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

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ABSTRACT

Introduction Cystic fibrosis (CF) is a multisystem condition that is complicated by recurrent pulmonary infections requiring aggressive antibiotic treatment. This predisposes the patient to complications such as sensorineural hearing loss, renal impairment, hypersensitivity and the development of antibiotic resistance. *Pseudomonas aeruginosa* is one of the more common organisms which cause recurrent infections and result in greater morbidity and mortality in people living with CF. Bacteriophages have been identified as a potential alternative or adjunct to antibiotics. We hypothesise that bacteriophage therapy is a safe and well-tolerated treatment in children with CF infected with *P. aeruginosa* infection in their airways.

Methods This single-arm, open-labelled, non-randomised trial will run for a maximum period of 36 months with up to 10 participants. Adolescents (≥12 years and <18 years of age) who continue to shed *P.aeruginosa* (within 3 months of enrolment) despite undergoing eradication therapy previously, will be considered for this trial. Non-genetically modified bacteriophages that have demonstrated obligate lytic activity against each of the study participants' *P. aeruginosa* strains will be selected and prepared according to a combination of established protocols (isolation, purification, sterility testing and packaging) to achieve close to good manufacturing practice recommendations. The selected bacteriophage will be administered endo-bronchially first under direct vision, followed by two times a day nebulisation for 7 days in addition to standard CF treatment (intravenous antibiotics, physiotherapy to be completed as inpatient for 10–14 days). Safety and tolerability will be defined as the absence of (1) fever above 38.5°C occurring within 1 hour of the administration of the nebulised bacteriophage, (2) a 10% decline in spirometry (forced expiratory volume in 1 s %) measured preadministration and postadministration of the first dose of nebulised bacteriophage. Clinical reviews including repeat sputum cultures and spirometry will be performed at 3, 6, 9 and 12 months following bacteriophage treatment.

Ethics and dissemination Our clinical trial is conducted in accordance with (1) good clinical practice, (2) Australian legislation, (3) National Health and Medical Research Council guidelines for the ethical conduct of research.

Trial registration number Australia and New Zealand Clinical Trial Registry (ACTRN12622000767707).

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Bacteriophages are demonstrating great potential in the treatment of resistant bacterial infections based on a compassionate use basis.

WHAT THIS STUDY ADDS

⇒ This trial will examine the safety of bespoke bacteriophage in the treatment of children with cystic fibrosis infected by *P. aeruginosa* to be delivered via endo-bronchial and inhalational routes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ If our hypothesis is supported, our study will pave the way for the use of bacteriophages in children outside of the compassionate access pathway and demonstrate that bacteriophages can be administered safely via inhalation or respiratory routes.

INTRODUCTION

Cystic fibrosis (CF) is a life-limiting genetic condition of the lungs, exocrine organs and gastrointestinal tract that occurs in 1 in 2500 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.[1 2] A child with CF infected with *P. aeruginosa* within their airway has 2.6 times higher risk of death within 8 years when compared with 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.[1 3 4] 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.[2]

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.[5–7] Additionally, there is the added risk of a change in the airway microbiota allowing for opportunistic infections and a rise in resistant organisms.[8 9] 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 with 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.[10 11] 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.[12]

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 used in multiple n=1 studies in different countries.[13–21] 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.[22] Although not persistently observed, phages have been shown to reverse antibiotic resistance and restore susceptibility to various classes of antibiotics in *P. aeruginosa*.[23] 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.[24] Phages have been used in children in multiple situations with the youngest child being 2 years of age, treated with intravenous bacteriophage against *P. aeruginosa*.[25] Phages are a part of the human microbiome and the environment.[26 27] Lytic phages are specific to a particular bacterial strain while some are known to affect multiple strains of the bacteria.[28–30] The microbiota has been shown to be less affected by phages when compared with antibiotics[31]; this has been shown in both murine and human studies (adults and children).[32 33] A trial performed in children with diarrhoeal disease demonstrated that phages pass the alimentary tract without any change in the microbiome.[34] Phages when inhaled are the least systemically absorbed compared with other methods of delivery.[35 36] 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.[37] Phages are able to stimulate an adaptive immune response. Dose,[38] duration[38–40] and route of administration may influence this with topical, enteral and inhaled routes of administration considered less immunogenic compared with intravenous and intraperitoneal administrations.[41] 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 effectively and therefore are not able to induce an inflammatory response.[42–44] Phages are used in the food industry to reduce the bacterial load in food used for human consumption.[45 46] Phages that are non-genetically modified organisms are used as a surrogate for viruses in aerosolisation studies to evaluate the efficacy of viral filters.[47]

