CYSTIC FIBROSIS PHAGE STUDY AT YALE (CYPHY)

Protocol Number

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Protocol Version

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Short Title:

CYstic fibrosis bacterioPHage study at Yale (CYPHY): A single-site, randomized, double-blind, placebo-controlled study of bacteriophage therapy YPT-01 for *Pseudomonas aeruginosa* infections in adults with cystic fibrosis

IND Number: 23427

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Study Phase: 2

REVISION HISTORY

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n/a (no protocol changes)	Provide updated Lab Manual and Investigator’s Brochure; submitted to FDA July 12, 2021 & subsequently included in IRB amendment	MOD00039066 June 23, 2021	SN0005 July 12, 2021
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n/a (no protocol changes)	IND Annual Review submitted to FDA	n/a	SN0008 Sept 02, 2022
9. October 05, 2022	Amendment #5, End the Double-Blind Randomized segment including ending enrollment Open the Open-Label Extension	MOD00055062 Nov 01, 2022	SN0009 Oct 21, 2022
10. October 26, 2022	Amendment #5, Additional Open-Label changes for IRB review	MOD00055062 Nov 01, 2022	SN0011 Nov 29, 2022
11. November 29, 2022	Amendment #5, response to IRB comments. Recruitment concluded Oct 21, 2022 The study is in the open-label stage status upon IRB approval of this amendment.	MOD00055062 Nov 30, 2022	SN0011 Nov 30, 2022

Synopsis

Primary Objective

The primary objectives of this Phase 2 study are to determine whether YPT-01 phage therapy reduces sputum bacterial load in cystic fibrosis subjects with *Pseudomonas aeruginosa*, and to evaluate short-term safety of phage therapy in this patient population.

Secondary Objectives (if applicable)

The secondary objectives of this study are to:

- Assess whether YPT-01 induces “trade-offs” between evolved phage resistance and decreased virulence factors present in sputum *P. aeruginosa*;
- Determine whether YPT-01 alters subject sputum microbiome;
- Understand whether YPT-01 changes subject lung inflammation and inflammatory markers;
- Describe clinical response in subjects to YPT-01, with respect to change in lung function (e.g., FEV1pp), as well as rates of pulmonary exacerbations, acute antibiotic use for exacerbations, and hospitalizations;
- Understand whether YPT-01 improves subject-reported quality of life (via CFQ-R);
- Obtain preliminary data on the long-term safety of phage therapy

Study Duration

Approximately 3 years from initial IRB approval

Study Design

Prospective, randomized, parallel, placebo-controlled, double-blinded with an optional open-label extension for subjects who initially received placebo.

Study Population

Clinically-stable (e.g., no exacerbations or medication changes within 4 weeks) individuals with cystic fibrosis (no solid organ transplant; FEV1pp > 40%) with chronic sputum *P. aeruginosa*.

Number of Participants

40 (screened), 36 (enrolled and randomized)

Number of Study Sites

Single center study at Yale University

Primary Outcome Variables

Change in *P. aeruginosa* sputum CFU from baseline to day number 14 (7 days after completion of phage therapy)

Short-term safety profile of inhaled phage therapy during the randomized portion of the study (within the first 56 days of the blinded portion of the trial):

Safety-related outcomes, i.e. frequency and severity of different types of adverse events as related to the study intervention, will be actively monitored throughout the study and summarized for the following days post-phage treatment during randomized blinded (experimental) period: *d7, d14, d21, d28, d56. Monthly thereafter until month 6.* Safety-related outcomes will be summarized post-phage treatment on *d7, d14, d28, and monthly thereafter for a total of 6 months (placebo and open-label time total)* during the open-label period.

Secondary and Exploratory Outcome Variables (if applicable)

Secondary end-points for response profiles at d3, d7, d14, d21, and d28:

1) Change over time from baseline in PsA sputum load
2) Change over time from baseline in "trade-offs" of PsA, as measured by change in PsA antibiotic sensitivity (after OMKO1 phage), motility and pyocyanin levels (after TIVP-H6 phage), and endotoxin levels (after LPS-5 phage);
3) Changes from baseline in sputum phage presence;

Secondary end-point at d14:

1) Change from baseline in lung inflammation as measured by sputum transcriptomics;

Secondary end-points for response profiles at d14 and d28:

1) Change from baseline in sputum inflammatory markers, as measured by inflammatory cytokines;

Secondary end-points for response profiles at d3, d7, d14, d21, d28, and d56:

1) Change from baseline in lung function (FEV1pp and absolute FEV1) from baseline;
2) Change from baseline in quality of life and respiratory symptoms as measured by CFQ-R at each clinic visit from baseline;
3) Rate(s) of pulmonary exacerbations and medication changes from baseline.

Secondary end-point for long-term safety at month 6:

1) Frequency and severity of different types of adverse events.

Tertiary end points for response profiles at d7, d14, d21, d28, and d56:

1) Change from d1 in anti-phage antibody titers.

Abbreviations

Abbreviation	Explanation
Ab	Antibodies
AE	Adverse Event
ANC	Absolute Neutrophil Count
ANCOVA	Analysis of Covariance
APT	Adaptive Phage Therapeutics
CAR	Carbenicillin
CF	Cystic Fibrosis
CFF	Cystic Fibrosis Foundation
CFQ-R	Cystic Fibrosis Quality-of-Life Revised Survey
CFR	Code of Federal Regulations
CFRSD	Cystic Fibrosis Respiratory Symptom Diary
CFTR	Cystic Fibrosis Transmembrane Receptor
CFU	Colony-forming units, a measure of bacterial load
CI	Confidence Interval
CIP	Ciprofloxacin
CLIA	Clinical Laboratory Improvement Amendments
CONSORT	Consolidated Standards of Reporting Trials (publishing requirements)
CRF	Case Report Form
CRISS	Chronic Respiratory Infection Symptom Score
CS	Compound Symmetry
CT	Computerized Tomography (scan)
CTCAE	Common Terminology Criteria for Adverse Events
CYPHY	CYstic fibrosis bacterioPHage study at Yale (this study)
d	day

DMC	Data Monitoring Committee
DSMC	Data Safety Monitoring Committee
eIND	emergency Investigational New Drug Application
EMR	Electronic Medical Record
EOP	Efficiency of Plaquing, an *in vitro* measure of phage efficacy against a pathogen
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume over 1 second
FEV1pp	Percent predicted of Forced Expiratory Volume over 1 second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GEE	Generalized Estimating Equations
GMP	Good Manufacturing Practice
HCl	Hydrogen Chloride
HIPAA	Health Insurance Portability and Accountability Act
HRPP	Human Research Protection Program (at Yale)
ICC	IntraClass Correlation
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICOI	Institutional Conflict of Interest
ICOIC	Institutional Conflict of Interest Committee
IDS	Investigational Drug Service, a branch of the Yale Pharmacy
IEC	Independent Ethics Committee
IND	Investigational New Drug Application
IO	Institutional Official
IP	Intraperitoneal

IQR	Interquartile Range
IRB	Institutional Review Board
ITT	Intent-To-Treat
IV	Intravenous
IVC	Inspiratory Vital Capacity
LB	Luria-Bertani medium, used to culture bacteria
LME	Linear Mixed Effects
LPS	Lipopolysaccharide
LPS-5	Lipopolysaccharide-targeting phage 5, one of the phages comprising YPT-01
LRT	Likelihood Ratio Test
LSFI	Leaders Significant Financial Interests
MAR	Missing at Random
MDR	Multidrug-resistant, often used in reference to bacteria or pathogens
$MgSO_4$	Magnesium Sulfate
MIC	Minimum Inhibitory Concentration
ML	Maximum Likelihood
NAL	Nalidixic acid
NCFB	Non-Cystic Fibrosis Bronchiectasis
OMKO1	Outer Membrane Knockout-1, an efflux pump targeting phage and one of the phages comprising YPT-01
OR	Odds Ratio
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PEF	Peak Expiratory Flow
PFT	Pulmonary Function Tests (e.g., spirometry)
PFU	Plaque-forming units, a measure of phage dosage

Phage	Bacteriophage, a virus of bacteria
PI	Principal Investigator
PsA	*Pseudomonas aeruginosa*
QIC	Quasi-likelihood under the assumption of independence criterion
qPCR	quantitative PCR
SAE	Serious Adverse Event
SN	Serial Number
SoA	Schedule of Activities
TDN	Therapeutics Development Network of the Cystic Fibrosis Foundation
TET	Tetracycline
TIVP-H6	Type IV Pilus Targeting Phage H6, one of the phages comprising YPT-01
U	Units, a measure of antibiotic concentration (e.g., meropenem)
UPIRSO	Unanticipated Problems Involving Risks to Subjects or Others
VC	Vital Capacity
WHO	World Health Organization
YCCI	Yale Center for Clinical Investigation
YPT-01	Yale Phage Therapy 01, a treatment algorithm composed of phages LPS-5, OMKO1, and TIVP-H6

Table of Contents

1 Introduction

1.1 Introductory Statement

This document is a protocol for a human research study. The purpose of this protocol is to ensure that this study is to be conducted according to ICH GCP guidelines, CFR 21 Part 312, and according to other applicable government regulations and institutional research policies and procedures.

2 Background

2.1 Background/prevalence of research topic

Cystic fibrosis (CF) is the most common life-limiting genetic disease among caucasians. CF is caused by mutation(s) in the cystic fibrosis transmembrane receptor (CFTR), which encodes a chloride channel. The majority of individuals with CF develop chronic lung disease characterized by recurrent bacterial infections, which cause pulmonary exacerbations that result in significant morbidity and mortality. In the lungs, exaggerated mucus production and inflammation cause bronchiectasis, which is a change in lung architecture that prevents effective bacterial clearance. *Pseudomonas aeruginosa* (PsA), a Gram-negative bacterium, is an important concern because it is the most prevalent bacterial pathogen in CF adults, and strongly correlates with lung function decline and increased morbidity and mortality (Parkins et al., 2018). Therapies targeting PsA improve clinical outcomes in CF patients, and the current focus is on antibiotics to treat chronic lung disease and pulmonary exacerbations caused by these bacteria (Mogayzel et al., 2013). Despite this multi-modal approach, PsA continues to cause significant disease because it cannot be effectively cleared from the lungs.

In addition, PsA is one of the emerging multi-drug resistant (MDR) bacteria identified by the World Health Organization (WHO). By 2050, MDR pathogens will cause 10 million human deaths worldwide (de Kraker et al., 2016; O'Neill, 2014), which will exceed cancer deaths. MDR PsA is already increasingly problematic for CF patient care. Therefore, development of novel therapeutic approaches that may reduce infection, increase antibiotic sensitivity, or decrease inflammation with limited off-target effects are highly desirable for the CF community. These goals may be accomplished with ***bacteriophage (phage) therapy***. Phages are lytic (virulent) bacteria-specific viruses that may be used as self-amplifying 'drugs' to target and kill specific bacteria. In addition to their antibacterial effects, phages can also be designed to manipulate bacteria to decrease lung inflammation.

2.2 Preclinical Experience

Bacteriophages (phages) are a potential treatment for infections in humans caused by bacterial pathogens. Lytic phages are viruses that infect bacteria, generally resulting in production of phage progeny, which cause bacterial cell death through lysis (**Figure 2-1**). Given the distinctions in cellular components and molecular machinery of prokaryotic versus eukaryotic cells caused by genetic divergence over long spans of evolutionary time, phages are restricted to infecting bacteria and cannot infectiously destroy human cells. In addition, a majority of phages are restricted in host range to a particular bacterial species (or subset of genotypes within the species), or in some cases limited to infecting closely-related species of bacteria.

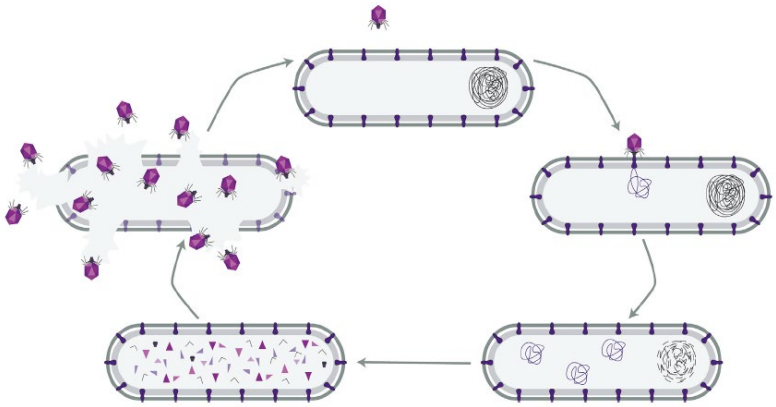


Figure 2-1 Lytic phage replication begins when the virus irreversibly binds to a receptor (protein or sugar) on the surface of a bacterial cell. The phage delivers its genomic content into the cytoplasm of the bacterial cell. Typically, host resources, including proteins and genomes are repurposed to fuel phage replication. Replication, transcription and translation of the phage genome begins usually through redirecting host metabolism to the production of new phage particles. Upon assembly of new phage particles, lysis of the bacterial cell allows newly replicated phage particles to escape the cytoplasm and go on to infect other phage-susceptible bacterial cells.

Yale's Phage Program has identified three environmentally-sourced phages for use in this study: OMKO1, TIVP-H6, and LPS-5. Limited preclinical studies have been performed on the phages, and pharmacokinetic and toxicology studies have not been completed on them. Each phage preparation used in subjects is tested for and meets quality specifications prior to clinical use. In addition, extensive use in humans over the last century has suggested that phage therapy is generally safe, while demonstrating anecdotal evidence of efficacy (see **Investigator's Brochure**). The degradation products of bacteriophages are proteins and nucleic acids, which are expected to be metabolized in an identical manner to what naturally occurs in the body. In addition, it is important to note that CF lung has a distinct microbial community that includes phages which may contribute to the spread of virulence or antibiotic-resistance genes (Brown-Jaque et al., 2018). OMKO1, TIVP-H6, and LPS-5 have been confirmed to be lytic, non-lysogenic phages, which minimizes the risk of integration into pathogen genomes and passage of virulence and antibiotic-resistance genes. Additionally, these three phages provide coverage of approximately 95% of isolates based on analysis of 96 adult CF sputum culture PsA isolates tested *in vitro* (see **Investigator's Brochure**).

OMKO1

Phage OMKO1 (family *Myoviridae*) targets the multidrug efflux pump MexAB-XY of PsA, killing susceptible bacterial cells via lysis while exerting selection for the bacterial population to evolve phage resistance that can coincide with decreased resistance (i.e., re-sensitivity) to certain antibiotics. Prior work demonstrated that phage OMKO1 was generally capable of attacking MDR PsA genotypes because it associated with the evolutionarily-conserved *oprM* gene (OprM membrane protein) of MexAB-XY efflux pumps in these bacteria (Chan et al., 2016). The targeting of OprM results in a tradeoff between efflux pump function and evolved

phage resistance, resulting in increased sensitivity of surviving PsA cells to antibiotics. **Table 2-1** shows the change in antibiotic sensitivity between MDR PsA isolates and spontaneous phage-resistant mutants that arise in the bacterial population following exposure to phage (Chan et al., 2016). Phage-resistant mutants demonstrate a 2- to 12-fold increase in sensitivity to antibiotics, which are known or suspected to be exported from the cell via functional MexAB-XY efflux pumps.

Table 2-1. Mean antibiotic sensitivity of PsA before and after phage-OMKO1-selection *in vitro*.

Antibiotic	Class	Strain	Isolate MIC[1] (mg/L)	Phage Resistant Isolate MIC[1] (mg/L)	Fold-increased Antibiotic Sensitivity
Tetracycline	Tetracycline	PA01	6.333	1.896	3.340189873
		PA14	10.67	3.4	3.138235294
		PAN	7.333	2.8	2.618928571
		1845	6.667	5.2	1.282115385
		1607	11.33	2.2	5.15
		PAPS	5.667	1.95	2.906153846
		PASk	58.67	14.67	3.999318337
		PADFU	48	10	4.8
Erythromycin	Macrolide	PA01	256	5.6	45.71428571
		PA14	34.67	10.8	3.210185185
		PAN	31.33	4.2	7.45952381
		1845	256	15.6	16.41025641
		1607	61.33	7.6	8.069736842
		PAPS	24.67	4.4	5.606818182
		PASk	149.3	22	6.786363636
		PADFU	256	55.67	4.598527034
Ciprofloxacin[2]	Fluoroquinolone	PA01	0.073	0.057	1.280701754
		PA14	0.21	0.047	4.468085106
		PAN	0.064	0.024	2.666666667
		1845	0.136	0.126	1.079365079
		1607	0.104	0.045	2.311111111
		PAPS	0.172	0.014	12.28571429
		PASk	5.333	1.666	3.201080432
		PADFU	3.667	1.417	2.58786168
Ceftazidime	Cephalosporin	PA01	0.667	0.663	1.006033183
		PA14	1	0.8	1.25
		PAN	1.5	0.6	2.5

		1845	0.917	0.7	1.31
		1607	1	0.55	1.818181818
		PAPS	0.667	0.35	1.905714286
		PASk	1.167	0.583	2.001715266
		PADFU	1.333	0.667	1.99850075

[1] Minimum Inhibitory Concentration (MIC) is the concentration of the drug needed to achieve efficient bacterial killing; includes data shown in Chan et al. 2016.

Phage OMKO1 also improved survival in a standardized lethal model of PsA infection in mice (**Figure 2-2**). In this infection model, immunosuppression was induced [cyclophosphamide (150 mg/kg IP)] prior to bacterial challenge. Mice were subsequently challenged with PsA strain UNC-D instilled intratracheally after isoflurane anesthesia (2-3% in O_2). Afterwards, OMKO1 phage particles ($1x10^{7.5}$ plaque-forming units, PFU) were administered alone (**Figure 2-2 left**), or in combination with meropenem [1250 U (**Figure 2-2 center**) or 1000 U (**Figure 2-2 right**) IV]. These experiments showed that the addition of OMKO1 rescued mice in the presence of the sub-therapeutic meropenem concentrations (**Figure 2-2 center & right**).

