A Phase 1b/2, Multi-Centered, Randomized, Double-Blind, Placebo-Controlled Trial of the Safety and Microbiological Activity of a Single Dose of Bacteriophage Therapy in Cystic Fibrosis Subjects Colonized with *Pseudomonas aeruginosa*

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DMID Clinical Project Manager: Erica Raterman, PhD

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human subjects research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [United States Code of Federal Regulations (CFR), 45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

The study trial will be carried out in accordance with the Protocol, Good Clinical Practice (GCP), and as required by the following:

- United States 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 [Investigational New Drug (IND) Application].
- International Council for Harmonisation (ICH): Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research (OER), Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance
- Applicable DMID Policies, Guidelines, and Plans to include but not limited to: Clinical Quality Management Policy, DMID Guidelines for Clinical Study Product Management, Guidelines for Writing Notes to the Study File, Study Product Management Plan (SPMP), Clinical Quality Management Plan (CQMP), etc.

SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements. I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

Name

Title

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LIST OF ABBREVIATIONS

AE	Adverse Event
APT	Adaptive Phage Therapeutics
ALT	Alanine Aminotransferase
ARLG	The Antibacterial Resistance Leadership Group
AST	Aspartate Aminotransferase
AUC	Area Under Curve
Bp	Base pairs
BPM	Beats per Minute
CF	Cystic Fibrosis
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CFR	Code of Federal Regulations
CFRSD	Cystic Fibrosis Respiratory Symptom Diary
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CFU	Colony Forming Units
cGMP	Current Good Manufacturing Practice
CMS	Clinical Material Services
CQMP	Clinical Quality Management Plan
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support
DAIDS	Division of AIDS
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DOOR	Desirability of Outcome Ranking
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
eIND	Emergency Investigational New Drug Application
ESI	Event of Special Interest
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in 1 second
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HLT	High Level Term
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Intent-to-Treat

IV	Intravenous
LFT	Liver Function Tests
LLN	Lower Limit of Normal
MDR	Multidrug-Resistant
MedDRA	Medical Dictionary for Regulatory Activities
MM	Medical Monitor
mmHG	Millimeters of Mercury
MO	Medical Officer
MOP	Manual of Procedures
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OER	Office of Extramural Research
PI	Principal Investigator
PID	Patient Identification
PFU	Plaque Forming Unit
PK	Pharmacokinetics
PVG	Pharmacovigilance Group
QA	Quality Assurance
QC	Quality Control
QOL	Quality of Life
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDCC	Statistical and Data Coordinating Center
SOC	System Organ Class
SOP	Standard Operating Procedure
SPMP	Study Product Management Plan
SUSAR	Suspected Unexpected Serious Adverse Reaction
US	United States
WRAIR	Walter Reed Army Institute of Research
WBC	White Blood Cell
YO	Year old

PROTOCOL SUMMARY

Title:	A Phase 1b/2, Multi-Centered, Randomized, Double-Blind, Placebo-Controlled Trial of the Safety and Microbiological Activity of a Single Dose of Bacteriophage Therapy in Cystic Fibrosis Subjects Colonized with *Pseudomonas aeruginosa*
Design of the Study:	Randomized, Double-Blind, Placebo-Controlled Trial
Study Phase:	1b/2
Study Population:	Up to 72 male and female subjects 18 years of age and older with Cystic Fibrosis (CF) and residing in the United States (US)
Number of Sites:	Up to 20 clinical sites in the US
Description of Study Product or Intervention:	A total of 4 x 10^7 to 4 x 10^9 plaque forming units (PFU) of a 4-component bacteriophage mixture administered intravenously
Study Objectives:	Primary: • Describe the safety of a single dose of intravenous (IV) bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum. • Describe the microbiological activity of a single dose of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum. • Describe the benefit to risk profile of a single dose of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum. Secondary: • N/A

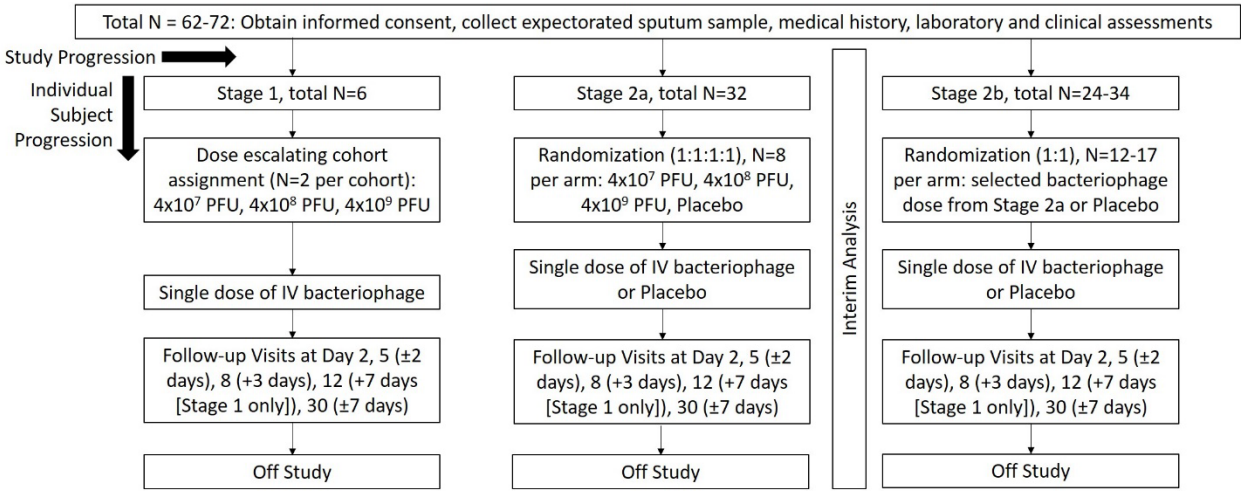
	Exploratory: • Characterize the serum and sputum pharmacokinetic (PK) profiles of a single dose of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum. • Describe changes in lung function from the administration of a single dose of IV bacteriophage therapy through Day 30 ± 7. • Characterize geographically diverse *P. aeruginosa* isolates susceptibility to bacteriophages. • Describe subjects' quality of life (QOL) before and after receiving bacteriophage therapy.
Duration of Individual Subject Participation:	Up to 44 days
Estimated Time to Last Subject/Last Study Day:	Approximately 48 months

STUDY SCHEMA

Figure 1: Treatment Arms

Stage 1 Sentinel Stage (unblinded, dose-escalation)		**Stage 2** Double Blind Comparison					
Dose	# of Subjects	Dose	**Stage 2a** # of Subjects		Dose	**Stage 2b** # of Subjects	Total # of subjects at the final selected dose (Combination of Stages 2a + 2b)
4×10^7 PFU	2	4×10^7 PFU	8	Interim Analysis			
↓							
4×10^8 PFU	2	4×10^8 PFU	8		Optimal Stage 2a dose	~12-17	~20-25
↓							
4×10^9 PFU	2	4×10^9 PFU	8				
		Placebo	8		Placebo	~12-17	~20-25

Figure 2: Study Flow Diagram Illustrating Study and Subject Progression



1 KEY ROLES

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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Multidrug-resistant (MDR) bacterial infections remain a pressing global concern. The CF population is particularly prone to MDR infections. These manifest as frequent lower respiratory tract infections that are increasingly challenging to treat as resident bacteria routinely acquire additional mechanisms of drug resistance and develop an increasingly mucoid phenotype, rendering most antibiotics ineffective over time.[1] *P. aeruginosa* remains the most common pathogen responsible for CF exacerbations.[2] Few novel antibiotic agents recently approved by the FDA have substantial activity against highly drug-resistant *P. aeruginosa*.[3] Because of the limited number of antibiotics available to treat respiratory infections in CF patients, particularly as they enter more advanced phases of disease, novel treatment approaches are desperately needed.

Bacteriophages are viruses that target and kill bacteria.[4] Since the 1940s, with the advent of modern antibiotics, their use in antimicrobial therapeutics in the western world has been largely abandoned. Although bacteriophage products have recently been approved in food safety applications (e.g., bacteriophages targeting *Listeria monocytogenes*),[5] their use in human medicine remains experimental. In recent years, bacteriophage administration as adjunct therapy to systemic antibiotics for the treatment of complex infections under compassionate use conditions has increased as is evident by a growing number of case reports in the literature. Organisms that have been targeted with bacteriophage therapy include *P. aeruginosa*, [6-8] *Acinetobacter baumannii* complex, [9,10] *Staphylococcus aureus*, [11-13] and *Mycobacterium abscessus*.[14] Bacteriophage therapy has been used to treat a variety of infectious processes including bacterial pancreatitis, pneumonia in lung transplant recipients [7] and in patients with CF,[6,14] left ventricular assist device infections,[12] staphylococcal sepsis,[15] burns,[16] chronic sinusitis,[13] and hardware-associated bone and joint infections.[10,17] The role of bacteriophages in decolonizing patients harboring MDR pathogens in the intestinal tract has also been explored.[18] These clinical experiences have noted very few safety concerns, as described in Section 2.3.1. Serious adverse events (SAEs) attributable to bacteriophages have not been reported in the published literature.

Unique features that make bacteriophages attractive for clinical use include: bactericidal activity, high specificity for target pathogens including MDR pathogens, biofilm penetration, avoidance of host tissue damage, preservation of the human microbiome, and potential synergy with antibiotics. However, much work remains before bacteriophage therapy can be routinely used in clinical practice. Major gaps in knowledge include: the optimal frequency, dosage, and duration of administration, availability of clinical laboratory testing technologies that reliably predict activity of bacteriophages for specific clinical applications, understanding factors affecting the kinetics of bacteriophage resistance during clinical use, optimal use of bacteriophages in combination with antibiotics and in combination with other bacteriophage regimens, and optimization of bacteriophage stability in pharmaceutical preparations.

Rigorous scientific studies are required to address these critical gaps in knowledge related to bacteriophage therapeutics. The overarching goal of the proposed clinical trial is to enhance the understanding of the safety and microbiological activity of bacteriophage therapy.

2.2 Scientific Rationale

2.2.1 Purpose of Study

This clinical trial is designed to describe the safety and microbiological activity of bacteriophages directed at *P. aeruginosa* in clinically stable CF subjects with *P. aeruginosa* respiratory colonization. This is a dose-ranging study of intravenous (IV) anti-pseudomonal bacteriophage therapy. Sentinel subjects will be enrolled first and receive a single IV dose of a 4-component anti-pseudomonal bacteriophage (see Section 4.1 for details); all subjects will be monitored for the subsequent 96 hours (Stage 1) before enrollment in Stage 2 can begin. Stage 1 subjects will continue to be monitored for 30 ± 7 days. After Stage 1 enrollment and the 96-hour monitoring period are complete, eligible subjects will be enrolled into Stage 2 and receive a single IV dose of a 4-component anti-pseudomonal bacteriophage or placebo and then will be monitored for 30 ± 7 days (Stage 2). Stage 2a will randomize subjects to placebo or one of three different doses of bacteriophage. After the last Stage 2a subject has been enrolled, study enrollment will be held for an Interim Analysis (Section 10.4) to determine the dose that will be used in Stage 2b. This trial will limit the route of administration of bacteriophages to the IV route. As the safest, yet still microbiologically active dosage of bacteriophages has yet to be defined, this will be a dose-ranging study evaluating 1 x 10^7 PFU, 1 x 10^8 PFU, and 1 x 10^9 PFU of each bacteriophage (equaling a total of 4 x 10^7 PFU, 4 x 10^8 PFU, and 4 x 10^9 PFU of bacteriophages in the 4-component bacteriophage mixture). It is expected that a single dose of bacteriophage will be safe and lead to at least a one-$\log_{10}$ decrease in *P. aeruginosa* colony forming units (CFU)/mL in sputum, compared to baseline.

2.2.2 Study Population

This is a Phase 1b/2 clinical trial that will enroll up to 72 CF subjects. Clinically stable volunteers will be recruited from up to 20 CF outpatient clinics in the US. All study visits will occur in the ambulatory setting. There will be no enrollment from international sites. A detailed review of the study will be discussed with potential subjects and consent will be obtained. Inclusion will be limited to subjects 18 years of age or older (at the time of the screening) who are capable of providing informed consent. Furthermore, subjects should be capable and willing to complete all study visits and perform all procedures required by this protocol. Individuals who are pregnant, planning to become pregnant at any time during the study period, or breastfeeding will not be included as the impact of bacteriophage therapy on pregnancy, fetuses, and neonates is unknown. Cystic fibrosis (CF) primarily affects persons of European backgrounds and generally has an unequal distribution across races and ethnicities. It is expected that the racial and ethnic distribution of enrolled subjects will reflect those of the local CF communities from where subjects are recruited but may not reflect the US population as a whole. Prisoners and other potentially vulnerable populations will not be enrolled.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The scientific literature regarding the use of bacteriophage therapy for clinical use has not indicated any clear safety risks. As subjects will be receiving bacteriophage IV, there may be risks associated with this route of administration. Placement of a peripheral venous catheter can cause discomfort, minor bleeding, and infections from skin flora.

