airway to review his/her lungs) and

subsequently through twice-daily nebulisation (adding a liquid medication into a device that will turn it into a mist in order to be inhaled by your child) over a period of 7 (seven) days. Your child will be admitted to the hospital for a standard CF tune-up for 10 to 14 days during the study. As you may already know a tune-up involves admission and will include the antibiotics delivered into the veins (intravenous, IV) and physiotherapy. Following the completion of treatment in hospital, your child will then have follow-up appointments at the CF clinic every 3 months for 9 months in the CF clinic as part of the routine clinic schedule.

All children in this study will receive this bacteriophage treatment (referred to as phage therapy) and there will not be any placebo involved as this is an open-labelled study

There are no additional costs associated with participation in this research project, nor will you or your child be paid. All medication, tests and medical care required as part of the research project will be provided free of charge.

If you decide that your child can participate in this research project, the study doctor will inform your child's doctor and/or Clinical Nurse Consultant.

Please also note that all treatment and follow-ups during the clinical trial period will be performed in the Children's Hospital at Westmead (CHW). If your child is under the care of the Sydney Children's Hospital, Randwick

team, your child will be admitted under a different doctor and clinical team at CHW (the CHW Respiratory team).

The following flow chart provides information on how the study will be conducted

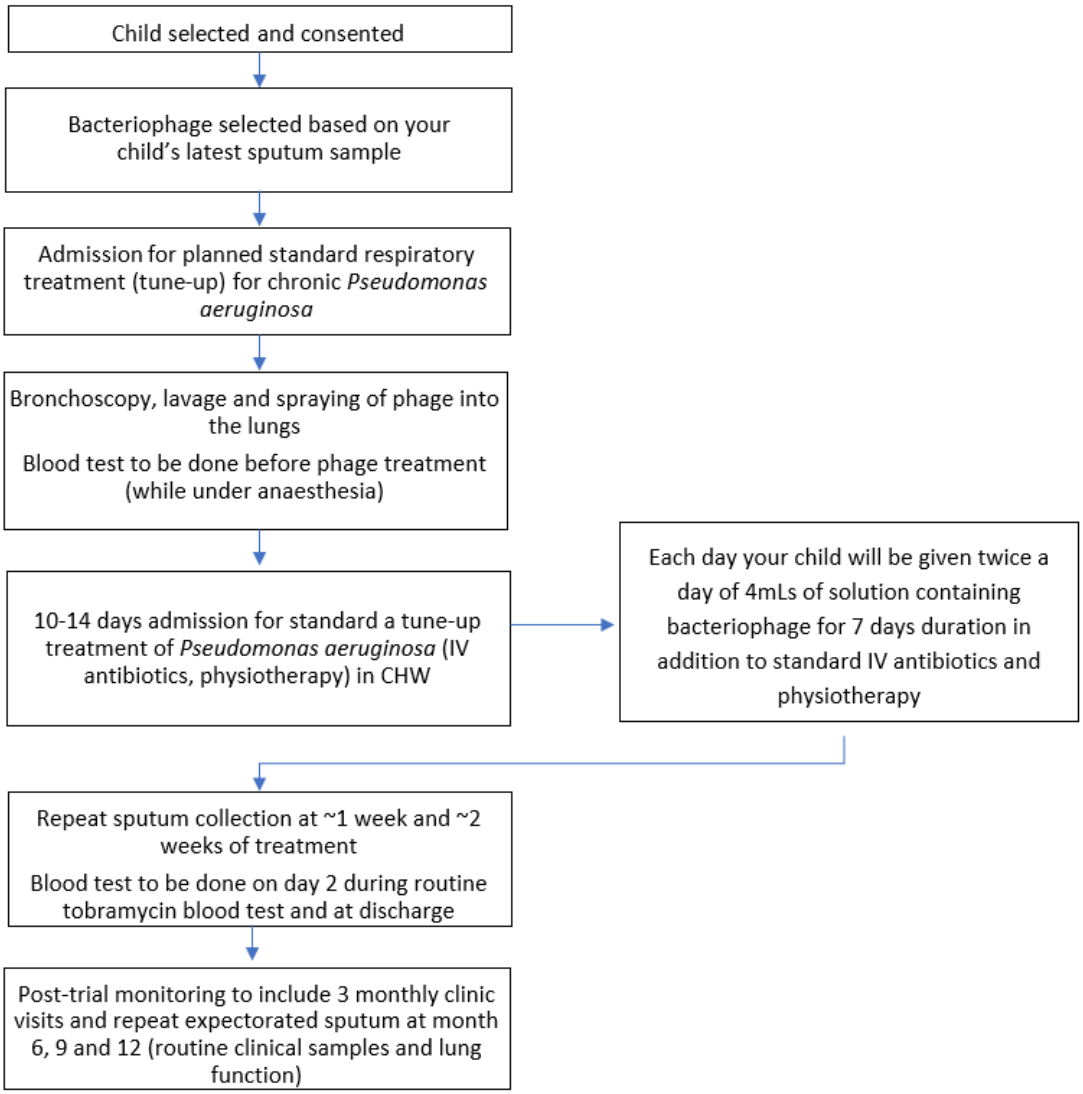


Figure 1: Summary of treatment

TIMEPOINT	Pre-admission	Day 0	Day 1	Day 2	Day 3-6	Day 7	Day 8 to 9 * or up to 13	Discharge (day 10-14)	Week 3	Month 3	Month 6	Month 9	Month 12
Eligibility screen	X												
Informed consent	X												
Bronchoscopy and instillation of bacteriophage		X											

