Clinical Research Protocol

OPTIMIZing Treatment for Early *Pseudomonas aeruginosa* Infection in Cystic Fibrosis

The OPTIMIZE Multicenter, Placebo-Controlled, Double-Blind, Randomized Trial

Protocol Number:	OPTIMIZE-IP-12
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Development Phase:	Phase 3
Sponsor:	Bonnie Ramsey, M.D. (Seattle Children's Research Institute)
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Anticipated Start Date	Winter 2013

Approval:

Sponsor-Investigator Signature (Bonnie Ramsey, MD) *Date* 9/2/16

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the Sponsor with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: OPTIMIZE-IP-12

Protocol Title: **OPTIMIZing Treatment for Early *Pseudomonas aeruginosa* Infection in Cystic Fibrosis: The OPTIMIZE Multicenter, Placebo-Controlled, Double-Blind, Randomized Trial**

Protocol Date: 22 August 2016

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Investigator Signature *Date*

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Print Name and Title

Site # _____

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	adverse event
AFB	acid fast bacilli
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BAL	bronchoalveolar lavage
BUN	blood urea nitrogen
CCC	Clinical Coordinating Center
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFR	Code of Federal Regulations
CFRSD	Cystic Fibrosis Respiratory Symptoms (or Signs) Diary
CFRSD-CRISS	CFRSD-Chronic Respiratory Infection Symptom Score
CFTR	cystic fibrosis transmembrane conductance regulator
CRF	case report form
CRP	C-reactive protein
CT	Computed tomography
CTCAE	Common Terminology Criterion for Adverse Events
dBHL	Decibels Hearing Level
DCC	Data Coordinating Center
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
eCRF	electronic case report from
EDC	electronic data capture
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	forced expiratory flow
FEV_1	forced expiratory volume over one second
FVC	forced vital capacity
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form

ICH	International Conference on Harmonisation
IGF	insulin growth factor
IL-8	Interleukin-8
IM	Inflammatory Biomarkers
IRB	Institutional Review Board
IRT	immunoreactive trypsinogen
IV	intravenous
LDH	lactate dehydrogenase
MD	Medical Doctor
MOA	mechanism of action
msec	millisecond
NHLBI	National Institutes of Health/National Heart, Lung, and Blood Institute
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NP	Nurse Practitioner
NTM	Non-tuberculous mycobacteria
OAE	Otoacoustic emissions
Pa	*Pseudomonas aeruginosa*
PA	Physician Assistant
PFT	pulmonary function test
PI	Principal Investigator
PR	Measure from beginning of P wave to onset of ventricular depolarization (R) - ECG measure
QRS	QRS interval; duration of the QRS complex in milliseconds – ECG measure
QT	QT interval; duration between start of **Q** wave and end of **T** wave in milliseconds - ECG measure
QTc	QT interval corrected
RC	Research Coordinator
RN	Registered Nurse
Sa	*Staphylococcus aureus*
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase

TCH CTRC	The Children's Hospital Clinical and Translational Research Center in Denver
TDNCC	Therapeutics Development Network Coordinating Center
TIS	tobramycin inhalation solution
VRA	Visual reinforcement audiometry

PROTOCOL SYNOPSIS

TITLE	OPTIMIZing Treatment for Early *Pseudomonas aeruginosa* Infection in Cystic Fibrosis: The OPTIMIZE Multicenter, Placebo-Controlled, Double-Blind, Randomized Trial
SPONSOR	Bonnie Ramsey, M.D.
FUNDING ORGANIZATION	National Institutes of Health/National Heart, Lung, and Blood Institute (NHLBI) Study Drug provided by Pfizer, Inc Study Drug provided by Novartis Pharmaceuticals Corp Study Compressors & Nebulizers provided by PARI Respiratory Equip, Inc
NUMBER OF SITES	Approximately forty-five (45)
RATIONALE	Cystic fibrosis (CF) is the most common autosomal recessively inherited disease among Caucasians, affecting approximately 25,000 to 30,000 individuals in the United States[1]. Although CF is a complex multi-system disease, 85% of the mortality results from lung disease, characterized by persistent infection and inflammation leading to progressive airway obstruction and bronchiectasis, and ultimately to respiratory failure[2]. *Pseudomonas aeruginosa* (*Pa*) is the most important pathogen and is acquired early in life, with an age specific prevalence of 30% by 10 years of age and ~ 70% by age 18[3, 4]. We have recently completed two multicenter trials demonstrating that chronic therapies in children with CF and mild disease may positively affect disease progression by different, potentially complementary mechanisms. First, the NIH-funded EPIC trial focused on identifying the optimal treatment strategy for early *Pa* infection in young children with CF[5]. The trial demonstrated that monotherapy with tobramycin inhalation solution (TIS) given only when quarterly cultures were *Pa* positive (termed "culture-based") was as efficacious as more intensive antibiotic regimens in terms of clinical and microbiologic efficacy, including pulmonary exacerbations and initial *Pa* eradication[5]. Yet, nearly 34% of EPIC trial participants still experienced *Pa* recurrence during the 18 month study and 50% experienced an exacerbation, one of the key signs of acute clinical worsening associated with a shorter time to recurrence of *Pa* infection[6] and poorer long-term clinical outcomes[7, 8]. Second, we conducted a randomized, placebo-controlled trial of oral azithromycin in children with

	CF uninfected with *Pa* that demonstrated a 50% reduction in pulmonary exacerbations in parallel with an anti-inflammatory effect among those treated with azithromycin[9, 10], similar to results in trials of azithromycin in older individuals with CF chronically infected with *Pa*[11-13]. Additional findings from the EPIC trial have led us to hypothesize that combining TIS and oral azithromycin, given their complementary routes and mechanisms of action (MOA), will be additive in their impact on early CF lung infection with *Pa*. First, among EPIC participants failing initial *Pa* eradication therapy, those with more frequent exacerbations were more likely to have chronic *Pa* infection[6]. In addition, persistent *Pa* strains had a phenotype consistent with early infection (highly antibiotic susceptible and non-mucoid), suggesting little exposure to the inhaled antibiotic, possibly due to inhibition of penetration by airway mucus, edema and inflammation. This theory is supported by the Australian longitudinal bronchoalveolar studies in infants with CF which demonstrated a significant inflammatory response in the first months of life and that *Pa* is the most pro-inflammatory of bacterial pathogens isolated[14, 15]. Based upon these findings, we hypothesize that a therapeutic approach that reduces the frequency of exacerbations and reduces airway inflammation may prolong the time to *Pa* recurrence. Specifically, **we hypothesize that chronic oral azithromycin will provide added clinical benefit to standardized anti-pseudomonal therapy with TIS among children with early *Pa* and will decrease the risk of pulmonary exacerbations, reduce inflammation, and reduce the rate of *Pa* recurrence.**
STUDY DESIGN	This is a multicenter, double-blind, randomized, placebo-controlled clinical trial in 274 children with CF ages 6 mos -18 years with early *Pa*, defined as either a first lifetime documented *Pa* culture or a *Pa* positive culture after at least two years of negative cultures. The study will assess the clinical and microbiologic efficacy and safety of azithromycin given three times weekly in combination with standardized TIS therapy among children with early *Pa*. TIS therapy is defined as an initial eradication treatment with 1-2 courses of 28 days TIS and subsequent 28 day treatments only at times a quarterly oropharyngeal culture is positive for *Pa*. Eligible participants will be randomized within 40 days from the date of collection of their *Pa* positive culture. Participants initiating TIS more than 14 days prior to the baseline visit for treatment of their *Pa* positive culture will be excluded. Participants will be

	randomized in a 1:1 fashion to receive one of the following two treatment strategies for 18 months: (1) oral placebo in addition to standardized TIS therapy, or (2) oral azithromycin in addition to standardized TIS therapy. Three times weekly administration of oral azithromycin or matching placebo will be continued throughout the 18-month trial. All participants will receive a 28-day course of TIS therapy during the first quarter of the trial and have a follow-up culture obtained to document *Pa* clearance. Participants who remain *Pa* positive after the first 28-day course of TIS will receive a second 28-day course of TIS. Subsequently, participants will receive TIS therapy (administered as a single 28-day course) only if their quarterly cultures are *Pa* positive. Cultures will be obtained at the start of each quarter for the duration of the 18-month study. Clinical and microbiologic outcomes will be assessed throughout the 18-month trial, including the occurrence of pulmonary exacerbations and *Pa* recurrence.
PRIMARY OBJECTIVE	To compare the time to pulmonary exacerbation between participants randomized to oral placebo and culture-based TIS versus oral azithromycin and culture-based TIS
SECONDARY OBJECTIVES	• Compare *Pseudomonas aeruginosa* (*Pa*) recurrence between treatment groups • Evaluate the safety profile of azithromycin in combination with culture-based TIS therapy as compared to culture-based TIS therapy alone, including the incidence of treatment emergent pathogens • Compare changes in plasma inflammatory markers between treatment groups
SECONDARY EXPLORATORY OBJECTIVES	• Compare hospitalization rates and other indices of clinical efficacy, including changes in height, weight, pulmonary function (in participants ≥4 years old) and respiratory symptoms, between treatment groups • Compare changes in the respiratory microbiome between treatment groups • Develop a comprehensive baseline predictive model of poor microbiologic or clinical response to therapy, including respiratory microbiome, inflammatory biomarkers, demographic and clinical characteristics in order to identify high risk children for future studies of alternative therapeutic approaches • Establish a linked data and specimen repository for future

	studies of disease mechanisms, potential novel therapeutic approaches and predicting response to therapy
NUMBER OF SUBJECTS	Approximately two-hundred seventy-four (274)
SUBJECT SELECTION CRITERIA	**Inclusion Criteria:** 1. Age ≥ 6 months to ≤ 18 years 2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype or positive CF Newborn Screening result for IRT/DNA or IRT/IRT **and** one or more of the following criteria: a. sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT) b. two well-characterized mutations in the cystic fibrosis transmembrane conductive regulator (CFTR) gene c. Abnormal nasal potential difference (change in NPD in response to a low chloride solution and isoproteronol of less than -5 mV) 3. Documented new positive oropharyngeal, sputum or lower respiratory tract culture for *Pa* obtained within 40 days of the Baseline Visit (Visit 1), defined as: a) first lifetime documented *Pa* positive culture; or b) *Pa* recovered after at least a two-year history of *Pa* negative respiratory cultures (≥ 1 culture/ year) 4. Clinically stable with no evidence of any significant respiratory symptoms at the Baseline Visit that would require administration of intravenous anti-pseudomonal antibiotics, oxygen supplementation, and/or hospitalization as determined by the study physician 5. Written informed consent obtained from participant or participant's legal representative (and assent when applicable) and ability for participant to comply with the requirements of the study **Exclusion Criteria:** 1. Macrolide antibiotic use within 30 days of the Baseline Visit 2. Initiation of current course of treatment with TIS >14 days prior to Baseline Visit

	3. Weight <6.0 kg at the Baseline Visit 4. History of aminoglycoside hypersensitivity or adverse reaction to inhaled aminoglycoside 5. History of azithromycin hypersensitivity or adverse reaction to azithromycin or allergy to macrolide antibiotics 6. History of positive respiratory culture for NTM or *B. cepacia* complex within 2 years of the Baseline Visit 7. History of unresolved, abnormal renal function (defined as serum creatinine greater than 1.5 times the upper limit of normal for age). 8. History of unresolved, abnormal liver function tests (defined as ALT and/or AST greater than 4 times the upper limit of normal range) or history of portal hypertension 9. History of unresolved, abnormal neutropenia (ANC ≤ 1000) 10. Abnormal ECG test at the Baseline Visit defined as a QTc (B) of ≥460 msec or history of ventricular arrhythmia 11. History of abnormal hearing sensitivity defined as hearing threshold levels >25 dB HL (decibels Hearing Level) for VRA (visual reinforcement audiometry) at any frequency (500-4000Hz) or >20 dB HL for play or standard audiometry at any two frequencies (500-8000Hz) in either ear, not associated with middle ear disease (including infection) or a flat (Type B) tympanogram 12. New initiation of chronic therapy (greater than 21 days) with drugs known to prolong QT interval (refer to Appendix III) within 30 days prior to the Baseline Visit or co-administration of nelfinavir or oral anticoagulants 13. Positive serum or urine pregnancy test at the Baseline Visit (to be performed on all females of child-bearing potential) or for females of child bearing potential: pregnant, breastfeeding, or unwilling to use barrier contraception during participation in the study 14. Administration of any investigational drug within 30 days prior to the Baseline Visit 15. Presence of a condition or abnormality (e.g., pre-existing heart disease) that in the opinion of the site investigator would compromise the safety of the participant or the quality of the data

TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Active treatment: azithromycin provided as 900 mg oral suspension in bottles. Product is reconstituted with 12 mL water. Final concentration = 200mg/5mL (final volume = 22.5 mL) Participant dose: Approximately 10 mg/kg up to a maximum of 500 mg per dose orally three times weekly
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Placebo: vehicle suspension. Product is reconstituted with 12 mL water. Final volume = 22.5 mL Participant dose: Equivalent volume to match test product dose of approximately 10 mg/kg up to a maximum of 500 mg per dose orally three times weekly
STANDARD OF CARE TREATMENT: **TOBRAMYCIN INHALATION SOLUTION**	Participants receive TIS (TOBI®) 300 mg BID. Each treatment course will be administered for 28 consecutive days by inhalation using the PARI LC PLUS™ Nebulizer. The initial two treatment courses of TIS will be provided as part of the study, unless TIS was prescribed and administered in the 14 days prior study enrollment. *Pa* positive cultures at subsequent study visits will be treated with TIS as standard of care and is required per protocol.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Participants will be on study for 18 months **Screening:** Eligible participants will be scheduled for Baseline Visit 1 within 40 days of their *Pa* positive culture and prior to receiving any anti-pseudomonal therapy other than TIS and ≤14 days from initiating TIS **Treatment:** 18 months (78 weeks ±2 weeks) **Follow-up:** Final Visit at Week 78 The total duration of the study is expected to be forty-nine (49) months: thirty-one (31) months for participant recruitment and eighteen (18) for final participant follow-up.
CONCOMITANT MEDICATIONS	**Allowed:** Standard therapy for CF is allowed except for treatments noted in the exclusion criteria and the prohibited medications section below. Ongoing chronic treatment (>30 days prior to the Baseline Visit) with inhaled dornase alpha, high dose ibuprofen, hypertonic saline, inhaled or systemic steroids, short and long-acting bronchodilators and airway clearance, and FDA-approved CFTR modulators is allowed. Acceptable chronic therapies initiated after study enrollment if clinically indicated include: inhaled dornase alpha, hypertonic saline, short/long acting bronchodilators, airway clearance, and FDA-approved CFTR modulators.