There have been several case reports and case series, as well as limited clinical trials investigating the safety of bacteriophage therapy. Although significant variability exists across published studies, there have not been SAEs related to bacteriophage therapy in the published literature. **Table 1** describes published studies on bacteriophage use in humans and includes reported adverse events (AEs). There are no consistent mild or moderate AEs across studies that have been associated with bacteriophage therapy.

From a theoretical point of view, the immediate lysis of bacteria following bacteriophage administration may result in skin flushing, hemodynamic changes in pulse and blood pressure, and respiratory difficulties.

From prior experiences with bacteriophage the following may occur, allergic reactions, temporary increases in liver enzymes, temporary shortness of breath or wheezing, temporary decrease in blood pressure, temporary feeling of being flushed or sweaty, development of fever, and temporary increase in heart rate.

A theoretical risk with bacteriophage therapy is the risk of transduction. This means the bacteriophage may serve as a vector to transfer DNA from one bacterium to another. The transferred DNA may include antibiotic resistance genes, as well as other clinically relevant genes. The likelihood of this occurring is unknown; however, experimental data from the current trial indicate this risk is very low. More specifically, whole genome sequencing analyses of paired *P. aeruginosa* isolates obtained from the sputum samples of 19 subjects pre and post bacteriophage or placebo treatment from Stage 2a were performed to assess whether there was any evidence of transduction by bacteriophage among subjects enrolled in the trial. The results indicate that there is no evidence of transduction by the bacteriophage within enrolled subjects.

The Investigator's Brochure (IB) provided by National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID)/Division of Microbiology and Infectious Diseases (DMID) with pharmaceutical support from Walter Reed Army Institute of Research (WRAIR) and Adaptive Phage Therapeutics (APT), Inc., describes experiences with providing bacteriophages under FDA emergency IND (eIND) and Compassionate Use. No AEs have been reported on the cardiovascular system, renal system, endocrine system, skin, central nervous system, or on lipase/amylase values. There was one case in which a patient developed fever, wheezing, and shortness of breath two hours following 2 consecutive doses of IV bacteriophage administration dosed at 1×10^{11} PFU. The symptoms resolved with acetaminophen, solumedrol, albuterol, and diphenhydramine. The same bacteriophage mixture was subsequently tolerated by the patient without further incident when the bacteriophage dosage was reduced to 1×10^{10} PFU.

Some patients receiving bacteriophage mixtures were noted to have asymptomatic isolated elevations in liver function tests (LFTs) including either aspartate aminotransferase (AST) or alanine aminotransferase (ALT), which normalized following cessation of bacteriophage therapy. For some of the patients with elevated AST/ALT, resolution could not be determined. No cases of phage-related drug-induced liver injury have been reported. Refer to the IB for additional details.

Table 1: Summary of Bacteriophage Safety Events Reported in the Peer-Reviewed Literature.

Publication	**Source of Bacteriophages**	**Brief Description**	**Bacteriophage Dosing (highest dose given, PFU)**	**Adverse Events (AEs)**
Aslam 2020[19] PMID: 33005701	Naval Medical Research Center, APT, Armata Pharmaceuticals, Roach Laboratory, Baylor College of Medicine, Walter Reed Army Institute of Research.	Describes 10 patients treated with intravenous bacteriophages for a variety of infections and caused by a variety of organisms.	Highest dose administered for a treatment course: **5 x 10^9 IV q6h** for 14 days. Two infusions up to 1 x 10^{11} were administered to one patient, but with adverse events.	In one case, an 82-year-old (YO) male developed **fever, wheezing, and shortness of breath** after 2 infusions of 1 × 10^{11} PFU/mL concentration. Symptoms resolved with acetaminophen, solumedrol, albuterol nebulization, and diphenhydramine, and the patient continued phage therapy at a lower dose without further incident. No AEs reported in other 9 patients.
Aslam, 2019[12] PMID: 30661974	AmpliPhi Biosciences 3 bacteriophages	65 YO male with LVAD infection with MSSA and open chest wound	**3 x 10^9 IV** q12h for 28 days	None reported

LaVergne, 2018[20] PMID: 29687015	Naval Medical Research Center provided phages 1 bacteriophage	77 YO male with traumatic brain injury with craniotomy site infection with MDR *A. baumannii*	**2.14 × 10^7** IV q2h for 8 days	Two hours after first dose became **briefly hypotensive** (no pressors)
Duplessis, 2019[21] PMID: N/A	US Navy and APT Personalized phage cocktails ranging from 1 to 3 phages	13 case studies of eIND bacteriophage use Two pediatric uses: 10 YO female and 2 YO males Eleven adult uses ranging from 18 – 77 YO, including males and females Duration of phage therapy ranged from 2 days to 19 weeks	Varied per case; highest reported case dose **1.0 x 10^{10}** PFU/mL IV	No identified adverse effects attributed to phage therapy
Cano, 2020[22] PMID: 32699879	APT 1 bacteriophage	62 YO male with a relatively susceptible *K. pneumoniae* prosthetic knee infection	**6.3 x 10^{10}** IV q24h for 40 days	None reported
Tkhilaishvili, 2020[8] PMID: 31527029	George Eliava Institute of Bacteriophages 1 bacteriophage	80 YO female with a MDR *P. aeruginosa* prosthetic knee infection & osteomyelitis	**1 x 10^8** q8h through each drain for local delivery for 5 days	None reported
Aslam, 2019[7] PMID: 31207123	AmpliPhi Biosciences, Naval Medical Research Center, and APT provided bacteriophages	Patient 1: 67 YO male lung transplant recipient (hypersensitivity pneumonitis) with 2 MDR *P. aeruginosa*	Patient 1: **5 x 10^9** IV q2-4h and nebulized q6-12h for 29 days and separated 56 days	None reported

	Patient 1: varied between 3-5 bacteriophages Patient 2: 4 bacteriophage cocktail Patient 3: 1 bacteriophage	pneumonia hospitalizations Patient 2: 52 YO female non-CF lung transplant recipient with MDR *P. aeruginosa* pneumonia Patient 3: 28 YO male CF lung transplant recipient with *Burkholderia* species pneumonia	Patient 2: **4 x 10^9** IV q12h for 4 weeks Patient 3: **3.5 x 10^7** IV q12-24h for 12 weeks	
Dedrick, 2019[14] PMID: 31068712	SEA-PHAGES program and U of Pittsburgh 3 bacteriophage cocktail	15 YO female CF lung transplant recipient with *M. abscessus* sternal wound infection with infected skin nodules	**1 x 10^9** IV q12h for ~4 months	During first 2 days patient felt **sweaty and flushed** but no other changes
Schooley, 2017[9] PMID: 28807909	AmpliPhi Biosciences, Naval Medical Research Center, and Texas A&M 9 bacteriophage custom cocktail (not all administered at same time)	68 YO male with MDR *A. baumannii* necrotizing pancreatitis with infected pancreatic pseudocysts	**5 x 10^9** IV for 2 weeks (varying intervals but mostly q6-8h; intra-cavitary q6h for ~12 weeks	None reported
Nir-Paz, 2019[10] PMID: 30869755	Naval Medical Research Center and APT 2 bacteriophage cocktail	42 YO male with MDR *A. baumannii* and *K. pneumoniae* osteomyelitis	**5 x 10^7** IV q8h for 11 days	None reported
Law, 2019[6]	AmpliPhi Biosciences	26 YO female with severe CF	**4 x 10^9** IV q6h for 8 weeks	None reported

PMID: 31102236	4 bacteriophage cocktail	exacerbation with MDR *P. aeruginosa*		
Ferry, 2018[23] PMID: 30060002	Pherecydes Pharma 4 bacteriophage cocktail	60 YO male with non-small lung cancer with MDR *P. aeruginosa* osteomyelitis near sacroiliac joint	**1.2 to 9.7 x 10^8** instilled into infected bone for 4h at a time q3days over 12 days	None reported
Jault, 2019[16] PMID: 30292481	Pherecydes Pharma 12 bacteriophage cocktail.	25 patients with burn wounds infected with *P. aeruginosa* randomized to topical bacteriophage cocktail vs. standard of care (silver sulfadiazine)	**1 x 10^6** topically qday for 7 days	1 death in each study arm determined to be unrelated to assigned treatment **Mild AEs unrelated to the study drug:** **In 3 of 13 patients in bacteriophage group**: hyperthermia (1), bacteremia (1), ear infection (1), pseudomonal sepsis (1), superinfection (1), urinary tract infection (1), decreased oxygen saturation (1), hematuria (1), hemorrhagic urinary tract (1), hypoxia (1) **In 7 of 13 patients in standard group**: pancytopenia (1), hyperthermia (1),

				impaired hearing (1), bacteremia (1), bronchitis (1), fascial infection (1), pneumonia (1), pseudomonas infection (1), septic shock (3), skin graft (2), post-procedural hemorrhage (1), lung disorder (1), urticarial (1), hemorrhagic shock (1)
Corballino, 2019[18] PMID: 31414123	George Eliava Institute of Bacteriophages 1 custom bacteriophage	57 YO male with Crohn's disease colonized with MDR *K. pneumoniae*	**1 x 10^6** oral and intra-rectal bacteriophages q12h for 3 weeks	None reported
Ooi, 2019[13] PMID: 31219531	AmpliPhi Biosciences 1 custom bacteriophage	Open-label study of 9 adults with chronic *S. aureus* sinusitis	3 patients received 3 x 10^8 nasal irrigations q12h for 7 days; 3 patients received 3 x 10^8 q12h for 14 days, and 3 patients received **3 x 10^9** q12h for 14 days	**6 mild AEs** in 6 subjects (diarrhea, epistaxis, oropharyngeal pain, cough, nose pain, deceased bicarbonate level)
Fabjian, 2020[24] PMID: 32139910	AmpliPhi Biosciences 3 bacteriophage cocktail	Open-label study of 13 patients with severe *S. aureus* infections	**1 x 10^9** IV q12h for ~14 days	None reported

2.3.2 Potential Benefits

There is no expected clinical benefit to subjects who enroll into this trial. The data from this trial will further the science of bacteriophage therapy for future patients with infectious processes, including potentially the CF population. The findings will determine if future clinical trials using bacteriophage therapy would potentially benefit patients with infections and inform the optimal dosage, safety concerns, and expected microbiological activity associated with bacteriophage therapy for such a trial.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

This is a Phase 1b/2, multi-center, randomized, placebo-controlled, double-blind study of a single dose of IV bacteriophage in approximately 72 clinically stable adult CF subjects colonized with *P. aeruginosa* in expectorated sputum. The primary study outcomes include safety and microbiological activity of IV bacteriophage therapy.

As part of screening, an expectorated sputum sample will be obtained from each subject and tested for the presence of *P. aeruginosa*. If *P. aeruginosa* is isolated from the sputum and all eligibility criteria are met, the subject will be eligible for the study. If *P. aeruginosa* is not isolated, the subject will be considered a screen failure. Screening for susceptibility of *P. aeruginosa* to the 4-component bacteriophage mixture will not occur prior to administration of study treatment.

Stage 1 (Unblinded Safety Assessment/Dose-Escalation in Sentinel Subjects): Following screening, eligible subjects will be assigned into one of the three IV bacteriophage dosing regimens (arms). A total of 6 sentinel subjects will be enrolled in Stage 1 of the study. The arms will be sequentially enrolled with dose escalation by one $\log_{10}$ starting at 4 x 10^7 PFU. In each dosing arm, two subjects will be enrolled to serve as sentinels. Each sentinel subject will receive a single dose of IV bacteriophage therapy, followed by a 30 ± 7 days observation period. Refer to Section 8.2.3.1 for details regarding dosing in sentinel subjects.

Stage 2 (Double Blind Comparison): If no SAEs (related to the study product) are identified during the 96 hours after bacteriophage administration for all Sentinel Subjects in Stage 1, the study will proceed to Stage 2. In Stage 2a, 32 subjects will be randomly assigned to one of four arms: one of three IV bacteriophage doses or placebo in a 1:1:1:1 allocation. The subjects and the protocol team will be blinded to active versus placebo preparations. An interim analysis will be performed to determine the bacteriophage dosing with the most favorable safety and microbiological activity profile (Section 10.4). This will occur after 8 subjects per arm have been randomized and completed their Day 8 + 3 days Follow-up Visit. The interim analysis will use data from Stage 2a to identify the bacteriophage dose and the sample size that will be used for Stage 2b (Section 10.4). During Stage 2b, subjects will be randomized into the bacteriophage (dose selected based on Interim Analysis following Stage 2a) or placebo arm. The final sample size is expected to be up to 72 subjects total with up to 25 subjects in the placebo arm and up to 25 subjects in the bacteriophage arm.