Nebulization of bacteriophage			X	X	X	X							
Standard IV		X	X	X	X	X	X	X					
Physiotherapy		X	X	X	X	X	X	X					
Sputum collection	X		X@			X		X		X	X	X	X
Anthropometry	X		X			X		X			X	X	X
Spirometry	X		X*			X		X		X		X	X
Blood investigation**	X			X				X					
Clinical review	X	Xa	X	X	X	X	X	X			X	X	X

Discharge review (telephone)									X				

Figure 2: Timetable of schedule, footnote: [@]= sputum on day 1 to be performed before the first dose of nebulised bacteriophage, Blood investigations are timed to coincide with standard blood taking practices; admission, day 2 to review tobramycin level and day 14 during removal of the central line. [a]= This clinical review will be conducted prior to performing the bronchoscopy.

4 WHAT DOES MY CHILD HAVE TO DO?

This trial will involve a minimum of 10 and a maximum of 14 days of hospital admission for treatment of *Pseudomonas aeruginosa* and will involve a bronchoscopy on the day of admission during the insertion of a catheter to deliver IV antibiotics (through midline, central line). If your child has an implanted access device (PORT), this will be accessed instead of inserting a separate line. This admission will be conducted in CHW. If your child is cared for by the Sydney Children's Hospital, Randwick (SCH) team, he or she will be admitted under a different CF team in CHW instead.

The reason a bronchoscopy is important for this research is to allow us to look at the lungs and evaluate the amount and site of maximum secretion of mucus within the lungs and to instill (spray) the bacteriophage solution on those 'problematic' areas that nebulised solutions may not reach. Although bronchoscopy is a relatively safe procedure there is still a risk of adverse effects from performing this procedure, this include a 1 in 500 change of bleeding from the airway (which includes coughing up blood or blood stained phlegm) and a 1 in 10,000 risk of death.

The admission will be a routine 'tune-up' admission in which your child will receive standard IV antibiotics as decided by thephysician (or if your child is followed up by the SCH team, the CHW Respiratory team will decide this instead). For this study, your child will be required to undergo lung function

testing mid-way (anywhere between day 5 to 9) into his/her admission and at the end of the admission (anywhere between day 10 to 14), which is a routine part of our normal clinical care. Your child's usual physician may request for additional lung function testing for their routine clinical care if necessary.

Bloods will be taken 4 times during this study. 2 of these will be performed while your child is under anaesthesia while 1 will be performed on day 2 during routine blood testing for tobramycin level checks. Another set of blood will be taken at the end of admission when the midline or central line is removed. In the unlikely event that the midline or central line is blocked or if we are unable to obtain blood sample during removal, then a blood test will be performed by pricking your child's vein. The blood samples taken before, during and after treatment is a vital part of this study to help us evaluate the effects of the treatment.

Your child will be prescribed twice daily phage treatment that will be delivered through nebulisation for 5 minutes each time for a period of 7 days during their admission. The IV antibiotic and physiotherapy will be continued after completion of the bacteriophage treatment up to a total duration from admission to discharge of 14 days. After the 10 days to 2 weeks of admission, your child will be discharged from the hospital.

Further information about your child will be obtained from their health records held at this and other health services, for the purpose of this

research. This will include data such as number of hospitalisations, previous lung function and results from previous sputum samples. By signing the consent form, you agree to the study team accessing health records if they are relevant to the child's participation in this research project.

As bacteriophages are very specific to only specific types or groups of *Pseudomonas*, there is a chance that we may not find a suitable bacteriophage to proceed with the study for your child. This will be known only after we have tested the initial sputum sample from your child that contains the bacteria. The admission and bronchoscopy will only proceed if we have a suitable bacteriophage to treat your child with. This does not mean that your child will never have the chance in the future to be treated with bacteriophages, it just means that we haven't been able to find the specific bacteriophage at this point in time.

THE SUMMARY OF THE TESTS OR ASSESSMENTS THAT WILL BE PERFORMED ARE AS FOLLOWS (PLEASE REFER TO TABLE 1 IN THE APPENDIX FOR A GRAPHICAL REPRESENTATION OF THE FOLLOWING);

A) BRONCHOSCOPY

- A bronchoscopy will be performed when your child receives general anaesthesia for his/her routine midline or central line insertion.

- This may result in an additional general anaesthetic that is not a routine part of most CF admissions.
- Although bronchoscopy is a relatively safe procedure there is still a risk of adverse effects from performing this procedure, this includes a 1 in 500 change of bleeding from the airway (which includes coughing up blood or blood stained phlegm) and a 1 in 10,000 risk of death.

B) BLOOD TEST

- This will be done 3 times as follows
    - *while under general anaesthesia during the bronchoscopy*
    - *during their routine tobramycin level check (usually on the second day of admission/before the third dose) and*
    - *when his/her midline is taken out on the last day of treatment.*
    - *This was specifically designed to minimize distress during blood taking.*

- In the event that your child develops fever within 1 hour of administration of the phage treatment, a set of bloods will need to be taken. This will be performed by pricking your child’s vein.

