Clinical Research Protocol

TEACH Trial: Testing the Effect of Adding CHronic Azithromycin to Inhaled Tobramycin. A randomized, placebo-controlled, double-blinded trial of azithromycin 500mg thrice weekly in combination with inhaled tobramycin

Protocol Number:	TEACH-IP-15
Version Date:	June 11, 2018
Investigational Product:	azithromycin
IND Number:	Not applicable
Development Phase:	Phase 4
Sponsor-Investigator:	Dave Nichols, MD Seattle Children's Hospital
Funding Organization:	National Institutes of Health/National Heart, Lung, and Blood Institute Cystic Fibrosis Foundation Therapeutics, Inc. (CFFT)
Medical Monitor:	TDNCC Medical Monitoring Group (800) 314-0961
Coordinating Center:	CFFT Cystic Fibrosis Therapeutics Development Network Coordinating Center Seattle, WA 98101 Telephone: (206) 987 – 5725 Fax: (206) 987 – 7505

Approval:

MD — June 29, 2018

PI or Sponsor Signature (Name and Title) *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Sponsor-Investigator 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: TEACH-IP-15

Protocol Title: TEACH Trial: Testing the Effect of Adding CHronic Azithromycin to Inhaled Tobramycin. A randomized, placebo-controlled, double-blinded trial of azithromycin 500mg thrice weekly in combination with inhaled tobramycin

Protocol Date: June 11, 2018

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Investigator Signature *Date*

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Print Name and Title

Site # _____

Site Name _____

Address _____

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
BAL	bronchoalveolar lavage
CFR	Code of Federal Regulations
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFFT	Cystic Fibrosis Foundation Therapeutics
CFRSD	Cystic Fibrosis Respiratory Symptom Diary
CFRSD-CRISS	CFRSD-Chronic Respiratory Infection Symptom Score
CFQ-R	Cystic Fibrosis Questionnaire - Revised
CFQ-R RSS	CFQ-R Respiratory Symptom Scale
CFTR	cystic fibrosis transmembrane conductance regulator
CRF	case report form
DSMB	Data Safety Monitoring Board
ECG	electrocardiogram
EDC	Electronic data capture
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
HIPAA	Health Insurance Portability and Accountability Act of 1996
IRB	Institutional Review Board
IV	intravenous
LFT	Liver function test
mEq	milliequivalent
Pa	*Pseudomonas aeruginosa*
PFT	pulmonary function test
SAE	serious adverse experience
TDNCC	Therapeutics Development Network Coordinating Center
TISP	Tobramycin inhalation solution or powder

PROTOCOL SYNOPSIS

TITLE	TEACH Trial: Testing the Effect of Adding CHronic Azithromycin to Inhaled Tobramycin. A randomized, placebo-controlled, double-blinded trial of azithromycin 500mg thrice weekly in combination with inhaled tobramycin
SPONSOR-INVESTIGATOR	Dave Nichols, MD (Seattle Children's Hospital)
FUNDING ORGANIZATION	National Institutes of Health/National Heart, Lung, and Blood Institute Cystic Fibrosis Foundation Therapeutics, Inc. (CFFT)
NUMBER OF SITES	Approximately 40
RATIONALE	Patients with cystic fibrosis (CF) commonly acquire a chronic airway infection with *Pseudomonas aeruginosa* (*Pa*). Both inhaled tobramycin and oral azithromycin have been proven beneficial in these patients and are currently used by the majority of eligible patients. 75% of US patients prescribed inhaled tobramycin are also prescribed chronic oral azithromycin. We believe that azithromycin may be inhibiting the anti-*Pa* effects and clinical benefits of inhaled tobramycin in clinically infected patients. This study is designed to compare monotherapy with inhaled tobramycin versus combination therapy adding chronic oral azithromycin to inhaled tobramycin.
STUDY DESIGN	This is a prospective, randomized, placebo-controlled, double-blinded study of azithromycin in subjects with chronic *Pa* airway infection using inhaled tobramycin. Subjects who have received two (2) or more cycles of inhaled tobramycin within the 24 weeks prior to enrollment will be recruited into the study. This study will investigate whether azithromycin is associated with poorer clinical and microbiologic outcomes as compared to placebo during concurrent administration of inhaled tobramycin. Eligible subjects will be enrolled and randomized to either azithromycin or placebo at Visit 1, approximately 14 days prior to the start day of their next planned 28-day nebulized solution or dry powder tobramycin (TISP) cycle. Both tobramycin inhalation solution (TIS) and tobramycin inhalation powder (TIP) will be allowed, based on clinical prescription. Tobramycin solution should be nebulized with an approved nebulizer and air compressor. Subjects will be randomized in a 1:1 fashion to azithromycin (500 mg three times per week) or matched placebo. Randomization will be stratified by FEV_1 % predicted (25%-50%, >50%-75%, >75%), chronic oral azithromycin use for the past 30 days, inhaled tobramycin formulation (TIS, TIP), and site. Between Visit 1 (Day -14) and Visit