3.2 Study Objectives

3.2.1 Primary

- Describe the safety of a single dose of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum.
- Describe the microbiological activity of a single dose of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum.
- Describe the benefit to risk profile of a single dose of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum.

3.2.2 Secondary

- N/A

3.2.3 Exploratory

- Characterize the serum and sputum PK profiles of a single dose of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum.
- Describe changes in lung function from the administration of a single dose of IV bacteriophage therapy through Day 30 ± 7.
- Characterize geographically diverse *P. aeruginosa* isolates susceptibility to bacteriophages.
- Describe subjects' quality of life (QOL) before and after receiving bacteriophage therapy.

3.3 Study Endpoints or Outcome Measures

3.3.1 Primary

- Number of grade 2 or higher treatment-emergent AEs through Day 30 ± 7.
- Change from baseline to Day 30 ± 7 days in $\log_{10}$ *P. aeruginosa* total colony counts in quantitative sputum cultures after administration of IV bacteriophages/placebo.
- Desirability of Outcome Ranking (DOOR) using the greatest reduction by Day 8 + 3 days Follow-up Visit.

More desirable	Rank 1	No SAE (related to study product) and > 2 $\log_{10}$ reduction in *P. aeruginosa* CFU/mL
↑	Rank 2	No SAE (related to study product) and 1-2 $\log_{10}$ reduction in *P. aeruginosa* CFU/mL
↓	Rank 3	No SAE (related to study product) and < 1 $\log_{10}$ reduction in *P. aeruginosa* CFU/mL
Less desirable	Rank 4	SAE (related to study product)

3.3.2 Secondary

- N/A

3.3.3 Exploratory

- Population mean PK parameter estimates and the magnitude of the associated inter-individual variability for IV bacteriophage therapy in serum and sputum.
- Individual post hoc PK parameter estimates and calculated exposure measures in serum for IV bacteriophage therapy.
- Individual post hoc PK parameter estimates and calculated exposure measures in sputum for IV bacteriophage therapy.
- Changes in the forced expiratory volume in 1 second (FEV1) from the administration of IV bacteriophages through Day 30 ± 7 days.
- Changes in $\log_{10}$ *P. aeruginosa* colony counts of each morphology in quantitative sputum cultures from administration of IV bacteriophages through Day 30 ± 7 days.
- Proportion of *P. aeruginosa* isolates susceptible to individual bacteriophages and the bacteriophage cocktail.
- Change from baseline through Day 30 ± 7 days Follow-up Visit of QOL as measured with the Cystic Fibrosis Questionnaire-Revised (CFQ-R) and the Cystic Fibrosis Respiratory Symptom Diary (CFRSD).

4 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

4.1 Study Product Description

Bacteriophage are viruses that infect bacteria and act by attaching to their bacterial host, releasing nucleic acid into the host, and replicating until host lysis. The product used in this study is designated WRAIR-PAM-CF1. WRAIR-PAM-CF1 is a cocktail of four bacteriophages: PaWRA01Phi11, PaWRA01Phi39, PaWRA02Phi83, and PaWRA02Phi87 (see **Table 2**) which are lytic against *P. aeruginosa*, naturally occurring, demonstrated to be pure, and free of known deleterious genes, including lysogeny (so theoretically should not incorporate into the chromosome of the bacterial host), antibiotic resistance genes, and toxin genes. Each bacteriophage lot is manufactured at APT in accordance with current Good Manufacturing Practices (cGMPs). Bacteriophage product is diluted with Plasma-Lyte A, pH 7.4. Glycerol is added as a cryoprotective agent to a final concentration of 20% (v/v). Residual purification agent (< 100 µg/mL), host cell protein (< 100 µg/mL), and endotoxin (< 250 EU/mL) may be present per product specification. All aforementioned residuals are present in levels that are considered acceptable per FDA recommendations. Refer to the IB for further details.

4.1.1 Formulation, Packaging, and Labeling

Bacteriophage

The bacteriophage will be packaged in two milliliter (2 mL) vials. Each vial will contain 1 mL of a single bacteriophage at a concentration of 1 x 10^9 to 1 x 10^{11} PFU/mL suspended in Plasma-Lyte A and 20% (v/v) of glycerol. Bacteriophage is a clear to slightly turbid suspension, essentially free of foreign particulates. The bacteriophage will be labeled according to manufacturer specifications and regulatory requirements and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use." Additional label contents are detailed in the protocol-specific Manual of Procedures (MOP).

The 4-component bacteriophage mixture will include a 1:1:1:1 combination of four individual bacteriophage (see **Table 2**) and will contain a total of 4 x 10^7 PFU, 4 x 10^8 PFU, or 4 x 10^9 PFU, depending on the target dose. The final phage combination to be administered to each subject will be diluted to the target dose with normal saline. Dosing will be based on the actual product potency, according to instructions that will be provided in the MOP, which will be updated as necessary, based on the most recent potency results.

Table 2: Components of WRAIR-PAM-CF1

Bacteriophage	Genome size, bp	Family	Genus
PaWRA01Phi11	66,800	*Myoviridae*	*Pbunavirus*
PaWRA01Phi39	66,708	*Myoviridae*	*Pbunavirus*
PaWRA02Phi83	45,439	*Podoviridae*	*Bruynoghevirus*
PaWRA02Phi87	44,893	*Podoviridae*	*Bruynoghevirus*

Placebo (Normal Saline, 0.9% Sodium Chloride, USP)

0.9% Sodium Chloride Injection, USP, is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection. Each mL contains sodium chloride 9 mg and contains no preservatives, antimicrobial agents, or added buffer. The solution is clear in appearance with a pH range of 4.5 to 7.0. Normal saline used for the placebo will be supplied in pre-filled infusion bags. Each infusion bag should only be used for one dosing preparation.

The placebo will be labeled according to manufacturer specifications and regulatory requirements and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use." Additional label contents are detailed in the protocol-specific MOP.

4.1.2 Product Storage and Stability

Bacteriophage

Bacteriophage must be stored and shipped at -80°C, with allowable fluctuation of ± 10°C, and remain frozen until dosing preparation. Prior to use, vials should be thawed at room temperature (20–25°C; 68–77°F; vials thaw within 30 minutes) and the bacteriophage preparation should be administered within 3 hours of thawing. Each vial should only be used for one dosing preparation and should not be frozen for multi-use. Any remaining bacteriophage following dilution should be discarded as biohazardous waste.

Placebo (Normal Saline, 0.9% Sodium Chloride, USP)

Store at 20ºC to 25ºC [See USP Controlled Room Temperature; excursions between 15°C and 30°C are permitted]. Protect from freezing.

4.2 Acquisition/Distribution

Bacteriophage

Bacteriophage will be provided by APT and distributed through the DMID Clinical Material Services, Fisher BioServices. Normal saline (0.9% Sodium Chloride for Injection, USP) for dilution, will also be distributed through the DMID Clinical Material Services, Fisher BioServices.

Placebo

Normal saline (0.9% Sodium Chloride for Injection, USP) used as the placebo, will be provided by DMID Clinical Materials Services (CMS, Fisher BioServices).

Refer to the protocol-specific MOP for shipping instructions.

4.3 Protocol-Specified Medications/Treatments Other Than Study Products

N/A

4.4 Dosage/Regimen, Preparation, Dispensing and Administration of Study Intervention/Investigational Product

Bacteriophage

Bacteriophage should be thawed at room temperature and diluted in normal saline to a final administration volume of 25 mL. The final dilution will be administered as a 30-minute IV infusion, with a range of 15-35 minutes.

Placebo

Placebo will be normal saline at a final volume of 25 mL and administered as a 30-minute IV infusion, with a range of 15-35 minutes.

All bacteriophage and placebo preparation should occur in an investigational pharmacy by an unblinded pharmacist and aliquoted appropriately for equal volume administrations in order to maintain the double-blind design. All bacteriophage preparation will occur in a Class II Biological Safety Cabinet. Refer to the protocol-specific MOP for information on the preparation, labeling, and administration of the study product.

4.4.1 Rationale for Dose Selection

The minimum and maximum doses that are being investigated in this study were informed by previous case reports, case series, and clinical trials, which generally used IV doses ranging from approximately 10^7 PFU to 10^{10} PFU (see **Table 1**). A single dose is being administered to evaluate the safety of the 4-component bacteriophage product prior to moving to a multi-dose regimen.

4.5 Pre-determined Modification of Study Intervention/Investigational Product for an Individual Subject

N/A

4.6 Accountability Procedures for the Study Intervention/Investigational Product(s)

Bacteriophage and placebo will be stored and shipped from the DMID contract Clinical Material Services (CMS) to the Clinical Sites. Once received, bacteriophage and placebo will be stored in and dispensed by the Investigational Pharmacy.

The FDA requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product disposition is maintained, and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the subject number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records must be available for inspection by the DMID monitoring contractors and are subject to inspection by a regulatory agency (e.g., FDA) at any time. An assigned Study Monitor will review the pharmacy records.

Any unused solution left in the IV infusion bag or IV administration tubing after administration to the subject should be discarded as biohazardous waste. Unused, thawed (with or without dilution) investigational bacteriophage vials will be discarded as biohazardous waste. Upon completion of the study and after the final monitoring visit, any remaining unused study product will either be returned or destroyed appropriately at the clinical site as per sponsor requirements and instructions, or in accordance with disposition plans. Refer to the MOP for complete drug accountability and monitoring.

5 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL

5.1 Eligibility Criteria

Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses. No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer (MO). If subjects are (1) in the process of receiving **or** (2) planning on receiving (in the next 30 days) the COVID-19 vaccine, eligibility screening should be deferred to at least 2 weeks after the last COVID-19 vaccination

5.1.1 Subject Inclusion Criteria

Subjects must meet all the inclusion criteria to be eligible to participate in the study:

1. Adult (≥ 18 years) at the time of screening.
2. Confirmed CF diagnosis based on a compatible clinical syndrome confirmed by either an abnormal sweat chloride testing or CFTR gene variations.*

   **Can be obtained from documentation in medical records; actual test results not necessary.*
3. Likely able to produce at least 2 mL of sputum during a 30-minute sputum collection following a hypertonic saline treatment or other approach to increase sputum production.*

   **Determined by investigator or their designee judgement. Approaches for obtaining sputum may include, but are not limited to, inhaled hypertonic saline (e.g., 3%, 7%, or 10%), inhaled hypertonic bicarbonate, inhaled mannitol, or spontaneously expectorated sputum. The same approach is recommended, whenever possible, for all sputum collections for a given subject, refer to the MOP for further details.*
4. *P. aeruginosa* (regardless of CFU/mL) isolated from a sputum, throat culture, or other respiratory specimen in the past 12 months.
5. Confirmed *P. aeruginosa* isolation from a sample of expectorated sputum at the Screening Visit.
6. Capable of providing informed consent.
7. Capable and willing to complete all study visits and perform all procedures required by this protocol.

5.1.2 Subject Exclusion Criteria

Subjects who meet any of the exclusion criteria will not be enrolled in the study:

1. Body weight < 30 kg.
2. FEV1 < 20% of predicted value at screening, using the Hankinson equations.[25]

3. Elevated LFTs obtained at screening.*

   a. Alanine aminotransferase (ALT) > 5 x the upper limit of normal (ULN) or aspartate transaminase (AST) > 5 x ULN or total bilirubin > 3 x ULN, **OR*

   b. Total bilirubin > 1.5 x ULN combined with either ALT > 3 x ULN or AST > 3 x ULN.

   ULN reflect local laboratory ranges.

4. Acute clinical illness requiring a new oral, parenteral, or inhaled antibiotic(s) ≤ 30 days prior to the Baseline Visit.*

   **Does not include chronic suppressive medications or cyclic dosing medications such as inhaled antibiotics.*

5. Women who are pregnant, planning to become pregnant during the study period, or breastfeeding.*

   **Women of childbearing potential must have a negative serum β-HCG test during screening and agree to use an effective method of contraception for the duration of the trial. A female is considered of childbearing potential unless postmenopausal, or surgically sterilized, and at least 3 months has passed since sterilization procedure.*

   a. *Female surgical sterilization procedures include tubal ligation, bilateral salpingectomy, hysterectomy, or bilateral oophorectomy.*
   b. *Female is considered postmenopausal if she is > 45 years old and has gone at least 12 months without a spontaneous menstrual period without other known or suspected cause.*
   c. *Effective methods of contraception include (a) abstinence, (b) partner vasectomy, (c) intrauterine devices, (d) hormonal implants (such as Implanon), or (e) other hormonal methods (birth control pills, injections, patches, vaginal rings).*

6. Active treatment of any mycobacterial or fungal organisms ≤ 30 days prior to baseline. Chronic treatment for suppression of fungal populations is allowable.