	Acute treatment with drugs categorized as known to prolong QT interval is allowed according to the following: • For drugs commonly used to treat CF patients (e.g. ciprofloxacin, levofloxacin, moxifloxacin, fluconazole, propofol, sevoflurane, ondansetron), study drug does not need to be stopped. • For all other drugs categorized as known to prolong QT interval, acute use is allowed, but study drug (azithromycin or placebo) **must** be temporarily stopped (Refer to Section 11.2). After acute treatment is completed, participants should re-start study drug on the next scheduled treatment day. Drugs known to prolong QT interval are described as follows: "Substantial evidence supports the conclusion that these drugs prolong the QT interval AND are clearly associated with a risk of torsades de pointes (TdP), even when taken as directed in official labeling" and listed on the CredibleMeds® Website (Composite List of All QT Drugs under the category "Known Risk of TdP"). Treatment for pulmonary exacerbations and as required for acute care is allowed except for treatments noted in the prohibited medications section below. Physicians are encouraged to prescribe acute antibiotic therapy only in the presence of symptoms. **Prohibited:** The following medications are prohibited until the end of study (Visit 8, Week 78): • Investigational therapies • Chronic inhaled antibiotics (e.g., Cayston®, colistin). Chronic treatment is defined as more than 1 course of therapy within a 6-month period. • Initiation of chronic treatment (defined as >21 days of treatment) with high dose ibuprofen and systemic steroids The following medications are prohibited when the participant is concurrently receiving study drug. **Therefore, study drug must be stopped during concurrent administration of these medications.** • Macrolides (other than study drug): Acute treatment with macrolides, including azithromycin is allowed however no acute macrolides may be prescribed without stopping study drug (azithromycin or placebo). After acute treatment is completed, participants should re-start study drug on the

	next scheduled treatment day. • Drugs known to prolong QT interval (Composite List of All QT Drugs under the category "Known Risk of TdP"). (Refer to Appendix III and Section 6.1). • Nelfinavir or oral anticoagulants •
EVALUATIONS	
PRIMARY ENDPOINT	Difference between treatment groups in the time to a protocol-defined pulmonary exacerbation. Pulmonary exacerbations will be defined according to an *a priori* sign and symptom-based definition.
SECONDARY ENDPOINTS	1. Time to *Pa* recurrence over the 18-month study period 2. Safety as measured by the incidence of adverse events and laboratory abnormalities over the 18-month study period 3. Changes from baseline in plasma inflammatory markers Secondary Exploratory Endpoints: 4. Proportion of participants with initial *Pa* eradication success, defined as a *Pa* negative respiratory culture at Week 13 5. Proportion of all cultures obtained that are positive for *Pa* during the 18-month study period 6. Prevalence of treatment-emergent *Staphylococcus aureus* (methicillin susceptible and resistant), *Haemophilus influenzae, Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and *Alcaligenes (Achromobacter) xylosoxidans* (from oropharyngeal and sputum cultures), and non-tuberculous mycobacteria (in sputum only) during the 18-month period 7. Proportion of participants hospitalized over the 18-month study 8. Proportion of participants prescribed acute oral, inhaled, and IV acute antibiotics over the 18-month study 9. Change from baseline to the end of the 18-month study in pulmonary function (FVC, FEV_1, $FEF_{25-75\%}$) in those old enough to perform spirometry (≥4 years of age) 10. Change from baseline to the end of the 18-month study in weight and height 11. Change from baseline to the end of the 18-month study in the self-report and parent-completed Cystic Fibrosis Respiratory Diary scores

	12. Changes from baseline in the respiratory microbiome 13. Association of baseline inflammatory marker values, microbial diversity measures and other baseline clinical and demographic characteristics with clinical and microbiologic outcomes over the 18-month study
PLANNED INTERIM ANALYSES	An independent data safety monitoring board (DSMB) will review comprehensive interim reports on a semi-annual basis. *A priori* interim stopping rules for efficacy and safety of the primary endpoint will be guided as outlined in the DSMB charter. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	An intent to treat analysis will be used for the primary analysis, including all participants who were randomized. The primary endpoint, time to pulmonary exacerbation, will be compared between treatment groups using Cox proportional hazards regression adjusting for randomization strata (age 6 mos-3 years, >3-6 years, >6-12 years, >12-18 years). Hazard ratios and 95% confidence intervals will be used to describe the treatment effect and p-values will be evaluated against a two-sided 0.05 level of significance.
Rationale for Number of Subjects	Assuming a two-sided 0.05 level type I error and a sample size of approximately 137 children randomized to each treatment group (culture-based TIS with oral placebo or culture-based TIS with oral azithromycin), the study has 90% power to detect a hazard ratio for the time to exacerbation of 0.53 or smaller (a 47% or greater reduction) between treatment groups and 80% power to detect a hazard ratio of 0.58 or smaller (a 42% or greater reduction). These estimates account for six planned interim analyses and one final analyses of the primary endpoint. With a maximum projected withdrawal rate of at most 9% over the course of the 18 month study based on the prior EPIC trial with similar duration of follow-up, it is conservatively estimated that the study still has at minimum 80% power to detect a hazard ratio of 0.56 or smaller between treatment groups (a 44% or greater reduction).

1 BACKGROUND AND RATIONALE

CF lung disease begins in the first few months of life and follows a course of recurrent lower airway bacterial infection and inflammation[16-18], early gas trapping and bronchiectasis[19], and progression of disease over years and decades at a variable pace. With the development of chronic lung infection, obstructive disease progressively worsens, ultimately leading to respiratory failure[3, 4, 20, 21]. *Pseudomonas aeruginosa* (*Pa*) is the most important pathogen infecting the CF lower airways[17, 22], and its acquisition early in life is associated with a pro-inflammatory effect[14], lower lung function, poor nutritional outcomes, and decreased survival[21]. Greater morbidity and cost of care is associated with chronic *Pa* infection including increased rate of hospitalizations and greater need for antibiotic therapy[2, 23]. In spite of aggressive therapy, more rapid decline in lung function and higher mortality rates ensue[24-31].

Pa infection of the CF airway typically proceeds from *early* infection to chronic infection, defined as >50% of cultures positive for *Pa* in a 12 month period[3, 32]. Early *Pa* strains have distinct characteristics more likely to respond to antibiotic therapy, including non-mucoid phenotype, broad antimicrobial susceptibility and significantly lower colony density [16]. Over time, *Pa* forms biofilms within the CF airway environment[33] and develops a mucoid phenotype [31] making it more refractory to treatment. Although some studies have shown that a minority of individuals with CF spontaneously clear early *Pa* infection[3, 34], data from multiple studies suggest that antibiotics are superior to no treatment in clearing *Pa* from respiratory cultures[3, 34-39]. Understanding the transition period from early to chronic *Pa* infection is thus of critical importance in identifying strategies to prevent this progression.

Therapeutic approaches to treatment of early *Pa* infection have varied by antibiotic type, route, duration of treatment, and use of single or multiple courses, making interpretation challenging for clinicians eager to adopt these treatment strategies[3, 34-39]. The focus of these trials was primarily towards the microbiologic endpoint of clearance of *Pa* from either the upper[34, 35, 37] or lower [40, 41]airway rather than clinical endpoints. Several studies examined the durability of microbiologic effect (i.e., time to *Pa* recurrence)[36, 37, 39, 40]. One of the largest studies was the ELITE trial[39], which compared the efficacy of tobramycin inhalation solution (TIS, TOBI®) given for 28 days as compared to 56 days for individuals with CF >6 months of age (90% were under 18 years of age) with early *Pa* infection and demonstrated that time to *Pa* recurrence did not differ by duration of initial therapy, with an average of 33% of individuals having *Pa* recurrence within the 27 months of follow-up. Two intervention studies of early *P*a infection have compared multiple anti-pseudomonal treatment strategies, one focused on *Pa* eradication after 28 days of therapy[42] and the other on clinical outcomes, in addition to microbiologic endpoints, over an 18 month period[5]. The 28-day study was a randomized, open label trial which compared inhaled colistin with oral ciprofloxacin (the CC group, n=105) versus TIS and oral ciprofloxacin (the TC group, n=118) at the time of initial *Pa* infection. At 28 days, the eradication rate was not significantly different between the 2 regimens (CC: 62.8% vs. TC: 65.2%). The second study was the EPIC clinical trial supported by NHLBI, NIDDK and CFF (Grant U01-HL080310)[5] in which 306 children with early *Pa* were randomized to TIS given at quarterly intervals vs. TIS given only when quarterly cultures were positive for *Pa* (culture-based therapy). The primary clinical outcome was time to pulmonary exacerbation. All children enrolled in the trial received an initial course of TIS (with or without oral ciprofloxacin) to induce eradication before proceeding to their randomized treatment arms. There were several key findings from this study. First, there was no evidence to suggest that more intensive quarterly treatment with TIS (with or without ciprofloxacin) was

more effective than therapy given only when cultures were positive for *Pa,* both in terms of time to pulmonary exacerbation and *Pa* recurrence[5]. Second, the initial TIS treatment course successfully resulted in negative *Pa* cultures in 85% of children although by the end of the study 34% had at least one *Pa* recurrence. The addition of ciprofloxacin to the TIS therapy conferred no additional benefit throughout the 18 month study. Third, 50% of all participants experienced a pulmonary exacerbation, and the subset of children failing initial eradication were at the highest risk for exacerbations and progression to chronic *Pa* infection[6].

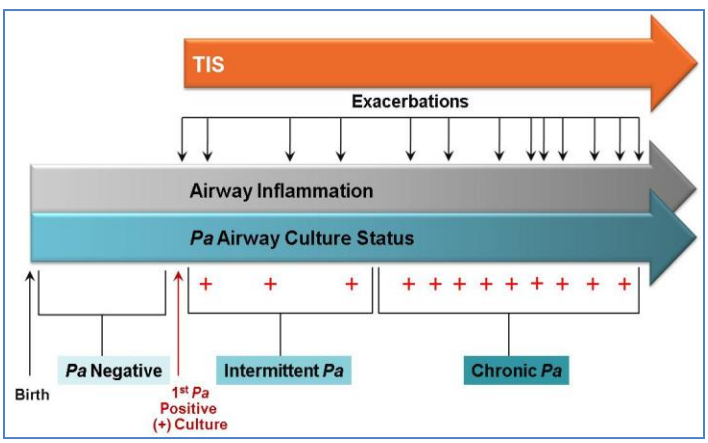
Based upon the accumulative published data from the studies outlined above, a working group of European and North American CF clinicians met in April 2012 to make recommendations for early *Pa* eradication therapy. The consensus of the group was that antibiotic treatment for first or recent *Pa* acquisition is indicated (consistent with a recent Cochrane Review in 2009[38]) and that either TIS monotherapy[5, 39] or inhaled colistin with oral ciprofloxacin are efficacious[42] and should be considered standard of care[43]. In general, inhaled colistin with oral ciprofloxacin is used at European sites and TIS is primarily used in the US. Among a sub-cohort of children enrolled in the U.S. Cystic Fibrosis Foundation (CFF) National Patient Registry[44] with first or recent acquisition of *Pa* in 2010, 82% were treated with TIS. By contrast, no clinical trials have demonstrated azithromycin to be bactericidal for *Pa* as evidenced by a decrease in bacterial load [13]. For this reason, azithromycin has not been considered as a potential therapy for eradication of early *Pa* infection and its use in this population is low. The 2010 Cystic Fibrosis Foundation (CFF) national patient registry reported only 27% of patients with first or recent *Pa* had been prescribed chronic azithromycin therapy (personal communication, Bruce Marshall, MD, CFF).

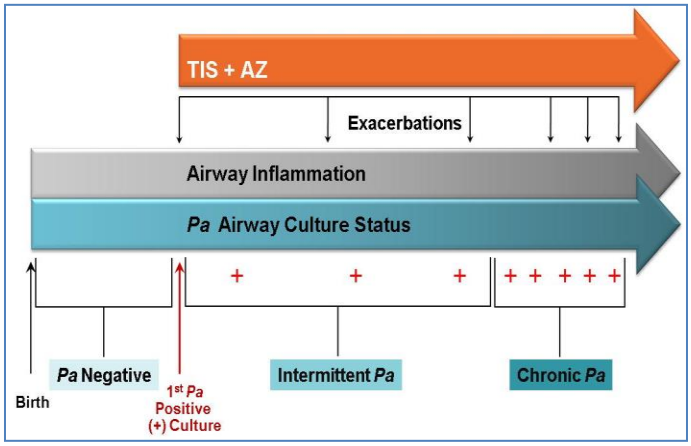
There have been, however, multiple trials assessing the clinical, rather than microbiologic impact of azithromycin for established *Pa* infection[11-13] with clear evidence of impact on clinical endpoints including significant reductions in exacerbations, improvement in pulmonary function, and weight gain as compared to placebo[11-13]. Part of this clinical impact may be due to an anti-inflammatory effect[10, 11, 45], as demonstrated by a reduction in a range of serum inflammatory markers including calprotectin, serum amyloid antigen, absolute neutrophil count and C-reactive protein (CRP) at both 28 and 168 days of chronic therapy[10]. Azithromycin has been studied in younger, healthy children with CF without chronic *Pa*[9] showing a 50% reduction in the rate of pulmonary exacerbations among those treated with azithromycin and improved body weight[9]. These are encouraging findings but no studies of macrolide antibiotics such as azithromycin have been focused on the question of enhancing eradication of early *Pa* infection even though the complementary mechanisms of action of azithromycin could enhance the efficacy of proven anti-pseudomonal therapies, such as TIS.