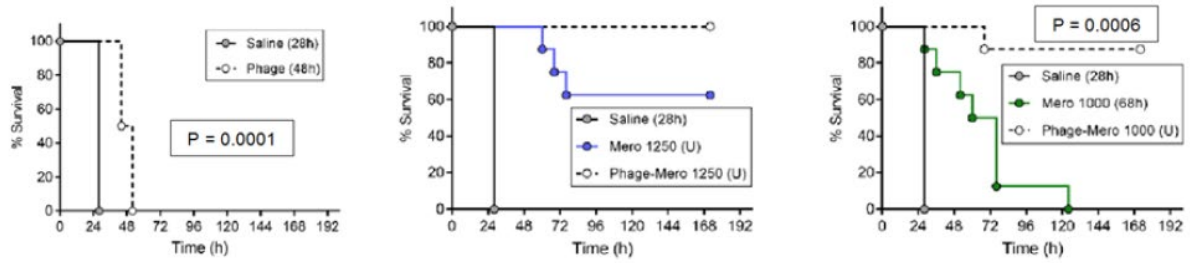


Figure 2-2. Assessment of phage therapy in a standardized lethal model of PsA infection. Mice infected with $1x10^6$ colony-forming units (CFU) PsA were treated with saline (solid line, grey circles) or OMKO1 phage ($1x10^{7.5}$ PFU; dashed line, white circles) 3 hours after infection (n=8 female mice in each group). Mice infected with $1x10^6$ cells of PsA treated with saline (solid line, grey circles), meropenem [1250 mg/kg/day; blue line & circles (LEFT), or 1000 mg/kg/day (green line & circles; RIGHT)], or OMKO1 phage ($1x10^{7.5}$ PFU; dashed line, white circles) plus meropenem 3 hours after infection (n=8 female mice in each group). Phage plus meropenem vs meropenem alone (p = 0.0006).

TIVP-H6

Phage TIVP-H6 (family *Myoviridae*) was discovered by searching for lytic phages that specifically impaired the ability for PsA bacteria to produce the virulence factor pyocyanin, such that evolved phage resistance resulted in reduced pyocyanin production. To do so, a large number of candidate phages were screened for their ability to infect a pyocyanin overproducing strain, and it was observed that: 1) phage TIVP-H6 infected this PsA strain, and 2) PsA mutants resistant to phage TIVP-H6 failed to produce pyocyanin. The impact of

sub-inhibitory concentrations of several antibiotics (ciprofloxacin, tetracycline, nalidixic acid, carbenicillin, and erythromycin) on PsA pyocyanin production was also tested in 48-hour cultures of bacteria (**Figure 2-3**). These results showed a significant ($p < 0.001$) decrease in pyocyanin production in PsA relative to controls, when bacteria were exposed to erythromycin alone, to phage TIVP-H6 alone, and to erythromycin plus phage TIVP-H6 (**Figure 2-3**). Interestingly, the antibiotic ciprofloxacin was found to increase production of pyocyanin, but this effect was reversed when bacteria were exposed to ciprofloxacin plus phage TIVP-H6 (**Figure 2-3,** column 6). Analysis of genomes from 10 independently isolated PsA mutant strains resistant to phage TIVP-H6 revealed numerous mutations in genes for the type IV pilus. Deletion of the type IV pilus in PsA was previously shown to impair pyocyanin production, twitching motility and biofilm formation, thus attenuating bacterial virulence (Persat et al., 2015).

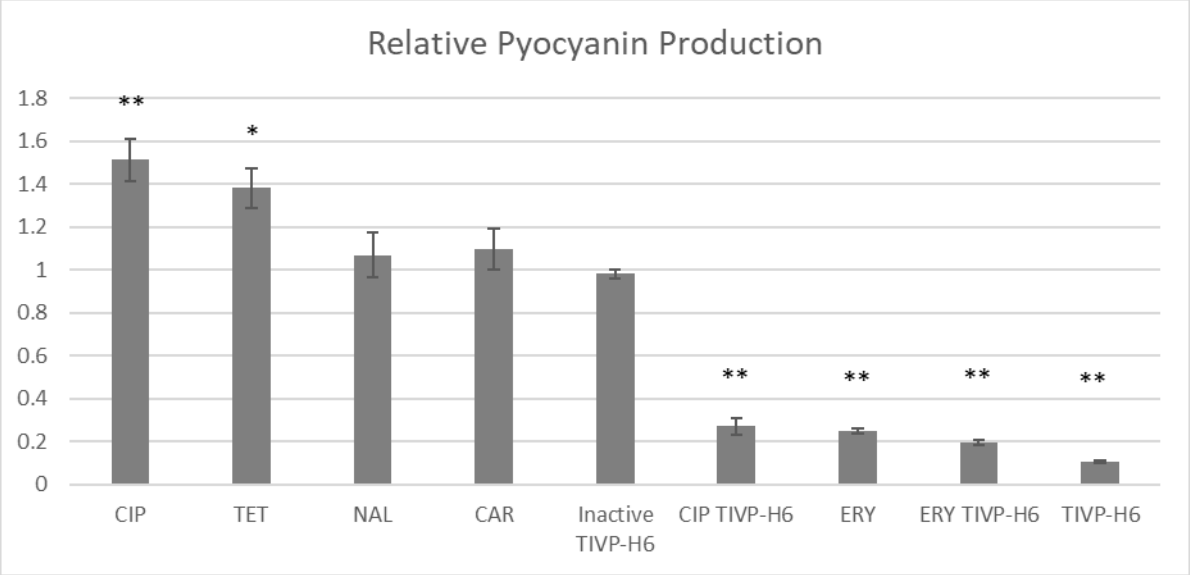


Figure 2-3. Impact of phage and sub-inhibitory concentration of antibiotics on production of pyocyanin in PsA. Results are expressed as fold-change in pyocyanin production relative to untreated control (y-axis) when PsA is exposed to traditional antibiotics or TIVP-H6 (x-axis). Representatives from four major drug classes were tested for their impact on pyocyanin production. Ciprofloxacin (CIP), Tetracycline (TET), Nalidixic acid (NAL), Carbenicillin (CAR), and heat-inactivated phage (Inactive) TIVP-H6 did not reduce pyocyanin production (left hand bars 1 through 5). In contrast, phage TIVP-H6 reduced pyocyanin production and significantly attenuates the increase pyocyanin production induced by CIP alone. (* $p < 0.05$; ** $p < 0.01$, significance compared to untreated control)

LPS-5

Phage LPS-5 (family *Podoviridae*) was discovered by searching for lytic viruses that specifically interacted with lipopolysaccharide (LPS) in PsA, such that evolved phage resistance resulted in reduced virulence due to changed LPS production. It was hypothesized that phage interaction(s) with LPS could have an impact on the production of outer membrane vesicles (Cryz et al., 1984; Kon et al., 1999). Therefore, extracellular endotoxin, measured by the Congo-endotoxin assay, would be reduced or absent in bacteria that evolved resistance to phage LPS-5. Consistent with this hypothesis, endotoxin production in 48-hour cultures of

phage-resistant PsA mutants was observed to be below the limit of detection, indicating that bacterial resistance to phage LPS-5 coincided with an evolutionary trade-off evidenced by reduced endotoxin production. Ten independently isolated PsA mutants resistant to phage LPS-5 were found to have many mutations in LPS synthesis genes, confirming the hypothesis that LPS is the primary cellular receptor for phage LPS-5.

2.3 Clinical Experience

Phages OMKO1, TIVP-H6, and LPS-5 have been previously used in phage "therapy" for 12 patients under single-patient emergency INDs (eINDs). Nine were CF patients, two had non-CF bronchiectasis (NCFB), and one had an infected aortic graft (Chan et al., 2018). The CF and NCFB patients tolerated nebulized phage therapy without any adverse side effects. One individual had a serious adverse event (SAE); however, it was deemed to be unrelated to phage treatment (See **Investigator's Brochure**). In all patients, bacterial loads were significantly reduced, and in many cases bacterial cultures showed increased antibiotic sensitivity or decreased inflammatory products (e.g., pyocyanin) after phage treatment, which were the 'trade offs' that the Yale investigators intended to leverage.

In addition, several patients' responses showed evidence of clinical improvement (e.g., improved lung function or decreased use of antibiotics, **Figure 2-4**). Patients FEV1% were assessed (**Figure 2-4A**) and post-phage treatment sputum samples were obtained to quantitate PsA titers seven days after completion of phage treatment (**Figure 2-4B**), to analyze antibiotic sensitivity (**Figure 2-4D**), and virulence factor (e.g., pyocyanin; **Figure 2-4C**) production. These results show log10 -2.66 CFU/mL (±1.17 CFU/mL) decrease in PsA (**Figure 2-4B**) seven days after completion of inhaled phage treatment. PsA produces pyocyanin, which contributes to lung inflammation. Post TIVP-H6 phage, pyocyanin decreased in patient samples 15.67-fold (**Figure 2-4C**). Treatment with OMKO1 phage resulted in decreased MIC for common CF antibiotics by 3.8-fold (**Figure 2-1D**). CF patients' pre-phage FEV1% of 35.5% (±0.18%) increased to 42.83% (±0.23%) (**Figure 2-4B**), which is a mean change of 7.3 percentage points. Narratives of each subject's experience are presented in the **Investigator's Brochure**. For these cases, a phage dose of 1e10 PFU provided daily for 7-10d was used, which was well-tolerated and is thus considered an effective target dose. These preliminary data informed the design of a single center trial to compare phage therapy to placebo.

Table 2-2. Summary of single-patient cases in CF and NCFB treated with OMKO1, TIVP-H6, or LPS-5 phages

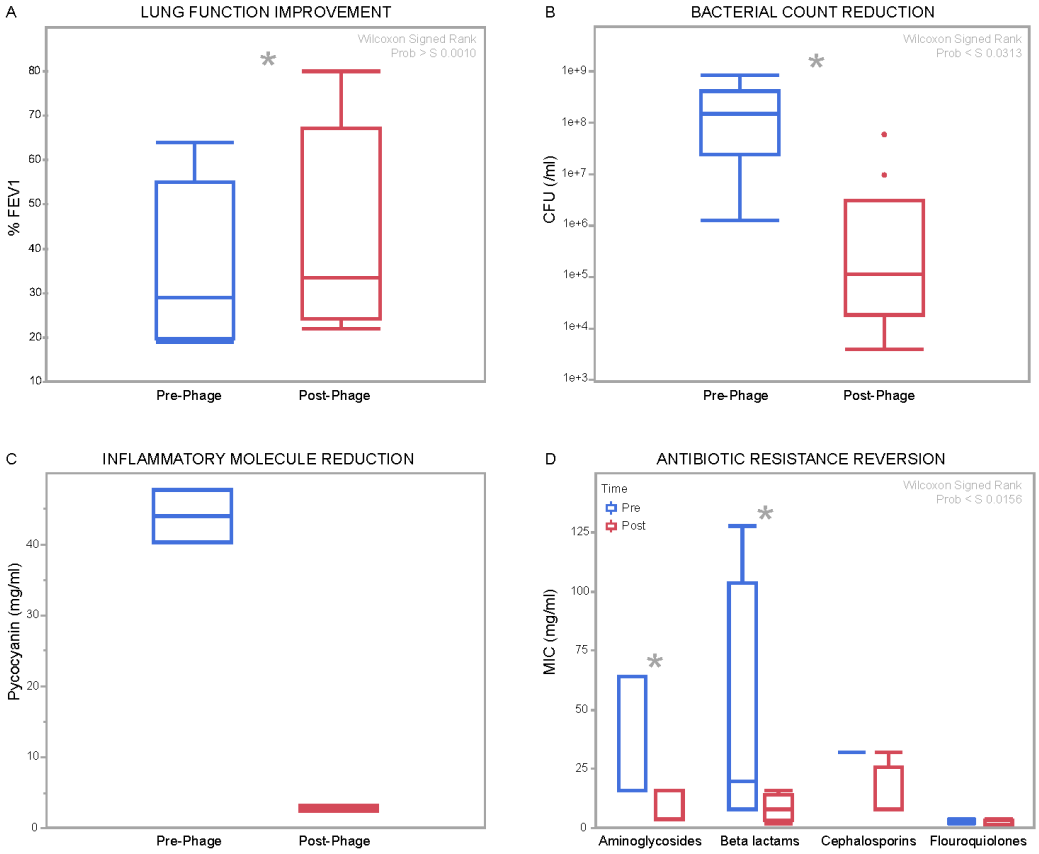
Patient	Background	Pathogen	Phage(s) Used	Primary Impact	Tradeoff[2]	Date
22 yo Female	CF	PDR PsA	OMKO1, TIVP-H6, LPS-5	CFU Decrease	Yes	Dec 2017
72 yo Male	NCFB	MDR PsA	TIVP-H6, LPS-5	CFU Decrease	Yes	Sep 2018
71 yo Male	NCFB	MDR PsA	TIVP-H6, LPS-5	Infection Resolved[1]	Yes	Oct 2018
16 yo Female	CF	MDR PsA	OMKO1, TIVP-H6, LPS-5	CFU Decrease	Yes	Nov 2018
38 yo Female	CF	PDR PsA	TIVP-H6, LPS-5	CFU Decrease	Yes	Dec 2018
26 yo Female	CF	MDR PsA	LPS-5	CFU Decrease	Yes	Jan 2019
38 yo Female	CF	MDR PsA	LPS-5	CFU Decrease	Yes	Apr 2019
46 yo Female	CF	MDR PsA	TIVP-H6, LPS-5	CFU Decrease	Yes	May 2019
27 yo Female	CF	MDR PsA	TIVP-H6, LPS-5	CFU Decrease	Yes	May 2019
26 yo Female	CF	MDR PsA	TIVP-H6, LPS-5	CFU Decrease	Yes	Apr 2019
35 yo Male	CF	MDR PsA	TIVP-H6, LPS-5	CFU Decrease	Yes	Apr 2019

CF = cystic fibrosis; NCFB = non-cystic fibrosis bronchiectasis; MDR = multi-drug resistant; PDR = pan-drug resistant; PsA = *Pseudomonas aeruginosa*

[1] Repeat sputum test PsA negative

[2] Sputum evidence for change in PsA virulence factor (e.g., antibiotic resistance, pyocyanin production)

Figure 2-4. Comparison of Clinical Measures Using Patient Sputum Pre- vs. Post-Phage Therapy. (A) Average lung function (FEV1%) improvement, (Wilcoxon Signed Rank, Prob>S 0.0010). (B) Average bacterial count (CFU/mL) reduction, (Wilcoxon Signed Rank, Prob<S 0.0313). (C) Average inflammatory molecule (Pyocyanin (mg/ml)) reduction (not enough data for statistical analysis). (D) Average antibiotic resistance (MIC (mg/ml)) reversion, (Wilcoxon Signed Rank, Aminoglycosides and Beta lactams Prob<S 0.0156, Cephalosporins and Fluoroquinolones ns).



3 Rationale/Significance

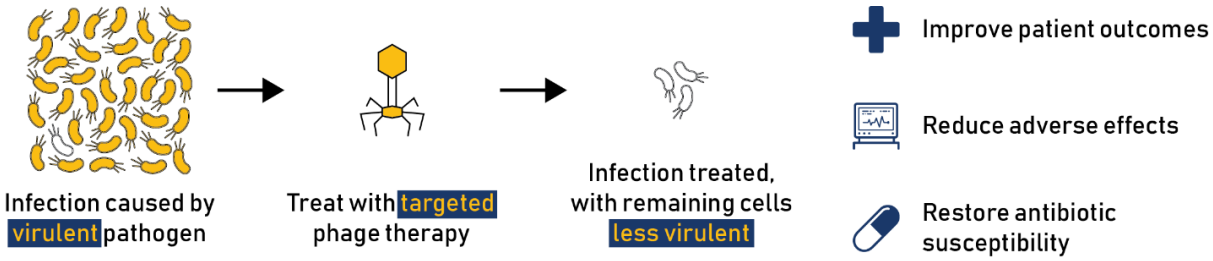
3.1 Problem Statement

While the emergence of CFTR modulators has the potential to dramatically change CF outcomes, the threat of multi-drug resistant pathogens is already a reality for many CF patients, and the probability and severity of such infections will only increase with continued reliance on traditional antibiotics alone. Therefore, new antibacterial and anti-inflammatory approaches to combat these pathogens and their effects are urgently needed. Inhaled phage therapy has such potential due to: 1) antibacterial properties that uniquely differ from antibiotics; 2) potentially-low side effects given that phages are naturally present (and therefore frequently encountered) in the environment, including in the CF lung; and 3) potential for administered phage to kill target bacteria while selecting for phage resistance that coincides with useful trade-offs, especially reduced lung inflammation and re-sensitivity of bacteria to traditional antibiotics. However, carefully designed clinical trials are required to develop this therapy.

Phage therapy is the use of lytic phages to treat bacterial infections. As one of the first antibacterial approaches discovered and developed in the early 20th century, phage therapy was largely eclipsed by development of chemical antibiotics following the discovery of penicillin, and the clinical potential of phage therapy was never fully embraced in Westernized countries. However, studies performed in the latter half of the 20th century, recent clinical trials, and individual case reports (see **Investigator's Brochure**), which demonstrate safety and potential efficacy, have renewed interest in phage therapy. As a class of antibacterials, phages are distinct from traditional chemical antibiotics in four potentially beneficial ways: 1) phages are self-amplifying in the presence of bacteria, and thus 2) are limited in the absence of their substrate (i.e., susceptible bacteria); 3) phages are often able to penetrate biofilms to reach infectious bacterial cells (Deshpande Kaistha & Devi Umrao, 2016; Pires et al., 2016; Szafrański et al., 2017); and 4) phage mechanisms for killing bacteria are distinct from those of traditional antibiotics. Exploiting the differences between antibiotics and phage therapy has been a driving force for continued research into the potential clinical utility of phage therapy.