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.[48] Phages have shown some potential to remain intact following nebulisation in a murine model.[12 49] Optimum dosing remains unclear, however, based on previous preclinical studies that include in-vitro and animal models, the PFU/mL to achieve treatment effect is commonly between 10^6 and 10^{10} PFU/mL.[15 50–52]

A review of human trials and the use of phage in children via respiratory routes are described in tables 1 and 2.

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-randomised, 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

Table 1 Existing human trials previously published (only relevant inhaled route is shown)

Reference	Year	Indication	Aetiology	Delivery method	Efficacy
Delacoste[63]	1959	Refractory coughs	NA	Inhalation	100%
Hoeflmayr et al[64]	1962	Bronchitis	*Streptococci* (2/3); *Staphylococci* (1/3)	Inhalation	90%
Garsevanishvili[65]	1974	Pneumonia	*Staphylococci, streptococci* and *enterococci* were targeted	Inhalation	N/A
Ioseliani et al[66]	1980	Lung infections	*Staphylococci* and/or others	Inhalation, bronchoscopy	>90%
Meladze et al[67]	1982	Parenchyma and pleura infections	*Staphylococcus*	Inhalation, topical, parenteral	>90% (223)
Kvachadze et al[68]	2011	Cystic fibrosis-associated infections	*Staphylococcus* and *Pseudomonas*	Inhalation	Improvement

This table was adapted from Abedon *et al.* 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.[69]

Network Ethics committee (ethics approval number 2022/ETH00241, version 3). 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
► Adolescents (≥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.[53]
► Continues to shed *P. aeruginosa* in sputum despite undergoing eradication therapy using two

antipseudomonal antibiotics and/or is currently on suppressive nebulised antibiotic treatment.
► The latest clinical isolates of *P. aeruginosa* taken within 3 months of enrolment are susceptible to (demonstrates lytic activity) available anti-*P. aeruginosa* phages.
► Ability to perform reliable spirometry.[54]

Exclusion criteria
► Children who have received more than 1 mg/kg of prednisolone continuously for more than 7 days before study enrolment or have a diagnosed immunosuppressive condition.
► Children that require ≥18 hours/day of non-invasive ventilatory support during admission.
► A current diagnosis of allergic bronchopulmonary aspergillosis (ABPA).
► History of haemoptysis in the past 12 months before study enrolment.
► Prior known inability to expectorate sputum.
► Has undergone solid organ transplantation.
► Positive sputum isolation of *Burkholderia cepacia* or non-tuberculous *Mycobacterium* within the past 1 year.

Table 2 Recent case reports of children with cystic fibrosis treated with bacteriophage[41]

Reference	Route of administration	Main safety outcomes
Lebeaux et al[70]	Inhaled phage against *Achromobacter xylosoxidans*	Well tolerated
Gainey et al[24]	Intravenous against *Achromobacter*	No adverse events
Dedrick et al[71]	Intravenous and topical	Weak phage-neutralisation antibody and cytokines. Diaphoresis and flushing without fever. No adverse reaction
Hoyle et al[72]	Inhaled and oral against *A. xylosoxidans*	No safety data, patient improved
Kvachadze et al[68]	Inhaled against *Pseudomonas aeruginosa*	No adverse events

- ► A positive COVID-19 PCR nasal swab with a confirmed COVID-19 infection (true positive) in the past 6 months before enrolment.[55]
- ► Within 3 months of having received a booster COVID-19 vaccine or within 6 months of receiving the first dose of vaccine.[56]