- Your child's CF physician may also request for additional bloods to be taken as part of the routine management of your child's CF, however, we will ensure that whenever possible these tests will be performed on blood that has already been taken in order to avoid repeated blood taking.

C) LUNG FUNCTION

- A lung function test will be done before admission, likely during your child's routine clinic visit. A lung function will be performed during the administration of the phage treatment to make sure that your child does not develop a wheeze.
- Further lung function will be performed on week 1 of admission and on week 2 of admission.
- Following completion of the phage treatment, 3 more lung function test will be performed on month 6, 9 and 12 (likely during your clinic visit).
- As usual, both height and weight (anthropometry) will be performed just before lung function is done in the CHW respiratory lab.

D) SPUTUM COLLECTION

- We will need to collect your child's sputum as follows;
    - Day 1 of admission (after bronchoscopy and right before the first dose of nebulised phage)
    - Day 7 (range 5 to 9 days)
    - Day 14 (range 10 to 14 days)
    - Then every 3 months and a final sputum at month 12.
- The sputum must be collected in 2 separate jars as one will be used for routine microbiology and the second sample will be used for testing the bacteriophages.

E) STOOL SAMPLE

We will need 2 stool samples

- 1 will need to be obtained before the trial is started (ideally in the morning of the bronchoscopy) and you will be provided with a collection jar for that and
- another 1 at the end of the 7-day treatment (range between day 7 to 10)

F) COVID TEST

In order to perform this trial, we will need to ensure that you and your child have not been exposed to COVID-19 prior to admission. The timing of isolation will depend on the most recent health orders

by the NSW government. You and your child (or any other adult that is accompanying your child) must have a COVID-19 PCR nasal swab 72 hours prior to admission.

G) TELEHEALTH FOLLOW-UP

Upon discharge from the hospital at the end of the 2 weeks, Dr Singh will give a call 1 week (week 3 of study) to troubleshoot and address any questions and concerns that may arise from the study.

5 OTHER RELEVANT INFORMATION ABOUT THE RESEARCH PROJECT

This is the first study of its kind in Australia and globally. Up to 10 children will be recruited for this trial.

6 DOES MY CHILD HAVE TO TAKE PART IN THIS RESEARCH PROJECT?

Participation in any research project is voluntary. If you do not wish for your child to take part, they do not have to. If you decide that they can

take part and later change your mind, you are free to withdraw your child from the project at any stage.

If you do decide that your child can take part, you will be given this Participant Information and Consent Form to sign and you will be given a copy to keep.

Your decision that your child can or cannot take part, or that they can take part and then be withdrawn, will not affect their routine treatment, relationship with those treating them, or their relationship with The Respiratory Department, Children's Hospital at Westmead.

8 WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

We cannot guarantee or promise that your child will receive any benefits from this research; however, possible benefits may include:

1. The ability to receive nebulised treatment of bacteriophages comfortably with minimal side effects.
2. The eradication or suppression of the *Pseudomonas aeruginosa* that has not been achieved through previous standard treatment with IV antibiotics.
3. A reduction of inflammation in the lung.

4. A change of the sensitivity of the Pseudomonas strain in your child's lungs to make it easier to treat with standard antibiotics in the future.

9 WHAT ARE THE POSSIBLE RISKS AND DISADVANTAGES OF TAKING PART?

Medical treatments often cause side effects. The participant may have none, some or all of the effects listed below, and they may be mild, moderate, or severe. If the participant has any of these side effects, or you are worried about them, talk with the study doctor. The study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell the study doctor immediately about any new or unusual symptoms that the participant gets.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long-lasting or permanent. If a severe side effect or reaction occurs, the study doctor may need to stop the child's treatment. The child's study doctor will discuss the best way of managing any side effects with you.

Side Effect	How often is it likely to occur?	How severe might it be?	How long might it last?
Fever occurring within an hour of administration of the bacteriophage	**Likely.** in the first 48 hours of treatment	Mild. Fever is expected in 1 in 5 children that have undergone a bronchoscopy in the first 48 hours. However, there is a possibility of fevers (>38.5°C) occurring within an hour of administering the medication. Your child will be given either paracetamol or ibuprofen to relieve the fever and will be closely monitored. This does not stop your child from getting subsequent doses of the medication unless it occurs more than 3 times after each	1-4 hours

		administration of the bacteriophage.	
Wheeze	**Unlikely.** Immediately after nebulised treatment	Mild. A test dose will be performed with a lung function test to assess if your child has any wheeze or drop in lung function after the first administration of the dose. Ventolin will be given if this happens and the test will be repeated. If your child continues to have a wheeze (bronchospasm on the lung function test) a diluted dose will be reattempted the next day. If there is still a wheeze (or bronchospasm) above the limits set by the study, no further doses will be given.	<1 hour
Mild to moderate	**Unlikely**	Mild to moderate. A mild to moderate allergic reaction may occur during or as soon as the	<1 hour