	2 (Day 0), a two-week run-in period will be used to begin administration of either azithromycin or placebo. Among those randomized to azithromycin, the 2-week run-in period will be used to initiate azithromycin and standardize uptake prior to the start of the next TISP cycle. Among those randomized to placebo, the run-in period will be used as a standardized washout prior to the start of the next TISP cycle. Subjects will begin TISP at Visit 2 in addition to their continued dosing of randomized study drug treatment (azithromycin or placebo). Subjects will continue both TISP and study drug dosing for 28 days (up until Visit 3), which corresponds with the end of a clinically prescribed 4-week cycle of TISP. Subjects completing the study through Visit 3 will be offered participation and consent to an 8-week open-label extension period during which azithromycin will be provided. During the open-label period, subjects will be instructed to remain off of TISP for the first 4-week period, followed by a 4-week period of TISP.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be enrolled in the randomized study for up to 42 days (6 weeks). • Treatment V1-V2: azithromycin or placebo without TISP: 14 days (-2 days/+4 days) followed by: • Treatment V2-V3: azithromycin or placebo with TISP: 28 days (-2 days/+4 days) Subjects participating in the optional open-label extension will be on study for up to 98 days (14 weeks). • Treatment V3-V4: azithromycin without TISP: 28 days (-/+4 days) followed by: • Treatment V4-V5: azithromycin with TISP 28 days (-/+4 days) The total duration of the study is expected to be approximately 33.5 months: 30 months for subject recruitment and 3.5 months for the final subject enrolled to complete the study.
PRIMARY OBJECTIVE	Determine if azithromycin impairs the previously-recognized clinical benefits of inhaled tobramycin by comparing changes in pulmonary function as measured by FEV_1 between subjects randomized to azithromycin versus placebo.

SECONDARY OBJECTIVES	The secondary objectives for the randomized period are to: • Compare changes in patient-reported quality of life between subjects randomized to azithromycin versus placebo The secondary exploratory objectives for the randomized period are to: • Compare the safety profile between subjects randomized to azithromycin versus placebo • Compare changes in additional spirometry measures, bacterial density, and weight between subjects randomized to azithromycin versus placebo • Compare rates of pulmonary exacerbations, hospitalizations, and acute antibiotic usage between subjects randomized to azithromycin versus placebo The objectives for those continuing in the open-label extension: • Among those randomized to azithromycin and remaining on azithromycin, obtain additional data to determine the long term effect of azithromycin on clinical outcomes • Among those randomized to placebo and switching to azithromycin, compare differences in clinical outcomes observed during the randomized and open-label periods
NUMBER OF SUBJECTS	Approximately 120 subjects randomized
SUBJECT SELECTION CRITERIA: **Inclusion Criteria**	<u>Inclusion Criteria for Randomized Period:</u> 1. Male or female ≥ 12 years of age at Visit 1 2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and one or more of the following criteria: o Sweat chloride equal to or greater than 60 mEq/L by quantitative pilocarpine iontophoresis test (QPIT) o Two mutations in the CFTR gene believed to be disease causing in the opinion of the site investigator o Abnormal nasal potential difference (change in NPD in response to a low chloride solution and isoproterenol of less polarizing than -7 mV) 3. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative 4. Two or more respiratory cultures (sputum, OP swab, BAL sample) growing *Pa* collected within the 12 months prior to randomization, at least 1 month apart, with at least one collected in the 6 months prior to randomization. 5. FEV_1% predicted between 25-100% at Visit 1