Additionally, knowledge of the cellular-binding targets that phage(s) utilize when attaching to pathogenic bacteria may enable prudent choices of phages used in patient treatment. By utilizing phages that bind to virulence factors and drug-resistance mechanisms, the phage should kill bacteria while selecting for evolution of phage resistance that coincides with lowered virulence and drug re-sensitivity (defined as evolutionary "trade-offs") (**Figure 3-1**). Trade-offs are often observed in biology, where organisms evolve one trait that improves fitness (a relative advantage in reproduction or survival), while simultaneously suffering reduced performance in another trait (Ferenci, 2016; Goldhill & Turner, 2014). Thus, phage therapy could be developed as an 'evolutionary-based strategy' that forces a trade-off between the evolution of phage resistance, and reduced virulence and/or drug re-sensitivity in target bacteria (**Figure 3-1**). Thus, using trade-off-based approaches in phage therapy can be doubly beneficial: 1) success is achieved when the phage kills the target bacterium, and 2)

success is also achieved when residual bacteria evolve phage resistance, because this causes increased bacterial sensitivity to clinically-approved antibiotics and/or decreased bacterial production of inflammatory products (Kortright et al., 2019).



The Yale Phage Research Team has identified, purified, and sequenced environmentally-sourced lytic phages that effectively target and kill PsA, while selecting for clinically-useful trade-offs. Research at Yale has shown that lytic phage treatment decreases bacterial densities *in vitro* (please see **Investigator's Brochure**), and in emergency therapy of a human patient (Chan et al., 2016, 2018). These results demonstrated the doubly-beneficial effects of using certain phages in therapy, owing to forced trade-offs between evolution of phage resistance and reductions in pathogenic traits, especially bacterial re-sensitivity to antibiotics (Chan et al., 2018) and decreased bacterial secretion of harmful inflammatory products. In particular, the Yale team discovered and characterized phages that utilize three distinct receptor binding sites expressed on the surface of PsA cells: multi-drug efflux pumps, type-IV pili, and lipopolysaccharide (LPS). When exposed to each of these phages, PsA cells are effectively killed, whereas the remaining bacterial population evolves in the predicted manner: increased phage resistance evolves at the expense of down-regulation or other functional changes in the traits associated with these phage receptors, creating trade-offs that lead to drug re-sensitivity and lowered virulence in PsA.

In unpublished experiments, 96 clinical strains of PsA isolated from adult CF sputum samples were examined *in vitro* for their susceptibility to each of the above-described phages and others in the Yale collection and it was observed that 1) PsA bacteria were effectively killed and 2) some phages were capable of broad-host-range killing across multiple PsA isolates. Using single-patient emergency investigational new drug applications (INDs) [eINDs], the Yale Phage Research Team has administered phages OMKO1, TIVP-H6 and LPS-5 via inhalation to CF and non-CF bronchiectasis (NCFB) patients to target their multi-drug and pan-drug resistant PsA infections. These individuals received nebulized phages for 7 to 10 days, and examination of their post-therapy sputum samples showed: 1) decreased bacterial densities; 2) reduced pathogenic traits in remaining bacteria (i.e., bacteria with increased antibiotic sensitivity and decreased pyocyanin production); and 3) evidence for improved clinical outcomes (e.g., increased lung function by spirometry measures, and decreased need for administered antibiotics). These supportive results prompted the design of the current clinical study.

Taken together, preclinical and eIND data suggest that phage therapy may be safe and efficacious against PsA infections in CF patients. However, these promising clinical data come from cases of personalized treatment, and truly convincing results should stem from a blinded, randomized, controlled trial. Therefore, CYstic fibrosis bacterioPHage study at Yale (CYPHY) proposes to test the safety and efficacy of phage therapy to treat PsA infections in CF patients in this investigator-initiated, double-blind, randomized-controlled trial.

3.2 Purpose of Study/Potential Impact

The main purpose of this study is to determine the short-term safety and efficacy of phages OMKO1, TIVP-H6, and LPS-5 to treat PsA infections in CF subjects. The use of phages in this experimental study may result in reduced bacterial burden. In addition, the strategy of Yale's Phage Program is to use specific phages with targeted ability to exploit cellular receptors responsible for bacterial drug-resistance and/or virulence. Therefore, the phage therapy approach will kill bacteria, while forcing bacterial evolution in the direction of increased sensitivity to traditional antibiotics, and/or decreased inflammation, on average. This research may provide data demonstrating that these phages are safe and effective in treating PsA infections in CF, building evidence for their general use as an alternative, or combined approach, to using conventional antibiotics. This outcome would provide an additional therapeutic option for patients. Data from this study may also demonstrate improvement in forced expiratory volume over one second (FEV1) or FEV1 percent predicted (FEV1pp) and reduction in patient pulmonary exacerbations, which would benefit CF patient outcomes, as well as their quality of life.

The primary outcomes will focus on efficacy, as measured by relative change in CFU of PsA in subject sputum, from before to after the intervention, as well as short-term safety as determined by number and severity of adverse events. Additional secondary endpoints will be clinical outcomes (e.g., FEV1, FEV1pp, CFQ-R), and additional understanding of the effect of phage therapy on the human lung (e.g., sputum microbiome, subject transcriptome, and phage antibody production). If our hypothesis is supported in this study, these data can be used to support further active commercial development of phage as a therapy for PsA in CF subjects.

3.2.1 Potential Risks

There are multiple AEs that may occur with phage therapy, although an SAE related to nebulized phage has not yet been reported. Because phages constitute biologic materials, human immunogenic reactions are possible. These responses may include fever, headache, and malaise following initial and subsequent phage treatments. Such reactions can be addressed with symptomatic treatment, and phage therapy may be continued provided the subject and their physician both agree that continuation of the therapy has potential benefit. Therefore, phage initial administration occurs under clinical supervision. It is possible, although presumed rare, that an allergic or anaphylactic reaction may occur, which would be treated in a similar manner as any such patient reaction to a new medication. However, it is observed that various largely-uncharacterized phages are commonly found in the CF lung (Brown-Jaque et al., 2018), reducing the likelihood that exposure to the characterized OMKO1, TIVP-H6, and LPS-5 phages would result in an unfortunate allergic reaction.

Additionally, as phages are produced *in vitro* on bacteria grown in nutrient media, phage therapy will not be used in subjects with known allergies to nutrient-media components of soy, yeast, egg, or meat. Allergic or anaphylactic reactions will be treated symptomatically (e.g., nebulized albuterol, oxygen, and epinephrine, if necessary). In the event of an allergic or anaphylactic response, phage therapy will be discontinued.

Additional potential adverse events of phage therapy administration include increased cough, shortness of breath, or wheezing, which may be treated with nebulized albuterol and oxygen if necessary. CF is a chronic progressive respiratory disease that is associated with pulmonary exacerbations, and potentially hemoptysis and pneumothorax. It is possible that phage therapy could exacerbate these conditions. If these conditions occur, they will require physician evaluation that may include chest imaging (e.g., chest X-ray or CT scan) or the administration of antibiotics, among other interventions.

One patient treated via eIND (18690), experienced an SAE after the third of three separate phage treatments, which resulted in their death. Review at this subject's institution, and at Yale, determined that this event was unrelated to phage therapy (see **Investigator's Brochure** for details).

Study drug (phages or placebo), clinical visits, and procedures required for data collection (e.g., lung function and blood tests) in this study are provided free of charge. Some costs of travel can be covered by the study team as described in section 7.1 of this protocol. Administration of this therapy may come with financial risks for subjects because funds will not be provided to offset other costs associated with study participation. Such costs might include subject travel beyond what the study team can offer, standard-of-care treatments for cystic fibrosis, contraception, or if there is a clinical change that requires additional treatment. Such costs are not covered by this study.

Breach of confidentiality is a potential risk of participating in this study. However, all efforts, within reason, will be made to keep subject health information private. Because treatment involves the use of an investigational drug (phage), Dr. Koff, or his team, may share information about subjects, as well as portions of subjects' medical records, with the federal government's Office of Human Research Protections, the Yale School of Medicine Human Investigation Committee, the Food and Drug Administration, and Yale University. Dr. Koff and his staff will keep health information in strict confidence and will comply with any and all laws regarding the privacy of such information.

More detailed information about the known and expected risks and reasonably expected adverse events of treatments using phages OMKO1, TIVP-H6, and LPS-5 may be found in the **Investigator's Brochure**.

3.2.2 Potential Benefits

Benefit(s) to science: The use of phages in this experimental study may result in data supporting the safety and efficacy of phage therapy as a treatment for PsA infections in CF patients. These data include the evaluation of phage safety, as well as the provision of preliminary efficacy data, such as reduction in sputum bacterial load, and improvement in lung function, exacerbations, or symptoms. In addition, the strategy of Yale's Phage Program is to

use phages that target bacterial receptors that function in antibiotic resistance and pathogen virulence. Therefore, the phage therapy approach kills bacteria, while selecting for phage-resistant bacteria with greater sensitivity to traditional antibiotics, and/or decreased ability to cause tissue inflammation. Lastly, this study is designed to explore both microbiologic changes and human-subject immune changes in response to phages, providing the most detailed picture, to date, of mechanisms and outcomes of phage therapy in patients.

Possible clinical benefits to the subject may include improvement in symptoms, reduction of PsA load in sputum, improvement in lung function, improvement in quality of life (as measured by CFQ-R), and potentially decreased exacerbation(s) or need for additional medications. More detailed information about the known and expected benefits of YPT-01 may be found in the **Investigator's Brochure**.

4 Study Objectives

4.1 Hypothesis

In this single-center study, randomized double-blind placebo-controlled trial, we will test the hypotheses that 7d of inhaled phage therapy will suppress PsA load and will decrease PsA antibiotic resistance and virulence factors while safety and potential clinical responses (e.g., lung function, respiratory symptoms, and CFQ-R) will be evaluated. More specifically, our primary hypothesis for efficacy is that 7d inhaled phage therapy will decrease PsA load from baseline to 7 days post completion of phage therapy (14d time on study) in subjects randomized to two different doses of phage therapy (active phage treatment arm), as compared to subjects randomized to the placebo arm. Our co-primary hypothesis is related to the safety profile of phage therapy, as we hypothesize it is safe with respect to the number and severity of adverse events attributable to treatment. Our secondary efficacy hypotheses are that inhaled phage therapy: (1) will decrease PsA antibiotic resistance and virulence factors, (2) will favorably modify sputum phage, host, and microbiome, (3) and will have positive impact on clinical and quality of life response in subjects.

4.2 Primary Objectives

Our primary objectives are to determine preliminary efficacy of phage therapy in reducing sputum bacteria in subjects by comparing changes from baseline to 7 days post completion of phage therapy (14d on study) in sputum bacterial load between subjects randomized to phage therapy versus placebo, and to obtain short-term safety data on inhaled phage therapy by comparing the frequency and severity of adverse events attributable to treatment between subjects randomized to phage therapy versus placebo during the blinded portion of the study (first 56 days on study).

4.3 Secondary Objectives

The secondary objectives for the randomized period of this study are to:

1) Compare change over time in PsA sputum load between subjects randomized to phage therapy versus placebo.
2) Compare “trade-off” between evolved phage resistance and decreased virulence factors, as measured by changes in re-sensitivity to chemical antibiotics (for OMKO1-treated subjects), PsA motility and production of pyocyanin (for TIVP-H6-treated subjects), and production of endotoxin (for LPS-5-treated subjects) in PsA from subject sputum prior to, and following, phage therapy, between subjects randomized to phage therapy versus placebo.
3) Compare changes in sputum phage and bacterial microbiome between subjects randomized to phage therapy versus placebo.
4) Compare changes in lung inflammation and inflammatory markers (e.g., sputum transcriptomics and inflammatory cytokines) between subjects randomized to phage therapy versus placebo.

5) Compare clinical response in subjects, with respect to change in lung function (e.g., FEV1pp), as well as rates of pulmonary exacerbations, hospitalizations, and acute antibiotic use for exacerbations between subjects randomized to phage therapy versus placebo.
6) Compare changes in subject-reported quality of life (via CFQ-R) between subjects randomized to phage therapy versus placebo.

The tertiary exploratory objectives for the randomized period are to:

- Compare change in levels of phage-specific antibodies from baseline to post-treatment between subjects randomized to phage therapy versus placebo

Objectives of Open-label extension (placebo subjects that subsequently receive phage) are to:

- Provide subjects who received placebo with phage therapy.
- Continue to gather additional safety data by monitoring for adverse events determined to be caused by the investigational product.
- Describe change in PsA sputum load over time
- Describe “trade-off” between evolved phage resistance and reduced virulence factors, as measured by changes in sensitivity to chemical antibiotics (for OMKO1-treated subjects), PsA motility and production of pyocyanin (for TIVP-H6-treated subjects), and production of endotoxin (for LPS-5-treated subjects) in PsA from subject sputum prior to, and following, phage therapy.
- Describe differences in FEV1pp and absolute FEV1 (as measured in mL) change prior to, and following, phage therapy.

Objectives of 6-month safety follow-up are to:

- Obtain preliminary data on the long-term safety of phage therapy

5 Study Design

5.1 General Design Description

This is a prospective, randomized, placebo-controlled, double-blinded, single-site study of Yale Phage Therapy (YPT) 01 in CF subjects with chronic PsA airway infections. The purpose of this study is to demonstrate preliminary efficacy and short-term safety of inhaled phage therapy YPT-01. Clinically stable subjects who have confirmed diagnosis of CF with PsA in sputum cultures on at least two occasions within past year, and in sputum at screening visit, will be recruited into this study.

Experimental design: At Yale, this single-center, parallel trial has been designed to compare phage therapy to placebo. This study will screen 40 clinically stable (e.g., no exacerbations or medication changes within 4 weeks) CF subjects (no solid organ transplant; FEV1pp > 40% to < 100%) with chronic sputum PsA, and enroll 36, of whom 30 are expected to complete the study and meet the sample size goal. These 36 subjects will be block randomized to the intervention of phage therapy, or placebo, with anticipated enrollment rate of approximately 2 subjects per week. Per DMC recommendation, the exact size of randomization blocks is not listed in the protocol and will only be known to the blinded research team statistician. Furthermore, per DMC request, the PI and research team will be blinded to the randomization block size during this study. In this study, the research team and subjects will be blinded to intervention group. Each subject will receive inhaled, GMP-grade OMKO1, TIVP-H6, or LPS-5 phages at standard dose ($\geq 1x10^8$ PFU/dose) concentrations or placebo for 7d (GMP manufactured by Adaptive Phage Therapeuticfs, Gaithersburg, MD). All solutions are colorless, odorless, and identical in taste. The first dose of phage is supervised in clinic, and effect on lung function is evaluated ($\geq$ 10% decline in absolute FEV1 between pre-phage and post-phage will be considered clinically significant, and subject will not continue to receive YPT-01 because of “bronchospasm” safety concerns). Subjects will continue daily nebulized phage for 7d total. Subjects will return for clinical evaluation on d3, primarily to confirm subject has not experienced any SAEs or $\geq$ 10% decline in absolute FEV1 that is at least probably related to YPT-01. Subjects will also visit clinic on d7 for clinical evaluation and spirometry. After completion of intervention (7 d), subjects will be evaluated weekly (±3d) until d28, after which subjects will be evaluated monthly (i.e., d56, d84, d112, d140, d168, with ±6 days for each) through clinic visits or phone calls through month 6 for long-term safety and adverse events.

Open-label extension: The open-label extension serves as an opportunity for subjects in the placebo group to receive YPT-01 if they are interested. Based upon a survey of cystic fibrosis patients at Yale’s Adult Cystic Fibrosis Program and similar discussions that the Cystic Fibrosis Foundation has had with cystic fibrosis patients nationally, the addition of the open-label extension will contribute to improved enrollment. Thus, after completion of visit #7 (d56), subjects in the placebo group will be offered open-label phage treatment. For all subjects that agree to participate, the open label extension will begin after 3 months (90 days) from dosing of first subject in the study. The start date at 90 days was chosen to minimize risk of unblinding

the clinical staff to which subjects received placebo. There is a concern that the clinical staff might be biased by recognizing subjects that went directly into the open-label extension. Therefore, 90 days was chosen to minimize this risk, yet allow for subjects to receive YPT-01, if they wished to, without waiting for the entire study to be completed. This would minimize subjects waiting to receive the open-label extension without biasing the clinical staff. However, DMC will review the relevant safety data and assist with deciding on 90 days for initiation of phage therapy. At 90 days after completing placebo, subjects in the placebo group will be identified by Yale Clinical Research Pharmacy and the research coordinator will be notified to arrange phage treatment As in the randomized trial, the first phage dose will be supervised in clinic, and have subsequent clinical follow-up at d7, d14, and d28, and monthly thereafter for a total of 6 months starting from initiating therapy in the blinded portion of the study. AE's and SAE's will be monitored throughout the study to assess the safety of YPT-01. Given that approximately 24 of the 36 subjects will be enrolled and have completed at least d3 safety visit by 90 days (assuming enrollment of approximately 2 subjects per week), the open-label extension will not include a d3 clinic visit. Should any subject exhibit an SAE or $\geq$ 10% decline in absolute FEV1 that is at least probably related to YPT-01 during the 7d of phage treatment, the d3 clinic visit will be re-instated for the open-label extension, or at the discretion of the DMC.

For both the research and open-label extension, pre- and post-phage sputum samples will be collected and processed for PsA bacterial density, antibiotic sensitivity, virulence factors and deep sequencing. In addition, clinical responses (e.g., lung function, respiratory symptoms, and pulmonary exacerbations) will be evaluated. Should COVID-19 resurge resulting in limited research clinic visit availability or subject is unable or unwilling to conduct in person visits, monthly follow-up visits for safety and adverse events will be converted to phone calls to maximize subject safety.

Phage administration has been carefully reviewed by Yale University Infection Prevention, and standard CF infection prevention practices are required for phage administration.

Endpoints for double-blind randomized-controlled study: The **primary efficacy endpoint** is change in sputum PsA load from baseline (pre-phage) to d14 (i.e. 7 days post completion of 7-day inhaled phage therapy).