It is very encouraging that the majority of children with early *Pa* infection will rapidly clear the pathogen from both upper[5, 39] and lower[40] airway secretions. Challenges, however, remain. First and foremost, *Pa* recurrence may occur within months[5, 39]. In both the EPIC and ELITE trials, one-third of participants had recurrence of *Pa* during the 18 month and 27 month follow-up periods, respectively. In the EPIC Observational study, an ongoing prospective study of risk factors for *Pa* acquisition in young *Pa*-negative children with CF[46], the rate of *initial Pa* acquisition among 889 children with a mean age of 4.5 (SD 3.5) years was 18 cases per 100 person-years (95% confidence interval [CI] 17, 20), while the rate of *subsequent Pa* acquisition was slightly higher at 23 cases (95% CI: 21, 25) per 100 person-years. These data suggest that children with a history of *Pa*, even following successful eradication, are a higher-risk population requiring close monitoring. There are likely multiple factors associated with re-infection. In the EPIC Clinical trial, a significant association was observed between the occurrence of an

exacerbation and *Pa* recurrence[6]. Notably since 50% of the children in the EPIC trial experienced an exacerbation requiring acute antibiotic intervention (in addition to the study drug) during the follow up across treatment arms, a therapeutic approach that reduces the frequency of exacerbations may prolong the time to re-infection and potentially delay chronic *Pa* infection (Figure 1).

Figure 1. Hypothetical Benefits of the Proposed Intervention. *We hypothesize that the addition of azithromycin to TIS in children and adolescents with CF and early Pa will decrease exacerbation frequency, resulting in a delay in time to Pa recurrence. Through this process it is hoped that progression to chronic infection will also be delayed, although the time course for progression to chronic infection is beyond the duration of this study.*





The current proposal is a multicenter, randomized, placebo-controlled trial designed to evaluate the efficacy and safety of combining two therapeutic mechanisms of action to address early *Pa* infection in CF. Participants will be randomized to either oral azithromycin or oral placebo, in addition to receiving a standardized anti-pseudomonal treatment regimen with TIS. For the purposes of this proposal, TIS therapy is defined as an initial eradication treatment with 1-2 courses of 28 days TIS and subsequent 28 day treatments only at times a quarterly oropharyngeal or sputum culture is positive for *Pa*. TIS, an aminoglycoside, is a well established bactericidal antibiotic in vitro[47] and in vivo[48] in CF respiratory secretions. Azithromycin, a macrolide antibiotic, has no demonstrated antimicrobial efficacy in vivo[13]or in vitro against *Pa* growing in culture in logarithmic phase[49], but may be effective in vivo at killing *Pa* in biofilms[50, 51]. In addition, it has a demonstrated anti-inflammatory effect[10, 11, 13, 45]. These two approaches are complementary and should be additive. It is additionally possible that the two antibiotic approaches may be synergistic in vivo because macrolides may reduce quorum-sensing, motility and biofilm formation, all critical to *Pa* virulence and its ability to evade killing by aminoglycosides[52-55]. A modest degree of in vitro synergy has also been demonstrated against *Pa* in CF [56]. Thus, the combined therapy, as compared to TIS monotherapy, is hypothesized to demonstrate improved efficacy both for clinical outcomes including decreasing the risk of pulmonary exacerbation, and microbiologic endpoints including delaying *Pa* recurrence (Figure 1). The primary goal of this proposed study is to evaluate the effectiveness of adjunctive therapy with azithromycin to improve clinical outcome. Based upon the findings of these 2 trials and our current understanding of the drugs' mechanisms of action, **we hypothesize that the use of chronic oral azithromycin will provide added clinical benefit to the use of standardized anti-pseudomonal therapy with TIS among children**

with early *Pa* and decrease the risk of pulmonary exacerbation, reduce inflammation, and reduce the rate of *Pa* recurrence.

1.1 Risk / Benefit Assessment

Risks of Study Drug: While the safety and efficacy of azithromycin treatment given for 6 months has been well studied, there is no published safety and efficacy data for azithromycin for longer periods in the context of a placebo-controlled clinical trial or among children with CF less than 6 years of age[57]. Overall, gastrointestinal side effects have been comparable in individuals with CF to those described in other populations[58]. One study reported 33% of azithromycin vs.16% of placebo participants had nausea and 23% of azithromycin vs. 8% of placebo participants had diarrhea[13] and a subsequent study performed in children and adolescents with CF, demonstrated comparable gastrointestinal side effects between azithromycin and placebo groups[9]. Hepatotoxicity associated with azithromycin has not been detected as measured by elevations in AST, ALT, or GGT [9, 13]. No increase in subjective hearing loss or tinnitus has been noted among azithromycin participants in the CF studies [9, 12, 13]. However, hearing loss has been reported in adults with chronic obstructive lung disease[59] and cardiovascular (CV) disease[60] who received chronic azithromycin therapy for one year. Hearing loss and/or tinnitus are noted under post-marketing experience in the drug label although causal relationship to drug product is not possible to ascertain. A report[61] of a retrospective analysis of Tennessee Medicaid records identified a small increase risk (HR 2.88,95%CI 1.79,4.63, p<0.001) for CV sudden death in older adults (mean age > 60 years) with CV risk factors. By contrast, 2 large randomized controlled trials[60, 62] of adults with known CV disease receiving chronic azithromycin therapy did not observe an increase in CV death and a large retrospective Danish study[63] found the risk of CV death comparable to penicillin, an antibiotic not known to have CV effects. Because of the concern raised by the Tennessee study[63] that azithromycin may have arrhythmogenic potential in patients with increased CV risk (i.e., history of documented prolonged QT interval, hypokalemia, hypomagnesemia, bradycardia, or co-administration of other known arrhythmogenic drugs), in 2013, the FDA revised the azithromycin label to reflect this information[64]. The eligible population for this proposed CF study, children with no known CV risk factors, should not be at increased risk for macrolide associated CV sudden death. An increased risk of treatment-emergent macrolide-resistant S. aureus and H. influenzae has been observed [9] but there are no data to suggest an impact on clinical outcome. Although a recent report presents data from experiments using an animal model and clinical data from one site about a potential link between macrolides and increased risk of *M. abscessus*[65], no increased risk of treatment-emergent non-tuberculous mycobacteria (NTM) has been observed in any controlled clinical trials[9, 13, 66]. A large epidemiologic study conducted using the CFF National patient registry found that patients with the greatest number of years of chronic macrolide usage were the least likely to develop incident NTM[67].

Risks of Study Procedures: All study procedures performed during the study are consistent with standard of clinical care and there are no perceived additional risks in regard to physical well-being. During the study, there will be additional blood draws and respiratory culture collections. The risk of collecting blood includes soreness, bruising, mild pain, mild bleeding or infection at the site. There is also risk of fainting or light-headedness. Topical application of a numbing agent to reduce the pain from the blood draws will be allowed. This may cause mild skin discoloration or swelling that resolves in 1 to 2 hours. The collection of the respiratory samples from oropharyngeal swab may cause minor brief discomfort, gagging or coughing. There is a small risk of wheezing, shortness of breath, and increased cough when

performing spirometry and redness of the skin or minor skin irritation may occur when performing ECG testing and oximetry.

Risks to Confidentiality: As with any research study, there is a potential risk of breach of confidentiality. Study personnel at each site will enter data from source documents into a protocol-specific electronic Case Report Form (eCRF). Study participants will not be identified by name in the study database or on any data capture screens, but will be identified by initials and a subject identification number unique to this study. Only authorized individuals will be able to link the study ID to the subject's name (site personnel at the individual sites and DCC clinical site monitors or auditors). All investigators and staff involved in the proposed research will have completed human subjects' protection training and be bound by the agreement of confidentiality.

Possible Benefit: The combined therapy of TIS and azithromycin, as compared to TIS monotherapy, is hypothesized to demonstrate improved efficacy both for clinical outcomes including decreasing the risk of pulmonary exacerbation and for microbiologic endpoints including delaying *Pa* recurrence.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to compare the time to pulmonary exacerbation between participants randomized to oral placebo with TIS versus oral azithromycin with TIS.

2.2 Secondary Objectives

The secondary objectives of this study are to:

1. Compare *Pseudomonas aeruginosa* (*Pa*) recurrence between treatment groups
2. Evaluate the safety profile of azithromycin in combination with culture-based TIS therapy as compared to culture-based TIS therapy alone, including the incidence of treatment emergent pathogens
3. Compare changes in plasma inflammatory markers between treatment groups

Secondary Exploratory Objectives

4. Compare hospitalization rates and other indices of clinical efficacy, including changes in height, weight, pulmonary function (in participants ≥4 years old) and respiratory symptoms, between treatment groups
5. Compare changes in the respiratory microbiome between treatment groups
6. Develop a comprehensive baseline predictive model of poor microbiologic or clinical response to therapy, including respiratory microbiome, inflammatory biomarkers, demographic and clinical characteristics in order to identify high risk children for future studies of alternative therapeutic approaches
7. Establish a linked data and specimen repository for future studies of disease mechanisms, potential novel therapeutic approaches and predicting response to therapy

3 STUDY DESIGN

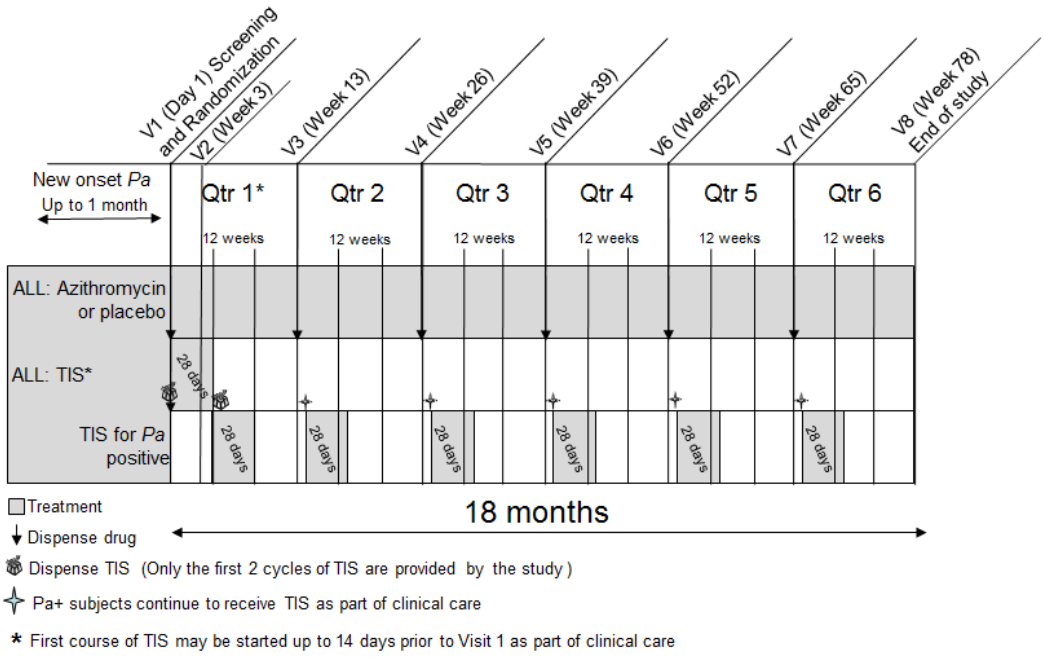
3.1 Study Overview

This is a multicenter, double-blind, randomized, placebo-controlled clinical trial in 274 children with CF ages 6 mos -18 years with early *Pa*, defined as either a first lifetime documented *Pa* culture or a *Pa* positive culture after at least two years of negative cultures. The study will assess the clinical and microbiologic efficacy and safety of azithromycin given three times weekly in combination with standardized TIS therapy among children with early *Pa*. TIS therapy is defined as an initial eradication treatment with 1-2 courses of 28 days TIS and subsequent 28-day treatments only at times a quarterly oropharyngeal culture is positive for *Pa*. Eligible participants will be randomized within 40 days from the date of collection of their *Pa* positive culture. Participants initiating TIS more than 14 days prior to the baseline visit for treatment of their *Pa* positive culture will be excluded. Participants will be randomized in a 1:1 fashion to receive one of the following two treatment strategies for 18 months: (1) oral placebo in addition to standardized TIS therapy, or (2) oral azithromycin in addition to standardized TIS therapy.

Three times weekly administration of oral azithromycin or matching placebo will be continued throughout the 18 month trial. All participants will receive a 28-day course of TIS therapy during the first quarter of the trial and have a follow-up culture obtained to document *Pa* clearance. Participants who remain *Pa* positive after the first 28-day course of TIS will receive a second 28-day course of TIS. Subsequently, participants will receive TIS therapy (administered as a single 28-day course) only if their quarterly cultures are *Pa* positive. Cultures will be obtained at the start of each quarter for the duration of the 18 month study (see Figure 2).

Clinical and microbiologic outcomes will be assessed throughout the 18-month trial, including the occurrence of pulmonary exacerbations and *Pa* recurrence.

Figure 2



The total duration of the study is expected to be forty-nine (49) months: thirty-one (31) months for participant recruitment and eighteen (18) for final participant follow-up.

4 CRITERIA FOR EVALUATION

4.1 Primary Efficacy Endpoint

The primary endpoint in the study is the difference between treatment groups in the time to a protocol-defined pulmonary exacerbation, comparing oral placebo with TIS versus oral azithromycin with TIS. Pulmonary exacerbations will be defined according to an *a priori* sign and symptom-based definition (Appendix II).

4.2 Secondary Endpoints

1. Time to *Pa* recurrence over the 18-month study period
2. Safety as measured by the incidence of adverse events and laboratory abnormalities over the 18-month study period
3. Changes from baseline in plasma inflammatory markers

Secondary Exploratory Endpoints

4. Proportion of participants with initial *Pa* eradication success, defined as a *Pa* negative respiratory culture at Week 13
5. Proportion of all cultures obtained that are positive for *Pa* during the 18-month study period
6. Prevalence of treatment-emergent *Staphylococcus aureus* (methicillin susceptible and resistant), *Haemophilus influenzae*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and *Alcaligenes (Achromobacter) xylosoxidans* (from oropharyngeal and sputum cultures), and non-tuberculous mycobacteria (in sputum only) during the 18-month period
7. Proportion of participants hospitalized over the 18-month study
8. Proportion of participants prescribed acute oral, inhaled, and IV acute antibiotics over the 18-month study
9. Change from baseline to the end of the 18-month study in pulmonary function (FVC, FEV_1, $FEF_{25-75\%}$) in those old enough to perform spirometry (≥4 years of age)
10. Change from baseline to the end of the 18-month study in weight and height
11. Change from baseline to the end of the 18-month study in the self-report and parent-completed Cystic Fibrosis Respiratory Diary scores
12. Changes from baseline in the respiratory microbiome
13. Association of baseline inflammatory marker values, microbial diversity measures and other baseline clinical and demographic characteristics with clinical and microbiologic outcomes over the 18-month study

5 PARTICIPANT SELECTION

5.1 Study Population

Children and adolescents with a diagnosis of CF who meet the inclusion and exclusion criteria will be eligible for participation in this study.