7. Anticipated need to change chronic antibiotic regimens during the study period.*

   **Subjects on cyclic dosing medications such as inhaled antibiotics, must be able and express willingness to keep the therapies at the time of screening constant (either remain on the therapy or not remain on the therapy) for the duration of the follow-up period (approximately 30 days). Subjects on chronic suppressive antimicrobial therapy must be able and express willingness to stay on the therapies for the duration of their follow-up period. This includes chronic azithromycin therapy.*

8. Known allergy to any component of the study product.

9. Any significant finding that, in the opinion of the investigator, would make it unsafe for the subject to participate in this study.

10. Enrolled in a clinical trial within ≤ 30 days of the Baseline/Dosing Visit, or participating in a clinical trial while enrolled in this clinical trial (inclusive of vaccine trials).
11. Currently or previously enrolled in this trial.

5.2 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination

5.2.1 Withdrawal from the Study or Discontinuation of the Study Product

Subjects may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled. An investigator may also withdraw a subject from receiving the study product for any reason. Follow-up safety evaluations will be conducted, if the subject agrees. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

The reasons, might include, but are not limited to the following:

- Subject no longer meets eligibility criteria
- Subject meets individual halting criteria (reference to Section 8.6.2)
- Subject becomes noncompliant
- Subject has a medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator, might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject lost to follow-up
- Subject determined by a physician's discretion to require additional therapy not indicated in the protocol to ensure subject's health and well-being (or treatment failure, if applicable)

The investigator should be explicit regarding study follow-up (e.g., safety follow-up) that might be carried out even though the subject will not receive further study product. If the subject consents, all AEs will be followed through the study defined follow-up period and all SAEs through resolution. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent or the investigator may seek subsequent informed consent using an IRB/IEC-approved consent form with the revised procedures.

The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study.

5.2.2 Subject Replacement

Subjects who withdraw, are withdrawn from this study, or are lost to follow-up after signing the Informed Consent Form (ICF) and after administration of the study product will not be replaced.

Subjects who withdraw, are withdrawn from this study, or are lost to follow-up after signing the ICF, but prior to administration of study product, may be replaced.

5.2.3 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

6 STUDY PROCEDURES

The following sections describe the study procedures and data that will be collected. For each procedure, subjects are to be assessed by the investigator or delegated site personnel. A Schedule of Events is located in Appendix A.

If a subject is prescribed antibiotic therapy with activity against *P. aeruginosa* after receipt of the study product, the subject will continue in the study and be included in an intent to treat analysis. Receipt of antibiotic therapy with activity against *P. aeruginosa* will be documented as a concomitant medication and the underlying condition for which the antibiotics were taken will be reported as an AE.

6.1 Screening

Screening, Visit 1 (Up to Day -7):

Informed consent must be obtained prior to the subject entering the study, and before any protocol specific procedures are performed. Subjects will be screened in accordance with pre-defined inclusion and exclusion criteria as described in Section 5.1. Subjects will be provided with a description of the study through the ICF (purpose and study procedures) and asked to read and sign the ICF. After the subject has provided informed consent to participate in the study, screening will include:

- Review eligibility criteria with the subject
- Serum HCG pregnancy test on all females of childbearing potential
- Complete medical history. The medical history will also include a full account of the subject's routine respiratory physiotherapy regimen.
- Complete medication history. Medication history will include medications taken within the past 30 days and those that remain active.
- Demographic information (age, gender, race, ethnicity)
- Height and weight measurement
- Review of systems
- Physical examination*
- Vital signs*
- Spirometry
- Sputum collection, to be collected following spirometry, as determined necessary by the local investigator to increase sputum production
- Liver function tests (see Section 7.2.1 for complete list of tests)

**Standard of care results obtained for clinical care purposes within 72 hours of screening are acceptable for screening.*

6.2 Baseline/Dosing (Day 1)

Baseline, Visit 2 (Day 1):

- Confirm eligibility criteria, as needed
- Urine pregnancy test on all female subjects of childbearing potential prior to administration of study product/placebo
- Review of systems
- Treatment assignment (**Stage 1 only**) *or* randomization **(Stage 2 only)**
- CFQ-R, prior to administration of study product/placebo and preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFQ-R.
- Administration of study product (bacteriophage or placebo)
- Vital signs (before administration of study product or placebo AND within 30-60 minutes after administration of study product or placebo is completed)
- Review and record concomitant medications
- Symptom directed physical examination (before administration of study product or placebo AND 30-60 minutes after administration of study product or placebo is completed)
- Spirometry (before administration of study product or placebo)
- Sputum collection (to be collected following spirometry, as determined necessary by the local investigator to increase sputum production) both pre- and post-infusion for microbiological and PK assessment. See Section 7.2.1 and 7.2.2 for timing of sample collection.
- Serum collection both pre- and post-infusion for PK assessment. See Section 7.2.2 for timing of sample collection.
- Hematology (see Section 7.2.1 for complete list of tests)*
- Clinical chemistry (see Section 7.2.1 for complete list of tests)*
- Liver function tests (see Section 7.2.1 for complete list of tests)*
- CFRSD, prior to administration of study product/placebo and preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection before administration of the CFRSD.
- Clinical assessment of AEs
- Events of Special Interest [ESIs] (i.e., pulmonary exacerbations), See Section 8.2.2

** Labs obtained at the Screening Visit do not have to be repeated at the Baseline Visit, if collected within 7 days from the Baseline Visit.*

6.3 Planned Follow-up Visits

6.3.1 Follow-up Visits, Days 2 - 12 (Day 2, Day 5 ± 2, Day 8 + 3, Day 12 + 7 [Stage 1 only])

Follow-up, Visit 3 (Day 2):

- Review of systems
- Review concomitant medications
- Symptom directed physical examination
- Spirometry
- Vital signs
- Sputum collection (to be completed after spirometry, as determined necessary by the local investigator to increase sputum production) for microbiological and PK assessments
- Serum collection for PK assessments
- Hematology (see Section 7.2.1 for complete list of tests)
- Clinical chemistry (see Section 7.2.1 for complete list of tests)
- Liver function tests (see Section 7.2.1 for complete list of tests)
- CFRSD, preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection before administration of the CFRSD.
- Clinical assessment of AEs
- Events of Special Interest [ESIs] (i.e., pulmonary exacerbations), see Section 8.2.2

Follow-up, Visit 4 (Day 5 ± 2 days):

- Review of systems
- Review concomitant medications
- Symptom directed physical examination
- Spirometry
- Vital signs
- Sputum collection (to be completed after spirometry, as determined necessary by the local investigator to increase sputum production) for microbiological and PK assessments
- Hematology (see Section 7.2.1 for complete list of tests)
- Clinical chemistry (see Section 7.2.1 for complete list of tests)
- Liver function tests (see Section 7.2.1 for complete list of tests)

- CFRSD, preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFRSD.
- Clinical assessment of AEs
- Events of Special Interest [ESIs] (i.e. pulmonary exacerbations), see Section 8.2.2

Follow-up, Visit 5 (Day 8 + 3 days):

In Stage 2, this is a **virtual** visit, see Section 6.4.

- Review of Systems
- Review concomitant medications
- Sputum collection for microbiological and PK assessments
- CFRSD, preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFRSD.
- Clinical assessment of AEs
- Events of Special Interest [ESIs] (i.e., pulmonary exacerbations), see Section 8.2.2

If the subject does not have the necessary equipment or internet access for a virtual visit, this visit may be conducted in-person, with the same procedures as the virtual visit, and this will not be considered a protocol deviation.

Follow-up, Visit 6 (Day 12 + 7 days):

Note: This visit is only applicable for **Stage 1**.

- Review of systems
- Review concomitant medications
- Symptom directed physical examination
- Spirometry
- Vital signs
- Sputum collection (to be completed after spirometry, as determined necessary by the local investigator to increase sputum production) for microbiological and PK assessments
- Hematology (see Section 7.2.1 for complete list of tests)
- Clinical chemistry (see Section 7.2.1 for complete list of tests)
- Liver function tests (see Section 7.2.1 for complete list of tests)
- Clinical assessment of AEs

6.3.2 Final Study Visit

The Final Study Visit is the Follow-up, Visit 7 that occurs on Day 30 ± 7 days and includes the following:

- Review of systems
- Review concomitant medications
- Symptom directed physical examination
- Spirometry
- Vital signs
- Sputum collection (to be completed after spirometry, as determined necessary by the local investigator to increase sputum production) for microbiological and PK assessments
- Hematology (see Section 7.2.1 for complete list of tests)
- Clinical chemistry (see Section 7.2.1 for complete list of tests)
- Liver function tests (see Section 7.2.1 for complete list of tests)
- CFRSD, preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFRSD.
- CFQ-R, preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFQ-R.
- Clinical assessment of AEs

6.3.3 Early Termination Visit

If a subject terminates or is withdrawn from the study prior to the final Follow-up Visit, all attempts will be made to collect the following as part of an Early Termination Visit:

- Review of systems
- Review concomitant medications
- Symptom directed physical examination
- Spirometry*
- Vital signs
- Sputum collection (to be completed after spirometry, as determined necessary by the local investigator to increase sputum production) for microbiological and PK assessments
- Hematology (see Section 7.2.1 for complete list of tests)*
- Clinical chemistry (see Section 7.2.1 for complete list of tests)*
- Liver function tests (see Section 7.2.1 for complete list of tests)*
- CFRSD, preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFRSD.
- Clinical assessment of AEs

- Events of special interest [ESIs] (i.e., pulmonary exacerbations), if this visit occurs prior to Visit 5, Day 8 + 3 days (see Section 8.2.2)

**If the visit occurs > 7 days after a scheduled visit. If the visit occurs ≤ 7 days after a scheduled visit, laboratory tests may be collected and spirometry may be performed at the discretion of the local investigator.*

If a subject is considered by the local investigator to be too ill to attend an in-person or virtual Early Termination Visit, this visit may be conducted by telephone call.

6.4 Virtual Study Visits

Virtual study visits will be allowable as substitutes for in-person study visits for circumstances precluding in-person visits (e.g., subject has a COVID-19 diagnosis, subject fractures a bone preventing ambulation). Prior to conducting a virtual study visit, the reason for the requested virtual visit should be reviewed and approved by the protocol PI. If a virtual study visit is conducted without prior approval from the protocol PI, it will be considered a protocol deviation.

Note: Visit 5 (Day 8 + 3 days) will always be conducted virtually in Stage 2 without the need for prior approval by the protocol PI.

Virtual study visits will only be allowed for follow-up visits (Day 2, Day 5 ± 2 days, Day 12 + 7 days), the Final Study Visit (Day 30 ± 7 days), or an Early Termination Visit. Virtual study visits will not be allowed for the Screening or Baseline/Dosing Visit. Virtual visits will not be allowed in cases of Unscheduled Visits for safety follow-up for AEs and SAEs related to the study product.

The following will be collected during the virtual study visits:

- Review of systems
- Review of concomitant medications
- Sputum collection for microbiological and PK assessments
- CFRSD (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFRSD.
- CFQ-R (**Stage 2, Visit 7 [Day 30 ± 7] only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFQ-R.
- Clinical assessment of AEs
- Events of special interest [ESIs] (i.e., pulmonary exacerbations), if this visit occurs prior to Visit 5, Day 8 + 3 days (see Section 8.2.2)

A pre-labeled sputum collection kit with instructions and shipping documents will be provided to the subject prior to the visit. Refer to the MOP for further details.

Study procedures that cannot be performed virtually (e.g., vitals, physical examination) will not be considered protocol deviations.