The **primary short-term safety** endpoints: Safety-related outcomes, i.e. frequency and severity of different types of adverse events as related to the study intervention, will be actively monitored throughout the study and summarized for the following days post-phage treatment during randomized blinded (experimental) period: *d7, d14, d21, d28, and d56.*

Secondary end-points for response profiles at d3, d7, d14, d21 and d28:

1) Change over time from baseline in PsA sputum load;
2) Change over time from baseline in "trade-offs" of PsA, as measured by change in PsA antibiotic sensitivity (after OMKO1 phage), motility and pyocyanin levels (after TIVP-H6 phage), and endotoxin levels (after LPS-5 phage);

3) Changes from baseline in sputum phage presence;

Secondary end-point at d14:

1) Change from baseline in lung inflammation as measured by sputum transcriptomics;

Secondary end-points for response profiles at d14 and d28:

1) Change from baseline in sputum inflammatory markers, as measured by inflammatory cytokines;

Secondary end-points for response profiles at d3, d7, d14, d21, d28, and d56:

1) Change from baseline in lung function (FEV1pp and absolute FEV1) from baseline;
2) Rate(s) of pulmonary exacerbations and medication changes from baseline.

Secondary end-points for response profiles at d3, d7, d14, d21, d28, d56, and monthly thereafter through month 6:

1) Change from baseline in quality of life and respiratory symptoms as measured by Cystic Fibrosis Questionaire Revised (CFQ-R), Cystic Fibrosis Respiratory Symptom Diary (CFRSD) and Chronic Respiratory Infection Symptom Score (CRISS©) at each clinic visit from baseline;

Tertiary end points for response profiles at d7, d14, d21, d28, and d56:

1) Change from d1 in anti-phage antibody titers.

Endpoints for Planned Unblinded study extension for subjects randomized to treatment: Monthly measurements through month 6 of total follow up for each subject will be collected on the following outcomes, i.e. the following secondary endpoints for longer-term efficacy and safety will be assessed:

1) Monthly Frequency and severity of adverse events;
2) Monthly Medication changes;
3) Monthly Quality of life and respiratory symptoms as measured by CFQ-R, CFRSD & CRISS
4) M4 and M6 change from baseline lung function (FEV1pp and absolute FEV1), and possible additional end-points at M3 and M5 follow up if subject has home spirometry;
5) M4 and M6 Change from baseline in "trade-off" of PsA virulence characteristics, as measured by change in PsA antibiotic sensitivity (after OMKO1 phage), motility and pyocyanin levels (after TIVP-H6 phage) and endotoxin levels (after LPS-5 phage).
6) Frequency and severity of adverse events at month 6 for preliminary long-term safety

Endpoints for Open-label portion for subjects originally randomized to placebo: To minimize investigator unblinding, 3 months (90 days) after the first subject in the trial has received the first phage therapy dose and following approval from the DMC, subjects who received placebo will be offered YPT-01 in an Open-label study extension. Endpoints will comprise assessment of subjects at the following days post phage-therapy for:

1) Frequency and severity of adverse events and relationship attributable to treatment: open-label d1, d7, d14, d28, and monthly thereafter up to 6 months of total follow up for each subject from their original randomization to placebo group;
2) Change in "trade-off" of PsA virulence characteristics, as measured by change in PsA antibiotic sensitivity (after OMKO1 phage), motility and pyocyanin levels (after TIVP-H6 phage) and endotoxin levels (after LPS-5 phage) from open-label d1 to open-label d14 and d28; monthly thereafter up to 6 months, if subject agrees and is able to provide expectorated or induced sputum.
3) Change in FEV1pp and absolute FEV1 from open-label d1 to open-label d14 and d28.
4) Rate(s) of pulmonary exacerbations and medication changes: open-label d1, d7, d14, d28, and monthly thereafter up to 6 months of total follow up for each subject from their original randomization to placebo group.
5) Change in Quality of life and respiratory symptoms as measured by CFQ-R, CFRSD & CRISS at each clinic visit: open-label d7, d14, d28, and monthly thereafter up to 6 months of total follow up for each subject from their original randomization to placebo group.
6) Frequency and severity of adverse events at month 6 for preliminary long-term safety

5.1.1 CYPHY Trial Design Tables

Blinded, Randomized, Controlled Study and Unblinded Safety Follow-up for subjects receiving phage (n = 36)

	Screening	Month 1*						Month 2*	Month 3#	Month 4#	Month 5#	Month 6#
Day	-7	1	3	7	14	21	28	56	84	112	140	168
Visit	X	X	X	X	X	X	X	X		X		X
Phone Call									X		X	
Informed Consent	X											
Randomization	X											
Medical History	X	X										
Height and Weight	X	X	X	X	X	X	X	X		X		X
Vital Signs	X	X	X	X	X	X	X	X		X		X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X
Medication Regimen	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X	X	X		X		X
Daily Temperature	X	X	X	X	X	X	X					
Urine Pregnancy Test[1]	X	X		X								
Pre-treatment PFT[2]		X										
Post-dose Observation		X										
Post-treatment PFT[2]		X										
PFT[2]	X		X	X	X	X	X	X		X		X
Sputum	X		X	X	X	X	X	X[5]		X[5]		X[5]
Blood for phage Ab[3]		X		X	X	X	X	X				
CFRSD, CRISS & CFQ-R[4]		X	X	X	X	X	X	X	X	X	X	X
Study intervention												
Dispense diaries		X										
Review diaries			X	X	X	X	X					

* Blinded Portion for Short-term follow up

[3] Ab: antibodies

# Unblinded Portion for Longer-term follow up [1] Administered to females of child-bearing potential [2] PFT: Pulmonary Function Tests (e.g., spirometry)	[4] CFQ-R: Cystic Fibrosis Quality-of-Life Revised Survey [5] Expectorated or induced (if the subject agrees)

Open-Label Extension and Monthly Safety Follow-up for placebo-treated Subjects (n = 12)

	Open Label days – if subject enrolled 90 days after study start, they will initiate open label in month 3)					**Month 2**	**Month 3 (6 months from Day 1 of Double-Blind Arm)**
Day	Screening	1*	7	14	28	56	84
Visit		X	X	X	X		
Visit or Phone Call	X					X	X
Informed Consent	X						
Medical History	X	X					
Height and Weight		X	X	X	X	X	
Vital Signs		X	X	X	X	X	
Adverse Events		X	X	X	X	X	X
Medication Regimen	X	X	X	X	X	X	X
Physical Exam		X	X	X	X	X	
Daily Temperature		X	X	X	X		
Urine Pregnancy Test[1]		X	X				
Pre-treatment PFT[2]		X					
Post-dose Observation		X					
Post-treatment PFT[2]		X					
PFT[2]			X	X	X	X	
Sputum	X[5]		X	X	X	X[5]	
Blood for phage Ab[3]			X	X	X	X	
CFRSD, CRISS & CFQ-R[4]		X	X	X	X	X	X
Study intervention							
Dispense diaries		X					
Review diaries			X	X	X		

	* Day numbering restarted for open-label patients (e.g., 'open-label d1') [1] Administered to females of child-bearing potential [2] PFT: Pulmonary Function Tests (e.g., spirometry) [3] Ab: antibodies	[4] CFQ-R: Cystic Fibrosis Quality-of-Life Revised Survey [5] Expectorated or induced (if the subject agrees)

5.1.2 Study Date Range and Duration

The total duration of the study is expected to be approximately 3 years: 6 months for study startup, 27 months for subject recruitment & Double-blind arm procedures, plus 3 months for final placebo subject to receive Open-label treatment.

5.1.3 Number of Study Sites

Single-site study, conducted at Yale University and Yale-New Haven Hospital.

5.2 Outcome Variables

5.2.1 Primary Outcome Variables

The primary efficacy endpoint in the study is the difference from baseline to d14 (7 days after completing 7-day intervention period) in PsA sputum load, which will be compared between the active treatment and placebo treatment groups. The primary short-term safety endpoints are the between-group differences in adverse event frequency and severity between the active treatment and placebo treatment groups at d7, d14, d21, d28, and d56.

5.2.2 Secondary and Exploratory Outcome Variables

Secondary Endpoints:

- Long-term safety as measured by frequency and severity of adverse events at m3, m4, m5, and m6 data for subjects randomized to active phage, and monthly measurements in the open-label portion for subjects originally randomized to placebo.
- Response profile: change from baseline to d3, d7, d14, d21, and d28 in PsA antibiotic sensitivity (if treated with OMKO1 phage), motility and pyocyanin production (if treated with TIVP-H6 phage), or endotoxin production (If treated with LPS-5 phage)
- Response profile: change from baseline to d3, d7, d14, d21, d28 and d56 in FEV1pp and absolute FEV1.
- Response profile: change from baseline to d3, d7, d14, d21, d28 and d56 in the rate of pulmonary exacerbations.
- Response profile: change from baseline to d3, d7, d14, d21, d28, d56, and monthly thereafter through month 6 in quality of life as measured by scales derived from the CFQ-R, CFRSD & CRISS.
- Differences in sputum phage, host (e.g., inflammatory cytokines and transcriptomics), and microbiome as determined by high-throughput sequencing phage therapy and placebo treatment groups compared to each other on d3, d7, d14, d21 and d28 for phage presence, on d14 for bacterial microbiome and human lung inflammation (transcriptomics), and on d14 and d28 for inflammatory cytokines.

Exploratory Endpoints:

- Response profile: change from d1 to d7, d14, d21, d28, and d56 in anti-phage antibody titers.

5.3 Study Population

Subjects diagnosed with cystic fibrosis (CF) who meet all of the inclusion and none of the exclusion criteria will be eligible for enrollment in this study.

5.3.1 Number of Participants

This study will aim to enroll 36 subjects with CF who will have sputum *PsA*. Pre-phage sputum will be screened because, even though phages OMKO1, TIVP-H6, and LPS-5 can infect ~95% of clinical isolates assessed *in vitro* (see **Investigator's Brochure**), a small subset of subjects may have PsA that cannot be treated with OMKO1, TIVP-H6, and LPS-5 phages. This indicates that screening approximately 40 subjects will enable us to enroll 36 subjects for this clinical trial. Based on the sample size calculation, we would need only 30 subjects, but accounting for attrition (approximately 10%) and equal number of subjects to be randomized across the three treatment arms, we propose to enroll 36 subjects.

5.3.2 Eligibility Criteria/Vulnerable Populations

Inclusion:

1. Capable of giving signed informed consent;
2. Stated willingness to comply with all study procedures and availability for the duration of the study;
3. Age ≥18;
4. CF diagnosis based upon genetics, sweat chloride testing, or clinical manifestations;
5. Able to provide repeated induced sputum samples;
6. Able to use a nebulizer;
7. PsA culture positive on one occasion within past 2 years and in sputum at screening visit;
8. FEV1 >40%;
9. Clinically stable lung disease, defined as no decrease in FEV1 >10% or pulmonary exacerbations in the 4 weeks prior to screening;
10. If on CF modulator therapy (e.g., ivacaftor, ivacaftor/elexacaftor/tezacaftor), then subject remains on the same modulator therapy for at least 2 months prior to enrollment;
11. For females of reproductive potential: use of effective contraception for at least 1 month prior to screening and agreement to use 2 methods of effective contraception during study participation, and for an additional 6 weeks after the end of YPT-01 administration;
12. Males of non-reproductive potential (e.g., documented congenital bilateral absence of vas deferens) or males of reproductive potential (e.g., non-vasectomized males or

males vasectomized less than 120 days prior to study start) that agree to use condoms with spermicide while engaging in sexual activity or be sexually abstinent.

Exclusion:

1. History of solid organ transplant (e.g., lung or liver);
2. Severe neutropenia, as defined by absolute neutrophil count (ANC) of < 500 per microliter;
3. No YPT-01 phage identified that effectively targets sputum PsA;
4. Treatment for pulmonary exacerbation within the prior 4 weeks;
5. Change in pulmonary medications within the prior 4 weeks;
6. Subjects who are pregnant, who intend to become pregnant, or who do not wish to use contraception;
7. Subjects who are breastfeeding;
8. Participation in another clinical research study concurrently or within the prior 2 months;
9. Known allergy to soy, egg, yeast, or meat.
10. Any genetic or acquired (including medication-induced) immunocompromised condition, beyond the level of immunocompromise typically associated with CF and its management.

5.3.3 Study Specific Tolerance for Inclusion/Exclusion Criteria

Subjects who fail to meet one or more of the inclusion criteria, or who meet any of the exclusion criteria will not be enrolled in this study. Waivers of any of the above study entry criteria will not be granted.

5.3.4 Screen Fail Criteria

Any consented subject who is excluded from the study before randomization is considered a screen failure. All screen failures must be documented with the reason for the screen failure adequately stated. If a subject screen fails prior to randomization, they can be rescreened once if the site staff feels they meet eligibility criteria, and following confirmation from the Sponsor-Investigator or designee. Rescreened subjects are required to complete all screening procedures (i.e., test results from previous screenings cannot be used).

5.3.5 Change in Medications or Therapies

There are no restrictions on concomitant therapy. However, subjects should not make changes to medication unless deemed medically necessary. Subjects will inform the investigator of any changes in medication regimen and this will be recorded in the subject's study record.

6 Methods

6.1 Treatment

6.1.1 Identity of Investigational Product

YPT-01 is a treatment algorithm comprising phage OMKO1, phage TIVP-H6, and phage LPS-5. Phages comprising YPT-01 are provided as a 1 mL solution of a single phage at standard dose of ≥$1x10^8$ phage particles in phosphate-buffered saline (PBS) and 10 mM $MgSO_4$ for administration via inhalation (nebulization), and have been GMP manufactured by Adaptive Phage Therapeutics (APT). As YPT-01 phage ages the potency will be monitored by APT with a minimum acceptable dose of $1x10^8$ PFU. If the potency drops below $1x10^8$ PFU/mL the number of vials will be increased to achieve a dose of at least ≥$1x10^8$ PFU/dose. Maximum number of vials is 10 vials.

Importantly Endotoxin levels will be monitored to ensure that no dose exceeds 5 EU/kg/hr.

YPT-01 is currently not FDA-approved, although prior administration of YPT-01 phages has occurred via emergency INDs for compassionate use (see **Investigator's Brochure**).

YPT-01 is implemented by analyzing subject sputum culture. Phage sensitivity of PsA cultured from subject sputum (**Figure 6-1**) will be used to identify the single phage (**Table 6-1**) to that will be administered.

Placebo control will be GMP-grade phosphate-buffered saline (PBS) and 10 mM $MgSO_4$, provided by Adaptive Phage Therapeutics.

Table 6-1. Phages that comprise YPT-01 phage therapy.

Bacteriophage ID	Target Pathogen	Receptor/Target	Impact on bacterial community
OMKO1	*Pseudomonas aeruginosa*	Efflux pump	Re-sensitization to small-molecule antibiotics
TIVP-H6	*Pseudomonas aeruginosa*	Type IV Pilus	Reduction of pyocyanin, impaired twitching motility, decreased biofilm formation
LPS-5	*Pseudomonas aeruginosa*	LPS (O-antigen)	Increased sensitivity to hydrophobic antibiotics, reduced extracellular endotoxin, impaired iron uptake

Figure 6-1. Phage therapy algorithm. Briefly, PsA bacteria cultured from subject sputum samples are isolated and Efficiency of Plaquing (EOP) assay is performed with each phage (OMKO1, TIVP-H6, and LPS-5) as described below. The phage with the greatest EOP will be utilized for treatment. In the

event of equal EOP between two or more phages, phage for treatment will be utilized in the following order: TIVP-H6, followed by OMKO1, followed by LPS-5.

Determination of Efficiency of Plaquing (EOP)

10-fold dilutions of each phage will be performed eight times (dilutions -1 through -8) from an initial phage stock of approximately 10e9 PFU on both PsA host utilized to amplify phage (amplification host) and on PsA isolate from subject (subject host). A 10 µl spot of each dilution of phage will be applied to a lawn of amplification host and a lawn of subject host. Following incubation for 24 hours at 37°C, plaques will be enumerated and plaquing titer will be determined. Titer on subject host will be compared to that on amplification host, the ratio of which is the EOP. A value of EOP = 1 would indicate that plaquing titer is identical on the amplification and subject hosts; EOP = 10 would indicate that plaquing titer is 10x higher on the subject host. Whichever phage has the highest EOP on the subject host will be chosen preferentially for deployment. In the event of an equal EOP value between two or more phages, administered phage will be chosen based on frequency of use in emergent settings: TIVP-H6, followed by OMKO1, followed by LPS-5.

6.1.2 Dosage, Administration, Schedule

Based on our prior experience in humans (see **Investigator's Brochure**), the dose of $1x10^{10}$ PFU/dose has been effective in prior treatment under emergency INDs.

Subjects will receive 7 containers of YPT-01 to be delivered via nebulization once daily for 7 days. Subject is provided compressor, nebulizer, and all relevant equipment (InnoSpire Elegance Compressor #1099969 with SideStream Reusable neb #HS860, Philips Respironics). First dose of nebulized phage or placebo (both are clear odorless suspensions) is supervised in clinic. For the first dose of phage therapy, pre- and post-phage spirometry is performed to ensure safety of phage administration. If there is >10% change in absolute FEV1, then phage administration will be considered "bronchospastic," and the subject will not proceed with additional treatments. Subjects will be observed for 30 minutes following the initial nebulization. Subject is provided daily containers for subsequent 6 d of treatment. Subjects will be asked to nebulize treatment after standard airway clearance therapies.

6.1.3 Method of Assignment/Randomization

Adaptive Phage Therapeutics (APT), Gaithersburg MD, will provide phages (at two concentrations) and placebo for the study. Randomization will occur once a subject meets all inclusion/exclusion criteria (including once sputum PsA phage sensitivity is obtained), and will be performed in block phage (n=24), and placebo (n=12) arms, for a total of 36 subjects. Per DMC recommendation, the exact size of randomization blocks will only be known to the blinded research team statistician. Furthermore, per DMC request, the PI and research team will be blinded to the randomization block size during this study.