	6. Off TISP and other inhaled anti-pseudomonal antibiotics for at least two weeks at randomization 7. Willingness to begin the next course of TISP at the scheduled Visit 2 8. Use of at least 2 cycles of TISP within 24 weeks prior to Visit 1 9. Ability to swallow capsules (similar in size to pancreatic enzyme capsules) 10. Most recent LFTs less than 4x ULN. Must be obtained within 12 months prior to Visit 1 11. Current azithromycin use at Visit 1 or prior chronic azithromycin use defined as four or more consecutive weeks. Subjects do not have to be using chronic oral azithromycin at Visit 1 12. Stable clinical status with no significant change in medication or airway clearance routine for four weeks prior to Visit 1, as determined by site investigator <u>Inclusion Criteria for Open-Label Period:</u> 1. Completion of the randomized, placebo-controlled period (up through Visit 3) 2. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative 3. Ability for subject to comply with the requirements of the study 4. Willingness to begin the next course of TISP at the scheduled Visit 4 5. Clinically stable at Visit 3 as assessed by the site investigator
SUBJECT SELECTION CRITERIA: Exclusion Criteria	<u>Exclusion Criteria for Randomized Period</u>: 1. Weight < 40 kg at Visit 1 2. For women of child-bearing potential: a. positive pregnancy test at Visit 1 or b. lactating or c. unwilling to practice a medically acceptable form of contraception (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal during the study 3. Inability to perform reproducible spirometry 4. Inability or unwillingness to cycle off TISP for one 4-week period 5. Respiratory culture with *Burkholderia cepacia* complex species within the 24 months prior to Visit 1 6. Respiratory culture with nontuberculous mycobacteria (NTM) within 18 months prior to Visit 1 7. Use of IV or oral anti-pseudomonal antibiotics within four weeks prior to Visit 1 (other than azithromycin)

	8. Use of an investigational therapy within four weeks prior to Visit 1 9. History of unresolved, abnormal neutropenia (ANC ≤ 1000 cells/mm^3) 10. Current use of systemic corticosteroids equivalent to a daily dose more than 10 mg prednisone at Visit 1 11. Current use of nelfinavir, warfarin, haloperidol, or methadone at Visit 1 12. Initiation of approved CFTR modulators less than 30 days prior to Visit 1 13. ECG abnormalities identified at Visit 1 that require prompt, further medical evaluation or QTc interval >480 msec for males and >486 msec for females at Visit 1 14. Any other condition that, in the opinion of the site investigator/designee, would preclude informed consent or assent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives <u>Exclusion Criteria for Open-Label Period</u>: 1. Weight < 40 kg at Visit 3 2. For women of child bearing potential: unwilling to practice a medically acceptable form of contraception (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal during the study 3. Identified respiratory culture positive for NTM or *Burkholderia cepacia* complex species during randomized study period 4. Use of an investigational therapy at Visit 3 5. Current use of systemic corticosteroids equivalent to a daily dose more than 10 mg prednisone 6. Current use of nelfinavir, warfarin, haloperidol, or methadone 7. Any other condition that, in the opinion of the site investigator/designee, would preclude informed consent or assent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Over-encapsulated azithromycin tablets will be provided during the randomized period of the study. Azithromycin will be dosed as one 500 mg capsule three times per week. During the open-label extension period, azithromycin 500 mg tablets (without over-encapsulation) will be provided by the site pharmacy and dosed three times per week.

CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Placebo, matching the appearance of the azithromycin capsules and comprised of the same capsule material as the test product will be provided during the randomized period of the study. Placebo will be dosed as one capsule three times per week.
STANDARD OF CARE TREATMENT: TISP	In this study, TISP is given as standard of care, will be based on clinical prescription for 28 days on, followed by 28 days off, and will conform to manufacturer administration guidelines.
CONCOMMITANT MEDICATIONS	**Allowed:** For both the randomized and open-label periods, standard therapy for CF is allowed except for treatments noted in the conditionally allowed and prohibited medications sections below. Ongoing chronic treatment (>30 days prior to Visit 1) with inhaled dornase alpha, hypertonic saline, short and long acting bronchodilators, FDA-approved CFTR modulators, high dose ibuprofen, low-dose inhaled or systemic steroids and airway clearance is allowed. **Conditionally Allowed:** 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 (azithromycin or placebo) or open-label azithromycin **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) or open-label azithromycin **must** be temporarily stopped during use of these medications. After acute treatment is completed, subjects should restart study drug or open-label azithromycin on the next scheduled treatment day. • Chronic treatment (>30 days prior to Visit 1) with antidepressant medications citalopram and escitalopram is allowed without affecting study participation if: o The subject has been using both chronic oral azithromycin AND citalopram (or escitalopram) consistently for greater than 30 days at Visit 1 AND o The Visit 1 ECG demonstrates QTc within the normal limits as defined by the protocol 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