6.5 Unscheduled Study Visits

Unscheduled Study Visits may occur at the investigator's discretion. If an Unscheduled Study Visit occurs, every attempt will be made to collect the following, but it will not be considered a protocol deviation if not collected:

- Review of systems
- Review concomitant medications
- Symptom directed physical examination
- Spirometry*
- Vital signs
- Sputum collection (to be completed after spirometry, as determined necessary by the local investigator to increase sputum production) for microbiological and PK assessments
- Hematology (see Section 7.2.1 for complete list of tests)*
- Clinical chemistry (see Section 7.2.1 for complete list of tests)*
- Liver function tests (see Section 7.2.1 for complete list of tests)*
- CFRSD, preferably before the collection of study data (**Stage 2 only**). If subject is ready to produce sputum, prioritize sputum collection over administration of the CFRSD.
- Clinical assessment of AEs
- Events of special interest [ESIs] (i.e., pulmonary exacerbations), if this visit occurs prior to Visit 5, Day 8 + 3 days (see Section 8.2.2)

**If the visit occurs > 7 days after a scheduled visit. If the visit occurs ≤ 7 days after a scheduled visit, laboratory tests may be collected and spirometry may be performed at the discretion of the local investigator.*

6.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. Any missing CFQ-R or CFRSD assessments **will not** be considered a protocol deviation. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions should be developed by the site and implemented promptly. It is the responsibility of the site PI and other study personnel to use continuous vigilance to identify and report protocol deviations. All individual protocol deviations will be addressed in subject study records. All protocol deviations will be collected and stored in a sponsor-determined location. Protocol deviations must be sent to the reviewing IRB/IEC per its guidelines. The site PI and other study personnel are responsible for knowing and adhering to their IRB/IEC requirements.

If an Unscheduled Study Visit occurs, per Section 6.5, any assessments/procedures that do not occur will not be considered protocol deviations.

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

7.1.1 Research Procedures

- **Demographics:** Demographics will be obtained by subject interview including age, race/ethnicity, and gender.
- **Height and weight measurement:** The subject's height (centimeters) and weight (kilograms) will be measured.
- **Medical history**: The detailed medical history will include completion of a standard template by the study team through a combination of medical record review and subject interview. Medical history will be obtained regarding significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. Medical history will also include a full account of the subject's routine respiratory physiotherapy regimen.
- **Medication history:** A complete medication history will be obtained to include prescription medications, over-the-counter drugs, herbals, vitamins, and supplements the subject has taken. Information regarding any prophylactic systemic or inhaled anti-infective regimens will also be collected. Medication history will be collected both by a review of the medical records as well as by subject interview.
- **Review of systems:** A subject interview will be performed by a study clinician who is licensed to make medical diagnoses. The review of systems will include specific issues with various systems including: general well-being, headaches, vision, hearing, balance, mental health, dizziness, breathing issues, palpitations, nausea, vomiting, diarrhea, abdominal pain, leg swelling, joint swelling, rashes, and urinary symptoms. Refer to the MOP for further details.
- **Vital signs:** The following vital signs will be obtained: systolic and diastolic blood pressures in millimeters of mercury (mmHg), heart rate [beats per minute (BPM)], respiratory rate, and body temperature in degrees Celsius (oral measurement).
- **Physical examination:** Complete physical examination (except for a breast, rectal, and genitourinary examination).
- **Symptom directed physical examination**: A symptom directed physical examination will include the general appearance, lung auscultation (including evaluating for symmetric chest rise, increased work of breathing, and listening for rhonchi or wheezing), and examination of any other body site as indicated by subject symptomatology.

- **Spirometry:** Spirometry will occur prior to required sputum collections, as considered necessary by the local investigator to increase sputum production. Spirometry will be performed according to the standard American Thoracic Society protocol.
- **Cystic Fibrosis Questionnaire-Revised (CFQ-R):** The CFQ-R is a quality of life (QOL) questionnaire designed to measure the impact that CF has on overall health, daily life, and perceived well-being and symptoms.[26] The CFQ-R (Appendix B), contains 50 items and will be administered during study visits for Stage 2 subjects[26]. For additional details on data collection, refer to the MOP.
- **Cystic Fibrosis Respiratory Symptom Diary (CFRSD):** The CFRSD is a QOL questionnaire, which evaluates the effect of treatment on respiratory infection symptom severity in patients with CF and chronic respiratory infection.[27] The CFRSD (Appendix C) will be collected during study visits for Stage 2 subjects. For additional details on data collection, refer to the MOP.
- Clinical assessment of AEs and ESIs (see Section 8)

7.1.2 Assessment of Concomitant Medications/Treatments Other Than Study Product

Active medications will be reviewed by subject interview and a review of subject medical records. Use of a new medication should prompt evaluation for the presence of a new AE or diagnosis of a new medical condition. Chronic medications, rescue medications, and over-the counter medications are allowed.

7.1.3 Assessment of Subject Compliance with Study Intervention/Investigational Product

Subjects will be directly observed while receiving a single dose of study product or placebo by a member of the study team who is licensed to administer the study product or placebo. Administration data will be recorded in the source and entered into the electronic case report form (eCRF).

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

Each site will maintain a list of the normal ranges and units of measurement for the laboratory parameters to be determined during this study, and the data and certification number of the laboratory. If the normal ranges change during the course of the study, the site investigator must update this list with the new ranges and effective dates. The normal laboratory reference ranges will also be retained in the regulatory binder at each site.

- **Serum HCG pregnancy test**: A serum HCG pregnancy test will be performed by the local laboratory for all women of childbearing potential at the Screening Visit and the results must be negative prior to treatment assignment/randomization.
- **Urine HCG pregnancy test:** A urine HCG pregnancy test will be performed by the local laboratory for all women of childbearing potential on the day of the Baseline Visit and the results must be negative prior to treatment assignment/randomization.

- **Sputum collection** for microbiological assessments (minimum of 2 mL sample per time point) will be obtained per standard clinical practice, following spirometry, as considered necessary by the local investigator to increase sputum production. Hypertonic saline treatment or other approaches to increase sputum production may be used, as needed, for sputum collection. Approaches for obtaining sputum may include, but are not limited to, inhaled hypertonic saline (e.g., 3%, 7% or 10%), inhaled hypertonic bicarbonate, inhaled mannitol, or spontaneously expectorated sputum. The same approach is recommended, whenever possible, for all sputum collections for a given subject; refer to the MOP for further details. At the Baseline/Dosing Visit, sputum collection will occur prior to administering investigational product/placebo and at 2 hours post completion of administering study product/placebo and flushing the IV line (± 1 hour). Samples will be collected by the local laboratory and will be sent to a central laboratory for analysis. Refer to the MOP for details.
- **Clinical chemistries** will be measured at the local laboratory and include serum creatinine, blood urea nitrogen, glucose, sodium, potassium, chloride, bicarbonate, and calcium.
- **Hematology** will be measured at the local laboratory and include complete blood count with red blood cell (RBC) count, hemoglobin, hematocrit, total white blood cell (WBC) count with differential counts, and platelet count.
- **Liver function tests** will be measured at the local laboratory and include AST, ALT, alkaline phosphatase, total bilirubin, lactate dehydrogenase, albumin, and total protein. Only AST, ALT, and total bilirubin are necessary at Screening.

7.2.2 Research Assays

- **Serum PK** studies (~3 mL sample of whole blood per time point) will be conducted per the directions in the MOP. At the Baseline/Dosing Visit, blood will be collected at any time (on the same calendar day) prior to the start of investigational product/placebo administration, and at 30 min, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, and 3.5 hours post completion of administering study product and flushing the IV line (all time points are ± 10 min). At Follow-up Visit 3 (Day 2), the PK serum sample will be collected once at any time, the time must be recorded, during the in-person visit. Samples will be collected by the local laboratory and sent to a central laboratory for analysis.
- **Sputum PK** studies (minimum of 2 mL sample per time point) will be obtained per standard clinical practice, following spirometry, as considered necessary by the local investigator to increase sputum production. Hypertonic saline treatment or other approaches to increase sputum production may be used, as needed, for sputum collection. Approaches for obtaining sputum may include, but are not limited to, inhaled hypertonic saline (e.g., 3%, 7% or 10%), inhaled hypertonic bicarbonate, inhaled mannitol, or spontaneously expectorated sputum. The same approach is recommended, whenever possible, for all sputum collections for a given subject, refer to the MOP for further details. At the Baseline/Dosing Visit, sputum collection will occur prior to administering investigational product/placebo, and at 2 hours post completion of administering study product/placebo and flushing the IV line (± 1 hour).

Samples will be collected by the local laboratory and will be sent to a central laboratory for analysis. Refer to the MOP for details.

- ***P. aeruginosa*** **colony counts** will be performed by the central lab using quantitative bacterial cultures on sputum specimens collected from the time of bacteriophage administration through Day 30 ± 7 days. Quantities of sputum (10 mg) will be measured precisely and added to 1 mL Sputolysin mucolytic agent to liquefy the sample. The liquefied sample will be diluted sequentially and plated onto MacConkey agar and Sheep's Blood Agar Plates using a 1 µL loop for each replicate. Colonies will be counted on the highest dilution in which individual colonies can be enumerated and colony counts adjusted appropriately based on the dilution factor and the mass of the sample to determine the true concentration of *P. aeruginosa*. Colonies representing different morphotypes will be definitively identified using MALDI-TOF to ensure that each enumerated colony type represents *P. aeruginosa*. Refer to the MOP for additional details.
- ***P. aeruginosa*** **susceptibility to bacteriophages** may be performed by the central lab on *P. aeruginosa* isolates in sputum cultures, depending on sample availability.

7.2.2.1 Laboratory Specimen Preparation, Handling, and Storage

Specific instructions are included in the MOP.

7.2.2.2 Laboratory Specimen Shipping

Specific instructions are included in the MOP.

7.2.3 Secondary Analysis of Stored Specimens and Data

The information and samples/specimens collected for this study may be used for future, currently unknown, research. The secondary research may include, but is not limited to additional work on bacteriophage treatment; cystic fibrosis; and infections of the blood, lung, skin, or other parts of the body. The research may include, but is not limited to further investigating bacteriophage susceptibility testing, serum neutralizing antibodies, whole genome sequencing the *P. aeruginosa* isolates, and changes to the respiratory microbiome. No human genetic testing or sequencing of human DNA will be performed using the samples/specimens collected.

8 ASSESSMENT OF SAFETY

8.1 Assessing and Recording Safety Parameters

This study will assist in enhancing the understanding of the safety and microbiological activity of IV bacteriophage therapy in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum. Safety will be assessed by the frequency and severity of AEs.

8.1.1 Adverse Events (AEs)

International Council for Harmonisation (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. The FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs of grade 2 or higher including lab abnormalities, abnormal clinical finding, and including local (infusion site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and eCRF regardless of causality or clinical significance.

Grade 1 AEs including lab abnormalities, abnormal clinical finding, and including local (infusion site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and eCRF only if assessed by the treating clinician as related to the study product.

Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product, potential alternate etiologies (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness, and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not be reported as an AE. However, if the frequency and/or severity of any pre-existing medical condition increases, it should be recorded as an AE.

8.1.1.1 Adverse Events Grading

AEs, including laboratory and clinical symptoms, will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50) and

assessed for relationship to study product. AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

Relationship to Study Product: The assessment of the AE's relationship to study product will be performed by a delegated, licensed study clinician listed on the Form FDA 1572. The assessment will be part of the documentation process. Whether the AE is related to the study product or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. The AEs will be assessed for relationship to the study product using the terms "related" or "not related":

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.
- Note: Pulmonary exacerbations are known to occur in individuals with CF with some frequency and will be considered **unrelated** to the study drug if they occur after Visit 5, Day 8 + 3 days. If a pulmonary exacerbation occurs during the ESI collection period (Baseline/Dosing through Visit 5), it may be considered related, at the discretion of the local investigator.

8.1.2 Serious Adverse Events (SAEs)

An AE or suspected AE is considered an SAE if, in the view of either the site PI or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening AE,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An SAE is classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR) if the event is related to study drug and not identified as an expected reaction to the drug per the IB.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a delegated, licensed study clinician listed on the Form FDA 1572.
- Recorded on the appropriate SAE data collection form and appropriate eCRF.
- Followed through resolution by a licensed study clinician (for IND studies, a physician listed on the Form FDA 1572 as the site PI or Sub-Investigator).
- Reviewed and evaluated by DMID, the Data and Safety Monitoring Board (DSMB) (during a periodic review unless related), and the IRB/IEC (per reporting requirements).

8.2 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of AEs from the administration of the study product through the follow-up period.

8.2.1 Unsolicited Events

Unsolicited events are AEs that occur following administration of study product. Subjects will be queried at each follow-up visit and during any follow-up telephone calls to evaluate occurrence of any AEs. Medical records should be reviewed at each visit for AEs.

8.2.2 Events of Special Interest

The following ESIs will be collected from the time of first administration of study product until Visit 5, Day 8 + 3 days:

Pulmonary exacerbations, defined as:

1) Initiation of oral or IV antibiotics by the treating clinicians for respiratory symptoms,

OR

2) Four or more of the following signs/symptoms[28]:

- Increase in sputum quantity or a change in quality;
- New or increased hemoptysis;
- Worsening cough;
- Increased shortness of breath;
- New onset malaise/fatigue/lethargy;
- Temperature > 38°C;
- Change in physical examination of the chest;
- Decrease in FEV1 > 10% from Baseline; and/or

- Radiographic changes consistent with a pulmonary exacerbation.