6.1.4 Blinding and Procedures for Unblinding

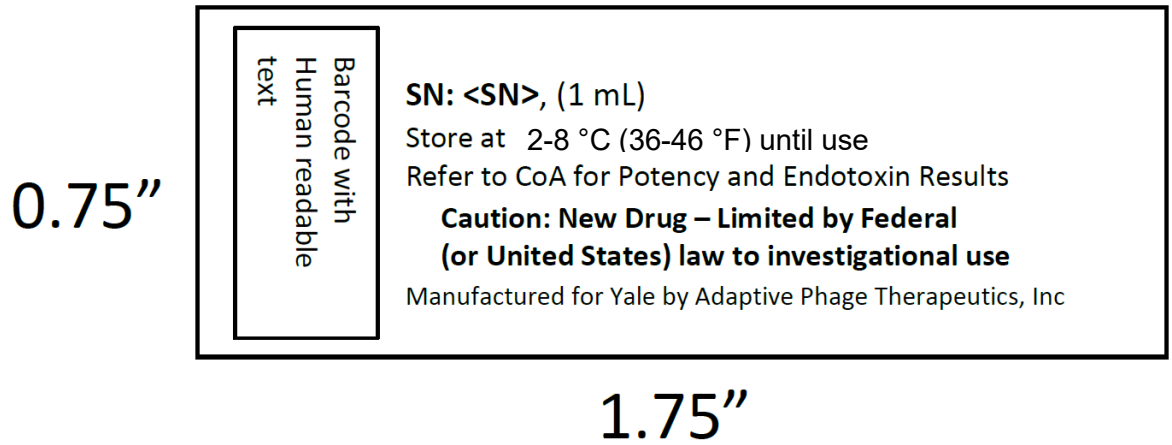
To preserve the blinding of the study, randomization table and treatment assignments are only accessible to the Investigational Drug Service (IDS) Pharmacy staff before the study is complete. The Investigators, study team managing the study, subjects, and any other personnel interacting directly with subjects will remain blinded the final enrolled subject in the blinded, randomized portion has completed the d56 study visit. The dispensing procedure will be carried out in such a manner that phage and placebo are identical in appearance, labeling, preparation time, expiration date and supplies used.

To provide subjects randomized to placebo a chance to receive YPT-01, subjects in the placebo group will be offered open-label phage treatment following all participants' completion of d56 visit. The subjects in the placebo group will be identified by IDS Pharmacy and phage will be provided, though the PI will be blinded to the dose of phage subject is receiving.

In case of an emergency, the PI has the sole responsibility for determining if unblinding of a subject's treatment assignment is warranted for medical management of the event. The subject's safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, investigator will reach out to IDS pharmacy to obtain treatment assignment information. It is the responsibility of the PI to promptly document the decision and rationale and notify the Sponsor as soon as an unblinding decision is made. It is also the PI's sole discretion to have subject(s) discontinued from the study or remain in the study.

6.1.5 Packaging/Labelling

The following label will be provided by APT on phage doses, which will be identified by serial number (SN). A list of serial numbers will be provided to Yale Pharmacy for randomization and dispensing:



Following administration of initial dose at clinic visit, 6 doses of phage therapy will be given to subjects during Visit 1. Subjects will be provided with the following information:

Storage and Handling Information on Individual Container:

CAUTION: Investigational New Drug – Limited by Federal law to investigational use

Active Ingredient (each container)*:*

Bacteriophage or Placebo

SN:

Storage and Handling Information provided with Outer Container:

CAUTION: Investigational New Drug – Limited by Federal law to investigational use

Active Ingredient (each container)*:*

Bacteriophage or Placebo

SN:

Number of containers:

Expiration Date:

Subject Name:

Storage

- Store tubes in the refrigerator (2 – 8 °C) and protect from direct sunlight
- *Do not* store in freezer
- Open each container just prior to use
- Do not open container when not in use

6.1.6 Storage Conditions

Single-use phage containers will be stored under refrigeration at 2 °C – 8 °C (36 °F – 46 °F) and protected from light until administration. If there is visual evidence for particulate matter in the solution, the phage preparation will not be used.

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply study intervention. Subjects self-administer study intervention under clinical team supervision for the first dose, and administer subsequent doses independently. Prior to dispensing to subject, all study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The PI, is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Unused doses and nebulizer provided during the study will be requested from subjects at the end of the study and disposed of through appropriate biosafety mechanisms as required by Environmental Health and Safety at Yale.

6.1.7 Concomitant therapy

There are no restrictions on concomitant therapy, however subjects should not make changes to medication unless deemed medically necessary. Subjects will inform the investigator of any changes in medication regimen and this will be recorded in the subject's study record.

6.1.8 Restrictions

No restrictions.

6.2 Assessments

6.2.1 Efficacy

Primary Objective

Sputum Bacterial Load

The parameter for the primary endpoint is change in bacterial load in subject sputum, as measured in colony-forming units (CFUs) of PsA, which is determined by serial dilution plating assays as described in the **Laboratory Manual**.

Induced Sputum Collection

Subject sputum is collected to assess the primary endpoint of reduction in PsA CFUs, and to measure secondary objectives of bacterial re-sensitivity to chemical antibiotics, assaying change in bacterial virulence factors, assessing human inflammatory and transcriptomic, and genomic changes in sputum microbiome. Sputum is induced as described in the **Laboratory Manual**, with sputum induction protocol repeated until the subject has produced at least 1 mL of sputum.

Secondary Objectives

Sensitivity of sputum PsA to chemical antibiotics

Sputum PsA are assessed for antibiotic sensitivity to determine the "trade-off" of re-sensitization to antibiotics. Antibiotic sensitivity testing is performed as described in the **Laboratory Manual** using commercially-available antibiotic resistance test kits (e.g., eTestSTrip from BioMerieux or similar product) and methods recommended by the manufacturer. The following antibiotics may be tested, but will be determined as clinically relevant for the strain based on initial PsA isolate resistance profile: cefepime, ceftazidime, piperacillin-tazobactam, meropenem, aztreonam, tobramycin, ciprofloxacin, and colistin.

Levels of bacteria virulence factors (pyocyanin or endotoxin)

Sputum PsA are assessed for level of pyocyanin and endotoxin production to determine the "trade-off" of reduced pyocyanin or endotoxin production. The pyocyanin quantification assay is performed as described in the **Laboratory Manual** and is adapted from (Ingledew & Campbell, 1969). The endotoxin quantification assay is performed utilizing a commercially-available endotoxin detection kit (e.g., Hyglos endoNext endoZyme kit or similar product) using methodology recommended by the manufacturer.

Assess change in PsA motility

Motility is quantified on PsA isolated from subject sputum using a "Macroscopic Twitching Assay" adapted from (Turnbull & Whitchurch, 2014) as described in the **Laboratory Manual.** Briefly, PsA cultures inoculated into the center of a 1% LB-Lennox Agar Plate and allowed to grow for 24 hours. The distance in millimeters between the inoculation point and outer halo of bacteria is then measured to determine distance traveled by PsA inoculate, informing motility function.

Pulmonary Function tests and Spirometry

Spirometry (pulmonary function tests, PFT) is assessed to determine change in pulmonary function (e.g., FEV1, FEV1pp) and will be measured by a spirometer using standard pulmonology methods, as described in the **Laboratory Manual**. This is a standard test for all cystic fibrosis subjects in our practice.

CF subjects at each clinic visit.

Spirometry results are provided as raw data, in liters per second, as well as percent predicted. Percent predicted is a comparison of the subject's test results to the *predicted values* for individuals of similar characteristics (e.g., height, age, gender, and ethnicity). Height is measured during screening visit per standard protocol.

Rate of pulmonary exacerbations

Pulmonary exacerbations will be assessed by collecting medical history data and recent changes to symptoms from subjects, and comparing the rate of exacerbations between phage treatments and the placebo group during the study, and to historical exacerbation rate from prior medical history in the Open-label extension.

The presence of a pulmonary exacerbation is established by:

(1) <u>One major criterion alone</u>, or (2) <u>Two minor signs/symptoms and fulfillment of symptom duration</u>

Major criteria: *(One finding alone establishes the presence of a pulmonary exacerbation)*

- Decrease in FEV1 %predicted (pp) of ≥ 10%
- Oxygen saturation < 90% on room air <u>*or*</u> absolute decrease of ≥ 5%
- New lobar infiltrate(s) or atelectasis on chest radiograph
- Hemoptysis (on more than one occasion in past week); hemoptysis is defined as 1) mild to moderate (5-240 mL), and 2) massive (>240 mL)

Minor signs/symptoms: *(Two minor signs/symptoms are required in the absence of major criteria. If at least 2 minor signs/symptoms are present, at least one needs to be 3 or more days in duration to meet the pulmonary exacerbation definition)*

- Increased work of breathing or respiratory rate
- New or increased adventitial sounds on lung exam
- Weight loss ≥ 5% of body weight
- Increased cough
- Decreased exercise tolerance or level of activity
- Increased chest congestion or change in sputum

<u>Subject Quality of Life</u>

Quality of life changes will be assessed using the Cystic Fibrosis Questionnaire Revised (CFQ-R), a subject-reported outcome report that has been validated to evaluate CF subject's disease-related quality of life (Ronit et al., 2017), as well as the Cystic Fibrosis Respiratory Symptom Diary (CFRSD), and the Chronic Respiratory Infection Symptom Score (CRISS). These assessments are meant to evaluate subject's gastrointestinal and respiratory symptoms and/or symptom progression, treatment burden, and overall daily functioning. These questionnaires will be key secondary efficacy endpoints to the study and can be administered as an electronic or written assessment, requiring approximately 20 minutes to complete.

<u>Inflammatory markers in sputum</u>

An aliquot of subject sputum will be analyzed for inflammation by measuring levels of inflammatory proteins (e.g., IL-8, IL-6, TNF-alpha, IL-1beta, MUC5AC, MUC5B, and other relevant chemokines and cytokines). Experimental methods for assessment of inflammatory markers in the sputum are described in the **Laboratory Manual**.

<u>PCR detection of treatment phage</u>

Polymerase chain reaction (PCR) and quantitative PCR (qPCR) will be performed on subject sputum to assess presence of phage used in treatment and will be performed as described in the **Laboratory Manual.**

Genomic and transcriptomic changes in sputum microbiome

Subject sputum will be assessed for genomic and transcriptomic changes in the sputum as adapted from (Hunter et al., 2011; Nelson et al., 2019) and (Cobián Güemes et al., 2019) and described in the **Laboratory Manual**.

Detection of anti-phage antibodies

Subject blood will be analyzed to assess presence and levels of anti-phage antibodies using standard ELISA assays, as described in the **Laboratory Manual.**

Detection of Bacterioal Load in Blood

6.2.2 Subject blood will be analyzed to assess bacterial load, as described in the Laboratory Manual.Safety

This study will collect all adverse events and serious adverse events that occur in subjects and record the severity and relationship to investigational product. These data will be collected during all clinic visits, through the End of Study Visit (Day 56), and through the end of the Open-Label phase for those subjects who enter that portion of the study.

Given prior experience with YPT-01 in subjects with FEV1 >40% predicted in eINDs (**Table 2-2**), incidence of adverse reactions is expected to be extremely low. However, the potential always exists for anticipated and/or unanticipated adverse events (serious or otherwise) to occur. Since it is not possible to predict with complete certainty the absolute risk in any given individual, the following plan is provided for monitoring the data and safety of the proposed study as follows:

Plan for Reporting Adverse Events
For the current study, the following individuals, funding, and/or regulatory agencies will be notified:

1) All co-investigators listed on the protocol
2) Data and Safety Monitoring Committee (DSMC)
3) Yale IRB
4) FDA

Adverse Events (AE)
An adverse event (AE) is any untoward medical occurrence in a subject during the study, which does not necessarily have a causal relationship with the treatment. This includes any newly occurring event or worsening of a pre-existing condition.

The study staff will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Subjects will be provided with a diary to record the occurrence of AEs during the study. The diary will be reviewed during each study visit. In addition to events spontaneously reported by subjects during the visit or in the diary, at each visit subjects will be specifically asked whether they have experienced increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and fever or chills since their last visit (or after administration of investigational product at the Day 1 visit). All subjects will be provided with a thermometer to monitor their temperature daily and a diary to record these measurements and subject symptoms.

Adverse events will be recorded in the subject case report form (CRF). Study assessments include physical exam(s), vital signs (including pulse oximetry), and spirometry/pulmonary

function testing (PFTs). Those deemed to have clinically significant change from baseline (e.g., absolute FEV1 10% change) will be documented as an AE. A clinical diagnosis will be provided, or if there is no diagnosis, an abnormal study result or assessment will be listed as the AE. All subjects will be queried about the occurrence of AEs at each study visit. For each AE, documentation will include: event description, classification as “serious” or “non-serious,” date of first occurrence and date of resolution (if applicable), severity, causal relationship to study intervention, action taken (e.g., diagnostic studies, medication, or treatment given), and outcome.

Adverse Events (AE) Severity
The National Cancer Institute’s Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 will be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. It should be pointed out that the term “severe” is a measure of intensity and that a severe AE is not necessarily serious.

Table 6-2. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Moderate (2)	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (e.g., preparing meals, using the telephone, managing money).
Severe (3)	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (e.g., bathing, dressing, feeding self, using toilet, taking medications).
Life-threatening (4)	Life-threatening consequences; urgent intervention indicated.
Death (5)	Death related to AE.

Table 6-3. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject’s clinical state or by other interventions. .

Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unlikely	There is little evidence to suggest a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication) or another reasonable explanation for the event (e.g. another clinical condition or other concomitant treatment)
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

Serious Adverse Events (SAE)
An SAE is defined as any AE occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect. Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

Serious Adverse Event (SAE) Reporting
SAEs that occur (whether or not related to study drug) will be reported on an SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained, and end after procedures for the final study visit have been completed. In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.

Assessment of Risks: Subjects will have an opportunity to participate in tests that are routinely used in clinical medicine. The risks associated with this study are the following:

Minimal risk: venipuncture, and sputum collection.

- Minimal risks of venipuncture: Approximately 16 mL of blood will be collected from subjects per draw, at each indicated visit in the **Study Schedule** (**Section 5.1**), for a total of approximately 96 mL of blood for subjects randomized to phage and 160 mL of blood for subjects randomized to placebo who choose to subsequently receive phage in the open-label portion. If a patient weighs less than 110 lbs the blood draw volume will be adjusted so as not to exceed the lesser of 50ml or 3ml/kg in an 8-week period. The procedure involved in obtaining blood specimens for laboratory analysis is conducted through the use of standard phlebotomy methods. The personnel obtaining these samples are highly trained nurses or technicians, well versed in this collection method, thereby imposing minimal risk. Occasionally, a subject will experience “light-headedness” during the phlebotomy procedure. To minimize the risk of this occurrence, all subjects will be placed in a supine position on a hospital bed, attended by trained personnel and monitored following the procedure. Should a bruise or swelling occur following the phlebotomy procedure, ice will be applied to the site, and trained personnel will monitor the site. This bruising/swelling is associated with minimal risk.
- Minimal risk of sputum collection. Expectorated sputum is routinely acquired from CF subjects at all clinic visits. If unable to spontaneously produce sputum, throat cultures are obtained from CF subjects at all clinic visits. No specific intervention occurs outside of usual care.

Moderate risk: nebulization of phage therapy, though no adverse events attributable to therapy have been seen in any of the adults who participated in eIND, to date.

- Risk of nebulization: YPT-01 is an experimental therapy. However, CF subjects nebulize several medications and to date there have been no adverse events associated with nebulization in CF subjects with mild to severe lung disease. If there is an issue with post-nebulization wheeze or coughing, nebulized albuterol will be provided.

For studies conducted under an IND, there are two types of Safety Reports submitted to FDA:

- 7-Calendar-Day FDA Telephone or Fax Report: The sponsor-investigator will directly notify the FDA, within 7 calendar days after initial receipt of the information, of any adverse event that is fatal or life-threatening, unexpected, and considered at least possibly related to the investigational product.
- 15-Calendar-Day FDA Written Report: The sponsor-investigator will directly notify the FDA within 15 calendar days after initial receipt of the information, of any serious adverse event (other than those that are fatal or life-threatening) that is unexpected and considered at least possibly related to the investigational product.

Note: Serious Adverse Events which do not meet the criteria for expedited reporting will be reported to the FDA in the IND Annual Report.

Stopping rules:

The following events would result in discontinuation in treatment of an individual subject:

- For first administration of phage in clinic, a 10% decrease in lung function (FEV1)
- For the duration of the trial, a Grade 3 or higher adverse event, or an SAE (regardless of grade) deemed at least probably caused by phage therapy

The following events would result in a pause in study enrollment and a pause in study treatment for all enrolled subjects:

- Any subject with an SAE that is at least probably related to phage therapy;
- Two or more subjects with the same SAE (regardless of attributability to phage therapy), or two SAEs (Grade 3 or higher) regardless of relatedness;
    - This stopping rule excludes expected hospitalizations for routine CF exacerbations (i.e. Pulmonary Exacerbations), which are reported in a monthly summary by sponsor to the DMC
- Two or more Grade 3 or higher AEs considered at least possibly related to phage therapy.

Following the imposition of a pause, the Data Monitoring Committee (DMC) created by the Cystic Fibrosis Foundation DMC, based at the University of Arizona, will be consulted to modify the protocol as needed, and to develop criteria to resume study. Any protocol changes would be submitted to the IRB for approval and communicated to the FDA. There are no *a priori* stopping criteria for this trial.

6.2.3 Biomarkers (if applicable)

N/A

6.3 Study Procedures

Some subjects may be receiving chronic inhaled antibiotic therapy (e.g., tobramycin, aztreonam, or alternating therapy) as part of their treatment regimen. Subjects whose treatment regimen includes inhaled tobramycin or alternating therapy between tobramycin and aztreonam will initiate nebulization of YPT-01 in the first 14 days of initiating a tobramycin treatment cycle. Subjects whose treatment regimen includes inhaled aztreonam will initiate nebulization of YPT-01 in the first 14 days of initiating an aztreonam treatment cycle.