	are listed on the CredibleMeds® Website (Composite List of All QT Drugs under the category "Known Risk of TdP"). Acute use of macrolide antibiotics may be required for certain infections and is allowed but also requires temporarily stopping the study drug or open-label azithromycin. The study drug or open-label azithromycin should be restarted on the next scheduled day following completion of the prescribed macrolide antibiotic. Treatment for pulmonary exacerbations 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 and initiate or increase the dose of acute systemic corticosteroids (defined as less than 10 days) only in the presence of increased symptoms suggesting a pulmonary exacerbation. **Prohibited:** For both the randomized and open-label periods, the following medications are prohibited (for the randomized period, between Visit 1 through Visit 3; for the open-label period, between Visit 3 and Visit 5). o Investigational therapies o Chronic inhaled antibiotics other than cycled TISP (e.g., Cayston®, colistin). Chronic treatment is defined as inhaled antibiotics prescribed as a maintenance therapy and not used temporarily to treat a pulmonary exacerbation. o Increase in chronic systemic corticosteroid dose beyond prednisone equivalent of 10 mg per day, or initiation of chronic systemic (oral or intravenous) corticosteroids at any dose. Chronic use is defined as greater than 10 days continuous use. o New or increase in ibuprofen dose of greater than 800 mg total dose per 24 hours for more than 5 days o Nelfinavir, warfarin, haloperidol, or methadone
PRIMARY ENPOINT	Difference between the azithromycin and placebo treatment groups in the relative change in FEV_1(liters) from randomization (Visit 1) to Visit 3.
SECONDARY ENDPOINTS	**Randomized Period** Secondary Endpoints: • Difference between the azithromycin and placebo treatment groups in the relative change in FEV_1(liters) during administration of TISP from Visit 2 to Visit 3. • Difference between the azithromycin and placebo treatment groups in the change from Visit 1 to Visit 3 in the Cystic Fibrosis Respiratory Symptom Diary (CFRSD) - Chronic Respiratory Infection Symptom Score (CRISS©)

	• Difference between the azithromycin and placebo treatment groups in the change from Visit 1 to Visit 3 in the scales derived from the CFQ-R Exploratory Secondary Endpoints: • Difference in adverse event rates and rates of abnormal QTc parameters from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups • Difference in the proportion of subjects hospitalized from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups Difference in the proportion of subjects prescribed acute oral, inhaled and IV antibiotics from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups • Difference in the proportion of subjects experiencing a protocol-defined pulmonary exacerbation requiring antibiotic therapy from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups • Difference between the azithromycin and placebo treatment groups in the absolute change from Visit 1 to Visit 3 and Visit 2 to 3 in FEV_1 (liters and % predicted), absolute and relative change in FVC (liters and % predicted), and absolute and relative change in $FEF_{25-75\%}$ • Difference between the azithromycin and placebo treatment groups in the change from Visit 1 to Visit 3 in weight • Difference in the change from Visit 1 to Visit 3 in sputum *Pa* bacterial density between the azithromycin and placebo treatment groups **Open-Label Period** • Among those randomized to azithromycin and remaining on azithromycin, longitudinal changes and/or rates in all clinical outcomes across the randomized and open-label periods • Among those randomized to placebo and switching to azithromycin, differences in all clinical outcomes between the randomized and open-label periods
PLANNED INTERIM ANALYSES	An independent data monitoring board (DSMB) will review comprehensive interim reports when approximately 50% and 75% of patients have completed the randomized period of the study. Regular reporting frequency to the DSMB is detailed in the DSMB charter. *A priori* interim stopping rules for futility and efficacy/harm with respect to the primary endpoint are outlined in the DSMB charter. Serious adverse experiences (SAEs) will be monitored by the committee on an ongoing basis throughout the study.