allergic reaction	Immediately after nebulised treatment	bacteriophage is administered. This can include swelling of the lips, face, eyes, presence of hives or welts or tingling mouth. An antihistamine agent will be given if this occurs and your child will be closely monitored should the reaction worsen.	
Severe allergic reaction	**Very unlikely** Immediately after nebulised treatment	Severe allergic reactions is extremely rare but potentially dangerous side effect from the treatment. This can manifest as swelling of the tongue, swelling or tightness in the throat, difficulty talking or a hoarse voice, persistent cough, persistent dizziness or collapse, pale or floppy, abdominal pain and vomiting. A reaction like this will result in immediate treatment with adrenaline	<1 hour to 72 hours

		administered via an injection into the muscles. In extremely rare situations, this can include multiple adrenaline injections and admission to the intensive care unit and a chance of requiring mechanical ventilation (breathing tube and machine)	
A change in the make-up of the bacteria in the lungs and/or gut	**Unlikely** After the treatment	Bacteriophages are very specific in terms of the bacteria they attack. Although some studies have shown that bacteriophages do not change the make-up of the other healthy bacteria (that forms part of our normal flora), it is important that we monitor this therefore we will be comparing the bacterial make-up of your child's lung and gut before and after treatment. These results will not be available during the	>24 hours

		hospital stay but the results will be communicated to you once it is available	

If you or your child become upset or distressed as a result of participation in the research, the study doctor will be able to arrange for counselling or other appropriate support. Any counselling or support will be provided by qualified staff who are not members of the research project team. This counselling will be provided free of charge.

These days, whilst general anaesthesia is generally very safe there are some risks associated with anaesthesia. The most common problems associated with anaesthesia are feeling unwell or vomiting, bruising at the site of injections, sore throat, or hoarse voice. Most patients do not have these problems. If these problems do happen, they usually get better very quickly. Damage to teeth may occur, but this is rare. The risk of brain damage or death due to anaesthesia is very rare.

The risk of problems from anaesthesia increases for patients who are having more major surgery, those with medical problems and those that require difficult anaesthetic procedures. If you have any concerns about these issues, you should discuss them with the study team.

10 WHAT WILL HAPPEN TO THE CHILD'S TEST SAMPLES?

Lavage, sputum, and blood samples will be stored following the finalisation of the respective test results. This is to allow for further analysis of the samples.

Samples of your child's blood and sputum obtained for this research project will be sent to the Children's Hospital at Westmead and the results will be part of your child's electronic medical records.

Your child's sputum obtained for this research project will be transferred to Iredell Laboratory, Westmead Institute of Medical Research, Westmead to be tested against the available phages. The Westmead Institute of Medical Research is not part of the Sydney Children's Hospital Network, but is part of the New South Wales Health Department.

Following this study, any remaining samples obtained will be stored under the NSW CF biobanking facility that is supervised by Prof Hiran Selvadurai. These samples may be used for future research in Bacteriophage and CF. However, your consent will be required in order for the remaining samples to be used in future research in the field of bacteriophage and CF. (as per the consent form below)

11 WHAT IF NEW INFORMATION ARISES DURING THIS RESEARCH PROJECT?

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, the study doctor will tell you about it and discuss with you whether you want the participant to continue in the research project. If you decide to withdraw your child, their study doctor will make arrangements for their regular health care to continue. If you decide that your child can continue in the research project, you will be asked to sign an updated consent form.

Also, on receiving new information, the study doctor might consider it to be in your child's best interests to withdraw them from the research project. If this happens, the doctor will explain the reasons and arrange for your child's regular health care to continue.

12 CAN MY CHILD HAVE OTHER TREATMENTS DURING THIS RESEARCH PROJECT?

Whilst your child is participating in this research project, they can continue all treatments they have been taking for their condition or for other reasons.

It is important to tell the study doctor or the study staff about all treatments or medications the participant may be taking, including over-

the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments.

You should also tell the study doctor about any changes to these during your child's participation in the research project.

13 WHAT IF I WITHDRAW MY CHILD FROM THIS RESEARCH PROJECT?

If you decide to withdraw your child from the project, please notify a member of the research team before you withdraw them. This notice will allow that person or the research supervisor to further discuss any health risks or special requirements linked to withdrawing.

If you do withdraw your child during the research project, the study doctor and relevant study staff will not collect additional personal information, although personal information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with the law. You should be aware that data collected by the study up to the time of withdrawal will form part of the research project results. If you do not want them to do this, you must tell them before your child joins the research project.

14 COULD THIS RESEARCH PROJECT BE STOPPED UNEXPECTEDLY?

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as unacceptable side effects for example persistent fevers related to the treatment, significant wheeze or breathing difficulties or severe allergic reaction.

15 WHAT HAPPENS WHEN THE RESEARCH PROJECT ENDS?

Once the project ends, there will be no further supply of bacteriophages. However, the ultimate aim of this study is to be able to provide information for the safe and routine use of bacteriophages in the future. A summary of the project results will be provided to the family of the participants.

PART 2 HOW IS THE RESEARCH PROJECT BEING CONDUCTED?

16 WHAT WILL HAPPEN TO INFORMATION ABOUT MY CHILD?

By signing the consent form you consent to the study doctor and relevant research staff collecting and using personal information about your child for the research project. Any information obtained in connection with this research project that can identify the child will remain confidential. *A code will be assigned to your child by the chief investigator for this study.* The

child's information will only be used for this research project and it will only be disclosed with your permission, except as required by law.