Screening visit: Day -7 (±5):

1. Review the study with the subject and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Collect urine for pregnancy test (if female of child-bearing potential).
3. Perform and record spirometry.
4. Induce sputum to obtain sputum culture and determine eligibility:
    a. Laboratory assessment of PsA culture susceptibility to YPT-01 as illustrated in **Figure 6-1**, and baseline antibiotic resistance through CLIA laboratory testing.
    b. If sputum culture pathogen meets inclusion criteria, assign the subject a unique study number randomized in block format as illustrated in **Table 6-4** in a format determined by the unblinded DMC statistician and research team statistician, which is unknown to the research study team, and schedule subsequent visit to complete Day 1 procedures.
    c. If sputum culture pathogen does not meet inclusion criteria, record as Screen Failure.
5. Record demographics.
6. Record medical history, including a history of CF diagnosis and date.
7. Record concomitant medications.
8. Perform a targeted physical examination.
9. Measure and record height and weight.
10. Obtain and record vital signs.

Following screening visit, subjects who meet screening criteria will be randomized into one of the following treatment arms:

Table 6-4. Randomization scheme

Arm Name	Phage	Placebo
Intervention Name	YPT-01	Vehicle solution
Type	Biologic	N/A
Dose Formulation	Liquid for nebulization	Liquid for nebulization

Unit Dose Strength(s)	≥1x10^8 phage particles units/dose	N/A
Double-Blind Arm Dosage Level(s)	One 1mL vial YPT-01 at a dose of >=1.0x10^8 PFU/mL plus two 1mL vials placebo, administered daily for 7 days	Three 1mL vials placebo, administered daily for 7 days
Open-Label Arm Dosage Level(s)	Up to ten 1mL vials YPT-01 phage as needed to achieve a dose of at least 1x10^8 PFU/mL once daily for 7 days. No more than 10 vials will be administered at a time to minimize the burden of administration. Placebo vials will be included as needed to reach a minimum volume of 3mL study drug per dose.	N/A
Route of Administration	Inhalation (nebulization)	Inhalation (nebulization)
Use	Experimental	Placebo control
Sourcing	Adaptive Phage Therapeutics	Adaptive Phage Therapeutics
Packaging and Labeling	Each Study Intervention container will be labeled as required per country requirement	Each Study Intervention container will be labeled as required per country requirement
[Current/Former Name(s) or Alias(es)]	YPT-01: OMKO1, TIVP-H6, or LPS-5	N/A

Treatment tracking
Subjects will be asked to track treatments in a study diary (**Appendix 3**).

Missed doses
Subjects are encouraged to administer phage at approximately the same time daily. However, missed doses may be made up as long as they are not taken within 8 hours of the next scheduled dose.

Voluntary discontinuation
A subject may, at any time, discontinue participation in the trial for any reason. If a subject chooses to discontinue treatment, they will be provided with the option to continue follow-up visits to monitor for adverse events.

Discontinuation due to protocol violation
A protocol violation may lead to discontinuation and occurs when the subject, investigator, or the Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet eligibility criteria following study enrollment
- Failure to obtain induced sputum at visits

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Primary Investigator (PI) will determine if a protocol violation should result in early discontinuation of study treatment for a subject.

When a protocol violation occurs, it will be discussed with the PI and a Protocol Violation Form detailing the violation will be generated. A copy of the form will be filed in the site's regulatory binder. The site will report the violation to their IRB in accordance with their IRB reporting requirements.

Pregnancy in subject or subject's partner
Should a subject or a subject's partner become pregnant while subject is on study drug, study drug will be discontinued and subject (or subject's partners') pregnancy and neonatal outcomes will be collected. Should a subject or subject's partner become pregnant after completion of study drug, but during study monitoring (e.g., in the experimental blinded or open-label portion of the study), subject pregnancy and neonatal outcomes will also be collected.

Subject replacement
Subjects who withdraw from the study will not be replaced.

6.3.1 Study Schedule

Following screening visit, the total number of expected visits is 10 in-person visits and 2 phone calls for phage-treated group and 15 in-person visits and 2 phone calls for placebo-treated group that continues to open-label, including consent and screening visit, treatment-visits, post-treatment follow-up visits, and end of study evaluation. However, should COVID-19 pandemic result in facility closures that prevent in-person visits as described in **Section 5.1**, the 2 in-person visits for safety follow-ups in month 4 and 6 for both phage-treated and open-label subjects will be converted to phone calls.

6.3.2 Informed Consent

Consent is required and will be acquired by a member of the research team using the Informed Consent form, which will be scanned and stored in the EMR. Physical copies will be provided to the subject as well as stored in a secure cabinet.

6.3.3 Screening (Day -7±5)

Screening visit will be performed by PI and/or investigator's clinical research team and will include medical history, physical exam, spirometry (PFT), CFQ-R, sputum collection, and informed consent. Sputum is obtained for research laboratory analysis (e.g., phage susceptibility) and baseline antibiotic sensitivity testing is assessed from subject medical records. Randomization is completed once sputum PsA phage susceptibility is obtained.

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. Rescreened participants should be assigned the same participant number as identified in the initial screen visit.

6.3.4 Enrollment

The investigator will enroll subjects who have consented and met the eligibility criteria during screening.

6.3.5 On Study Visits

Treatment visit 1: Day 1:

1. Confirm medical history and any changes in status since screening visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R.
4. Perform an abbreviated physical examination.
5. Obtain and record vital signs.
6. Collect urine for pregnancy test (if female of child-bearing potential).
7. Blood collection.
8. Perform and record pre-treatment spirometry.
9. Dispense study drug, compressor, nebulizer, (i.e., InnoSpire Elegance Compressor #1099969 with SideStream Reusable neb #HS860, Philips Respironics), and all relevant equipment (e.g., thermometer) to subject.
10. Review study drug administration with subject.
11. Supervise subject self-administer first dose of study drug in clinic.
    a. Observe subject for 30 minutes following nebulization for any potential adverse events.
12. Perform and record post-treatment spirometry. If > 10% decline in FEV1 compared to pre-treatment spirometry, this will be considered evidence for an adverse response to treatment and subject will not continue in the study.
13. Ask subject about AEs of interest (increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and chills or fever).

14. Review study drug dose, schedule, and storage conditions with subject.
    a. Subjects are asked to nebulize treatment after standard airway clearance therapies at the same time daily.
15. Provide subject medication and AE diaries and instructions for completion.
16. Remind subject NOT to discontinue therapy if they experience improvements or feel better.
17. Instruct subject to bring empty study drug containers, and study diary to subsequent visit.
18. Schedule subject for subsequent visit.

Treatment visit: Day 3 (±1):

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R.
4. Review subject study diaries and perform container count on returned containers.
5. Record any AEs. Query for increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and chills or fever since their last visit.
6. Perform an abbreviated physical examination.
7. Obtain and record vital signs.
8. Perform and record spirometry.
9. Induce and collect sputum sample.
10. Instruct subject to bring empty study drug containers, equipment provided for study (e.g., nebulizer), any unused doses of study drug, and study diary to subsequent visit.
11. Schedule subject for subsequent visit.

Treatment visit: Day 7 (±1):

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R.
4. Review subject study diaries and perform container count on returned containers.
5. Record any AEs. Query for increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and chills or fever since their last visit.
6. Perform an abbreviated physical examination.
7. Obtain and record vital signs.
8. Collect urine for pregnancy test (if female of child-bearing potential).
9. Blood collection.
10. Perform and record spirometry.
11. Induce and collect sputum sample.
12. Request return of equipment provided for study, empty containers of study drug, and any unused containers of study drug for disposal.
13. Schedule subject for subsequent visit.

Follow-up visits: Days 14 (±3), 21 (±3), and 28 (±3):

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R.
4. Review subject diary
5. Record any AEs. (Day 14 only: Query for increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and chills or fever since their last visit.)
6. Perform an abbreviated physical examination.
7. Obtain and record vital signs.
8. Blood collection.
9. Perform and record spirometry.
10. Induce and collect sputum sample.

11. Schedule subject for subsequent visit.

Abbreviated follow-up visit: Day 56 (±7):

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R.
4. Record any AEs.
5. Perform an abbreviated physical examination.
6. Obtain and record vital signs.
7. Blood collection.
8. Perform and record spirometry.

Monthly safety follow-up: M3, M4, M5, M6 (±7 days), alternating in person & phone visit:

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R.
4. Record any AEs.
5. Perform an abbreviated physical examination if subject is in clinic.
6. Obtain and record vital signs if subject is in clinic.
7. Perform and record spirometry if subject is in clinic.
8. Collect sputum if subject is in clinic and can expectorate sputum or agrees to undergo induced sputum.

Subjects from the placebo group who continue onto the Open-label portion will undergo the following schedule:

Open-Label Treatment Screening Visit (Day -7, ±7 days)

1. Open Label Consent is required and will be acquired by a member of the research team using the Open Label Consent form, which will be scanned and stored in the EMR. Photocopies of signed forms will be provided to the subject, and the wet ink versions will be stored in a secure cabinet.
2. Collect sputum sample, either in person or shipped overnight on ice packs. Sputum may be induced or produced spontaneously. Sputum is obtained for research laboratory analysis (e.g. phage susceptibility and bacterial load), and baseline antibiotic sensitivity testing is assessed from subject medical records. Open Label Day 1 dosing can occur once PsA phage susceptibility is obtained.
3. Confirm medical history and any changes in status since last visit.
4. Record any changes to concomitant medications.

Open-Label Treatment visit 1: Day 1

1. Confirm medical history and any changes in status since open label screening.
2. Record any changes to concomitant medications.
3. Administer CFQ-R and CFRSD.
4. Perform an abbreviated physical examination.
5. Obtain and record vital signs.
6. Collect urine for pregnancy test (if female of child-bearing potential).
7. Perform and record pre-treatment spirometry.
8. Review study drug administration with subject.
9. Supervise subject self-administer first dose of study drug in clinic.
    a. Observe subject for 30 minutes following nebulization for any potential adverse events.
10. Perform and record post-treatment spirometry. If > 10% decline in FEV1 compared to pre-treatment spirometry, this will be considered evidence for an adverse response to treatment and subject will not continue in the study.

11. Ask subject about AEs of interest (increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and chills or fever).
12. Review study drug dose, schedule, and storage conditions with subject.
    a. Subjects are asked to nebulize treatment after standard airway clearance therapies at the same time of day.
13. Provide subject medication and AE diaries and instructions for completion.
14. Remind subject NOT to discontinue therapy if they experience improvements or feel better.
15. Instruct subject to bring empty study drug containers, and return equipment provided for study (e.g., nebulizer), any unused doses of study drug, and study diary to subsequent visit.
16. Schedule subject for subsequent visit.

Open-label Treatment visit: Day 7 (±2):

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R and CFRSD.
4. Review subject study diaries and perform container count on returned study drug.
5. Record any AEs. Query for increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and chills or fever since their last visit.
6. Perform an abbreviated physical examination.
7. Obtain and record vital signs.
8. Collect urine for pregnancy test (if female of child-bearing potential).
9. Blood collection.
10. Perform and record spirometry.
11. Induce and collect sputum sample.
12. Request return of equipment provided for study, empty containers of therapy, and any unused containers of therapy for disposal.
13. Schedule subject for subsequent visit.

Open-Label Follow-up visit: Day 14 (±3):

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R and CFRSD.
4. Review subject diary
5. Record any AEs. Query for increased sputum production, shortness of breath, hemoptysis, chest pain, cough, and chills or fever since their last visit.
6. Perform an abbreviated physical examination.
7. Obtain and record vital signs.
8. Blood collection.
9. Perform and record spirometry.
10. Induce and collect sputum sample.
11. Schedule subject for subsequent visit.

Open-Label Follow-up visit: Day 28 (±3):

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R and CFRSD.
4. Record any AEs.
5. Perform an abbreviated physical examination.
6. Obtain and record vital signs.
7. Blood collection.
8. Perform and record spirometry.
9. Induce and collect sputum sample.

Open-Label Monthly safety follow-up: M2, M3(±7 days) in person or on the phone*:*

1. Record any changes in status since last visit.
2. Record any changes to concomitant medications.
3. Administer CFQ-R and CFRSD.
4. Record any AEs.
5. Perform an abbreviated physical examination, if subject is in clinic.
6. Obtain and record vital signs if subject is in clinic.
7. Perform and record spirometry if subject is in clinic.
8. Collect sputum if subject is in clinic and can expectorate sputum or agrees to undergo induced sputum.

6.3.6 End of Study and Follow-up

6.3.7 ***End of blinded study follow-up*** will occur at the end of day 56 for all randomized subjects, following which, subjects randomized to active phage treatment will continue being followed monthly up to 6 months of total follow up for each subject, and subjects who were originally randomized to placebo, and who will choose to initiate active phage therapy, will be followed for 28 days, and then monthly up to a total of 6 months follow up from the time of original randomization. Any remaining containers of intervention that were not used, and nebulizer used during the study will be collected for disposal.

Removal of subjects

In rare instances, it may be necessary for a subject to permanently discontinue (definitive discontinuation) study intervention. If study intervention is discontinued in such a manner, the participant **will** remain in the study to be evaluated for **adverse events**. See the SoA (CYPHY Trial Design, pages 32 & 33) for data to be collected at the time of discontinuation of study intervention and follow-up, and for any further evaluations that need to be completed.

Should any serious immune-related adverse event occur (e.g., anaphylactic response), the SAE will be reported, and subject will be discontinued from trial.

A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the PI for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.

At the time of discontinuation from the study, if possible, an early discontinuation visit should be conducted per schedule on day 28 as shown in the SoA (CYPHY Trial Design, pages 32 & 33). See SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed (CYPHY Trial Design, pages 32 & 33). The participant will be permanently discontinued both from the study intervention and from the study at that time.

If the participant withdraws consent for disclosure of future information, the sponsor may retain, and continue to use, any data collected before such a withdrawal of consent. If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

Subjects who are discontinued from the study will not be replaced.

6.4 Statistical Method

6.4.1 Statistical Design

Hypotheses:

Primary for efficacy: We hypothesize that subjects randomized to active phage therapy will have significantly higher change from baseline in PsA sputum load, as compared to subjects randomized to the placebo group during the non-Open-label portion of the study.

The **primary efficacy endpoint** is change in PsA sputum load from baseline (pre-phage) to d14 on study, i.e. 7 days after completion of phage or placebo treatment in the randomized and blinded (non-Open-label) portion of the study.

Primary for safety: We hypothesize that the short-term safety profile of active phage therapy, as measured during the first 2 months of follow up, will show that treatment with phage is safe.

Primary safety endpoints: frequency and severity of adverse events (AEs and SAEs) on d1, d3, d7, d14, d21, d28, d56.

Secondary: We hypothesize that treatment with active phage : 1) will decrease PsA sputum load over time, 2) will decrease PsA antibiotic resistance and virulence factors, 3) will favorably modify sputum phage, host, and microbiome, and 4) will have positive impact on clinical and quality of life responses in subjects..

Secondary outcome measures are: 1) monthly tracking for a total of 6-months of individual follow up from randomization of AEs and SAEs with active monitoring post d56 measurement in subjects randomized to treatment, and monthly measurements in open-label extension among subjects originally randomized to placebo who chose active phage therapy; 2) post-treatment change from baseline in PsA sputum load to d3, d7, d14, d21 and d28; 3) post-treatment change from baseline in PsA “trade-off” [e.g., decreased resistance to antibiotics, inflammatory products (pyocyanin or endotoxin), or motility] to d3, d7, d14, d21 and d28; 4) sputum phage presence at d3, d7, d14, d21, d28, host sputum inflammatory markers change from baseline to d14 and d28, lung inflammation transcriptomic change from baseline to d14, and microbiome changes from baseline to d14; and 5) change from baseline in lung function (e.g., FEV1, FEV1pp), pulmonary exacerbations, medication changes, and respiratory symptoms (CFQ-R)[14] to d3, d7, d14, d21, d28, d56, and monthly thereafter through M6 following initiation of phage therapy.

Method:

Using the intent-to-treat (ITT) approach for all analyses, whereby subjects are analyzed according to the group to which they were assigned, data will be examined to ascertain whether there is a significant difference in the reduction of PsA sputum load between two treatment conditions (phage vs. placebo) on day 14 of study (7 days after completion of treatment phase). As this is the only efficacy-related primary outcome, there is no need to adjust for either multiple comparisons or false discovery rate, and the significance will be established at the two-sided alpha level of 0.05.

Descriptive analyses of the study participants, as well as all primary and secondary/exploratory endpoints will be tabulated by randomization group, and will include mean (standard deviation) or median (IQR: 25th percentile, 75th percentile), for Gaussian (Normal) and non-normal distributions of continuous variables, respectively; and using count (percent) for discrete/categorical data. Between-group differences will be summarized graphically over time (as pertains to the measurement schedule for each outcome of interest) using estimated means of change from baseline or Odds Ratios (ORs) and 95% confidence intervals (95%CIs).

The primary unadjusted statistical analysis for the between-group change in PsA sputum on the log10 scale from baseline to day 14 on study (7 days after completing assigned treatment) will be based on the Student's t-test. In the adjusted analysis, we will use Analysis of Covariance (ANCOVA), where we will model the change in PsA on the log10 scale from baseline to day 14 on study, with the baseline PsA level as a covariate, as well as the following predictors (as the sample size permits): sex, age (a continuous variable), and phage type (a 3-level categorical variable).