5.2 Inclusion Criteria

1. Age ≥ 6 months to ≤ 18 years
2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype or positive CF Newborn Screening result for IRT/DNA or IRT/IRT and one or more of the following criteria:
    a. sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT)
    b. two well-characterized mutations in the cystic fibrosis transmembrane conductive regulator (CFTR) gene
    c. Abnormal nasal potential difference (change in NPD in response to a low chloride solution and isoproteronol of less than -5 mV)
3. Documented new positive oropharyngeal, sputum or lower respiratory tract culture for *Pa* obtained within 40 days of the Baseline Visit (Visit 1), defined as: a) first lifetime documented *Pa* positive culture; or b) *Pa* recovered after at least a two-year history of *Pa* negative respiratory cultures (≥ 1 culture/ year)
4. Clinically stable with no evidence of any significant respiratory symptoms at the Baseline Visit that would require administration of intravenous anti-pseudomonal antibiotics, oxygen supplementation, and/or hospitalization as determined by the study physician
5. Written informed consent obtained from participant or participant's legal representative (and assent when applicable) and ability for participant to comply with the requirements of the study

5.3 Exclusion Criteria

1. Macrolide antibiotic use within 30 days of the Baseline Visit
2. Initiation of current course of treatment with TIS >14 days prior to Baseline Visit
3. Weight <6.0 kg at the Baseline Visit
4. History of aminoglycoside hypersensitivity or adverse reaction to inhaled aminoglycoside
5. History of azithromycin hypersensitivity or adverse reaction to azithromycin or allergy to macrolide antibiotics
6. History of positive respiratory culture for NTM or *B. cepacia* complex within 2 years of the Baseline Visit
7. History of unresolved, abnormal renal function (defined as serum creatinine greater than 1.5 times the upper limit of normal for age).
8. History of unresolved, abnormal liver function tests (defined as ALT and/or AST greater than 4 times the upper limit of normal range) or history of portal hypertension
9. History of unresolved, abnormal neutropenia (ANC ≤ 1000)
10. Abnormal ECG test at the Baseline Visit defined as a QTc (B) of ≥460 msec or history of ventricular arrhythmia

11. History of abnormal hearing sensitivity defined as hearing threshold >25 dB HL (decibels Hearing Level) for VRA (visual reinforcement audiometry) at any frequency (500-4000Hz) or >20 dB HL for play or standard audiometry at any two frequencies (500-8000Hz) in either ear, not associated with middle ear disease (including infection) or a flat (Type B) tympanogram
12. New initiation of chronic therapy (greater than 21 days) with drugs known to prolong QT interval (refer to Appendix III) within 30 days prior to the Baseline Visit or co-administration of nelfinavir or oral anticoagulants
13. Positive serum or urine pregnancy test at the Baseline Visit (to be performed on all females of child-bearing potential) or for females of child bearing potential: pregnant, breastfeeding, or unwilling to use barrier contraception during participation in the study
14. Administration of any investigational drug within 30 days prior to the Baseline Visit
15. Presence of a condition or abnormality (e.g., pre-existing heart disease) that in the opinion of the site investigator would compromise the safety of the participant or the quality of the data

5.4 Study Specific Tolerance for Inclusion/Exclusion Criteria

Participants who fail to meet all 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.

5.5 Screen Fail Criteria

Any consented participant 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. Participants who fail screening can be rescreened once if the site investigator feels they meet eligibility criteria and following confirmation from the study monitor. Rescreened participants will complete all screening procedures (e.g., results from previous screening tests cannot be used).

6 CONCURRENT MEDICATIONS

All participants should be maintained on the same medications throughout the entire study period, without introduction of new chronic therapies, unless medically indicated.

6.1 Allowed Medications and Treatments

Standard therapy for CF is allowed except for treatments noted in the prohibited medications section below.

Ongoing chronic treatment (>30 days prior to the Baseline Visit) with inhaled dornase alpha, high dose ibuprofen, hypertonic saline, inhaled or systemic steroids, short and long-acting bronchodilators, airway clearance, and FDA-approved CFTR modulators is allowed. Acceptable chronic therapies initiated after study enrollment if clinically indicated include: inhaled dornase alpha, hypertonic saline, short/long acting bronchodilators, airway clearance, and FDA-approved CFTR modulators.

Acute treatment with drugs categorized as known to prolong QT interval is allowed according to the following:

- For drugs commonly used to treat CF patients (e.g. ciprofloxacin, levofloxacin, moxifloxacin, fluconazole, propofol, sevoflurane, ondansetron), study drug does not need to be stopped.
- For all other drugs categorized as known to prolong QT interval, acute use is allowed, but study drug (azithromycin or placebo) **must** be temporarily stopped (Refer to Section 11.2). After acute treatment is completed, participants should re-start study drug on the next scheduled treatment day.

Drugs known to prolong QT interval are described as follows: "Substantial evidence supports the conclusion that these drugs prolong the QT interval AND are clearly associated with a risk of torsades de pointes (TdP), even when taken as directed in official labeling" and listed on the CredibleMeds® Website (Composite List of All QT Drugs under the category "Known Risk of TdP").

Treatment for pulmonary exacerbations and as required for acute care is allowed except for treatments noted in the prohibited medications section below. Physicians are encouraged to prescribe acute antibiotic therapy only in the presence of symptoms.

6.2 Prohibited Medications and Treatments

The following medications are prohibited until the end of study (Visit 8, Week 78):

- Investigational therapies
- Chronic inhaled antibiotics (e.g., Cayston®, colistin). Chronic treatment is defined as more than 1 course of therapy within a 6-month period.
- Initiation of chronic treatment (defined as >21 days of treatment) with high dose ibuprofen and systemic steroids

The following medications are prohibited when the participant is concurrently receiving study drug. Therefore, study drug must be stopped during concurrent administration of these medications.

- Macrolides (other than study drug): Acute treatment with macrolides, including azithromycin is allowed however no acute macrolides may be prescribed without temporarily stopping study drug (azithromycin or placebo). After acute treatment is completed, participants should re-start study drug on the next scheduled treatment day.
- Drugs known to prolong QT interval (Composite List of All QT Drugs under the category "Known Risk of TdP"). (Refer to Appendix III and Section 6.1)
- Nelfinavir or oral anticoagulants

7 STUDY TREATMENTS

7.1 Method of Assigning Participants to Treatment Groups

Study personnel at the investigative site will use the Medidata Rave® and Balance™ systems to randomize each participant. An adaptive randomization (dynamic allocation based on minimization[68]) will be employed with the goal of ensuring equal representation in each study arm for each age strata. Participants will be randomly assigned in a 1:1 ratio to one of two arms: 1) oral placebo with TIS therapy, or 2) oral azithromycin with TIS therapy. The adaptive randomization algorithm will include

baseline age group (age 6 mos-3 years, >3-6 years, >6-12 years, >12-18 years). The dynamic allocation algorithm seeks to optimize randomization balance by minimizing a weighted average of the marginal imbalance[69] of treatment allocation for each factor and for the study overall. A random element is added to the otherwise deterministic minimization algorithm to reduce allocation predictability by using a biased coin[70] to include a chance of allocation to a treatment arm other than the arm that optimizes balance. After baseline data collection, participants will be randomized to one of the two study groups. To randomize a new participant, study personnel at the investigative site will complete the electronic case report form (eCRF) in the Medidata Rave system required to provide the stratification information and the participant will then be assigned to a study arm.

7.2 Blinding

Due to the objectives of the study, the identity of azithromycin and placebo treatments will not be known to participants, parents/legal guardians of the participants, and site research staff. The following study procedures will be in place to ensure double-blind administration of test and control (azithromycin/placebo) study treatments:

- Randomization assignments will be stored in a secure database and appropriately protected and backed up
- Access to the randomization code will be strictly controlled and limited to select members of the Data Coordinating Center (DCC) who are involved in the generation of interim reports for the DSMB
- Azithromycin and placebo will be manufactured to be identical in appearance and taste-matched
- Packaging and labeling of treatments will be identical

During the study, the blind may be broken for individual participants in emergencies when knowledge of the participant's treatment group is necessary for further patient management or when the event meets the FDA expedited reporting requirements as a suspected adverse reaction that is serious and unexpected (21 CFR 312.32(c)(1)(i)). In those cases, knowledge of the treatment received is necessary for interpreting the event. If the blind is broken and the subject was receiving placebo, an IND safety report would not be submitted. However, if an IND safety report is submitted to FDA, all participating investigators would be notified. Emergency unblinding procedures are provided in the Study Procedures Manual (Refer to Protocol Section 10.3 for Serious Adverse Experience reporting).

The study blind will be broken on completion of the clinical study and after the study database has been locked. The site investigators will be provided with each participant's treatment assignment after the statistical report is finalized.

7.3 Formulation of Test and Control Products

7.3.1 Formulation of Test Product

Active treatment: Azithromycin, 900 mg oral suspension will be provided by Pfizer, Inc. The product must be reconstituted with 12 mL water before use. Final concentration = 200mg/5mL (final volume = 22.5 mL).

7.3.2 Formulation of Control Product

Placebo: Vehicle suspension, taste- matched to the azithromycin will be provided by Pfizer, Inc. The placebo is reconstituted with 12 mL water before use. Final volume = 22.5 mL

7.3.3 Packaging and Labeling

Azithromycin and Placebo- Product will be provided as 900 mg bottles by the manufacturer. Bottles will be labeled and assembled into kits containing 30 bottles each. Kits will be labeled as blinded study drug. A 30-bottle kit will provide sufficient study drug for 12 weeks + 3 weeks.

7.4 Supply of Study Drug to the Site

The Investigational Drug Service at Seattle Children's Hospital (Central Pharmacy) will ship study drug to the investigational sites. The initial study drug shipment will be shipped after site activation (i.e., all required regulatory documentation has been received by the Sponsor and a contract has been executed). Subsequent study drug shipments will be made as needed.

7.4.1 Dosage/Dosage Regimen

Azithromycin or Placebo – All participants receive azithromycin or placebo for the 18 months of study enrollment. Each participant administers a dose of approximately 10mg/kg/dose up to maximum of 500 mg per dose orally three times weekly (e.g. Monday-Wednesday-Friday) per the Table 1 below.

Table 1

Weight (kg)	Dose volume	Dose (mg)
5.0-7.9	1.5 mL	60 mg
8.0-9.9	2.5 mL	100 mg
10.0-14.9	3 mL	120 mg
15.0-27.9	5 mL	200 mg
28.0-39.9	7.5 mL	300 mg
40.0-49.9	10 mL	400 mg
≥ 50.0 kg	12.5 mL	500 mg

7.4.2 Dispensing

Azithromycin or Placebo - Kits of a 12 (+3)-week supply will be dispensed to the participant by the site pharmacist (or designee) at enrollment and every 3-month visit for a total of 6 visits. Kits will be dispensed with a measuring syringe and/or cup to help with reconstitution of the suspension and with dosing.

7.4.3 Administration Instructions

Azithromycin and placebo: Bottles of study drug are reconstituted with water weekly by the participant or parent. Each bottle is reconstituted with 12 mL of water and should be shaken well before each use.

The correct amount of reconstituted study drug, based on the participant's weight, should be taken 3 times weekly (refer to Table 1). This study medication may be administered without regard to meals (on either a full or empty stomach).

Further information is provided in the Pharmacy Manual.

7.4.4 Storage

Azithromycin and placebo: Oral powder for suspension is stored at room temperature 15 - 30°C (59 - 86°F). Reconstituted suspension is stored between 5º to 30ºC (41-86ºF) and must be used within 10 days of mixing. The bottle should be kept tightly closed.

All study drug should be stored by the study site at controlled temperatures in a secure area under restricted access according to local regulations. If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee. Participants will be instructed to store study drug in the original packaging according to the instructions outlined above.

7.5 Dose Modification

Additional guidelines for study drug dose modifications are provided in the Study Procedures Manual.

7.5.1 Dose Modification Due to Study Drug Intolerance

If a study participant has moderate to severe intolerance (refer to Study Procedures Manual) the following schedule for dosage adjustment can be made at the discretion of the site investigator:

- If a study participant receiving study drug TIW (e.g. Monday-Wednesday-Friday) experiences drug-related intolerance, the dosing INTERVAL of the study drug will be extended to BIW (e.g. Monday and Thursday).
- If a study participant does not tolerate receiving study drug BIW, the dosing INTERVAL will be extended to once weekly (e.g. Monday).
- If a dose modification other than an interval change is needed, the modification should be discussed with the Medical Monitor or Sponsor.

Refer to Section 11 for permanent and temporary discontinuation of study drug, including guidelines for permanent study drug discontinuation for chronic treatment with azithromycin for persistent *Pa* infection.

7.5.2 Missed Dosages

Missed doses may be made up as long as they are not taken within 24 hours of the next scheduled dose (refer to Study Procedures Manual).

7.6 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each participant will be maintained on an ongoing basis by a member of the study site staff. The number of bottles dispensed

will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

7.7 Measures of Study Drug Treatment Compliance

Participants will be asked to return all unused study drug and both used and unused study drug bottles. Treatment compliance will be based on the number of doses reported taken by the participant.

Further information is provided in the Study Procedures Manual and Pharmacy Manual.

7.8 Tobramycin Inhalation Solution (TIS)

All participants receive a 28-day course of TIS therapy (300 mg/5 mL) administered twice daily, with the initial course starting no more than 14 days prior to the Baseline Visit.

Participants who remain *Pa* positive after the first 28-day course of TIS will receive a second contiguous 28-day course of twice daily TIS (56 continuous days of treatment). For participants who received TIS in the 14 days prior to enrollment, Visit 2 should be conducted within the protocol-specified windows. However, if those participants require a second course of TIS, the administration may not be continuous but should be within 7-14 days of the last dose of the previous TIS 28-day course.

After the first quarter, participants will continue to receive culture-based TIS therapy (one 28-day, twice daily administration) according to *Pa* culture results available at the start of each quarter and for the duration of the 18 month study.

The initial two treatment courses of TIS will be provided as part of the study, unless TIS was prescribed and administered in the 14 days prior to study enrollment. Participants with *Pa* positive cultures at subsequent study visits (Visits 3 through 7) will be treated with TIS as standard of care. TIS will not be provided at these subsequent visits as part of the study.