All data will be entered electronically into your child's electronic medical notes in the hospital system. Data entry will be performed by Dr Singh onto a data collection sheet. This sheet will use a special code name assigned to your child in order to protect his/her privacy.

The data will be stored as per the SCHN data retention policy for clinical trials which requires for data to be stored for 15 years or until the youngest participant turns 25 years of age.

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that the child cannot be identified, except with your permission.

Information about the child's participation in this research project may be recorded in their health records.

In accordance with relevant Australian and NSW health privacy and other relevant laws, you have the right to request access to your child's information collected and stored by the study team. You also have the right to request that any information with which you disagree be

corrected. Please contact the study team member named at the end of this document if you would like to access your child's information.

Any information obtained for the purpose of this research project and for the future research that can identify the participant will be treated as confidential and securely stored. It will be disclosed only with your permission, or as required by law.

17 COMPLAINTS AND COMPENSATION

If the participant suffers any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment for the participant. If the participant is eligible for Medicare, they can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

In the event of loss or injury, the parties involved in this research project have agreed to receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

18 WHO IS ORGANISING AND FUNDING THE RESEARCH?

- This research project is being conducted by Dr Jagdev Singh. The results of this research will be used by the study doctor *Dr Jagdev Singh* to obtain a *PhD* degree with the University of Sydney.
- This research has been funded by The Respiratory Department, The Children's Hospital at Westmead.
- This research is being conducted through the co-operation between the Respiratory Department, The Children's Hospital at Westmead, The Iredell Laboratory, Westmead Institute of Medical Research and The Barr Laboratory, University of Monash, Melbourne

19 WHO HAS REVIEWED THE RESEARCH PROJECT?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of The Sydney Children Hospital Network

This project will be carried out according to the National Statement on Ethical Conduct in Human Research (2007). This statement has been developed to protect the interests of people who agree to participate in human research studies.

20 FURTHER INFORMATION AND WHO TO CONTACT

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if the participant has any medical problems which may be related to their involvement in the project (for example, any side effects), you can contact the principal study doctor on 98453395 or any of the following people:

Clinical contact person (in-hours)

Name	*Dr Jagdev Singh*
Position	*Paediatric Respiratory Physician*
Telephone	*98453395*
Email	*Jagdev.Singh@health.nsw.gov.au*

Clinical contact person (out of-hours)

Name	*Respiratory On-Call*
Position	*Paediatric Respiratory Physician/ Fellow*
Telephone	*98450000 (switch then respiratory On-Call)*

For matters relating to research at the site at which the child is participating, the details of the local site complaints person are:

Complaints contact person

Name	Sydney Children's Hospitals Network HREC
Study reference number	2021/ETH00241, version 4, March 2024
Position	HREC Executive Officer
Telephone	(02) 9845 1253 or (02) 9845 3066
Email	SCHN-ethics@health.nsw.gov.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Name	Sydney Children's Hospitals Network HREC

Study reference number	2021/ETH11436
Position	HREC Executive Officer
Telephone	(02) 9845 1253 or (02) 9845 3066

Reviewing HREC approving this research and HREC Executive Officer details

Single Site -Research Governance Officer

Name	Sydney Children's Hospitals Network Research Governance Office
Study reference number	2021/ETH00241, Version 3, April 2022
Position	Research Governance Officer
Telephone	(02) 9845 1253 or (02) 9845 3066

Consent Form – Parent/Guardian

Title	A Single-Arm, Open-Labelled, Safety, and Tolerability of Intrabronchial and Nebulised Bacteriophage Treatment in Children with
Short Title	The evaluation of Intrabronchial and nebulised bacteriophage treatment in
Protocol Number	2022/ETH00241, version 3, April 2022
Project Sponsor	Respiratory Department, The Children's Hospital at Westmead
Coordinating Principal	Dr Jagdev Singh

Associate Investigator(s)	Professor Hiran Selvadurai Professor Jon Iredell (Iredell Laboratory, Westmead Institute of Medical Research) Professor Adam Jaffe Dr Jeremy Barr (Barr Laboratory, University of Monash) Professor Dominic Fitzgerald A/Prof Paul Robinson
Location	The Children's Hospital at Westmead or Sydney Children's Hospital, Randwick

Declaration by Parent/Guardian

I have read the Participant Information Sheet or someone has read it to me in a language that I understand.

I understand the purposes, procedures, and risks of the research described in the project.

I give permission for the child's doctors, other health professionals, hospitals or laboratories outside this hospital to release information to The Children's Hospital at Westmead concerning the child's disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to the child participating in this research project as described and understand that I am free to withdraw them at any time during the research project without affecting their future health care.

I understand that I will be given a signed copy of this document to keep.

Name of Child _____

Signature of Child

Date _____ _____

Name of Parent/Guardian _____

Signature of _____ Date _____

Name of Witness* to
Parent/Guardian's Signature
(please print)

_____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older

I consent to the storage and use of blood and sputum samples taken from the child for use, as described in the relevant section of the Participant Information Sheet, for (Tick which instance consent is provided for):

- ☐ This specific research project
- ☐ Other research that is closely related to this research project
- ☐ Any future research

Name of Child _____

Signature of Child

Date

_____ _____

Name of Parent/Guardian _____

Signature of _____ Date _____

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the research project, its procedures and risks and I believe that the parent/guardian has understood that explanation.