In the additional supportive (secondary) analyses for mean change from baseline to day 3, to day 7, to day 14, to day 21, to day 28, and to day 56 (when relevant) in primary and secondary continuous outcomes, we will use linear mixed effects (LME) modeling. In the LME models, we will approach the effect of time as a continuous variable, thereby allowing the examination of the parametric trajectories of change in the outcomes over time and furthermore, we will be able to obtain a measure of the within-subject correlation in the response, using the IntraClass Correlation (ICC). The latter is particularly useful, as it will allow us to describe the degree of heterogeneity in the response due to unmeasured subject characteristics and can be very helpful for the future larger trial planning. If parametric trajectories of mean change are not feasible, or for continuous outcomes with only 2 time points, we will also consider mean response profile (MRP) modeling, with time as a categorical variable, which allows an arbitrary change in the means (instead of a parametrically defined change in the mean) of outcomes over time. The within-person correlation will be implemented using the covariance pattern modeling. Estimation of parameters in the mean response model will be performed using maximum likelihood (ML), allowing us to use the Likelihood Ratio Test (LRT) to compare nested models. Estimation of covariance parameters in the LME and the covariance pattern model will be done with restricted maximum likelihood (REML), in order to use the LRT to select the best covariance structure. We realize that our sample size may not allow us to include all potential predictors of interest, and we are also aware of not over-fitting any given final model to the observed data; therefore, we will select the most parsimonious final models.

Weekly trade-offs of PsA and phage presence, as well as number of PEs and medication changes, these secondary outcomes will be summarized descriptively as numbers (counts) and proportions, which will allow us to select optimal statistical tests and models, based on the empirical distributions of these outcomes. As these are discrete outcomes, we propose to use marginal models implemented via generalized estimating equations (GEE).

For the analysis of change in lung inflammation transcriptomics change, differentially expressed genes (DEGs) related to inflammation at baseline and day 14 on study will be compared within each treatment group using paired-samples t-test in the unadjusted analyses,

and using ANCOVA (similar to the primary outcome) in the adjusted analyses, which will also compare the change in individual DEGs between two groups.

For the microbiome analysis, within each treatment group, active members of microbial community will be classified by bacterial taxonomical assignments using KAIJU at the genus level (Cobián Güemes et al., 2019) and will be plotted by baseline and day 14 on study, using color-coded by phylum bar charts. For each subject, we will output percent with certain top 5 phyla, and compare the change in these between baseline and day 14 using RPM (as described for the primary outcome above), using two time points as a categorical variable, treatment group and their interaction. Bacterial rank abundance plots will be generated using relative abundance at the genus level and stratified by baseline and day 14, as well as by treatment group; and 4 evenness statistics (for treatment group at baseline, for treatment group at day 14, for placebo group at baseline and placebo group at day 14) will be calculated as Shannon diversity index divided by the natural log of the total number of species (Cobián Güemes et al., 2019). Between-group comparisons of the trajectories in relative abundance by the bacterial rank will be performed by either using LME (as for the primary outcome above), treating the bacterial rank as a polynomial effect.

Statistical analyses for the blinded portion of the study (through d56 measurement) will be done separately from the extended through month 6 of follow up unblinded portion for those randomized to active phage, and separately from the data obtained from the open-label extension for subjects originally randomized to placebo who chose to receive active phage.

Statistical analyses will be conducted using SAS 9.4 (Cary, NC). Statistical significance will be established at the two-sided alpha=0.05 for non-transcriptomics and non-metagenomics outcomes. However, we will also report statistical trends (alpha 0.10) because we are interested in exploring associations between the outcomes and other predictors (e.g., subject characteristics other than the treatment effect), as well as between secondary outcomes and treatment effect, for which we are not specifically powered in this study.

False discovery rate (FDR) using a permutation-based method will be used in transcriptomics and metagenomics analyses adjusted for the multiple testing error, so as to preserve the alpha level of 0.05.

6.4.2 Sample Size Considerations

For the primary efficacy objective, based upon preliminary (eIND) data of ~2.5 log10 decrease in PsA post-phage (**Figure 2-4B**), a sample size of 10 subjects in the placebo group, and 20 subjects in the phage group will have 80% power at the two-sided alpha of 0.05 to reject the null hypothesis of equal means when the population mean difference is $\mu_{phage} - \mu_{placebo}$ =2.5-0.3, with an estimated standard deviation of 2.0, using a two-sided Student's t-test.

6.5 Planned Analyses

The statistical analysis plan will be finalized prior to unblinding and it will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints including primary and key secondary endpoints. All analyses will be performed using the intent-to-treat (ITT) approach.

6.5.1 Primary Objective Analysis

The primary unadjusted statistical analysis for the between-group change in PsA sputum (continuous variable on the log-10 scale) from baseline to day 14 on study (7 days after completing assigned treatment) will be based on the Student's t-test. In the adjusted analysis, we will use Analysis of Covariance (ANCOVA), where we will model the change from baseline in PsA at 14 days on study, with the baseline PsA level as a covariate, as well as the following predictors (as the sample size permits): sex, age (a continuous variable), and phage type (a 3-level categorical variable). Results will be summarized as the estimated mean changes in PsA sputum from pre-treatment to day 14 on study, with surrounding 95% Confidence Intervals.

In the additional supportive analyses for mean change from baseline to day 3, to day 7, to day 14, to day 21 and to day 28 in primary and secondary continuous outcomes (plus to day 56), we will use linear mixed effects (LME) modeling, with a random intercept for subject, and the following fixed effects: treatment arm (phage [coded as '1'] vs. placebo [coded as '0'] in one set of models), baseline values of log-10 PsA, time (in days), an interaction of time by treatment arm, subject age at randomization, and gender. The joint hypothesis test of the effect of treatment arm and treatment arm by time interaction, using the Wald-test, will help us ascertain whether there are significant between-treatment arm differences in the adjusted changes from baseline in the mean of PsA load across time. Prior to testing the significance of fixed effects, we will also test for the random effect for slope using the LRT (using the restricted maximum likelihood, REML) with a mixture of Chi-square distributions. In the LME models, we will approach the effect of time as a continuous variable, thereby allowing the examination of the parametric trajectories of mean change in the outcomes over time and, furthermore, obtain a measure of the within-subject correlation in the outcome variable (change from baseline in PsA), using the IntraClass Correlation (ICC). The latter is particularly useful, as it will allow us to describe the degree of heterogeneity in the response due to unmeasured subject characteristics and can be very helpful for the future larger trial planning.

If the effect of time in the LME cannot be parameterized as a parametric effect, we will also consider mean response profile (MRP) modeling, with time as a categorical variable, which allows an arbitrary change in the means of outcomes over time. The model for the mean response and the primary hypothesis test will be the same as in the LME. The within-person correlation will be implemented using the covariance pattern modeling, testing different within-person correlation structures, such as Compound Symmetry (CS), Ar(1), Toeplitz, Exponential, and unstructured. As this supportive analysis is designed to get better estimates for a larger Phase II efficacy trial, we will not apply a correction to our significance level, as we would normally do so when comparing multiple means (e.g., change from baseline to day 7 vs. to day 14 vs. to day 21 vs. to day 28, etc).

Estimation for both, LME and MRP with covariance pattern modeling, will be performed using maximum likelihood (ML and REML), allowing us to use the Likelihood Ratio Test (LRT) to compare nested models and Akaike Information Criterion (AIC) for non-nested models. Thus, the final LME model can be compared to the final covariance pattern model to choose the best fit to the data. We realize that our sample size may not allow us to include all potential predictors of interest, and we are also aware of not over-fitting any given final model to the observed data; therefore, we will select the most parsimonious final models.

The estimated adjusted mean changes from baseline and 95% Confidence Intervals (95%CIs) at day 3, day 7, day 14, day 21 and day 28 will be plotted by treatment arm.

Statistical analysis for the primary safety-related objective is summarized in **Section 6.5.3** Safety – see below.

6.5.2 Secondary Objectives Analyses

Differences between phage and placebo treatment arms with respect to the change in continuous secondary and tertiary endpoints (FEV1, FEV1pp, CFQ-R, and anti-phage antibody titers) will be analyzed in the similar fashion as described for the primary endpoint. For example, sustained effects of treatment over time will be examined using LME or MRP with covariance pattern modeling. Estimated effect sizes and 95%CIs, in particular for FEV1, FEV1pp and CFQ-R, will be useful for designing a larger multi-site trial.

Weekly trade-offs in PsA and phage presence, number of PEs and medication changes – these secondary outcomes will be summarized descriptively as proportions and numbers (counts), which will allow us to select optimal statistical tests and models, based on the empirical distributions of these outcomes. As these are discrete outcomes, we propose to use marginal models implemented via generalized estimating equations (GEE), with a separate model for the transformed mean response (link=logit for a binary outcome, link=log for a count outcome) and a model-based (naïve) or robust (sandwich) estimation of standard errors. For example for the probability of a compound binary outcome (e.g., 1=gaining sensitivity/decreased pyocyanin/endotoxin or motility, 0=no improvement) over the course of the study follow up in each treatment arm, we propose to separately model the transformed mean response using the logit link and the main effects of treatment arm (phage [coded as '1'] vs. placebo [coded as '0'] in one set of models), categorical time (e.g., baseline, day 3, day 7, day 14, day 21 and day 28 – as relevant for a specific secondary outcome), an interaction of time by treatment arm, subject age at randomization, and gender. The time by treatment arm interaction will be tested using the Wald test, and will allow us to ascertain whether the probability of a response differs across the follow up time between the two treatment arms. Since our sample size is relatively small to take complete advantage of the robust (sandwich estimator) standard errors from GEE, we will implement the Wald Test using both types of standard errors, the empirical/robust standard errors and model-based or naïve standard errors using the 'best' working correlation. The within-subject association will be modeled on the log-odds scale, and we will use the Quasi-likelihood under the assumption of independence criterion (QIC) to select among the 'best' working correlations (Compound Symmetry, Unstructured and Toeplitz). Results will be summarized using estimated Odds Ratios (ORs) and surrounding 95%CIs.

For the analysis of change in lung inflammation transcriptomics, differentially expressed genes (DEGs) related to inflammation at baseline and day 14 on study will be compared within each treatment group using paired-samples t-test in the unadjusted analyses, and using ANCOVA (similar to the primary outcome) in the adjusted analyses, which will also compare the change in individual DEGs between two groups.

For the analysis of microbiome, within each treatment group, active members of microbial community will be classified by bacterial taxonomical assignments using KAIJU at the genus level (Cobián Güemes et al., 2019) and will be plotted by baseline and day 14 on study, using color-coded by phylum bar charts. For each subject, we will output percent with certain top 5 phyla, and compare the change in these between baseline and day 14 using RPM (as described for the primary outcome above), using two time points as a categorical variable, treatment group (phage vs. placebo), and their interaction. Bacterial rank abundance plots will be generated using relative abundance at the genus level and stratified by baseline and day 14, as well as by treatment group; and 4 evenness statistics (for treatment group at baseline, for treatment group at day 14, for placebo group at baseline and placebo group at day 14) will be calculated as Shannon diversity index divided by the natural log of the total number of species (Cobián Güemes et al., 2019). Between-group comparisons of the trajectories in relative abundance by the bacterial rank will be performed by either using LME (as for the primary outcome above), treating the bacterial rank as a polynomial effect.

Tertiary/exploratory endpoint analysis of anti-phage antibody titers will follow similar analytical plans for continuous outcomes - as for the primary and secondary efficacy endpoints.

Results from the open-label period of the study and from the extended unblinded portion of the study will be summarized using descriptive statistics with surrounding 95%CIs, and will be modeled over the follow up time, as the data permit, using similar statistical approaches as described for the primary and secondary efficacy endpoints, but we will not conduct between-treatment comparisons. Furthermore, these data will not be combined with the data obtained from the blinded portion of the study.

6.5.3 Safety

All safety analyses will be made on the Safety Population: *all subjects who received any study treatment (including placebo), but excluding subjects who drop out prior to receiving any treatment*.

Safety will be evaluated with summary of adverse events for the safety population (see description under “Section 6.2.2, “Safety”). The summary statistics will be produced in accordance with the types and groupings of AE and SAEs of the study protocol. Treatment emergent adverse events (AEs) are those events that occur after the baseline assessment.

A tabular summary of AE will present: Number of subjects with any AE; Number of SAEs with outcome death; Number of subjects with SAE; Number of subjects with AEs leading to discontinuation of study intervention; Number of subjects with AEs leading to discontinuation of study; Total number of AEs; Total number of SAEs.

The Adverse Events summary tables will include number of adverse events, the number of subjects in each treatment group in whom the event occurred, and the incidence of occurrence and should be grouped by system organ class, preferred terms and/or other interested variables (e.g., relatedness, intensity and seriousness).

We will provide tabulated summaries for the short-term and longer-term safety profiles. The short-term safety profile from the randomized blinded portion of the study will include data from d1, d3, d7, d14, d21, d28, and d56. The longer-term safety profile will come from the monthly measurements of the extended unblinded portion for subjects originally randomized to active phage. We will also tabulate short-term safety profile, separately, for the open-label portion of the study for subjects originally randomized to placebo who chose to receive active phage after the blinded portion; and the open-label extension monthly measurements will also have additional longer-term safety data.

All formal testing of adverse effects will be based on the subject as the experimental unit. Thus, for comparing incidence of AE within system organ by treatment group, a one-sided Fisher's exact test will be conducted at 0.05 level (higher incidence in experimentally treated group is the alternative hypothesis). Also, highest AE grade will be determined for each subject and compared by treatment group using a 2 sample Mann-Whitney test (one-sided at 0.05). No correction for multiple testing will be employed in order that the statistical power is maintained.

6.5.4 Analysis of Subject Characteristics

Descriptive analyses of the study participants will be tabulated by randomization group, and will include mean (standard deviation) or median (IQR: 25th percentile, 75th percentile), for Gaussian and non-normal distributions of continuous variables, respectively; and using count (percent) for discrete data. We will report the following subject characteristics: age at randomization, gender, height, weight, race, FEV1, FEV1 percent predicted, BMI, CF-related diabetes (CFRD), pancreatic insufficiency (PI), number of pulmonary exacerbations during previous six months, most recent treatment for pulmonary exacerbation prior to first study dose, baseline pathogen isolation (e.g., PsA, *Staphylococcus*, etc.), concomitant medications or therapies at study start, use of inhaled antibiotic and which type, type of CFTR mutation, use of CFTR modulator, and which CFTR modulator.

6.5.5 Covariates

We will include the following covariates in the adjusted models: gender (male vs. female) and age, as these are known to be significantly associated with a number of our outcomes of interest. For example, age could be a proxy for the length of time diagnosed with CF, and hence can indirectly describe CF severity. Gender can also an important predictor of FEV1 and quality of life outcomes (based on CFQ-R).

Safety data will be stratified by (1) short-term safety obtained on all randomized subjects from the blinded portion of the study (through d56 measurement), which will allow us the between-treatment group comparison; (2) short-term safety data obtained from the open-label portion (through d28), which will give us descriptive information, (3) longer-term safety data obtained

from monthly follow up during the unblinded portion of the study on subjects originally randomized to the active phage, which will also give us descriptive information, and (4) longer-term safety data obtained from the monthly follow up data in the open-label portion of the study on subjects originally randomized to placebo who chose to receive active phage post the randomized portion of the study (also, for descriptive analyses).

6.5.6 Handling of Missing Data

The primary approach will be to minimize the occurrence of missing data by implementing follow-up procedures that ensure a close to 100% follow-up for all subjects, especially for the primary endpoint. In addition, we propose the following two approaches from a methodological perspective: (1) we anticipate the attrition to be at most 10%, therefore, we will inflate the sample size from 10 subjects per group to 10/0.9=12 subjects per group, and (2) our analytic approach for the primary outcome will treat missing outcomes as missing at random (MAR). The latter assumption for the missing data mechanism cannot be tested statistically, however we will check whether subjects with missing outcomes are significantly different from those with complete observations based on important subject characteristics. Even if we find such differences, the assumption of MAR will also be reasonable, especially when we incorporate variables predictive of the probability of missingness (e.g., available data: outcomes at other time points) into the statistical analyses, whereby the inference becomes conditional on what we know about our subjects, which generally meets the definition of MAR.

GEE estimation relies on the assumption that outcomes are missing completely at random (MCAR). While this assumption cannot be verified, we can test for departures from this assumption by comparing characteristics and available outcome data for subjects with/out missing outcomes using standard statistical tests used for between-group comparisons. If we find such departures, we will then approach our inference using generalized linear mixed effects modeling (GLMM). This approach is similar to the methods described for LME, but we will use the transformed means (link=logit or link=log) in the analyses and will report effect sizes using the marginalized (integrated over the distributed of random effects) estimates of the means on the original scale (e.g., proportions and mean counts).

7 Trial Administration

7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

This study will be conducted in accordance with the protocol and with the following:

o Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines

o Applicable ICH Good Clinical Practice (GCP) Guidelines

o Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB by the investigator and reviewed and approved by the IRB before the study is initiated.

· Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

· The investigator will be responsible for the following:

o Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB

o Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures

o Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

Travel Accomodations

In an effort to make study participation attainable for more patients, the trial will cover some of the costs associated with travel & hotel accommodations.

If patietns are traveling >200 miles they will be compensated up to $5,000. If patients are traveling <=200 miles they will be compensated up to $600.

Costs will be covered either by the study team booking directly through the Yale University portal Egencia or otherwise, or via compensation provided in the form of a Bank of America Card loaded remotely with the value indicated in travel & hotel receipts and/or bank statements.

Costs will be covered by the Yale Phage Center. Prior approval will need to be obtained for all costs associated with travel & hotel accommodations. Please feel free to discuss this with the study staff.

7.2 Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol or study

team will require an approved IRB amendment before implementation. The IRB will determine whether informed consent and HIPAA authorization are required.

The IRB will conduct continuing review at intervals appropriate to the degree of risk, but not less than once per year.

A study closure report will be submitted to the IRB after all research activities have been completed.

Other study events (e.g. data breaches, protocol deviations) will be submitted per Yale University's IRB's policies.

7.3 Subject Confidentiality

Subject confidentiality will be protected in accordance with Yale University IRB Policy 400. The investigator will make all efforts, within reason, to keep subject health information private and confidential.