Any ESI that meets the criteria of an SAE (Section 8.1.2) will be recorded and reported as an SAE instead of an ESI.

8.2.3 Dose Escalation Criteria

Dose escalation will occur in the sentinel subjects in Stage 1 of this trial (see Section 8.2.3.1). Individual subjects will not undergo dose escalation.

8.2.3.1 Sentinel Subjects

Following subject screening for Stage 1, eligible subjects will be assigned into one of the three IV bacteriophage dosing regimens. The arms will be sequentially enrolled with dose escalation by one $\log_{10}$ starting at 4×10^7 PFU. In each dosing arm, two subjects will be enrolled to serve as sentinels. Each sentinel subject will receive a single dose of IV bacteriophage, followed by a 96-hour observation period. Enrollment will be held between each of the three dose levels; if no SAE (related to study product) occurs and no halting criteria are met (Section 8.6) during the 96-hour observation period for both subjects on the given dose level, the next two Stage 1 sentinel subjects will be enrolled. If an SAE (related to study product) occurs during the 96-hour observation period, the study will be temporarily suspended and discussed with the DMID Medical Officer, DMID Medical Monitor (MM), and the DSMB on how to proceed. Sentinel subjects will be followed for approximately 30 ± 7 days according to Appendix A.

8.3 Reporting Procedures

8.3.1 Reporting Serious Adverse Events

All AE/ESI/SAEs will be collected in the eCRF.

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined criterion as an SAE that is assessed to be <u>related</u> to the study product must be submitted within 24 hours of site awareness on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)

6500 Rock Spring Dr. Suite 650

Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the Statistical and Data Coordinating Center (SDCC) system. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID pharmacovigilance group (PVG) and should be provided as soon as possible.

The DMID MM and DMID Clinical Project Manager will be notified of study product-related SAE by the DMID PVG. The DMID MM will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

SAEs assessed as not related to the study product will be captured on the appropriate AE eCRF but do not require separate reporting to the DMID Pharmacovigilance Group. Both related and not related SAEs will be captured in the database.

8.3.2 Regulatory Reporting for Studies Conducted Under DMID Sponsored IND

Following notification from the site PI or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected, unexpected SAE, or SUSAR, to the regulatory authorities. The DMID will report an AE as a suspected, unexpected AE only if there is evidence to suggest a causal relationship between the study intervention and the AE. The DMID will submit an IND safety report to the FDA and will notify all participating site PIs (i.e., all PIs to whom the sponsor is providing drug under its IND(s) or under any PI's IND(s)) of potential serious risks from clinical studies or any other source, as soon as possible. The DMID will report SUSARs to the FDA per regulations and after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to the FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All SAEs designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

8.3.3 Reporting of Pregnancy

Female subjects of childbearing age who become pregnant during the study after the administration of study drug will continue in the study as this is a single-dose study. Pregnancy occurring during a clinical investigation, although not considered an SAE, must be reported within the same timelines as an SAE. The positive pregnancy test will be recorded in the database within 5 days of site awareness, on the Pregnancy Report form. The report will include pregnancy outcome (e.g., any premature terminations, elective or therapeutic, any spontaneous abortions or stillbirths), as well as the health status of the mother and child, including date of delivery and infant's sex and weight. Any subject with a positive pregnancy test, who has received study product(s), will be followed through eight (8) weeks post live delivery or elective or natural termination of the pregnancy, whichever occurs first. If the database is locked at the time of pregnancy, a supplemental report will be generated and completed after birth, which will

be appended to the database. Any occurring AEs or SAEs that occur to the mother or fetus will be recorded in the eCRF in the database and on the SAE Report form.

8.4 Type and Duration of Follow-up of Subjects After Adverse Events

AEs will be assessed and followed from initial recognition of the AE through end of the protocol defined follow-up period for each stage.

SAEs will be followed through resolution even if the duration of follow-up goes beyond the protocol-defined follow-up period.

Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

8.5 Procedures to be Followed in the Event of Abnormal Laboratory Test Values

Any Grade 2 or higher abnormal laboratory value will be reported as an AE, regardless of the relationship to the study product or clinical significance. Grade 1 laboratory abnormalities will be reported as AEs if assessed by the investigator as related to the study product. Laboratory abnormalities reported as AEs will be followed until the abnormality stabilizes, normalizes or returns to a baseline level, whichever comes first, at the discretion of the investigator.

In the approach to grading laboratory values, if the Lower Limit of Normal (LLN) entered at the site falls within CTCAE defined graded values, the CTCAE ranges will be used to grade an AE, rather than using local laboratory ranges for determining normal and abnormal laboratory values.

Intermittent abnormal laboratory values such as electrolyte abnormalities or blood glucose levels that are part of the same clinical diagnosis (e.g., uncontrolled diabetes) can be recorded once with the highest grade for each fluctuating AE (Grade 2 or higher for this trial), including the start and stop dates of the intermittent syndrome. If the event has a clear resolution for a duration of at least 48 hours and then recurs, it should be treated as a separate AE. An AE resolution is defined as return to pre-treatment status or baseline for duration of more than 48 hours.

8.6 Halting Rules

8.6.1 Study Halting Criteria

If any of the following events occur, enrollment and dosing for all subjects will be suspended until the event is assessed by the DSMB:

- Any subject develops an SAE related to the study product through the last study visit.
- Two (2) or more subjects in the study (cumulative among all dose levels) experience a Grade 3 (severe) AE (including clinical, vital signs, and laboratory AEs identified from blood tests) that is related to study product and is of the same type [High Level Term (HLT)] through the last study visit.

- Any subject develops anaphylaxis within 24 hours after receiving the study product.
- Any subject reports two or more pulmonary exacerbations during the ESI collection period, from the time of study product administration through Visit 5, Day 8 + 3 days.

8.6.2 Individual Infusion Halting Criteria

An individual infusion will be stopped if a drug-related hypersensitivity is suspected, including anaphylaxis during the infusion. Symptoms of anaphylaxis may begin within seconds or minutes of infusion initiation. Infusion of the investigational product will be halted and will not be restarted if any of the following occur:

- Skin or mucous membrane manifestations: hives; Grade 2 or higher pruritus; flushing; swollen joints; swollen lips, tongue, or uvula
- Respiratory compromise: dyspnea, wheezing, or stridor
- A decrease in systolic blood pressure to < 90 mmHg or a > 30% reduction in the systolic blood pressure
- Development of a ventricular dysrhythmia; or bradycardia < 45 BPM that is associated with complaints of dizziness, nausea, or feeling faint
- Syncope
- Confusion and/or slurred speech
- Any other condition that the site PI judges to unduly increase the risk to the subject

8.7 Safety Oversight

8.7.1 Data and Safety Monitoring Board (DSMB)

Safety oversight will be conducted by a DSMB that is an independent group with expertise to interpret data from this clinical trial and will monitor subject safety and advise DMID. The DSMB members will be separate and independent of study personnel participating in this study and will not have scientific, financial, or other conflict of interest related to this study. The DSMB must consist of at least three voting members, including a biostatistician experienced in statistical methods for clinical trials and clinicians with relevant expertise in CF and bacterial infections.

The DSMB will operate under the rules of a DMID-approved charter that defines the data elements to be assessed and the procedures for data reviews and will be written at the organizational meeting of the DSMB. Procedures for DSMB reviews/meetings will be defined in the charter. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, ESIs, and unsolicited AE/SAEs. The DSMB will review SAEs on a regular basis and ad hoc during this trial. The DMID MM and MO will be responsible for reviewing SAEs in real time.

As defined in the charter, the DSMB will review data at pre-specified intervals during the course of the study for subject and overall study progress and will conduct ad hoc reviews as appropriate when a halting rule is met or for immediate concerns regarding observations during this study.

Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB will receive data in aggregate and presented by treatment arm. The DSMB will review grouped and unblinded data in the closed session only. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study, and to continue, modify, or terminate this trial.

8.7.2 PHAGE Advisory Group

The PHAGE Advisory group will consist of a small group of investigators and clinicians affiliated with this clinical trial and/or the ARLG and at least one DMID representative who will review the unblinded data provided by the SDCC during the interim analysis between Stage 2a and Stage 2b and make a recommendation to DMID on the sample size and the optimal bacteriophage dose to be considered for Stage 2b. After completion of Day 8 + 3 days Follow-up Visit for all subjects in Stage 2a, the PHAGE Advisory Group will review safety and microbiological data, unblinded to treatment arm, comparing the benefit to risk profile of each of the treatment groups, taking into consideration AEs and log reductions in *P. aeruginosa* CFU/mL through Day 8. Then, the PHAGE Advisory Group will recommend to DMID the most appropriate sample size (See Section 10.2) and the most effective and safest dose to administer in Stage 2b. Refer to Section 10.4 for more details on the Interim Analysis.

9 HUMAN SUBJECTS PROTECTION

The investigator will ensure that this study is conducted in compliance with 21 CFR part 50 and in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6 (R2).

9.1 Institutional Review Board/Independent Ethics Committee

Each site PI will obtain IRB approval for this protocol to be conducted at his/her research site(s), and send supporting documentation to the DMID (through a Coordinating Center such as the ARLG) before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with OHRP as applicable to the research. The DMID/ARLG must receive the documentation that verifies IRB/IEC approval for this protocol, associated informed consent documents, and - upon request - any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects at the site.

Any amendments to the protocol or consent materials must be approved by the IRB/IEC before implementation. The IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects, and may cease if annual review is no longer required by applicable regulations and the IRB/IEC. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

Each institution engaged in this research will hold a current FWA issued by the OHRP for federally funded research.

A single IRB of record will be accountable for compliance with regulatory requirements for this multi-center study, at participating sites. A formal Reliance Agreement will be required between the single IRB and participating sites. The formal Reliance Agreement will set forth the specific responsibilities of the IRB and each participating site. Participating sites will then rely on the IRB of record to satisfy the regulatory requirements relevant to the IRB review. Participating sites will maintain essential required documentation of IRB reviews, approvals, and correspondence, and must provide copies of any agreements and essential documentation to the DMID/Coordinating Center (such as the ARLG) or regulatory authorities upon request.

9.2 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual’s trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will sign one of two consents depending on the study stage (Stage 1 or Stage 2) that is open for enrollment at the time of informed consent. Subjects will receive a concise and focused presentation of key information

about the clinical trial, verbally and with a written consent form. The explanation will be organized, and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects. The key information about the purpose of the study; the procedures and experimental aspects of the study, risks, and discomforts; any expected benefits to the subject; and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. The explanation will include aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, and other risks that are currently unforeseeable), the expected duration of the subject's participation in the trial, alternative procedures that may be available, and the important potential benefits and risks of available alternative procedures.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact for answers to any questions relating to the research.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason and at any time without penalty or loss of benefits to which the participant is otherwise entitled.

The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority (ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the subject is authorizing such access.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial, and have the opportunity to discuss this trial with their family and/or friends prior to agreeing to participate.

Informed consent forms (ICFs) will be IRB-approved and subjects will be asked to read and review the consent form. Subjects must sign the ICF prior to starting any study procedures performed specifically for this trial.

Once signed, a copy of the ICF will be given to subjects for their records. Subjects may withdraw consent at any time throughout the course of the trial. The rights and welfare of subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site clinical staff may pre-screen via chart review and refer potential subjects to the Research staff. Research staff will obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site PI to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all ICFs that they sign.

9.2.1 Other Informed Consent Procedures

Illiterate Subjects

If illiterate**,** subjects will be consented according to the procedures approved by the reviewing IRB.

9.3 Consent for Secondary Use of Stored Specimens and Data

Residual samples/specimens are those that are left over after protocol-specified testing and this study has been completed. As part of the main consent form, subjects enrolled in this study will consent to remaining (residual) blood and sputum specimens being kept for possible use in secondary research studies. Residual specimens will be stored coded in the ARLG Biorepository, until released for secondary research. The researchers using the samples/specimens will not be able to link the samples back to subjects or directly identify subjects. No human genetic testing will be performed on these residual samples/specimens. The recipients of specimens will be informed that these specimens have an NIH certificate of confidentiality. Use of the specimens for secondary research unrelated to this study will require review by an IRB.

9.4 Exclusion of Women, Minorities, and Children (Special Populations)

Children will be excluded from this trial as the study entry criteria only allows enrollment of adults. Non-English speakers, illiterate individuals, and non-writing individuals will be allowed to participate in the study, provided the reviewing IRB approves methods and materials given to subjects, to include but not limited to the written informed consent written in the language understandable to the subject. Women and minorities will not be excluded from participation provided all study entry criteria are met. Investigational sites should follow the direction of the reviewing IRB prior to enrollment of subjects considered to be "vulnerable".