Name of Study Doctor/

_____

Senior Researcher[†]

(please print)

Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of, and information concerning, the research project.

I understand that, if I decide to discontinue the child's study treatment, a request may be made for them to attend follow-up visits to allow collection of information regarding their health status. Alternatively, a member of the research team may request my permission to obtain access to the child's medical records for collection of follow-up information for the purposes of research and analysis.

Form for Withdrawal of Participation – Parent/Guardian

Title	A Single-Arm, Open-Labelled, Safety and Tolerability of Intrabronchial and Nebulised Bacteriophage Treatment in Children with
Short Title	The evaluation of Intrabronchial and nebulised bacteriophage treatment in children with chronic *Pseudomonas*
Protocol Number	2022/ETH00241, version 4, March 2024
Project Sponsor	Respiratory Department, The Children's Hospital at Westmead
Coordinating Principal	Dr Jagdev Singh
Associate Investigator(s)	Professor Hiran Selvadurai Professor Jon Iredell (Iredell Laboratory, Westmead Institute of Medical Research) Dr Jeremy Barr (Barr Laboratory, University of Monash) Professor Dominic Fitzgerald A/Prof Paul Robinson
Location	The Children's Hospital at Westmead

Declaration by Parent/Guardian

I wish to withdraw the child from participation in the above research project and understand that such withdrawal will not affect their routine treatment, relationships with those treating them or the relationship with The Children's Hospital at Westmead

Name of Child _____

Signature of Child

Date

_____ _____

Name of Parent/Guardian _____

Signature of _____ Date_____

In the event that the parent/guardian's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher†

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the parent/guardian has understood that explanation.

Name of Study Doctor/

Senior Researcher†

(please print) _____

Signature _____ _____

† A senior member of the research team must provide the explanation of, and information concerning, withdrawal from the research pro

Appendix 3: Executive summary for parent information sheet

EXECUTIVE SUMMARY OF THE

PARTICIPANT INFORMATION STATEMENT

THE EVALUATION OF INTRA-BRONCHIAL AND NEBULISED BACTERIOPHAGE TREATMENT IN CHILDREN WITH *PSEUDOMONAS*. (CHIP-CF)

Your child is invited to participate in the above research study because your child continues to grow *Pseudomonas aeruginosa* in their phlegm that has not been eliminated with antibiotics and your doctor believes this could be a suitable treatment option for you. A detailed Information Statement about the study is attached and this is a summary of the essential information about the trial and where to find the relevant detailed information later in the Information Statement. You should read the Information Statement in full and discuss it with your family and medical practitioners before deciding to participate in this study. You may contact the study staff **Dr Jagdev Singh** to discuss or asks questions about the study on **98453395** or by email **Jagdev.singh@health.nsw.gov.au**

This study aims to *use a* tiny virus called bacteriophages (phages) that only consumes bacteria against the pseudomonas in your child's lungs.

Participation in this study is voluntary and refusal to participate or withdrawal from the study at a later stage will not affect the treatment

your child receives at The Respiratory Department, The Children's Hospital at Westmead.

If you decide to participate in this study your child will need to be admitted to the hospital for 2 weeks and subsequently be seen in the CF clinic at 3,6 and 9months after starting the study *(Figure 1, Page 5)*. During these visits your child will have a number of tests and assessments, such as a physical examination and lung function. The schedule for study visits and a full list of all tests and procedures is on the appendix of this document *(Figure 2, Page 6, details in pages 7 to 9).*

The most common risks to participants from the phage nebulisation might be fever, wheeze or rash. These are rare side effects and temporary in nature. A full list of the side-effects from the study drug and other risks associated with the study procedures is included *(pages 9 to 12)*

There is additional information about what information or samples will be collected about your child during the study *(page 12 to 14),* how that information will be used, and your child's privacy protected *(subsection 16 page 14).*

Please make sure you have completely understood what the study involves before you decide to participate.

Ethics Approval number: 2022/ETH00241

Appendix 4: Young person information sheet

YOUNG PERSON INFORMATION SHEET

Study Title	The evaluation of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with *Pseudomonas*. (CHIP-CF)
Principal Investigator/s	Dr Jagdev Singh (Dev), Respiratory Department, The Children's Hospital at Westmead.
Main Study Contact Person	Dr Jagdev Singh

1. INTRODUCTION

You are invited to participate in a research study titled- **The evaluation of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with *Pseudomonas***. This information sheet tells you about the study. It will help you choose if you want to join or not. You can ask your parents, carer, friend, or doctor if you need. You do not have to join this study if you do not want to.

Before you think of joining this study, you should know why and how the research is being done. Please read this information sheet carefully.

2. WHAT IS THE PURPOSE OF THIS STUDY?

- **We are performing this research to see if bacteriophages or phages (which are tiny viruses that like to eat bacteria) can be used safely to help children that have grown pseudomonas in their lungs.**
- **Dr Jagdev Singh (Dev) is also using this opportunity to complete his Ph.D.**

3. WHY HAVE I BEEN INVITED TO THIS STUDY?

You are invited to take part in this study because you have been growing a bacteria known as pseudomonas in your lungs for more than 1 year. We hope that this treatment will help to kill these bad bacteria.