7.4 Deviations/Unanticipated Problems

Adverse events, including Serious Adverse Events (SAEs) and Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) will be handled and reported to the Yale University IRB in accordance with IRB Policy 700 and 710.

7.5 Data Collection

Data collection and storage will be conducted in compliance with Yale University IRB Policies 440 and 445 and HRPP Policy 730. Briefly:

- Participants will be assigned a unique identifier. Any participant records or datasets that are transferred to the sponsor will contain this identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant will be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure will also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent
- The participant will be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.
- When University human research records are no longer deemed useful following the proscribed record retention period, the records will be de-identified or destroyed in accordance with University media control and/or biological specimen policies.

7.6 Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

7.7 Study Records

Study records are defined in the Yale University HRPP Policy 730 as any information preserved in a fixed medium, whether on paper, electronically, or otherwise. These include but are not limited to regulatory documents, protocols, consent forms, case report forms, subject medical records, and surveys.

7.8 Access to Source Documents

· Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

· Data reported on the CRF, or entered in the eCRF, which are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

· Definition of what constitutes source data can be found in Yale University IRB Policy 440 under "Materials": Data, films, biological specimens, or other recorded information that may be useful for research. Examples include vials of blood, medical records, tumor specimens, scans, and videotapes of interviews. Note that sources of "information" or "data" (e.g., facts contained in articles or books) are not affected by this policy because they do not identify any human subjects or contain PHI, or otherwise implicate any unique privacy concerns.

· Source documents will be available to the Coordinator, DMC, and Data Quality Monitor, Investigator, and Investigator's clinical team (e.g., nurses, clinical fellows). Data will be collected from them and entered into the site's electronic system (e.g., eCRF, ONCOR).

· Source documents will be kept at a secure, locked site by the investigator for a minimum of three (3) years as required by Yale University IRB Policy 730.1.

7.9 Data or Specimen Storage/Security

Data and specimens will be stored in a secure manner in accordance with Yale University IRB Policy 440 and 730. When data or specimens are no longer deemed useful following the proscribed record retention period, the records will be de-identified or destroyed in accordance with University media control and/or biological specimen policies.

7.10 Retention of Records

Study documents will be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

7.11 Study Monitoring

Study site monitoring is necessary to assure adequate protection of the rights of human subjects and the safety of all subjects involved in clinical investigations and the quality and integrity of the resulting data submitted.

The Study Principal Investigator-designated monitor(s) conducts monitoring visits to ensure that clinical investigators and study team members are compliant with the protocol, ICH good clinical practice, federal, state and local regulations and institutional policies and procedures, that data are of high quality and integrity, and that the facilities and staffing are adequate for continued study participation. This will be performed by conducting monitoring visits including a site initiation visit, regularly scheduled interim monitoring visits and/or remote interim monitoring visits while subjects are on study, and a site close-out visit at the site. Following each site visit, a visit report will be generated containing information on site activities and a summary of pertinent points and action items. The report will be provided with a follow-up letter. Site-specific data status reports will be distributed to the site regularly to outline planned, missing or incomplete case report forms and any outstanding data queries.

During monitoring visits, the following may be reviewed:

- Protection of the rights, safety and welfare of subjects through review of informed consent process and documentation, adverse events (AEs) and serious adverse events (SAEs) and safety procedures
- Subject eligibility
- Source verification
- Protocol compliance
- Deviations and Non-compliance
- Investigator Site File
- GCP compliance
- If applicable, include: Investigational Drug/ Device Storage and Accountability (including quantity and disposal procedures)
- If applicable, include: Laboratory Facilities
- If applicable, include: Equipment maintenance and calibration
- Additional study supplies inventory and assessment
- Study progress and/or follow-up on issues with Site Principal Investigator (PI) and relevant members of the study team

The Study PI and YCCI will define the required study monitoring activities in a Study Monitoring Plan.

7.12 Data Safety Monitoring Plan

Data Safety Monitoring Plan

The Investigator has communicated with Miriam Hunt-Bennett, the CFF DMC Senior Program Coordinator at the University of Arizona Health Sciences. This meeting with Miriam and Dr. Wayne J. Morgan (Professor of Pediatrics and Physiology at the University of Arizona) initiated the process for DMC and completion of a DMC Charter. Since then, the DMC has met to review the CYPHY protocol, and their requests and suggestions have been incorporated into this protocol.

A formal data review will occur after the first 5 participants have completed visit 7; A full interim data review will occur after 50% completed visit 7, or 6 months after the first randomization.

Unblinding in the event of an SAE

The data monitoring comittee (DMC) will be responsible for subject safety and will have the authority to stop or modify this study. The DMC chair has the option to unblind him/herself or the entire DMC for SAEs. This will be done on an individual SAE-basis, thus ensuring that the DMC is not unblinded for the whole study.

7.13 Study Modification

Study modifications will be made as study amendments to the protocol in accordance with Yale University IRB Policy 100 PR.1. The change will be implemented in the study upon approval by the IRB and submission to the IND.

7.14 Study Discontinuation

The sponsor designee reserves the right to terminate the study at any time for any reason at the sole discretion of the sponsor. Reasons for the early termination of the study by the sponsor or investigator may include but are not limited to:

· Failure of the investigator to comply with the protocol, the requirements of the IRB or local health authorities, the sponsor's procedures, or GCP guidelines

· Inadequate recruitment of participants by the investigator

· Discontinuation of further study intervention development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the subject and should assure appropriate subject therapy and/or follow-up.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

7.15 Study Completion

Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The primary date of completion will be defined as “the date the final subject was examined or received an intervention for purposes of final collection of data for the primary and secondary outcome measures and adverse events (e.g., last subject’s last visit), whether the clinical trial concluded according to the pre-specified protocol or was terminated.” Data analysis will occur following primary date of completion, after which the study will be complete. The IRB and FDA will be notified of study completion through annual reporting.

7.16 Conflict of Interest Policy

Institutional conflict of interest will be handled according to Yale IRB Policy 501, and will be defined as “a significant external relationship that creates the opportunity and incentive to directly and significantly bias decision-making or compromise the professional judgment of the IRB, the HRPP, the researchers, or others in the conduct, review, or oversight of human subjects research.”

The Institutional Conflict of Interest Committee (ICOIC) is responsible for the implementation of this policy. The ICOIC establishes and oversees procedures to collect information relating to SERs and relevant significant financial interests of University leaders (LSFIs). Additionally, any individual employed by the University or engaged in the review or conduct of University research who becomes aware of an SER is obligated to bring it to the attention of the ICOIC, or to the attention of the Institutional Official (IO) or an Institutional Review Board (IRB) Chair, who will in turn forward it to the ICOIC. The ICOIC reviews SERs and LSFIs to determine whether they pose the risk of creating ICOIs. If the ICOIC determines that a risk of ICOI exists in relation to human-subjects research, it will decide upon actions to eliminate or manage the ICOI, and it will report the ICOI along with those decisions to the Human Research Protections Program (“HRPP”). The HRPP and the IRBs may conduct additional reviews specific to human-subjects research and may impose measures, in addition to those determined by the ICOIC, to protect human research subjects and ensure the integrity of research.

7.17 Funding Source

The study will be funded through donations from non-profit entities, the Blavatnik Fund at Yale University and the Cystic Fibrosis Foundation. Salary support for this study is provided by Yale University’s Department of Pulmonology, Critical Care, and Sleep Medicine and the Department of Ecology and Evolutionary Biology.

7.18 Publication Plan

· The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

· The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

· Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

8 List of Tables

9 References

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10 Appendices

Appendix #	Title
1	Cystic Fibrosis Quality-of-Life Questionnaire Revised
2	Study Drug Diary
3	Symptoms Diary

1. Cystic Fibrosis Quality-of-Life Questionnaire Revised

Source:(Quittner et al., 2005), adapted from https://cfqr.github.io/

Understanding the impact of your illness and treatments on your everyday life can help your healthcare team keep track of your health and adjust your treatments. For this reason, this questionnaire was specifically developed for people who have cystic fibrosis. Thank you for your willingness to complete this form.

The following questions are about the current state of your health, as you perceive it. This information will allow us to better understand how you feel in your everyday life. Please answer all the questions. There are no right or wrong answers! If you are not sure how to answer, choose the response that seems closest to your situation.

Please fill-in the information or check the box indicating your answer.

What's your birth date?

Enter a date...

_____

_____

What's your gender?

- Male
- Female

During the past two weeks, have you been on vacation or out of school for reasons NOT related to your health?

- Yes
- No

What is your current marital status?

- Single/never married
- Married
- Widowed
- Divorced
- Separated
- Remarried
- With a partner

Which of the following best describes your racial background?

- Caucasian
- African American
- Hispanic
- Asian/Oriental or Pacific Islander
- Native American or Native Alaskan
- Other

- Prefer not to answer this question

What is the highest grade of school you have completed?
- Some high school or less
- High school diploma/GED
- Vocational school
- Some college
- College degree
- Professional or graduate degree

Which of the following best described your current work or school status?
- Attending school outside the home
- Taking educational courses at home
- Seeking work
- Working full or part time (either outside the home or at a home-based business)
- Full time homemaker
- Not attending school or working due to my health
- Professional or graduate degree

Please check the box indicating your answer.

During the past two weeks, to what extent have you had difficulty
Performing vigorous activities such as running or playing sports
- A lot of difficulty
- Some difficulty
- A little difficulty
- No difficulty

Walking as fast as others
- A lot of difficulty
- Some difficulty
- A little difficulty
- No difficulty

Carrying or lifting heavy things such as books, groceries, or school bags
- A lot of difficulty
- Some difficulty
- A little difficulty
- No difficulty

Climbing one flight of stairs
- A lot of difficulty
- Some difficulty
- A little difficulty
- No difficulty

Climbing stairs as fast as others
- A lot of difficulty

- Some difficulty
- A little difficulty
- No difficulty

During the past two weeks, indicate how often:

You felt well
- Always
- Often
- Sometimes
- Never

You felt worried
- Always
- Often
- Sometimes
- Never

You felt useless
- Always
- Often
- Sometimes
- Never

You felt tired
- Always
- Often
- Sometimes
- Never

You felt energetic
- Always
- Often
- Sometimes
- Never

You felt exhausted
- Always
- Often
- Sometimes
- Never

You felt sad
- Always
- Often
- Sometimes
- Never

Thinking about the state of your health over the last two weeks:

To what extent do you have difficulty walking?
- You can walk a long time without getting tired
- You can walk a long time but you get tired
- You cannot walk a long time because you get tired quickly
- You avoid walking whenever possible because it's too tiring for you

How do you feel about eating?
- Just thinking about food makes you feel sick
- You never enjoy eating
- You are sometimes able to enjoy eating
- You are always able to enjoy eating

To what extent do your treatments make your daily life more difficult?
- Not at all
- A little
- Moderately
- A lot

How much time do you currently spend each day on your treatments?
- A lot
- Some
- A little
- Not very much

How difficult is it for you to do your treatments (including medications) each day?
- Not at all
- A little
- Moderately
- Very

How do you think your health is now?
- Excellent
- Good
- Fair
- Poor

Thinking about your health during the past two weeks, indicate the extent to which each sentence is true or false for you.

I have trouble recovering after physical effort
- Very true
- Somewhat true
- Somewhat false
- Very false

I have to limit vigorous activities such as running or playing sports
- Very true
- Somewhat true
- Somewhat false
- Very false

I have to force myself to eat
- Very true
- Somewhat true
- Somewhat false
- Very false

I have to stay at home more than I want to
- Very true
- Somewhat true
- Somewhat false
- Very false

I feel comfortable discussing my illness with others
- Very true
- Somewhat true
- Somewhat false
- Very false

I think I am too thin
- Very true
- Somewhat true
- Somewhat false
- Very false

I think I look different from others my age
- Very true
- Somewhat true
- Somewhat false
- Very false

I feel bad about my physical appearance
- Very true
- Somewhat true
- Somewhat false
- Very false

People are afraid that I may be contagious
- Very true
- Somewhat true
- Somewhat false
- Very false

I get together with my friends a lot
- Very true
- Somewhat true
- Somewhat false
- Very false

I think my coughing bothers others
- Very true

- Somewhat true
- Somewhat false
- Very false

I feel comfortable going out at night
- Very true
- Somewhat true
- Somewhat false
- Very false

I often feel lonely
- Very true
- Somewhat true
- Somewhat false
- Very false

I feel healthy
- Very true
- Somewhat true
- Somewhat false
- Very false

It is difficult to make plans for the future (for example, going to college, getting married, advancing in a job, etc.)
- Very true
- Somewhat true
- Somewhat false
- Very false

I lead a normal life
- Very true
- Somewhat true
- Somewhat false
- Very false

The next questions are about school, work, or other daily tasks.

To what extent did you have trouble keeping up with your schoolwork, professional work, or other daily activities during the past two weeks?
- You have had no trouble keeping up
- You have managed to keep up but it’s been difficult
- You have been behind
- You have not been able to do these activities at all

How often were you absent from school, work, or unable to complete daily activities during the last two weeks because of your illness or treatments?
- Always
- Often

- Sometimes
- Never

How often does CF get in the way of meeting your school, work, or personal goals?
- Always
- Often
- Sometimes
- Never

How often does CF interfere with getting out of the house to run errands such as shopping or going to the bank?
- Always
- Often
- Sometimes
- Never

Indicate how you have been feeling during the past two weeks.

Have you had trouble gaining weight?
- A great deal
- Somewhat
- A little
- Not at all

Have you been congested in your chest?
- A great deal
- Somewhat
- A little
- Not at all

Have you been coughing during the day?
- A great deal
- Somewhat
- A little
- Not at all

Have you had to cough up mucus?
- A great deal
- Somewhat
- A little
- Not at all

Has your mucus been mostly:
- Clear
- Clear to yellow
- Yellowish-green
- Green with traces of blood
- Don’t know
- I don’t cough up mucus

How often during the past two weeks:

Have you been wheezing?
- Always
- Often
- Sometimes
- Never

Have you had trouble breathing?
- Always
- Often
- Sometimes
- Never

Have you woken up during the night because you were coughing?
- Always
- Often
- Sometimes
- Never

Have you had problems with gas?
- Always
- Often
- Sometimes
- Never

Have you had diarrhea?
- Always
- Often
- Sometimes
- Never

Have you had abdominal pain?
- Always
- Often
- Sometimes
- Never

Have you had eating problems?
- Always
- Often
- Sometimes
- Never

2. Study Drug Diary

CYPHY

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Yale New Haven Hospital

Study Participant Self-Administration Instructions

The study staff will explain how to take the study drug YPT-01 & observe Day 1 administration. Here are points to remember:

1. Take once daily, approximately 24 hours apart, following your standard airway clearance therapies.
2. If you miss taking your dose at the usual time, you may take it so long as your next dose will be in more than 8 hours.
3. Store the drug in the refrigerator.
4. Do not nebulize in the same room as any other individuals with cystic fibrosis or bronchiectasis

Please call your doctor or research nurse before taking any new prescription or over-the-counter medications/supplements other than the study drugs.

For any problems, issues, or questions you may have, please contact:
Enter name, title and contact number

Study Participant Self-Administration Study Drug Diary

Please record the time you take each vial of YPT-01 and any comments. Please bring the completed Study Drug Diary to each study visit; this will help us keep track of your study drug and how well you are tolerating it.
Please dispose of vials in the trash when you are done with them.

Participant Identifier: CYPHY-##
Protocol #: 200029160
Doctor: *enter name and phone number*
Nurse: *enter name and phone number*

You will nebulize 3 vials (1 dose) each day as listed here:

Study Drug Name	# of vials to take per time/dose	# of times/doses each day	Approximate time to take drug
YPT-01	*3*	*1*	____:____ ☐ a.m. ☐ p.m.

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To be completed by Study Participant:				
Day	Date	Numbers of YPT-01 vials nebulized	Time of dose	Comments or problems with dose, if any
1			____:____ ◻ a.m. / ◻ p.m. ◻ Dose Not Taken Why: _____	
2			____:____ ◻ a.m. / ◻ p.m. ◻ Dose Not Taken Why: _____	
3			____:____ ◻ a.m. / ◻ p.m. ◻ Dose Not Taken Why: _____	
4			____:____ ◻ a.m. / ◻ p.m. ◻ Dose Not Taken Why: _____	
5			____:____ ◻ a.m. / ◻ p.m. ◻ Dose Not Taken Why: _____	
6			____:____ ◻ a.m. / ◻ p.m. ◻ Dose Not Taken Why: _____	
7			____:____ ◻ a.m. / ◻ p.m. ◻ Dose Not Taken Why: _____	

3. Symptoms Diary

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Yale New Haven Hospital

Study Participant
Symptoms Diary

Directions: Please record any changes in your health (i.e. any worsening in health or new symptoms). Please remember to also record any medications used to treat these symptoms.

Symptom	Date Started	Date Stopped or Resolved	Medication taken to treat symptoms, if any
Example: Headache	*1 / 19 / 21*	*1 / 20 / 21*	*Tylenol 500mg*
	___/___/___	___/___/___	
	___/___/___	___/___/___	
	___/___/___	___/___/___	
	___/___/___	___/___/___	
	___/___/___	___/___/___	
	___/___/___	___/___/___	
	___/___/___	___/___/___	
	___/___/___	___/___/___	
	___/___/___	___/___/___	

To be completed by study staff:	
Participant Identifier: CYPHY-_____	Page _____ of _____

CYPHY

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Notes/Questions for your next visit:

To be completed by study staff:	
Participant Identifier: CYPHY-_____	Page _____ of _____

4. Daily Temperature Diary

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Study Participant
Daily Temperature Diary

- Carefully place the tip of the thermometer under your tongue
- With your mouth closed, leave the thermometer in place for about 1 minute until you hear the "beep"
- Remove the thermometer and read the temperature
- Record temperature on the appropriate date

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Yale New Haven Hospital

February 2021

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
Jan 31	Feb 1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	Mar 1	2	3	4	5	6

To be completed by study staff:	
Participant Identifier: CYPHY-_____	Page _____ of _____