STATISTICS **Primary Analysis Plan**	All analyses will be performed using a modified intent-to-treat (m-ITT) population, which is defined as all randomized subjects who received at least one dose of study drug. All tests will be two-sided and evaluated at a 0.05 level of significance. The primary endpoint will be the difference between the azithromycin and placebo treatment groups in the relative change from Visit 1 to Visit 3 in FEV_1 (liters). The primary endpoint will be compared between treatment groups using linear regression adjusted for randomization strata. Least squares means and the treatment effect for the relative change will be presented as well as corresponding 95% confidence interval and p-values. The p-values will be evaluated against a two-sided 0.05 level of significance.
Rationale for Number of Subjects	The primary endpoint is the difference between the azithromycin and placebo treatment groups in the relative change from Visit 1 to Visit 3 in FEV_1 (liters). Data from prior published studies of similar duration are available to estimate sample size and power for the study including a randomized, placebo-controlled trial of azithromycin in individuals with CF chronically infected with *Pa* and a randomized, placebo controlled trial of aztreonam lysine similarly among those with chronic *Pa*. These studies suggest a standard deviation of the relative change in FEV_1 ranging from 10 to 16. Based on these prior studies, we anticipate the relative change in FEV_1 from Visit 1 to Visit 3 to be -2% in the azithromycin arm and +6.85% in the placebo arm for an overall treatment effect of 8.65%. A sample size of 110 provides 85% power to detect a treatment effect of 7.5% or greater assuming a standard deviation of 13. 110 subjects provides 93% power to detect a treatment effect similar to what was observed in the preliminary data (8.65%) assuming a standard deviation of 13. It is noteworthy that the standard deviation from more recently completed studies CF studies have observed standard deviations in short term relative change in FEV (liters) ranging from 8-11, confirming our conservatism in the standard deviation estimate used for our sample size calculations. Assuming a standard deviation of 11, a sample size of 110 provides 85% power to detect a treatment effect of 6.3% or greater in favor of the placebo group. It is anticipated from prior CF trials conducted through the TDNCC that the attrition rate will be less than 10%, and thus a sample size of 120 will ensure at least 110 subjects will complete the trial.

1 BACKGROUND

Projected median survival of those with cystic fibrosis (CF) has dramatically improved in recent decades, largely as a result of new medications and therapies. *Pseudomonas aeruginosa* (*Pa*) is the most common pathogen in the airways of people with CF. By early adolescence, half of all patients with CF are chronically infected with *Pa*[1]. This prevalence increases to 70% or more by early adulthood, and persistent *Pa* airway infection is linked to more rapid loss of lung function and earlier mortality[2,3]. Inhaled antibiotics achieve high airway concentrations while avoiding many safety concerns associated with prolonged systemic drug exposure[4,5]. Strategies to eradicate initial *Pa* airway infection or treat chronic infection have proven beneficial[6-9].

The aminoglycoside tobramycin was the first inhaled anti-pseudomonal antibiotic commercially developed for CF and remains the most prescribed inhaled antibiotic used in the US[1]. Roughly two thirds of all US patients with CF and chronic *Pa* are prescribed inhaled tobramycin—typically as 28 days of on-off cycles. Long-term use of oral azithromycin has also been shown to improve lung function and reduce exacerbations in CF patients with chronic *Pa* airway infection[10,11]. While the mechanism of action is uncertain and potentially multifactorial, azithromycin has immunomodulatory effects and may function as an anti-inflammatory agent in CF[12-15]. Azithromycin is widely prescribed and is now the most common chronic antibiotic used in CF patients with persistent *Pa*[1]. In queries of recent clinical trials and the US CF Foundation National Patient Registry, we find that ~70% of all patients with *Pa* and 75% of those prescribed inhaled tobramycin are now also prescribed oral azithromycin on a chronic basis.

Potential interactions between concomitant medications and mechanisms for antibiotic resistance in CF are poorly understood. These issues have been cited in current CF consensus treatment guidelines as two key unanswered questions in need of further study[16]. Based on recent pre-clinical research demonstrating antagonism between azithromycin and tobramycin, but not the fluoroquinolone, ciprofloxacin[17], we hypothesized that azithromycin may selectively inhibit tobramycin when targeting *Pa* airway infection in CF lung disease. We have now observed a selective, antagonistic drug interaction between azithromycin and tobramycin in two in vitro models of biofilm growth with *Pa*, two animal models of biofilm-based *Pa* infection, and two non-overlapping CF clinical trials when retrospectively analyzing the impact of concomitant azithromycin use on clinical and microbiological outcome measures.