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 (intravenous antibiotics, physiotherapy to be completed inpatient for 10–14 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 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 10–14 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.
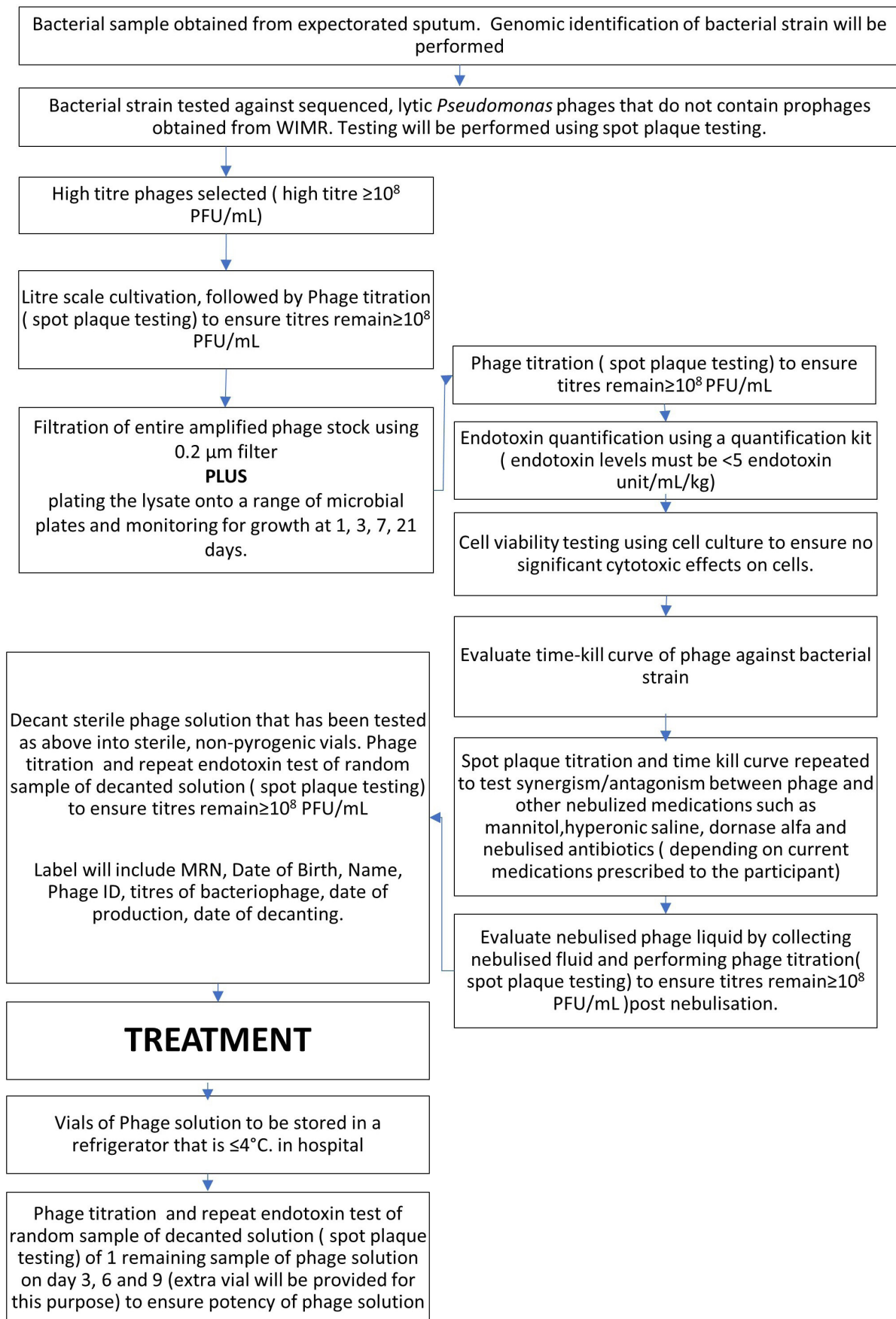
The phage solution will undergo regular monitoring throughout the entire study following processes that may reduce the phage titres (figure 1).