9.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the sponsor. Subject confidentiality will be maintained when study results are published or discussed in conferences.

All information provided by the sponsor and all data and information generated by the participating sites as part of this trial (other than a subject's medical records) will be kept confidential by the site PI and other study personnel to the extent permitted by law. This information and data will not be used by the site PI or other study personnel for any purpose other than conducting this trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site PI or other study personnel, (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of this trial, (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject, or (4) study results which may be published as described in Section 15 Publication Policy.

The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study sites will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

9.6 Certificate of Confidentiality

To protect privacy, the NIH provides a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information

that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the US Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the FDA.

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the participant's consent, information that would identify the participant as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, for the purposes of other research that is compliant with applicable Federal regulations governing the protection of human subjects in research.

9.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the subject, subject's insurance, or third party.

If it is determined by the site PI that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the ARLG, NIAID, NIH, by the participating sites, or other sponsors for any injury suffered due to participation in this trial.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

The study does not include formal hypothesis testing for the three primary endpoints.

10.2 Sample Size Considerations

The sample size for Stage 2 (Stage 2a/2b) was determined using the DOOR outcome measure. The sample size was calculated to provide desired precision of the estimate of the DOOR probability in order to describe the benefit to risk profile of a single dose of IV bacteriophage in clinically stable CF subjects with *P. aeruginosa* in expectorated sputum. If the true DOOR probability comparing IV bacteriophage and placebo is 70%, when the total sample size in each arm is 20 (combining subjects from Stages 2a and 2b), the two-sided normal approximate 95% confidence interval for DOOR probability is calculated at 51% and 89%, respectively, with the lower limit larger than 50% (see Section 10.5.5).

An interim analysis will be performed when the 8 subjects per arm in Stage 2a (40% = 8/20) have completed their Day 8 + 3 days Follow-up Visit (Section 10.4.2). Based on the observed data, two of the doses of IV bacteriophage therapy will be dropped from the study at the interim analysis, and the planned sample size will be re-evaluated as to whether it provides the desired precision of estimates of the DOOR probability for a selected dose and placebo. If necessary, after completion of Stage 2a and per details in Section 10.4.2, the sample size will be increased up to 17 subjects for each of the two arms in Stage 2b (a total of up to 25 for the placebo arm and up to 25 for the selected bacteriophage dose in Stage 2).

10.3 Treatment Assignment Procedures

10.3.1 Randomization Procedures

According to a computer generated, randomized allocation list using permuted block design, in Stage 2a, subjects will be randomly assigned to one of four arms: one of three IV bacteriophage doses or placebo in a 1:1:1:1 allocation. In Stage 2b, subjects will be randomly assigned to one of two arms: the selected IV bacteriophage dose after interim analysis or placebo in a 1:1 ratio.

The list of randomized allocations will be generated by statisticians at the SDCC (The Emmes Company). The SDCC will assign each subject a randomization code and assignment from the list. The randomization code will be released at the Baseline Visit according to Appendix A.

Instructions for use of the enrollment module are included in the EDC System User's Guide.

10.3.2 Masking Procedures

A double-blind/masking technique will be used in Stage 2 of the study. The three IV bacteriophage doses and placebo will be packaged identically for administration so that treatment blind/masking is maintained. The subject and the investigator or delegate(s) who are involved in the treatment or clinical evaluation of the subjects will be unaware of the treatment group

assignments. The study investigational pharmacist will remain unblinded throughout the study and will be informed of the appropriate dose for subjects according to procedures detailed in the MOP.

To maintain the overall quality and legitimacy of the clinical trial, randomization code breaks should occur only in exceptional circumstances when knowledge of the actual treatment is absolutely essential for further management of the subject. Investigators are encouraged to discuss with the study team if he/she believes that unblinding is necessary.

The investigator is encouraged to maintain the blind as much as possible. The actual allocation must NOT be disclosed to the subject and/or other study personnel including other site personnel, monitors, corporate sponsors or project office staff. There should not be any written or verbal disclosure of the code in any of the corresponding subject documents.

The investigator must report all code breaks (with reason) as they occur on the corresponding CRF page.

Unblinding will not be in and of itself a reason for termination from the study.

10.4 Planned Interim Analyses

There will be one planned interim analysis of the primary endpoints after all subjects in Stage 2a have completed their Day 8 + 3 days Follow-up Visit, or sooner if early withdrawal. Enrollment will be held until the dose for Stage 2b is determined from the planned interim analysis. The statistical methods for the interim analysis will be fully specified in advance in a statistical analysis plan (SAP).

10.4.1 Interim Safety Review

The DSMB will evaluate safety at pre-specified intervals. Ongoing review and summary of subjects' safety will occur to allow for early detection of a safety signal that may result from an AE. The DSMB will advise the trial sponsor on whether to continue, modify, or terminate the trial based on risk assessment.

10.4.2 Interim Microbiological Activity Review

An interim analysis will be performed to select the IV bacteriophage dose with the most favorable benefit to risk profile compared to placebo for further evaluation in Stage 2b, and to exclude proceeding with bacteriophage doses that do not reduce pseudomonal colony counts or are associated with safety issues, when all subjects in Stage 2a have completed their Day 8 + 3 days Follow-up Visit or last study visit if early withdrawal.

Continuous variables will be summarized by number of subjects, mean, standard deviation (SD), median, quartiles (1st and 3rd quartiles), minimum, and maximum values. Categorical variables will be summarized by number and percentage of subjects. The interim analysis will consist of a quantitative evaluation of potential effect sizes and associated precision using a predicted intervals and predicted interval plots approach.[29] Briefly, predicted intervals for DOOR probability will be calculated under a range of assumptions including: (1) the trends in outcomes

observed at interim analysis will continue to end of study, (2) both DOOR distributions are identical, and (3) best and worst case scenarios for remaining outcomes. By relying on prediction intervals, no statistical hypothesis testing is required and no power is lost at interim analysis. In addition, as supplementary information, (predictive) conditional power will be calculated to quantify the probability of rejecting the null hypothesis, i.e., DOOR probability ≤ 50%, at the final analysis under a range of assumptions.

Similar to DOOR, changes in $\log_{10}$ *P. aeruginosa* CFU/mL (total and each morphology) in sputum cultures from administration of IV bacteriophages through Day 8 + 3 days, will be evaluated, using predicted interval plots to assess the precision for measuring differences in $\log_{10}$ CFU/mL count.

The planned sample size (20 subjects per Stage 2b arm) will be reevaluated after completion of Stage 2a. If necessary, the sample size will be increased up to 25 subjects for the two arms in Stage 2b (up to 25 subjects for the placebo arm and up to 25 subjects for the selected bacteriophage dose) to provide the desired precision of estimates.

An unblinded statistician at the SDCC will report to the PHAGE Advisory Group. The PHAGE Advisory Group will make a recommendation to the DMID on the sample size (see Section 10.2) and the optimal dose to be considered for Stage 2b. This will only be a recommendation. Ultimately, DMID will review the recommendation and make the final decision on how to proceed regarding Stage 2b. Blinding to treatment assignment will be maintained at all investigational sites, and the results of the interim analysis will not be shared with the site investigators prior to the completion of the trial. The DMID will receive recommendations throughout the trial from the DSMB. DMID is responsible for deciding actions based upon the recommendations of the DSMB.

The blinded statistician at the SDCC will be involved in SAP revisions before and after the interim analysis and before the final analysis as part of the study team. This blinded statistician will be involved in discussions regarding modifications to the protocol, statistical methods, identification of protocol violations, or data validation efforts that occur after the interim analysis. A separate unblinded statistician at the SDCC who reports to the PHAGE Advisory Group will perform the interim analyses and generate the interim report but will not be involved in discussions about modifications to the protocol, statistical methods, identification of protocol violations, or data validation efforts.

10.5 Final Analysis Plan

10.5.1 General Considerations

The SAP, which includes more technical and detailed elaboration of the principal features stated in the protocol, will be prepared separately and finalized before the interim analysis.

Safety will be assessed by estimating the occurrence of Grade 2 or higher treatment-emergent AEs in the IV bacteriophage therapies compared to placebo through Day 30 ± 7 days.

Microbiological activity will be assessed by estimating changes in $\log_{10}$ *P. aeruginosa* CFU/mL (total and each morphology) in sputum cultures from baseline through Day 30 ± 7 days.

The benefit to risk profile will be assessed by comparing the DOOR of IV bacteriophage arms and placebo arm through Day 8 + 3 days. The DOOR will be analyzed by estimating the DOOR probability (Section 10.5.5), (i.e., the probability of a randomly selected subject having a better DOOR if assigned to receive an IV bacteriophage therapy compared to placebo).

All statistical calculations will be performed using up-to-date versions of SAS version 9.4 or higher (SAS Institute Inc., Cary, NC, USA) or language and environment for statistical computing 3.6.1 or higher (R Foundation for Statistical Computing, Vienna, Austria), unless otherwise specified. All PK analyses will be performed using NONMEM version 7.3 or higher (ICON Development Solutions, Ellicott City, MD). For continuous variables, summary statistics will include sample size, mean, SD, median, quartiles, minimum, and maximum values. Frequencies and percentages will be calculated for categorical variables. All reported confidence intervals will be two-sided 95%. Graphical displays will be produced.

10.5.2 Analysis Sets

Four analysis sets will be considered in the statistical analysis of the study:

1. *Screened Set*: The screened set will consist of all subjects who undergo the Screening Visit and receive a patient identification (PID) number.
2. *Intent-to-treat/Randomized Set*: The intent-to-treat (ITT) set will consist of all randomized subjects regardless of whether or not they received study product or placebo.
3. *Safety Set*: The safety set will consist of all randomized subjects who received at least one dose of study product or placebo. Subjects will be analyzed based on the treatment received.
4. *Pharmacokinetics Set*: The PK set will consist of all randomized subjects who contributed at least two of each a sputum and a blood sample.

On the basis of the ITT principle, all of the primary endpoints will be analyzed on the ITT set.

10.5.3 Subject Disposition

The number of subjects in two of the study sets (ITT and Safety) will be summarized by groups; the screened set will only be summarized by study center.

Screen failures (i.e., subjects screened but not randomized) and the associated reasons for failure will be tabulated overall. The number and percentage of subjects who complete the treatment period (including Day 30 ± 7 days) and of subjects who prematurely discontinue during the same period will be presented for each group and pooled across groups for the ITT set. The reasons for premature discontinuation as recorded on the termination pages of the eCRF will be summarized (number and percentage) by treatment groups for ITT set.

10.5.4 Demographics and Other Baseline Characteristics

Demographic variables and other baseline characteristics will be summarized by treatment arm for the Safety and ITT sets.

Prior medication is defined as any medication taken before the date of study product or placebo. Concomitant medication is defined as any medication started on or after the date of study product or placebo dose.

Concomitant medication use will be summarized by the number and proportion of subjects in each arm receiving each medication within each therapeutic class for the safety set. If a subject took a specific medication multiple times or took multiple medications within a specific therapeutic class, that subject would be counted only once for the coded drug name or therapeutic class.

10.5.5 Analysis of Primary Endpoints

Grade 2 or higher treatment-emergent AEs will be summarized descriptively. The type of AE, number of events (n) and number (%) of subjects with events in each arm will be tabulated. The type of AE, number of AEs and the number (%) of subjects with events will be presented by system organ class (SOC), HLT, and preferred term. Subjects are counted once in each SOC, HLT, and preferred term. The difference in proportion of the number (%) of subjects with events between IV bacteriophage and placebo arms will be calculated with the corresponding exact 95% confidence interval.

Changes in $\log_{10}$ *P. aeruginosa* CFU/mL (total and each morphology) in quantitative sputum cultures from administration of IV bacteriophages through Day 30 (± 7 days), Visit 7, will be summarized descriptively. Descriptive statistics will be presented by arms. The difference in mean change between IV bacteriophage and placebo arms will be estimated with the corresponding 95% confidence interval ($\log_{10}$-transformed and original scales). Area Under Curve (AUC) calculated using the trapezoidal rule will be used to summarize $\log_{10}$ *P. aeruginosa* CFU/mL the over the time. The difference in AUC between IV bacteriophage and placebo arms will be calculated with the corresponding 95% confidence interval.

The DOOR will be summarized by estimating the DOOR probability, using the equation

$$\text{DOOR probability} = \Pr[\text{DOOR}_{\text{IV}} > \text{DOOR}_{\text{P}}] + \frac{1}{2}\Pr[\text{DOOR}_{\text{IV}} = \text{DOOR}_{\text{P}}],$$

where DOOR_{IV} and DOOR_{P} are the DOOR for bacteriophage and placebo arms, respectively, and $\Pr[\text{DOOR}_{\text{IV}} > \text{DOOR}_{\text{p}}]$ is the proportion of a DOOR from IV bacteriophage dose exceeding a DOOR from placebo and $\Pr[\text{DOOR}_{\text{IV}} = \text{DOOR}_{\text{P}}]$ is the proportion of two DOOR outcomes being same. The corresponding 95% confidence interval will be calculated.