TIS, 300 mg/5mL (TOBI®, Novartis Pharmaceutical Corp) is commercially packaged in open-label cartons containing 56 single dose ampules containing 300 mg tobramycin. The ampules are packaged in sets of 4 enclosed within a laminated foil pouch. Fourteen pouches will be contained in each carton. Each TIS dose will be administered using the PARI LC PLUS™ Nebulizer and a suitable compressor. The nebulizers will be provided by the study. Compressors will be provided if the participant does not already have one.

Young children will be evaluated at the study site to determine if a PARI baby mask is required for dosing and to fit the child with an appropriate mask (size 1, 2, or 3) as needed.

The first dose of TIS may be administered at the study site if deemed appropriate by the site investigator.

Instructions for administration and for cleaning of the nebulizer and compressor will be reviewed at the first visit and copies of the instructions will be provided to the participant or parent.

TIS should be stored by the study site at controlled temperatures (2-8ºC or 36-46ºF) in a secure area under restricted access according to local regulations. If the temperature of study drug storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee.

Participants will be instructed to store TIS in the original packaging in the refrigerator. However, ampules may be stored at room temperature (25ºC/77ºF) for up to 28 days.

7.8.1 Use of TOBI® Podhaler and other nebulized tobramycin products

Use of both the TOBI Podhaler and other commercially available nebulized tobramycin formulations prescribed by the participant's clinician is allowed in place of the TIS described above. Only TIS is provided as part of the study. Participants will be required to use the study-provided TIS (TOBI®) for the first two cycles (if applicable) of dosing unless they were prescribed another formulation in the 2 weeks prior to enrollment. If a participant enrolls while on another inhaled tobramycin formulation for less than 14 days, they will be allowed to complete that 28 day course but will be asked to use TIS for further therapy. All participants will be encouraged to use TIS throughout the study period.

7.8.2 TIS Dose Modification Due to Persistent Infection

At the discretion of the primary CF care clinician, treatment with TIS may be revised to a 28-day on/ 28-day off regimen if required for treatment of persistent *Pa* infection. Persistent infection is defined in this study as any two respiratory cultures collected at study visits, positive for *Pa* from study Qtr 2 (Visit 3) onward. If the positive *Pa* culture occurs outside of a study visit, site investigators will be asked to consult with the Medical Monitor or study investigator-sponsors. The participants will be encouraged to continue in the study and complete all remaining scheduled visits and procedures. Refer to section 11.3 for guidelines for permanent TIS discontinuation.

Refer to section 11.1.1 for guidelines for permanent study drug discontinuation for chronic treatment with azithromycin for persistent *Pa* infection.

7.8.3 TIS Accountability

Drug accountability for TIS will only be performed for TIS provided as part of the study. The number of kits and ampules dispensed will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

7.8.4 Measures of TIS Treatment Compliance

Participants will be asked to return all used and unused TIS ampules provided as part of the study.

8 STUDY PROCEDURES AND GUIDELINES

8.1 Clinical Assessments

All assessments will be performed as shown in the Schedule of Events, Appendix I.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the participant or the participant's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

8.1.1 Concomitant Medications

All concomitant medication and concurrent therapies ongoing and started at the Baseline Visit and through Visit 8 will be documented. Dose, route, unit, frequency of administration, indication for administration and dates of medication will be captured. History of antibiotic use in the year prior to enrollment and any chronic macrolide use will also be collected for the study.

8.1.2 Demographics

Demographic information (date of birth, sex, race) will be recorded at the Baseline Visit.

8.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at the Baseline Visit.

8.1.4 Microbiology History

Results of the study-qualifying *Pa* positive respiratory culture will be entered into the study database. Additional historic culture results will be recorded for study purposes as specified in the Study Procedures Manual.

8.1.5 Recording of Clinical Care Microbiology Results

Information regarding the microbiology results from cultures that are collected during unscheduled assessments (refer to Appendix I Schedule of Events) will be extracted from the clinical record and entered into the study database for the duration of the study.

8.1.6 Physical Examination

Physical examinations (complete and abbreviated) will be performed by either the site investigator or investigator-designated qualified staff (MD, NP, RN, PA). A complete physical exam will be performed at the Baseline Visit, and the End of Study Visit (or Early Withdrawal), if applicable. An abbreviated physical exam will be performed at all other study visits. The abbreviated exam includes respiratory, cardiovascular, and abdominal assessments. After the Baseline Visit, new clinically significant abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

8.1.7 Weight and Height/Length

Height/Length and Weight measurements will be obtained at all study visits.

Height/Length

Recumbent length should be measured on all participants approximately ≤ 18 months of age. Height should be measured on all participants approximately > 18 months of age. Consistent equipment for each type of measurement (standing or recumbent) should be used.

Weight

Participants who are approximately ≤ 18 months of age should be weighed without any clothing or diapers. Participants who are approximately > 18 months of age can be weighed in street clothing, with

a dry diaper (if applicable), and no shoes. Weight may be measured using either a standing or sitting scale (as appropriate) and should be obtained using consistent equipment for each type of measurement (standing or sitting)**.**

8.1.8 Vital Signs

Resting (minimum of 5 minutes) measurements of pulse and respirations will be performed and recorded at all study visits. Body temperature and blood pressure will be performed as clinically indicated.

8.1.9 Oximetry

A resting (minimum of 5 minutes) measurement of oximetry will be measured on room air and recorded at all study visits.

8.1.10 Spirometry

Spirometry will be performed at all study visits by all participants 4 years of age and older at the time of the visit who are able to reproducibly perform spirometry[71]. Procedures will be performed in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests at all study visits. For preschool age participants performing spirometry, a single satisfactory maneuver is acceptable.

The same spirometry equipment should be used for the duration of the study whenever possible. Raw lung function numbers will be recorded along with percent of predicted calculated centrally.

Participants who routinely use bronchodilators should use them prior to spirometry as noted below:

- Participants who routinely use short acting inhaled bronchodilators should use them 15 minutes to 2 hours prior to PFTs during study visits.
- Participants who routinely use long acting bronchodilator agents should use them 15 minutes to 6 hours prior to PFTs during study visits.

8.1.11 Audiometry

Hearing testing will be performed at the Baseline Visit, Visit 5, and the End of Study Visit (or at Early Withdrawal). Testing will be conducted at the site or at a qualified testing laboratory identified by the study site by a licensed audiologist with experience testing children. Because scheduling of audiology testing may be difficult at some sites, testing can be conducted up to 2 weeks after the Baseline Visit and up to 2 weeks before or after Visit 5, and up to 2 weeks before the End of Study Visit (or at Early Withdrawal).

Participants a) unable to complete audiology; b) with acute middle ear disease; c) with a flat (Type B) tympanogram; or d) with an abnormal audiogram at Visit 1 or 5, can remain in the study on study drug if they meet all other eligibility and protocol criteria.

Testing will include visual reinforcement audiometry (VRA) using sound field testing or earphones (supraural or insert) for participants approximately 6 to 30 months old, play audiometry with earphones for participants approximately 30 months to 5 years (developmental age), and standard audiometry for those approximately 5 to 18 years of age. The specific test procedure(s) used for each participant is left

to the discretion of the audiologist. For young infants and for special populations where behavioral audiometry cannot be completed, otoacoustic emissions testing will be performed.

Audiometric responses will be measured from 500 – 6000 Hz for VRA and 250 to 8000 Hz for play and standard audiometry. When performing VRA, it is not necessary to test below 15dBHL. Bone conduction testing shall be performed if air conduction test results are elevated. Tympanometry will be performed with all audiometry testing.

Abnormal hearing sensitivity will be defined as hearing threshold levels >25 dB HL for VRA (visual reinforcement audiometry) at any one frequency (500-6000Hz) and >20 dB HL for play or standard audiometry at any two frequencies (250-8000Hz) in either ear.

All abnormal test results, as reported by the site audiologist, should be forwarded in real time to an independent audiologist to be over-read. If a determination of sensorineural hearing loss is made, the site will be notified and the study drug must be stopped for the duration of the study. These participants will be encouraged to continue in the study and complete all study visits. Treatment and follow-up will be determined at the discretion of the primary CF care clinician. Instructions for the over-reading reporting process are included in the Study Procedures Manual.

Subjective hearing loss, tinnitus, and vertigo as reported by the participant or parent, should be recorded as adverse events and managed at the discretion of the primary CF care clinician.

Sensorineural hearing loss, confirmed by the over-reader, will be reported as Serious Adverse Events. (Refer to Section 10.2)

8.1.12 Electrocardiogram (ECG)

Standard 12-lead ECG tracing will be obtained at the Baseline Visit, Visit 2, and the End of Study Visit (or Early Withdrawal). Testing will be conducted and reviewed at the site by trained personnel with experience interpreting electrocardiograms in children. Any test results of clinical concern, should be reported to the study PI to determine follow up actions as described below.

Testing will be performed using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. All tests will be measured in Lead II using Bazett correction factor and QTc(B) will be reported.

Abnormal baseline measurement for QTc(B) is defined as ≥ 460 msec. Abnormal results at the time of follow-up testing are defined as QTc(B) >500 msec, an absolute QT >500 msec, or an increase in QTc(B) of >30 msec.

Abnormal test results, as defined in the protocol, will be over-read by an independent cardiologist and should be forwarded in real time as instructed in the Study Procedures Manual.

Participants with abnormal ECG findings of QTc >500msec or QTc change of >60msec at V2 and V8 will be instructed to temporarily stop study drug (at V2) and the PI will continue to observe the participant for clinical signs of arrhythmia (specifically torsades de pointes) while the tracings are reviewed by the over-reader. Refer to the Study Manual for additional instructions on follow up and requirements for permanent discontinuation of study drug.

Abnormal test results, as defined by the protocol and confirmed by over-reading, will be reported as Serious Adverse Events. (Refer to Section 10.2)

The ECG tracing may be collected for additional safety review.

8.1.13 Signs and Symptoms Evaluation for Pulmonary Exacerbations

Key changes in signs and symptoms will be reviewed and documented at all visits and when unscheduled assessments occur throughout the duration of the study. Unscheduled assessments will be conducted when a participant initiates a new antibiotic therapy, has a change in antibiotic therapy, or as determined by the site investigator to assess possible symptoms of a pulmonary exacerbation.

The review of pulmonary signs and symptoms will be standardized for assessment of acute pulmonary exacerbations and will be used to derive a protocol defined acute pulmonary exacerbation. Criteria for the protocol defined acute pulmonary exacerbation were based on the definition used in both the Early Pseudomonas Infection Control (EPIC001) clinical trial (NIH U01HL080310)[25] and recent multicenter randomized trial of azithromycin in children and adolescents uninfected with *Pa*[9] Refer to Appendix II for the criteria for a pulmonary exacerbation.

8.1.14 Cystic Fibrosis Respiratory Questionnaires

The self-report Cystic Fibrosis Respiratory Symptom Diary (CFRSD) - Chronic Respiratory Infection Symptom Score (CRISS©) and the parent-completed Cystic Fibrosis Respiratory Symptom Diary (CFRSD) will be completed at each study visit and the responses will be entered into the electronic case report forms. Both questionnaires take less than 5 minutes to complete. The CFRSD-CRISS has now been used in over 700 patients with CF.

The CFRSD-CRISS contains 8 questions and the CFRSD includes 16 questions (8 symptom items, 4 emotional impact items, and 4 activity impact items) for parent symptom reporting[72-74]. The parent/guardian will complete the CFRSD for participants ≤ 11 years of age and participants ≥ 12 years of age will complete the CFRSD-CRISS self-report questionnaire.

Additionally, parent/guardians will be asked to complete a new version of the questionnaire which is currently being evaluated. The Cystic Fibrosis Respiratory Signs Diary for ages 0-6 years (CFRSD $_{0\text{-}6©}$ - 18 questions) or ages 7-11 (CFRSD $_{7\text{-}11©}$ - 15 questions).

8.1.15 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity, outcome, treatment and relation to study drug will be recorded on the case report form (CRF). If an adverse event occurs, at the discretion of the site investigator, the study drug can be stopped.

If a participant has study drug discontinued because of an adverse event, the participant will be followed and treated by the site investigator until the abnormal parameter or symptom has resolved or stabilized. Participants who discontinue study drug early will be encouraged to complete all remaining scheduled and follow-up visits and procedures.

8.1.16 Hospitalization

Information regarding occurrence of hospitalizations will be captured throughout the study.

8.1.17 Hematology

At the Baseline Visit, Visit 5 (Week 39), and the End of Study Visit (or Early Withdrawal, if applicable), blood will be obtained and sent to each site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count).

8.1.18 Blood Chemistry Profile

At the Baseline Visit, Visit 5 (Week 39), and the End of Study Visit (or Early Withdrawal, if applicable), blood will be obtained and sent to each site's clinical chemistry lab for determination of serum sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, total bilirubin, direct bilirubin, gamma-glutamyl transferase (GGT), albumin and LDH.

8.1.19 Pregnancy Test

At the Baseline Visit and the End of Study Visit (or Early Withdrawal), a urine or serum pregnancy test will be performed and the results reported for female participants who are of childbearing potential.

8.1.20 Respiratory Cultures for CF Pathogens including *P. aeruginosa* and *S. aureus* and non-tuberculous mycobacteria (NTM)

Respiratory specimens will be obtained at all study visits and cultured qualitatively at the site clinical laboratory for identification of *Pa* and *Sa (*methicillin susceptible and resistant), *Haemophilus influenzae*, *Burkholderia* species, *Stenotrophomonas maltophilia*, and *Alcaligenes (Achromobacter) xylosoxidans* (from oropharyngeal swabs or sputum, expectorated or induced, or BAL, if available), and non-tuberculous mycobacteria (for sputum-producers only). The respiratory specimen will be collected no sooner than 24 hours after the last dose of TIS.

Collection: Oropharyngeal specimens will be collected with a swab from the posterior oropharyngeal wall and tonsillar pillars. Established standardized procedures for collection of OP swabs developed by the CCC will be utilized. Participants will be encouraged to cough prior to collection of the OP specimen. If a participant is able to produce sputum or induced sputum is collected at any visit, specimens will also be cultured for non-tuberculous mycobacteria (NTM).

Detailed instructions for specimen collection, processing, storage and shipping of samples intended for research laboratory analysis will be provided in the Study Laboratory Manual.