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 Therapeutic Goods Administration-approved mesh nebulisers. To confirm the viability of the phage-nebuliser pairing, all candidate phages will undergo testing to ensure titres remain $\geq 10^8$ 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 (ie, lipopolysaccharides), 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:

- ► Standardised bacteriophage purification for personalised phage therapy, Luong *et al*.[57]
- ► 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.[58]

Bacterial sample obtained from expectorated sputum. Genomic identification of bacterial strain will be performed

Bacterial strain tested against sequenced, lytic *Pseudomonas* phages that do not contain prophages obtained from WIMR. 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 for growth at 1, 3, 7, 21 days.

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,hyperonic saline, 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

Phage titration and repeat endotoxin test of random sample of decanted solution (spot plaque testing) of 1 remaining sample of phage solution on day 3, 6 and 9 (extra vial will be provided for this purpose) to ensure potency of phage solution

Figure 1 Summary of bacteriophage processing. MRN, medical record number; PFU, plaque forming units; WIMR, Westmead Institute of Medical Research.

► 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.[59]

► Packaging selection is based on Guidance for industry, Container Closure Systems for packaging human drugs and biologics by Food and Drug Administration (FDA).[60]

► Nasal spray and inhalation solution, suspension, and spray drug products—chemistry, manufacturing, and controls documentation; guidance for industry.[61]

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 (1 mL of 1×10^8 PFU/mL of phage and added with 3 mL 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 2×10^8 PFU/8 mL in the total solution or two sterile vials of phage solution that contain 1×10^8 PFU/4 mL). Therefore, 2 mL/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 0.2 mL/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 1×10^8 PFU/4 mL and be administered two times a day. The ideal timing of phage dosing should be 12 hours apart (range 10–14 hours).

Concomitant therapy for intravenous antipseudomonal 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 10–14 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 1 and 2. Results will be transcribed onto a data collection sheet that will be deidentified and stored under a password-protected file. The data will be available for

TIMEPOINT	Pre-admission	Day 0	Day 1	Day 2	Day 3-6	Day 7	Day 8 to 9 * or up to day 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	
Discharge review (telephone)								X					

Figure 2 Timetable of schedule. @Sputum on day 1 to be performed before first dose of nebulised bacteriophage, *first spirometry will be based on procedure outlined in appendix 7, **full blood count, electrolytes, liver function test, C reactive protein, blood culture, interleukin 6, tumour necrosis factor-alpha, IgG, IgA, IgM, RNA nanostring study, neutralising 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. aThis clinical review will be conducted before performing the bronchoscopy.

review by the TSC and DSMB at the stipulated time points as per the individual charters. Data points collected will be standardised using:

▶ Phage identification, isolation, purification, viability testing and sterility data template.
▶ Bronchoscopy report.
▶ 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.
▶ 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 on 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 PCR (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), IgG, IgA, IgM, phage neutralising antibodies); blood sample (full blood count, renal and liver profile, C reactive protein, blood culture, interleukin 6, TNFα, IgG, IgA, IgM, nanoString RNA sequencing, phage neutralising 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 2). Bacterial diversity (16s RNA and, pretreatment 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 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:

▶ Fever >38.5°C over three consecutive temporally related administration or three 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.

▶ Bronchospasm defined as a 10% decline in forced expiratory volume in 1 s % measured preadministration and postadministration of the first dose of nebulised bacteriophage despite a reduction of dosing and pretreatment with inhaled salbutamol.[62]

Secondary outcomes
A reduction in sputum bacterial culture titres of *P. aeruginosa* from pretreatment to day 7 of treatment.

Indications to stop treatment on the participant
▶ Report of fevers >38.5°C that fit the adverse event criteria stated above.
▶ Severe allergy within 1 hour of the administration towards the nebulised liquid that requires inhaled salbutamol (×3, 20 minutely) or intramuscular epinephrine.
▶ Severe bronchospasm despite a reduction in the dosing of the nebulised bacteriophage and pretreatment inhaled salbutamol is administered.
▶ 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% CI. 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 analysis of variance (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 two committees that will be convened for this trial which include the 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 CF. 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 intravenous 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.

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