4. DO I HAVE TO BE IN THIS STUDY?

You do not have to participate in this study if you don't want to. The doctors and nurses will take the best care of you as they have in the past, regardless of whether you are in the study or not. If you choose to participate, you can stop being in it at any time. All you need to do is tell

one of the researchers or your parents/carers that you don't want to take part anymore.

5. WHAT WILL HAPPEN TO ME IN THIS STUDY?

In order to be a part of this study, you will need to be in the hospital between 10 days to 2 weeks. On the first day of your stay in the hospital, we will put you to sleep for a few minutes and we will perform a bronchoscopy (putting a tiny video camera through your mouth to see the inside of your lungs). Because you will be asleep, you will not feel anything. Through this camera (bronchoscope), we will be able to spray these bacteriophages directly into your lungs.

During the time when you are asleep, we will also take some blood sample from your veins. After that, you will be given the usual antibiotics and physiotherapy that you get during your previous hospital stay for a tune-up.

The next day, we will teach you how to inhale the bacteriophages. This will be very similar to when you take your hypertonic saline and/or pulmozyme. You will need to take this phage medication twice a day for 10 days only. On the second day, when it is time to take your blood test to look at the amount of antibiotics in your blood (just like we do in your previous hospital stay), we will take some extra blood during the test.

During your stay in the hospital, you will undergo a lung test (lung function test that you have done before during your previous CF clinic

visits or previous hospital stay) that will require you to blow into a special computer. This test will be repeated at the middle (day 7) and end (day 14) of your hospital stay.

At the end of your stay in the hospital, we will need to take another blood sample from your veins. This is important so that we can compare any changes or improvements before and after being given the treatment.

Another thing that we will need is your phlegm (the sticky stuff you cough up) on the first day, around the 7th day, and on the last day of your admission. We will also need to collect a poo sample before and after treatment with the bacteriophage.

After that, we will continue to see you in your usual CF clinic to make sure everything is going well.

We will also need to look at your medical records from your previous visits to the hospital, for example; looking at the number of times you have been sick with a cough, your height and weight, your previous lung function and the bacteria that are present in your phlegm.

The blood and phlegm that we will use for the study can also be used for further research in CF and bacteriophages in the future. However, we will be asking for your permission, and you can always say no if you would only like your blood and phlegm to be used in this particular study.

6. IF I CHOOSE TO JOIN THE STUDY, CAN ANYTHING BAD HAPPEN?

Phage treatment is generally very safe. Some bad things that may happen would be fever, or rash. These are expected to be extremely rare, but we will be monitoring you regardless.

As you remember, sometimes after being put to sleep (anaesthesia), you may wake up feeling sick. This feeling may last for 1-2 hours. Sometimes, you may also have fever and cough after the bronchoscopy, this is a common effect after undergoing a bronchoscopy. However, we will monitor you, nevertheless.

If anything during the research makes you upset, you can stop being part of the research. You should let your parents/carers know and you will be given the names of people you can talk to about what is making you upset, if that is what you want to do. The researchers can help you do that.

If you feel sick or if you notice any strange or bad feelings during the study, especially if they are unexpected or severe, you should let your parents or doctors know right away. You can call the doctor who is treating you or the study coordinator, [Dr Jagdev Singh at 98453395]. You can call at any time, day or night, to tell them.

You can also call the Kids Helpline (telephone number: 1800 551 800) or Lifeline (telephone number: 13 11 14) at any time."

7. WILL THERE BE ANY BENEFITS FOR ME IN THIS STUDY?

We cannot promise that you will receive any benefits from this research; however, possible benefits may include a reduction in your cough, the amount of phlegm that comes out or even the reduction of the bacteria in your lungs.

8. HOW WILL MY PRIVACY BE PROTECTED?

Your privacy and confidentiality will be protected at all times in this study. Unless you allow us, we will not tell anybody else you are or have been a part of this study. We will not release any information to anybody else that could be used to identify you, unless we are required to do so by law. For example, researchers are required to report if a participant is believed to be at risk of harm.

In order to protect your privacy, the study team will remove any information that may be used to identify you from any study documents, and instead of your name appearing on them, you will be identified by a specific study code number that applies only to you. Only this code number will be used on any research-related information collected about you for this study, so that your identity as part of the study will be kept completely private. Only the study team at **The Children's Hospital at Westmead** will have the ability to link this code number with your personal information, and the linking information will be kept in **the**

Respiratory Department, The Children's Hospital at Westmead server.

Your data will be stored for a minimum of 15 years after the study finishes or until the youngest participant turns 25.

If you decide to leave the study, we will not collect any more information about you. We would like to keep the information we have already collected about you to help us ensure that the results of the research project can be measured properly. Please let us know if you do not want us to do this.

9. WHAT WILL HAPPEN TO THE STUDY RESULTS?

We would like to share the study results by publishing them in relevant journal articles and / or presenting them at different conferences. We will make sure that information is published /presented in such a way that you are not identifiable unless you have given us permission to do so.