If *B. cepacia* complex is isolated from respiratory cultures during the study, the participant is allowed to continue use of study drug.

If NTM is isolated from sputum cultures during the study, the participant must permanently discontinue study drug, but should be encouraged to continue with all remaining study visits. If the AFB smear is positive from sputum cultures during the study, the subject will temporarily discontinue study drug pending the culture results. If NTM is isolated, the participant must permanently discontinue study drug. If the culture is negative, study drug may be restarted on the next scheduled treatment day.

8.1.21 Subject Diary

Participants will be requested to complete a diary to document each dose of study drug and TIS taken and to record changes in concomitant medications and symptoms from Visit 1 through the End of Study Visit (or Early Withdrawal). A diary will be dispensed at the Baseline Visit and again at Visits 3 through 7 and reviewed at every study visit after the Baseline Visit. Adverse events will be recorded in the CRF, as applicable.

8.2 Research Laboratory Assessments

8.2.1 Plasma for Inflammatory Biomarker Analysis and Banking

Blood Collection and Processing: At the Baseline Visit, Visit 5 and End of Study Visit (or Early Withdrawal), approximately 4mL blood will be collected for plasma samples. Blood processing instructions will be provided in the Study Laboratory Manual. At the end of the processing, the sample will be split into aliquots, which will be placed immediately into a –70^{o}C freezer for storage until shipment.

Specimen Shipment: At the end of the study or upon request, all samples will be shipped on dry ice via overnight courier to The Children's Hospital Clinical and Translational Research Center (TCH CTRC) in Denver for analysis and banking.

Inflammatory Biomarker Analysis: Plasma collected from participants will be used for measurement of C-reactive protein (CRP), myeloperoxidase (MPO), and calprotectin.

Banking: Plasma collected from participants will also be stored for indefinite banking. Separate informed consent regarding this process will be obtained from each study participant.

8.2.2 OP swabs for Microbiome Analysis

When specimens for respiratory culture are collected at all study visits, an additional oropharyngeal swab will be collected for microbiome analysis, using the same collection procedure. Swabs will be placed into a –70^{o}C freezer for storage until shipment.

Specimen Shipment: At the end of the study or upon request, all swabs will be shipped on dry ice via overnight courier to The Children's Hospital Clinical and Translational Research Center (TCH CTRC) in Denver for analysis and banking.

Banking: A suspension from the swabs collected from participants will be stored for indefinite banking. Separate informed consent regarding this process will be obtained from each study participant.

9 EVALUATIONS BY VISIT

Underlined procedures are performed as part of standard of care at the quarterly clinical care visit.

9.1 Baseline Visit 1 – Screening and Randomization (Day 0)

1. Review the study with the participant (participant's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. Ensure participant has opted either in or out of CFF Registry ID collection and long-term specimen banking.
2. Assign the participant a unique participant number.

3. Record demographics data.
4. Record medical history, including antibiotic use and CF diagnosis and date
5. Record microbiology history.
6. Review and record concomitant medications.
7. Complete CF Respiratory Diaries (participant or parent).
8. Review Pulmonary Symptoms.
9. Perform a complete physical examination.
10. Measure and record height/length and weight.
11. Obtain and record vital signs.
12. Obtain and record oximetry.
13. Collect blood for clinical laboratory testing (chemistry and hematology) at site lab.
14. Collect blood or urine for pregnancy test (if female of child-bearing potential).
15. Obtain results from chemistry and hematology drawn at this visit.
16. Collect plasma for inflammatory marker analysis and banking (if banking consent was obtained).
17. Perform and record spirometry (if participant is ≥ age 4 years)
18. Perform audiometry at or within 2 weeks after visit. Obtain signed copies of testing and evaluation.
19. Perform ECG testing. Obtain signed copies of testing and evaluation.
20. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
21. Collect additional OP swab for microbiome.
22. Randomize participant to azithromycin or placebo (blinded).
23. Dispense azithromycin/placebo and dosing instructions.
24. Dispense TIS study medication, nebulizer cleaning instructions and dosing instructions, unless first course was taken by the participant prior to enrollment in the study.
25. Administer first dose of TIS in the clinic, unless first course was taken by the participant prior to enrollment in the study. (The first dose of TIS may be administered at the study site if deemed appropriate by the site investigator.)
26. Dispense nebulizer (and masks if applicable) and compressor for TIS as needed.
27. Dispense Subject Diary and review completion instructions.
28. Schedule participant to return in 3 weeks (19-23 days after Screening Visit).

9.2 Visit 2 (Week 3, Day 21 ± 2 days)

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Record any adverse events.
5. Record any hospitalizations.
6. Record changes to concomitant medications.

7. Perform an abbreviated physical exam.
8. Measure and record height/length and weight.
9. Obtain and record vital signs.
10. Obtain and record oximetry.
11. Perform and record spirometry (if participant is ≥ age 4 years).
12. Perform ECG testing. Obtain signed copies of testing and evaluation.
13. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
14. Collect additional OP swab for microbiome.
15. Schedule participant to return in 11-15 weeks (Week 13 Visit)
16. Advise the participant that RC will contact them if their respiratory culture results are positive for *Pa* and ask them to begin treatment without interruption of their TIS if possible.
17. Following Visit 2: For participants with Visit 2 respiratory cultures **positive for *Pa*, prescribe TIS within one week of visit**.

9.3 Visit 3 (Week 13 ± 2 weeks)

Underlined procedures are performed as part of standard of care at the quarterly clinical care visit.

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Perform study drug and study-dispensed TIS accountability.
5. Record any adverse events.
6. Record any hospitalizations.
7. Record changes to concomitant medications.
8. Perform abbreviated physical examination.
9. Measure and record height/length and weight.
10. Obtain and record vital signs.
11. Obtain and record oximetry.
12. Perform and record spirometry (if participant is ≥ age 4 years).
13. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
14. Collect additional OP swab for microbiome.
15. Dispense azithromycin/placebo.
16. Dispense Subject Diary.
17. Schedule participant to return in 11-15 weeks for Visit 4 (Week 26)
18. Advise the participant that RC will contact them if their respiratory culture results are positive for *Pa* and ask them to begin treatment within one week of visit.
19. Following Visit 3: For participants with Visit 3 respiratory cultures **positive** for *Pa*: prescribe TIS within one week of visit.

9.4 Visit 4 (Week 26 ± 2 weeks)

Underlined procedures are performed as part of standard of care at the quarterly clinical care visit.

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Perform study drug accountability.
5. Record any adverse events.
6. Record any hospitalizations.
7. Record changes to concomitant medications.
8. Perform abbreviated physical examination.
9. Measure and record height/length and weight.
10. Obtain and record vital signs.
11. Obtain and record oximetry.
12. Perform and record spirometry (if participant is ≥ age 4 years).
13. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
14. Collect additional OP swab for microbiome.
15. Dispense azithromycin/placebo.
16. Dispense Subject Diary.
17. Schedule participant to return in 11-15 weeks for Visit 5(Week 39).
18. Advise the participant that RC will contact them if their respiratory culture results are positive for *Pa* and ask them to begin treatment within one week of visit.
19. Following Visit 4: For participants with Visit 4 respiratory cultures **positive** for *Pa*, prescribe TIS within one week of visit.

9.5 Visit 5 (Week 39 ± 2 weeks)

Underlined procedures are performed as part of standard of care at the quarterly clinical care visit.

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Perform study drug accountability.
5. Record any adverse events.
6. Record any hospitalizations.
7. Record changes to concomitant medications.
8. Perform abbreviated physical examination.
9. Measure and record height/length and weight.
10. Obtain and record vital signs.
11. Obtain and record oximetry.

12. Collect blood for clinical laboratory testing (chemistry and hematology) at site lab.
13. Collect plasma for inflammatory marker analysis and banking (if banking consent was obtained).
14. Perform and record spirometry (if participant is ≥ age 4 years).
15. Perform audiometry at or within 2 weeks of visit. Obtain signed copies of testing and evaluation.
16. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
17. Collect additional OP swab for microbiome.
18. Dispense azithromycin/placebo.
19. Dispense Subject Diary.
20. Schedule participant to return in 11-15 weeks for Visit 6 (Week 52).
21. Advise the participant that RC will contact them if their respiratory culture results are positive for *Pa* and ask them to begin treatment within one week of visit.
22. Following Visit 5: For participants with Visit 5 respiratory cultures **positive** for *Pa*, prescribe TIS within one week of visit.

9.6 Visit 6 (Week 52 ± 2 weeks)

Underlined procedures are performed as part of standard of care at the quarterly clinical care visit.

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Perform study drug accountability.
5. Record any adverse events.
6. Record any hospitalizations.
7. Record changes to concomitant medications.
8. Perform abbreviated physical examination.
9. Measure and record height/length and weight.
10. Obtain and record vital signs.
11. Obtain and record oximetry.
12. Perform and record spirometry (if participant is ≥ age 4 years).
13. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
14. Collect additional OP swab for microbiome.
15. Dispense azithromycin/placebo.
16. Dispense Subject Diary.
17. Schedule participant to return in 11-15 weeks for Visit 7(Week 65).
18. Advise the participant that RC will contact them if their respiratory culture results are positive for *Pa* and ask them to begin treatment within one week of visit.
19. Following Visit 6: For participants with Visit 6 respiratory cultures **positive** for *Pa*, prescribe TIS within one week of visit.

9.7 Visit 7 (Week 65 ± 2 weeks)

Underlined procedures are performed as part of standard of care at the quarterly clinical care visit.

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Perform study drug accountability.
5. Record any adverse events.
6. Record any hospitalizations.
7. Record changes to concomitant medications.
8. Perform abbreviated physical examination.
9. Measure and record height/length and weight.
10. Obtain and record vital signs.
11. Obtain and record oximetry.
12. Perform and record spirometry (if participant is ≥ age 4 years).
13. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
14. Collect additional OP swab for microbiome.
15. Dispense azithromycin/placebo.
16. Dispense Subject Diary.
17. Schedule participant to return in 11-15 weeks for Visit 8 (Week 78).
18. Advise the participant that RC will contact them if their respiratory culture results are positive for *Pa* and ask them to begin treatment within one week of visit..
19. Following Visit 7: For participants with Visit 7 respiratory cultures **positive** for *Pa*, prescribe TIS within one week of visit.

9.8 Visit 8 - End of Study (Week 78 ± 2 weeks)

Underlined procedures are performed as part of standard of care at the quarterly clinical care visit.

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Perform final study drug accountability.
5. Record any adverse events.
6. Record any hospitalizations.
7. Record changes to concomitant medications.
8. Perform a complete physical examination.
9. Measure and record height/length and weight.
10. Obtain and record vital signs.
11. Obtain and record oximetry.

12. Perform audiometry at or within 2 weeks of visit. Obtain signed copies of testing and evaluation.
13. Perform ECG testing. Obtain signed copies of testing and evaluation.
14. Collect blood for clinical laboratory testing (chemistry and hematology) at site lab.
15. Collect blood or urine for pregnancy test (if female of child-bearing potential).
16. Collect plasma for inflammatory marker analysis and banking (if banking consent was obtained).
17. Perform and record spirometry (if participant is ≥ age 4 years).
18. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
19. Collect additional OP swab for microbiome.
20. If the participant has any unresolved adverse events at this visit, advise them that RC will contact them for additional follow-up.

9.9 Unscheduled Assessment – Change in Antibiotics or as determined by Investigator

If the participant notifies the study site of a change in antibiotic therapy, the study site should collect the following information.

1. Review Pulmonary Symptoms.
2. Record any adverse events.
3. Record changes to concomitant medications.
4. Obtain clinical records of any respiratory cultures collected as part of the assessment.

9.10 Early Withdrawal from Study

If the participant withdraws early from the study and attends a final visit, the following procedures should be completed if possible:

1. Complete CF Respiratory Diaries (participant or parent).
2. Review Pulmonary Symptoms.
3. Review Subject Diary.
4. Record any adverse events.
5. Perform final study drug accountability.
6. Record any hospitalizations.
7. Record changes to concomitant medications.
8. Perform complete physical examination.
9. Measure and record height/length and weight.
10. Obtain and record vital signs.
11. Obtain and record oximetry.
12. Collect blood for clinical laboratory testing (chemistry and hematology) at site lab.
13. Collect blood or urine for pregnancy test (if female of child-bearing potential).
14. Collect plasma for inflammatory marker analysis and banking (if banking consent was obtained).
15. Perform and record spirometry (if participant is ≥ age 4 years).

16. Perform audiometry at or within 2 weeks of visit. Obtain signed copies of testing and evaluation.
17. Perform ECG testing. Obtain signed copies of testing and evaluation.
18. Obtain respiratory culture for processing at site microbiology lab (OP swab or sputum).
19. Collect additional OP swab for microbiome.
20. If the participant has any unresolved adverse events at this visit, advise them that RC will contact them for additional follow-up.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Prescribing or Product Information of greater severity or frequency than expected based on the information in the Prescribing or Product Information.

The Investigator will probe, via discussion with the participant, for the occurrence of AEs during each participant visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, as modified for cystic fibrosis, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. 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

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. 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.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

10.2 Serious Adverse Experiences (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 participant or require intervention to prevent one of the outcomes listed.

For this study, important medical events that will be reported as an SAE include:

- Sensorineural hearing loss, determined by audiometry and confirmed by the over-reader
- ECG testing results of QTc(B) >500 msec, an absolute QT >500 msec, or an increase in QTc(B) of >30 msec, that has been confirmed by over-reading (Refer to Section 8.1.12).

10.3 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) 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.

All SAE Report Forms will be reviewed by the site investigator and sent to the DCC within one business day of the site learning of the event. Sites will scan and email or fax the SAE report to:

Email address: cfsaesfacsys@seattlechildrens.org

Direct dial fax number: 206-985-3278

The site will notify the DCC of additional information or follow-up to an initial SAE Report as soon as relevant information is available. Follow-up information is reported on an SAE Report Form.

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.

10.4 Medical Monitoring

The Medical Monitor for the Data Coordinating Center should be contacted directly at this number to report medical concerns or questions regarding safety.

Pager: (800) 341-0961

11 DISCONTINUATION AND REPLACEMENT OF PARTICIPANTS

A participant may be discontinued from study drug (azithromycin or placebo) at any time if the participant, the site investigator, or the Sponsor feels that it is not in the participant's best interest to continue.