In addition, the DOOR will be analyzed as follows: DOOR distribution by arms, cumulative difference (point estimate and confidence band) in DOOR categories for bacteriophage vs. placebo, point estimate and confidence interval of difference in mean partial credit vs. placebo, and DOOR distribution for bacteriophage vs. placebo arm.

Each of the components of the DOOR will be compared between the IV bacteriophage and placebo arms: presence/absence of SAE and reduction in *P. aeruginosa* CFU/mL (> 2 $\log_{10}$, 1-2 $\log_{10}$ reduction, < 1 $\log_{10}$ reduction).

All analyses are summarized in the **Table 3** below.

Table 3: Summary of Analysis of Primary Endpoints

Outcome/Variable	Summary Measures	Analysis of Methods	Analysis Set
1) Grade 2 or higher treatment-emergent AEs	• The number of events • The number of subjects with events • Evaluated until Day 30	• Descriptive statistics (frequency and percentage) in each arm • Difference in bacteriophage vs. placebo arm and the corresponding 95% confidence interval	ITT
2) Log_{10} *P. aeruginosa* CFU/mL	• Mean change from baseline • Evaluated until Day 30	• Descriptive statistics (the number of subjects, mean, SD, quartiles, minimum, median, and maximum) • Confidence interval for mean change from baseline in each arm • Difference in mean change from bacteriophage vs. placebo arm and its corresponding 95% confidence interval ($\log_{10}$-transformed and original scales) • Plot median (Q1,Q3) over time by arm	ITT
	• AUC over times	• Difference in AUC of bacteriophage vs. placebo and its corresponding 95% confidence interval.	
3) DOOR	• DOOR Probability	• 95% confidence interval for DOOR Probability of bacteriophage vs. placebo	ITT

Outcome/Variable	Summary Measures	Analysis of Methods	Analysis Set
	• DOOR Categories distribution • Evaluated until Day 8	• Descriptive statistics (frequency and percentage) in each arm • Cumulative difference in DOOR categories for bacteriophage vs. placebo (point estimate and confidence band) • Point estimate and confidence interval of difference in mean partial credit of bacteriophage vs. placebo arm • DOOR distribution of bacteriophage vs. placebo arm	

10.5.6 Subgroup and Adjusted Analyses for Primary Endpoints

As an explanatory analysis, subgroup analyses of the primary outcomes may be conducted to explore whether estimated treatment effects vary significantly between subcategories of trial subjects, including, but not limited to, bacteriophage susceptibility and presence of co-infection with other organisms recovered on sputum cultures collected during the study period. In addition, the primary endpoints will adjust for pre-randomization variables, which might reasonably be expected to be predictive of favorable outcomes.

10.5.7 Analysis of Exploratory Endpoints

Exploratory endpoints will be summarized by appropriate statistics. Continuous variables will be summarized by number of subjects, mean, SD, median, quartiles, minimum, and maximum values. Categorical variables will be summarized by number and percentage of subjects. Blood and sputum samples collected from the sentinel subjects in Part 1 will be used for exploratory analysis purposes. Phage PK will be analyzed using compartmental population PK models to obtain population mean estimates of clearances and volumes as well as estimates of inter-individual variability. Individual estimates of clearances and volumes will be obtained from post hoc estimates and used to estimate exposure in the central compartment and a peripheral compartment representing the sputum. Additional details regarding exploratory analysis will be included in the SAP.

10.5.8 Missing Data

The endpoints could be missing for subjects who withdraw from the trial or for other reasons. The reasons for withdrawal will be reported and compared qualitatively by arm. The effect that any missing data might have on results will be assessed via sensitivity analysis. If the pattern of missing data is different to that envisaged at the design stage, further sensitivity analyses will be provided that are tailored to the missing data pattern observed. The amount of missing data will be compared between randomized arms. The baseline characteristics of subjects with missing data will be compared to subjects without missing data.

10.5.9 Safety Data

An event that occurs during the treatment period will be considered a treatment-emergent AE if it was not present before the first dose of investigational product or was present before the first dose of investigational product and increased in severity during the treatment period. Adverse events (AEs) will be coded by primary system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA).

The type of AE, number of events (n) and number (%) of subjects with events in each group will be tabulated. The type of AE, number of AEs and the number (%) of subjects with AEs will be presented by SOC, HLT, and preferred term. Subjects are counted once in each SOC, HLT, and preferred term. In addition, summaries of AEs by grouped causal relationship to treatment (all-cause/treatment-related) and grade will be provided by system organ class and preferred term.

The incidence of treatment-emergent AEs, on-therapy SAEs, and AEs leading to premature discontinuation will be summarized by preferred term and treatment group and will be sorted by decreasing frequency for the investigational product. In addition, the incidence of fatal on-therapy SAEs (i.e., events that caused death) will be summarized separately by treatment arm and preferred term. An SAE will be defined as an on-therapy SAE if it occurred during or after the infusion of investigational/placebo product.

Listings will be presented for subjects with SAEs, subjects with AEs leading to discontinuation, subjects with ESIs, and subjects who die (if any).

10.5.10 Clinical Laboratory Parameters and Vital Signs

Descriptive statistics for clinical laboratory values and changes from the baseline values at each assessment time point will be presented by treatment arm for each clinical laboratory parameter. Descriptive statistics for vital signs and changes from baseline values at each visit and at end of study will be presented by arm.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate research records for this trial, in compliance with §21 CFR 312.62, ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance (QA) reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site is responsible for conducting routine QA and quality control (QC) activities to internally monitor study progress and protocol compliance. The site PI will provide direct access to all study-related source data/data collection forms, reports, training documentation, and regulatory files for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the participating site(s) for clarification and resolution.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue permanent ink is required to ensure clarity of reproduced copies. When making changes or corrections, the original entry should be crossed out with a single line, and the change should be initialed and dated. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Data reported in the eCRF derived from source data collection forms/source documents should be consistent or the discrepancies should be explained.

13.2 Statistical and Data Coordinating Center/Biostatistician Responsibilities

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site PI. During the study, the site PI must maintain complete and accurate documentation for the study.

The Emmes Company will serve as the SDCC for this study and will be responsible for data management, quality review, analysis, and reporting of the study data.

13.3 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs/ESIs, concomitant medications, medical history, physical assessments, and clinical laboratory values) will be collected on data collection forms, as appropriate, by study personnel then entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

All data collection forms/source documents (including laboratory reports) must be reviewed by the clinical team and data entry staff, who will ensure that the data are accurate and complete. Responses to QOL questionnaires will be collected on paper and entered by designated study staff into the eCRF for scoring and analysis. For additional details on data entry and reporting, refer to the MOP.

13.4 Types of Data

Data for this trial will include clinical, safety, QOL questionnaires, and outcome measures.

13.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study product disposition records will be retained for 2 years after a marketing application is approved for the study product for the indication for which it is being

investigated; or, if no application is to be filed or if the application is not approved for the study:product, until 2 years after the investigation is discontinued and the FDA has been notified.

These documents will be retained for a longer period; however, if required by local regulations. Informed consent forms (ICFs) for future use will be maintained as long as the sample/specimen exists.

No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the site PI when these documents no longer need to be retained.

14 CLINICAL MONITORING

A separate clinical monitoring plan will describe the conduct of the monitoring, to include but not limited to the monitoring frequency and the level of detail of the monitoring.

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures (SOPs). The sponsoring agency, DMID, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits (in-person or remote, as appropriate) will be made at intervals defined by the clinical monitoring plan and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site PIs to discuss any problems and actions to be taken and will document site visit findings and discussions.

15 PUBLICATION POLICY

Results from primary and exploratory endpoint analyses may be distributed by the SDCC to key study team members (protocol PIs, protocol statisticians, and other necessary study team members) after database lock and prior to the generation of all the Clinical Study Report Tables, Listings, and Figures. These analyses may be used by the PIs for manuscript and abstract development while the Clinical Study Report is being finalized.

Following completion of the study, the lead PI is expected to publish the results of this research in a scientific journal. All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of OER Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first participant. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting, a copy of this protocol (and its amendments) and a copy of the SAP will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party *does not plan* to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

16 LITERATURE REFERENCES

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17 APPENDICES

Appendix A. Schedule of Events

Visit	Screen	Baseline/ Dosing	Follow-up Visits					Early Termination Visit[1]/Unscheduled Study Visit
Visit Number	**1**	**2**	**3**	**4**	**5**[18]	**6**[19]	**7**	**N/A**
Day (Window)	**Up to Day -7**	**1**	**2**	**5 ± 2**	**8 + 3**	**12 + 7**	**30 ± 7**	**Variable**
Informed Consent	X							
Eligibility Criteria	X	X						
Serum HCG Pregnancy Test	X[2]							
Urine Pregnancy Test		X[2]						
Demographics	X							
Height and Weight Measurement	X							
Medical History[3]	X							
Medication History[4]	X							
Review of systems	X	X	X	X	X	X	X	X
Treatment Assignment (**Stage 1**) /Randomization (**Stage 2**)		X						
Administration of Study Product		X						
Concomitant Medications		X	X	X	X	X	X	X
Physical Examination	X[5]							
Symptom Directed Physical Examination		X[6]	X	X		X	X	X
Vital Signs	X[5]	X[6]	X	X		X	X	X
Spirometry	X	X[7]	X	X		X	X	X[8]
Sputum Collection[9]	X	X[10]	X	X	X	X	X	X

Visit	Screen	Baseline/ Dosing	Follow-up Visits					Early Termination Visit[1]/Unscheduled Study Visit
Visit Number	**1**	**2**	**3**	**4**	**5**[18]	**6**[19]	**7**	**N/A**
Day (Window)	**Up to Day -7**	**1**	**2**	**5 ± 2**	**8 + 3**	**12 + 7**	**30 ± 7**	**Variable**
Clinical Chemistry		X[11,15]	X	X		X	X	X[12]
Liver Function Tests	X[13]	X[11,15]	X	X		X	X	X[12]
Hematology		X[11,15]	X	X		X	X	X[12]
Serum for PK		X[14]	X					
CFQ-R (**Stage 2 only**)[17]		X[15]					X	
CFRSD (**Stage 2 only**)[17]		X[15]	X	X	X		X	X
Safety Assessment		X	X	X	X	X	X	X
Events of Special Interest		X	X	X	X			X[16]

[1]May be conducted by telephone call if subject is too ill to attend in-person or virtual visit, per local investigator.
[2]Pregnancy testing will be performed for all female subjects of childbearing potential and the results of both the HCG test at screening and urine test at baseline must be negative prior to treatment assignment/randomization.
[3]Including full account of the subject's routine respiratory physiotherapy regimen.
[4]Medication history within the past 30 days and those that remain active.
[5]Standard of care results obtained for clinical care purposes within 72 hours of screening are acceptable for screening.
[6]Before administration of study product or placebo AND 30-60 minutes after completed administration of study product or placebo.
[7]Spirometry to occur prior to study product/placebo administration.
[8]If the visit occurs > 7 days after a scheduled visit. If the visit occurs ≤ 7 days after a scheduled visit, spirometry may be performed at the discretion of the local investigator.
[9]At in-person visits to be completed after spirometry, as considered necessary by the local investigator to increase sputum production.
[10]Sputum will be obtained pre-infusion and post-infusion for microbiological and PK assessments (see Sections 7.2.1 and 7.2.2 for details).
[11]Labs obtained at the Screening Visit do not have to be repeated at the Baseline Visit, if collected within 7 days from the Baseline Visit.
[12]If the visit occurs > 7 days after a scheduled visit. If the visit occurs ≤ 7 days after a scheduled visit, laboratory tests may be collected at the discretion of the local investigator.
[13]AST, ALT, and total bilirubin only.
[14]Serum will be obtained pre-infusion and post-infusion for PK assessments (see Section 7.2.2 for details).
[15]Prior to administration of Study Product/Placebo.
[16]If the visit occurs prior to Visit 5, Day 8 + 3 days.
[17]Preferably before the collection of study data. If the subject is ready to produce sputum, prioritize sputum collection over administration of the CFQ-R/CFRSD.
[18]In Stage 2, this is a virtual visit.
[19]This visit is only applicable for Stage 1.

Appendix B. Cystic Fibrosis Questionnaire – Revised (CFQ-R)

Appendix C. Cystic Fibrosis Respiratory Symptom Diary (CFRSD)