You can also tell us on the consent form if you want to receive a simple summary of the study findings for information.

10. WHO SHOULD I CONTACT IF I HAVE ANY QUESTIONS?

If you have any questions or want more information about this study before or during participation, you can talk to **Dr Jagdev Singh** on **98453395**

You can also ask your parents/carers to talk to us.

11. WHO DO I CONTACT IF I HAVE CONCERNS ABOUT THE STUDY?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). This study has been approved by the Sydney Children's Hospitals Network HREC **(approval number: 2022/ETH00241)**.

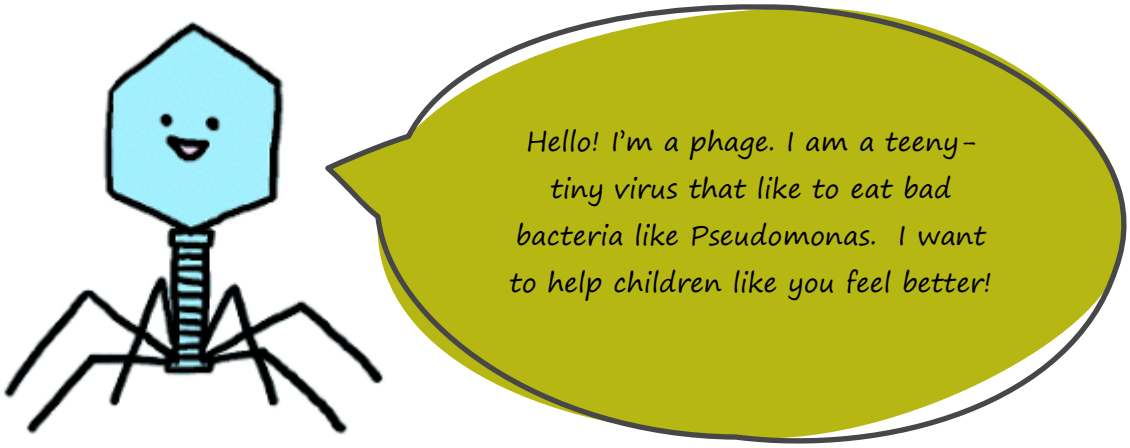
Please talk to your parents/carers if you are worried about being in this study, or you have a complaint. They can talk to **Dr Jagdev Singh** on **98453395** or they can contact the Human Research Ethics Committee on (02) 9845 1253 or SCHN-Ethics@health.nsw.gov.au.

Appendix 5: Child information sheet

Child Information Sheet

Study Title	CHIP-CF: The evaluation of Intra-bronchial and Nebulised Bacteriophage Treatment in Children with Pseudomonas.
Principal Investigator/s	Dr Jagdev Singh (Dev), Respiratory Department, The Children's Hospital at Westmead
Contact Details	Dr Jagdev Singh

This information sheet has been put together to help you choose if you would like to take part in our research study about phages in CF.



1. What is this study about?

We are researchers at **The Children's Hospital at Westmead**.

We are trying to find out if we can use a special type of virus called phages to destroy a type of bacteria called Pseudomonas that causes a lot of health problems in children.

Finding out these things maybe able to help us use phages in the future to treat infections in the lungs.

2. Do I have to be in this study?

No you don't. If you say no, that is ok. You and your parents/carers will decide if being in this study is the right thing for you.

Even if you take part at the beginning and change your mind later and don't want to be a part of the study anymore; that is okay as well. All you need to do is tell the researcher or your parents/carers that you don't want to take part anymore. We will still look after you in the best way we can.

3. What will happen to me in this study?

If you agree to this study, you will spend 2 weeks with us in the hospital for your usual tune-up.

On the first day, you will come into the hospital and be put to sleep for a few minutes for us to have a look at your lungs with a tiny camera.

The next day, you will then be taught to breathe in the phage medication (nebuliser). It will be the same as your pulmozyme or hypertonic saline that you do every day. You will need to do this twice a day after your physiotherapy. After 2 weeks (or between 10days to 14 days) you will go home.

After going home, Dr Dev will give your parents a call in 1 week to check up on how you are going. If everything is well, we will continue to see you in the clinic every 3 months for a check-up.

4. Is there anything that could make me upset if I take part in the research?

Breathing in the medication will not be upsetting. However, in order for us to look into your lungs, you will be put to sleep and sometimes you will feel

a bit sick when you wake up. Usually, this feeling gets better after 1-2 hours.

You may also feel a little sick or unwell during or after breathing in the medication. You must tell your doctor/parent/carer if you feel unwell.

If anything you talk about during the research does make you upset you can stop the research. Your parents/carers will be told and you will be given the names of people you can talk to about what is making you upset, if that is what you want to do. The researchers can help you do that.

You can also call the Kids Helpline at any time. Their telephone number is 1800 551800.

5. What will happen to my information?

Your information will only be used by the researchers. You can tell them whatever you want and no one will know that it came from you. The only time the researchers would have to tell someone is if anyone hurt you or upset you in any way. The researchers would also have to tell someone if you said you might hurt yourself or someone else.

6. Who can answer my questions?

If you have any questions, you can talk to **Dev** on **9845000**.

You can also ask your parents/carers to talk to us.

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