11.1 Permanent Discontinuation of Study Drug

A participant must be permanently discontinued from study drug (azithromycin or placebo) for the following reasons:

- Pregnancy
- Isolation of NTM from a respiratory culture during the study
- Determination of sensorineural hearing loss (determined by audiometry and confirmed by the over-reader). Participants will be encouraged to continue in the study and complete all remaining scheduled visits and procedures (See Section 8.1.11).
- Abnormal ECG findings at a study visit after Visit 1, defined as a QTc(B) >500 msec, an absolute QT >500 msec, or an increase in QTc(B) of >30 msec that has been confirmed by over-reading.

Additional reasons for study drug discontinuation are listed below. Participants should continue with all remaining study visits if possible.

- Abnormal clinically significant laboratory safety test results, as determined by the site investigator

- Adverse event, as determined by the investigator*
- Subject decision
- Protocol violation, as determined by the investigator or Sponsor
- Death

*If a participant is discontinued from study drug due to an adverse event, the participant will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized. All dose modifications should be recorded appropriately in the source documents and CRFs.

11.1.1 Permanent Discontinuation of Study Drug Due to Persistent *Pa* Infection

At the discretion of the primary CF care clinician and in consultation with the participant, treatment with study drug (blinded placebo or azithromycin) can be discontinued and chronic azithromycin can be initiated if required for treatment of persistent *Pa* infection. Persistent infection is defined in this study as any two respiratory cultures collected at study visits, positive for *Pa* from study Qtr 2 (Visit 3) onward. The participants will be encouraged to continue in the study and complete all remaining scheduled visits and procedures.

If one or more of the positive *Pa* cultures occurs outside of a study visit, investigators will be asked to consult with the Medical Monitor or study investigator-sponsors.

Refer to section 7.8.2 for guidelines for TIS dose modifications due to persistent *Pa* infection.

11.2 Temporary Discontinuation of Study Drug

Study drug (azithromycin or placebo) must be temporarily stopped if acute treatment with azithromycin or any macrolide is required. After acute treatment is completed, participants are allowed to re-start study drug (azithromycin or placebo) on the next scheduled treatment day. (See Section 6.2)

Study drug must be temporarily stopped if a sputum AFB smear result is AFB positive. If the culture is negative, study drug may be restarted on the next scheduled treatment day. (See Section 8.1.20)

Study drug must be temporarily stopped if acute treatment with any drug known to prolong QT interval is required (refer to Appendix III). After acute treatment is completed, participants are allowed to re-start study drug on the next scheduled treatment day.

Study drug may also be temporarily stopped or dose-modified due to an abnormal clinically significant laboratory safety test results, as determined by the site investigator. Refer to protocol Section 7.5 for dose modification information.

11.3 Discontinuation of TIS

Participants who are discontinued from use of TIS during the study may continue use of study drug (azithromycin or placebo). All participants who permanently discontinue use of TIS should be encouraged to complete all remaining scheduled visits and procedures.

If a participant is discontinued from TIS use due to an adverse event, the participant will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized. All dose modifications should be recorded appropriately in the source documents and CRFs.

Refer to Section 7.8.2 for TIS Dose Modification Due to Persistent Infection

11.4 Withdrawal of Participants from the Study

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. This may include participants who withdraw from study treatment early and who decline to continue to come in for the remaining study visits.

Reasonable attempts will be made by the site investigator to provide a reason for participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents. Participants who withdraw from the study should be encouraged to come in for a final visit (Section 9.10: Early Withdrawal from Study).

11.5 Replacement of Participants

Participants who withdraw from the study treatment or from the study will not be replaced.

12 PROTOCOL VIOLATIONS

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

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Inappropriate administration of study medication that impacts the safety of the participant or inappropriate dispensing of study drug (e.g., wrong container # dispensed)

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation.

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

13 DATA SAFETY MONITORING

An independent data safety monitoring board (DSMB) appointed by the NHLBI will review comprehensive interim enrollment, safety, and efficacy data reports on a semi-annual basis. *A priori* interim stopping rules for efficacy and safety with respect to the primary endpoint will be in place and evaluated as outlined in the DSMB charter, with approximately 6 planned interim analyses and a final analyses. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study. Further details regarding the timing and content of the interim reviews is included in the statistical section below and in the DSMB charter.

14 STATISTICAL METHODS AND CONSIDERATIONS

A detailed statistical analysis plan will be written that will describe all analyses, tables, figures, and data listings that will be generated for this study. All analyses will be performed using SAS® (SAS Institute, Inc., Cary, NC, USA) or R (R Foundation for Statistical Computing, Vienna, Austria).

14.1 Data Sets Analyzed

All analyses will be performed using an intent-to-treat (ITT) population, which is defined as all randomized participants. The primary efficacy analyses will be repeated in the per-protocol population, which is defined as participants having completed ≥80% of doses (azithromycin or placebo). Participants who are discontinued from study drug are encouraged to complete all remaining study visits and will remain in the analyses population according to ITT.

14.2 Demographic and Baseline Characteristics

Treatment groups will be described and compared with respect to baseline demographic and clinical characteristics including age, gender, CFTR genotype, race, height, weight, randomization strata (6 mos-3 years, >3-6 years, ≥6-12 yrs, >12-18 yrs), lifetime history of *Pa* positivity (ever versus never positive greater than 2 years prior to enrollment), presence of *Sa* and/ or other gram-negative bacteria, FEV_1% predicted among those able to perform spirometry, and use of chronic medications (inhaled dornase alfa and hypertonic saline). For all analyses, baseline will be defined as the measurement prior and closest to the administration of the first dose of study drug (azithromycin or placebo).

14.3 Analysis of Primary Endpoint

The primary endpoint will be time to a protocol-defined pulmonary exacerbation, comparing the two treatment groups: oral placebo and culture-based TIS treatment versus oral azithromycin and culture-based TIS treatment. Follow-up time will begin at first dose of study drug (Day 0) and end after completion of the final study visit (at approximately 18 months). Time to exacerbation will be presented using Kaplan-Meier survival estimation and compared between treatment groups using a Cox proportional hazards model with effects for treatment group and randomization strata (age group), with a test of the treatment effect at a two-sided significance level of 0.05. The hazard ratio and its 95% confidence interval will be presented. Additional secondary analysis may employ a stratified log-rank test of treatment arm adjusting for the randomization strata. The proportion having an event at each study visit will be presented by treatment group using Kaplan-Meier estimation.

Estimates of the hazard ratio and associated 95% confidence interval for the primary endpoint will also be reported by subgroups defined by demographic and disease specific baseline characteristics of interest. No adjustments for multiple comparisons will be made.

14.4 Analysis of Secondary Endpoints

Descriptive analyses and graphical displays will be used to summarize all secondary endpoints, including changes from baseline in continuous endpoints and rates over the follow-up period for event based endpoints. The ITT population will be used for all secondary analyses.

Safety

All reported SAEs and AEs will be coded using MedDRA and grouped by body system. (S)AEs will be tabulated by treatment group using standard coding terms sorted by body system. The incidence of AEs in each treatment arm will be tabulated by seriousness, severity, and relationship to study drug. If an adverse event is reported more than once during the study period for a given participant, the greatest severity and the worst-case relationship will be presented in tables. The number of (S)AEs will be summarized for each treatment group as follows: (i) The proportion of participants with at least one (S)AE, (ii) The average number of (S)AEs per patient, and (iii) The rate of (S)AEs per patient month of follow-up. Histograms showing the frequency of the number of (S)AEs in each treatment group will be included. Rates of (S)AEs by System Organ Class (SOC) will be presented by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% confidence intervals for each SOC. The rate ratios will be compared using a two-sided 0.05 level test for Poisson count data.

Clinical laboratory data at each study visit and changes from baseline will be summarized by treatment group. In addition, the following clinical laboratory summaries will be presented by treatment group: (i) the incidence of clinically significant abnormalities at each study visit; and (ii) tables summarizing the frequencies of participants below, within, and above the normal reference ranges at baseline and end of study; (iii) tables displaying baseline to end of study shifts in each laboratory value (shifts between below, within or above normal range).

The proportion of participants permanently or temporarily discontinuing study drug will be tabulated by treatment group. Drug discontinuation events will be categorized as: (1) Permanently discontinued study drug, (2) Permanently discontinued study drug and withdrew from study, and (3) Temporarily discontinued study drug. Reason for permanent drug discontinuation will be summarized. Compliance measures will be computed separately for the inhaled tobramycin and oral azithromycin therapies. Those patients with less than 80% average compliance for either azithromycin or placebo will be excluded from the per protocol population.

Microbiology

Descriptive analyses and graphical displays will be used to summarize the emergence and disappearance of microbiologic organisms identified throughout the study. In the event that both an OP culture and an expectorated sputum culture are available from a participant at the same visit and produce discordant results, the positive result will be used in the analysis for this visit. The proportion of participants with *Pa* positive cultures after the first quarter of treatment will also be summarized for each treatment group with differences between treatment groups tested using a 0.05 level of significance Chi-square test.

Time to *Pa* recurrence from weeks 13 through 78 will be presented using Kaplan-Meier survival estimation and compared between treatment groups using a Cox proportional hazards model with effects for treatment arm and randomization strata, with a test of the treatment effect at a two-sided significance level of 0.05. The hazard ratio and its 95% confidence interval will be presented. The proportion of *Pa*-positive respiratory cultures among all respiratory cultures taken from weeks 13 through 78 will also be modeled. The response will be binary (positive or negative culture). The proportion of *Pa*-positive respiratory cultures at each visit will be graphically displayed by treatment group. A generalized estimating equation (GEE) model using a logit link will be used to model this data with robust variance estimation and an independence working correlation matrix, including effects for both treatment and

randomization strata[75]. The significance of the treatment group variable will be tested using a two-sided 0.05 level of significance. The treatment associated odds ratio from this model and corresponding 95% confidence interval will be the measure of treatment effect. The estimated treatment effect will be interpreted as the marginal odds ratio of *Pa* positive respiratory cultures during the 18 months.

Inflammatory Markers

Inflammatory markers will be appropriately transformed (e.g. $\log_{10}$) and will be descriptively summarized and graphically displayed for each visit and for changes from baseline. Differences between treatment groups in the 39 and 78 week change from baseline will be estimated with corresponding 95% confidence intervals and tested using a two-sided 0.05 level two-sample t-test. Several exploratory regression models will be developed to investigate the association between the inflammatory markers and clinical outcomes including time to pulmonary exacerbation. Baseline inflammatory markers in addition to other baseline characteristics will be included in a Cox proportional hazards model for time to exacerbation with a goal of identifying a set of baseline inflammatory markers predictive of clinical outcome. Separate models may be generated for each treatment group to reduce the number of potential interactions with treatment. Additional models exploring specific microbiologic outcomes including time to *Pa* recurrence will be similarly developed. Repeated measures regression models will also be generated investigating the association between baseline inflammatory markers (adjusting for other baseline predictors) and changes from baseline in continuous outcomes such as spirometry, anthropometric measures.

Hospitalization and Acute Antibiotic Usage

Endpoints for hospitalization, intravenous antibiotic usage, inhaled antibiotic usage, and oral antibiotic usage will be descriptively summarized. Graphical displays for the proportion of participants with each event and corresponding event rates will also be provided. For each endpoint, the proportion of participants experiencing the event will be compared between treatment groups using a logistic regression model including effects for treatment group and randomization strata. Odds ratios and 95% confidence intervals will be used to describe the treatment effect and the significance of the treatment group variable will be tested using a two-sided 0.05 level of significance.

Pulmonary Function

Baseline clinical and demographic characteristics will be summarized among all participants with spirometry values available at the baseline visit. Spirometry analyses will only include the subset of participants who are 4 years of age or greater and for whom a spirometry measure at baseline is available. FEV_1, FEV_1 % predicted, FVC, and $FEF_{25\%-75\%}$ will be descriptively summarized and graphically displayed for each visit and for changes from baseline. Predicted values at each visit will be calculated centrally. For each spirometry measure, a repeated measures linear regression model will be used to model the change in the measure over time (in days). Robust variance estimation of the coefficients will be implemented within the generalized estimating equation framework with an independence working correlation matrix. The models will include effects for treatment and randomization strata. The difference between treatment groups in the 18-month change from baseline in each spirometry variable will be estimated via the repeated measures model with corresponding 95%

confidence interval. The models will include effects for treatment and randomization strata. The significance of the treatment group variable will be tested using a two-sided 0.05 level of significance.

Anthropometric Measures

Height/length, height/length percentile, weight, and weight percentile will be descriptively summarized and graphically displayed for each visit and for changes from baseline. For each anthropometric measure, a repeated measures linear regression model will be used to model the change in the measure over time (in days). Robust variance estimation of the coefficients will be implemented within the generalized estimating equation framework with an independence working correlation matrix. The models will include effects for treatment and randomization strata. The difference between treatment groups in the 18 month change from baseline in each anthropometric variable will be estimated via the repeated measures model with corresponding 95% confidence interval. The models will include effects for treatment and randomization strata. The significance of the treatment group variable will be tested using a two-sided 0.05 level of significance.

Respiratory Symptoms

The CFRSD and CFRSD-CRISS will be analyzed as composite scores calculated based on "yes" responses to a specified set of the CFRSD and CFRSD-CRISS questions. Component responses and composite scores will be descriptively summarized at baseline in addition to changes from baseline. Change from baseline in each composite score will be analyzed using a repeated measures linear regression model with time from baseline (in days) included in the model. Robust variance estimation of the coefficients will be implemented within the generalized estimating equation framework with an independence working correlation matrix. The models will include effects for treatment and randomization strata. The difference in the scores between treatment groups in the 18 month change from baseline will be estimated via the repeated measures model with corresponding 95% confidence interval. The significance of the treatment group variable will be tested using a two-sided 0.05 level of significance.

Respiratory Microbiome

Respiratory microbial communities are complex multivariate systems and will be descriptively summarized at both baseline and end of study using OP swab samples. Total bacterial load (16S rDNA copy per OP swab) will be calculated for each sample and averaged. Sequencing and phylogenetic-based clustering techniques classify the bacteria into operational taxonomic units (OTUs, e.g. genus) and their relative proportion of the entire community (in a given sample) can be calculated[76]. True diversity of varying orders will calculated for each sample and graphically investigated to evaluate the evenness and richness across the treatment groups[77, 78]. Shannon diversity, or true diversity of order 1, will be used for statistical comparisons across treatment groups and over time using a two-sample t-test and paired t-test, respectively. Microbial community data contain only relative information due to the fact that they are constrained to a unit sum. Therefore, we propose to use an isometric log ratio transformation (ilr) applied to the relative abundance measures prior to statistical analysis[79, 80]. Relative abundance of each OTU will be summarized via graphical displays, which will include principal component analysis and biplots. Change from baseline in the dominant OTUs will be summarized by treatment group and compared using bootstrap methodology[81]. Both baseline and

change in the respiratory microbiome will be correlated with clinical and microbiologic outcomes using exploratory regression models to estimate the association between microbiome characteristics in the respiratory tract and both the risk of a pulmonary exacerbation and risk of *Pa* recurrence.

Comprehensive Baseline Predictive Model of Eradication Failure

A multivariable logistic regression model will be developed to identify key baseline predictors associated with initial eradication failure, defined by the *Pa* status at Week 13. Baseline demographic, clinical, microbiologic, inflammatory, and respiratory microbiome variables will all be assessed for inclusion in the model. Stepwise regression methods will be used for variable selection. The positive predictive value, negative predictive value, sensitivity, and specificity based on the model will be evaluated and corresponding ROC curves generated. The ultimate goal is to develop a robust predictive model to identify children with early *Pa* who are at high risk for eradication failure. This model could then be used in future studies to identify this high risk subset and evaluate alternative first line treatment strategies to promote *Pa* eradication.

14.5 Missing Data

With respect to the primary endpoint, missing data may arise from missing exacerbation symptom assessment data or due to early withdrawal. Site training, automatic queries in the electronic data capture system, and thorough clinical site monitoring will be performed in order to minimize the occurrence of missing data. Participants who discontinue treatment will be encouraged to remain in the study while completing all study procedures. Participants who discontinue study will be censored at the date of last contact. The impact of missing data on the primary endpoint will be assessed through sensitivity analysis. A sensitivity analysis of the primary endpoint using only participants with complete follow up data will be performed. Furthermore, the potential influence of acute respiratory events not meeting the primary endpoint definition will also be assessed by censoring participants with acute antibiotic usage prior to meeting the primary endpoint. Further details regarding the handling of missing data will be provided in the statistical analysis plan.

14.6 Interim Analysis

An independent data safety monitoring board (DSMB) appointed by the NHLBI will review comprehensive interim enrollment, safety, and efficacy data reports on a semi-annual basis. *A priori* stopping rules for efficacy and safety with respect to the primary endpoint will be guided as outlined in the DSMB charter. Serious adverse events will be monitored by the committee on an ongoing basis throughout the study. All study personnel and investigators will remain blinded to results of the interim analyses, with the exception of designated Data Coordinating Center (DCC) personnel who will prepare the interim reports. In addition to interim DSMB reports, semi-annual reports will also be generated for the Steering Committee to the study. This Committee will remain blinded, and will only be provided enrollment and site performance data.

14.7 Sample Size

Results from a recently completed randomized trial in children with CF uninfected with *Pa* demonstrated a 51% reduction in exacerbations in the group receiving azithromycin as compared to those receiving placebo[9]. Further, results from a randomized trial in children and adults chronically

infected with Pa, the majority receiving concurrent TIS, demonstrated a 40% reduction in exacerbations in the group receiving azithromycin as compared to those receiving placebo[13]. It is felt that a 40-50% reduction in exacerbations is the minimal effect size that would need to be observed to justify the addition of a second antibiotic regimen for the treatment of children with CF and new onset *Pa*. Data from the original EPIC trial indicates that approximately 50% of children with new onset *Pa* will experience a protocol-defined pulmonary exacerbation over the 18-month follow up period[5]. Thus, even in the presence of standard of care treatment with TIS, we expect a high rate of exacerbation among those randomized to receive oral placebo with TIS. Assuming a two-sided 0.05 type I error and a sample size of approximately 137 children randomized to each treatment group (culture-based TIS with oral placebo or culture-based TIS with oral azithromycin), the study has 90% power to detect a hazard ratio for the time to exacerbation of 0.53 or smaller (a 47% or greater reduction) between treatment groups and 80% power to detect a hazard ratio of 0.58 or smaller (a 42% or greater reduction). With a maximum projected withdrawal rate of at most 9% over the course of the 18-month study based on the prior EPIC trial with similar duration of follow-up, it is conservatively estimated that the study still has at minimum 80% power to detect a hazard ratio of 0.56 or smaller between treatment groups (a 44% or greater reduction). These power estimates account for six planned interim analyses and one final analyses, assuming an O'Brien-Fleming type boundary with boundary parameter equal to 1.0[82]. Further, it is anticipated that approximately 34% of the children in the placebo group will experience *Pa* recurrence over the 18-month study based on estimates from the EPIC trial (Section 2.2.2). The study has 80% power to detect a hazard ratio of 0.52 or lower (48% or greater reduction) in the time to *Pa* recurrence between the azithromycin and placebo groups assuming a two-sided 0.05 type I error.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

An electronic data capture system, Medidata Rave, will be utilized for collection of study data. The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each participant who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a participant's visit or assessment into the protocol-specific electronic Case Report Form (eCRF). Participants will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, participant number and initials.

If a correction is required for an eCRF, the time and date stamp will track the person entering or updating eCRF data and creates an electronic audit trail.

The Investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

15.2 Data Management Procedures

The data will be entered into a validated database. The DCC will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices for the handling and analysis of data for clinical trials.

15.3 Data Quality Control and Reporting

Once data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented in an audit trail.

15.4 Security and Archival of Data

The EDC system is hosted by Medidata; the data are stored at Medidata's primary data center in Houston, Texas, with fail-safe data centers in New Jersey. Data are regularly backed up by Medidata and stored with Iron Mountain.

Medidata maintains 21 CFR Part 11-compliant electronic systems, with procedures in place to safeguard against unauthorized acquisition of data. Any authorized communication with the Medidata servers at the Houston Data Center is conducted via SSL (128-bit) encryption. Robust password procedures, consistent with 21 Part 11, are in place. Robust physical security procedures are in place at the Houston Data Center to prevent unauthorized personnel physical access to the server rooms. EDC account access is maintained and monitored by the Data Coordinating Center.

Other databases will be stored on Seattle Children's servers and are safeguarded against unauthorized access by established security procedures. Network accounts are password protected and maintained and monitored by the Seattle Children's Information Services group. Data is backed up regularly according to the Information Technology group's procedures.

Note that there is an intention to make banked biospecimens and associated data available to investigators for future exploration. The biospecimens will be collected under Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approval, processed according to a standard operating procedure and stored at a central facility, with appropriate procedures to enable long term, stable storage. Researchers may apply, via a standardized process, for use of de-identified data and specimens for research purposes. Applications will undergo a scientific review process. When applying for use of data or specimens, the applicant must agree to: (1) use the data and specimens only for research purposes and to not make any attempts to try to identify any individual subject; (2) securing the data and specimens using appropriate methods; and (3) destroy or return the data (and specimens) in accordance with the specimen/data use agreement after analyses are completed. Before data or specimens will be released to an investigator, documentation of IRB exemption or approval from their institution must be provided.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each participant must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that participant. The Investigator

must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (e.g., patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of one year after database lock and accessible for 10 years following database lock. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Part 312 and ICH Guidelines for GCP (E6) and to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP. By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

15.7 Participant Confidentiality

In order to maintain participant confidentiality, only a site number, participant number and participant initials will identify all study participants on CRFs and other documentation submitted to the Sponsor. If specific consent is given, the participant's CFF patient registry number will also be collected. Additional participant confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to participants. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will

keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, prescribing information, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB's unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the participants or the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Participants (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each participant (or their legal representatives) prior to entering the participant into the trial. Information should be given in both oral and written form and participants (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the participant will also be obtained. If a participant is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the participant. A copy of the signed consent form (and assent) will be given to the participant or legal representative of the participant and the original will be maintained with the participant's records.

During the course of the study, if modifications are made to the consent form that impact the participant, the participant will be re-consented as described above.

16.3.1 Consent for Collection and Use of CFF Registry ID Number

To facilitate possible future evaluation of retrospective and prospective information from all participants who screen for this study, consent will also be sought at the Baseline Visit to collect the participant's CFF Registry ID number. The CFF registry collects data on all patients with CF followed at CFF-accredited care centers at each clinical encounter, at each hospitalization or course of antibiotics, and via a year-end survey. Data include microbiology results, spirometry results, CF genotype and other information.

16.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of participants.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to participants or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/participants.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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18 APPENDIX I: SCHEDULE OF EVENTS

	Quarter 1		Quarter 2	Quarter 3	Quarter 4	Quarter 5	Quarter 6	End of Study or Early Withdrawal	
	Baseline Visit 1 Screening (Day 0)	Visit 2 (Week 3 ± 2 days)	Visit 3 (Week 13 ± 2 weeks)	Visit 4 (Week 26 ± 2 weeks)	Visit 5 (Week 39 ± 2 weeks)	Visit 6 (Week 52 ± 2 weeks)	Visit 7 (Week 65 ± 2 weeks)	Visit 8 (Week 78 ± 2 weeks)	Unscheduled Assessment[8]
Informed Consent	X								
Consent for CF Registry ID & Banking	X								
Respiratory Questionnaires [1]	X	X	X	X	X	X	X	X	
Demographics & Medical History	X								
Concomitant Medication	X	X	X	X	X	X	X	X	X
Microbiology History	X								
Complete Physical Exam	X							X	
Abbreviated Physical Exam		X	X	X	X	X	X		
Height & Weight	X	X	X	X	X	X	X	X	
Vital Signs & Oximetry	X	X	X	X	X	X	X	X	
Spirometry[2]	X	X	X	X	X	X	X	X	
Collect plasma for IM and Banking[3]	X				X			X	
Chemistry and Hematology performed at site lab	X				X			X	
Audiometry[4]	X				X			X	
12-lead ECG Testing	X	X						X	
Pregnancy Test (urine or serum)	X							X	
Respiratory Culture (processed at site lab)[5]	X	X	X	X	X	X	X	X	
OP swab collection for	X	X	X	X	X	X	X	X	

	Quarter 1		Quarter 2	Quarter 3	Quarter 4	Quarter 5	Quarter 6	End of Study or Early Withdrawal	
	Baseline Visit 1 Screening (Day 0)	**Visit 2 (Week 3 ± 2 days)**	**Visit 3 (Week 13 ± 2 weeks)**	**Visit 4 (Week 26 ± 2 weeks)**	**Visit 5 (Week 39 ± 2 weeks)**	**Visit 6 (Week 52 ± 2 weeks)**	**Visit 7 (Week 65 ± 2 weeks)**	**Visit 8 (Week 78 ± 2 weeks)**	**Unscheduled Assessment[8]**
microbiome sample[3]									
Randomization to azithromycin or placebo	X								
Dispense TIS	X[6]	[X] [6]	[X][7]	[X] [7]	[X] [7]	[X] [7]	[X] [7]		
Dispense azithromycin/placebo	X		X	X	X	X	X		
Dispense Subject Diary	X		X	X	X	X	X		
Review Subject Diary		X	X	X	X	X	X	X	
Drug Accountability			X	X	X	X	X	X	
Adverse Events Review		X	X	X	X	X	X	X	X
Hospitalization Review		X	X	X	X	X	X	X	
Pulmonary Symptom Review	X	X	X	X	X	X	X	X	X
Clinical Care Micro Results (respiratory cultures)									X

[1] Completed at each study visit
[2] Spirometry is only performed among participants 4 years of age or older able to reliably perform the procedure.
[3] Samples will be shipped at the end of study or upon request
[4] Audiometry can be performed up to 2 after weeks of Visit 1, up to 2 weeks before or after Visit 5, and up to 2 weeks before Visit 8. Testing will include visual reinforcement audiometry (VRA) using sound field testing for participants approximately 6 to 30 months old, play audiometry with earphones for participants approximately 30 months to 5 years (developmental age), and standard audiometry for those approximately 5 to 18 years of age. Tympanometry will be performed with all audiometry testing.
[5]Respiratory cultures are processed at site microbiology laboratory. Sputum will additionally be cultured for NTM at the site microbiology laboratory.
[6] All participants will receive a 28 day course of TIS at Visit 1, unless TIS was prescribed and administered in the 14 days prior study enrollment. At Visit 2, participants whose respiratory culture are *Pa* + based on site microbiology lab results will receive a second 28-day TIS course during the first treatment quarter.
[7] At Visits 3, 4, 5, 6, and 7, participants whose respiratory culture is *Pa* + based on site microbiology lab results will receive a 28-day TIS course during the treatment quarter as standard of care.
[8] An unscheduled assessment will be conducted when a participant initiates a new antibiotic therapy, has a change in antibiotic therapy or as determined by the investigator to assess possible symptoms of a pulmonary exacerbation.

19 APPENDIX II: DEFINITION OF PULMONARY EXACERBATION

The presence of a pulmonary exacerbation is established by the following:

(1) One of the major criteria alone
or
(2) Two of the minor signs/symptoms and fulfillment of symptom duration

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

(1) Absolute decrease in FEV_1 %predicted of ≥10%
(2) Oxygen saturation <90% on room air *or* absolute decrease of ≥ 5%
(3) New lobar infiltrate(s) or atelectasi(e)s on chest radiograph
(4) Hemoptysis (more than streaks on more than one occasion in past week)

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 PE definition)*

(1) Increased work of breathing or respiratory rate
(2) New or increased adventitial sounds on lung exam
(3) Weight loss ≥5% of body weight or decrease across 1 major percentile in weight percentile for age in past 6 months
(4) Increased cough
(5) Decreased exercise tolerance or level of activity
(6) Increased chest congestion or change in sputum

20 APPENDIX III: COMPOSITE LIST OF DRUGS THAT PROLONG QT AND/OR CAUSE TORSADES DE POINTES (TDP)

The list of drugs that prolong QT and/or cause torsades de pointes (TdP) may be updated during the study.

Refer to the most recent version of the list at this website: Composite List of All QT Drugs under the category "Known Risk of TdP."

Drugs known to prolong QT interval are described as follows: "Substantial evidence supports the conclusion that these drugs prolong the QT interval AND are clearly associated with a risk of torsades de pointes (TdP), even when taken as directed in official labeling."

Instructions for searching for specific QT drugs will be posted on the OPTIMIZE study website.