In-vitro **Data**

Initial studies were conducted with the laboratory strain of *Pa* PA01[18]. Using a flow cell biofilm culture method, we observe significant antibiotic effects, manifested as reduced biofilm biomass, with antibiotics individually (Figure 1a). However, when azithromycin was added, we observed neutral to increased effect with ciprofloxacin (Figure 1b) but antagonistic effects with tobramycin (Figure 1c).

Figure 1a

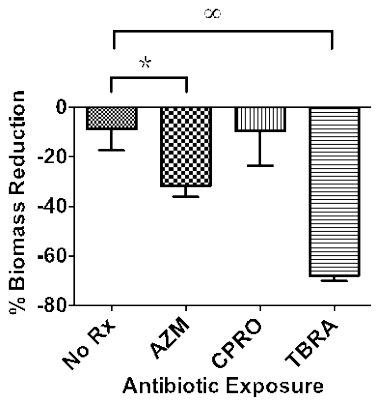


Figure 1b

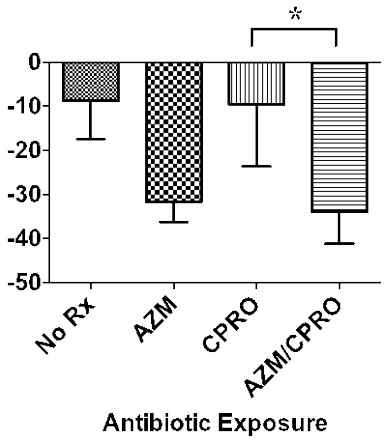
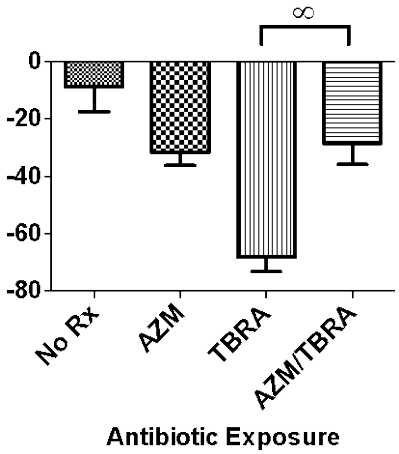


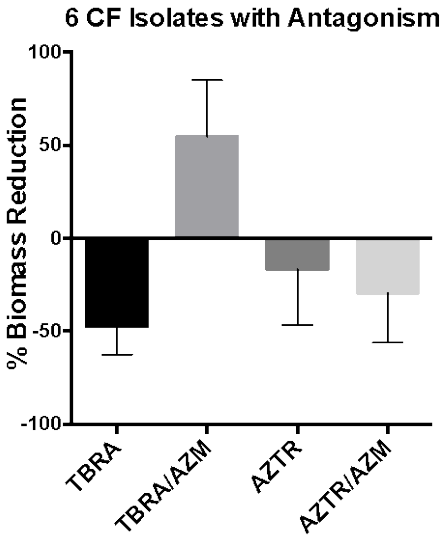
Figure 1c



*P<0.01 ∞P<0.001

Additional studies in the flow-cell biofilm model were conducted using 15 CF clinical isolates of *Pa*[19]. Twelve of the isolates grew sufficient biofilms to be tested in the antibiotic comparison studies. Of those 12, six showed antagonism when azithromycin was added to tobramycin. We then tested aztreonam in those six isolates and observed neutral effects in three isolates and additive effects in three isolates (Figure 2). The flow-cell system is devoid of any mammalian tissue. These data suggest that the suspected drug interaction occurs directly between the antibiotics in the context of bacterial infection rather than indirectly through altered host response.

Figure 2



More recently, we have developed a biofilm aggregate model that incorporates lysed human neutrophils to enhance biofilm growth[20]. In this model, *Pa* develops non-surface attached biofilms suspended in nutrient poor media containing the lysed neutrophils, and may more closely resemble the bacterial communities that have been documented in airway secretions from people with CF. We have tested 30 CF clinical isolates in this model. Ten isolates were collected from a clinical trial, 10 were collected from patients at National Jewish Health, and 10 were obtained from the CF repository at Seattle, WA.

In the biofilm aggregate model, we observe highly significant reduction in bacterial killing when azithromycin is added to tobramycin, with 2-3 log antagonism (100-1000 fold; Figure 3a). To date, we do not observe antagonism with other antibiotics commonly used for inhalation therapy in CF (Figure 3b; effect of azithromycin when added to anti-*Pa* antibiotics).

Figure 3a

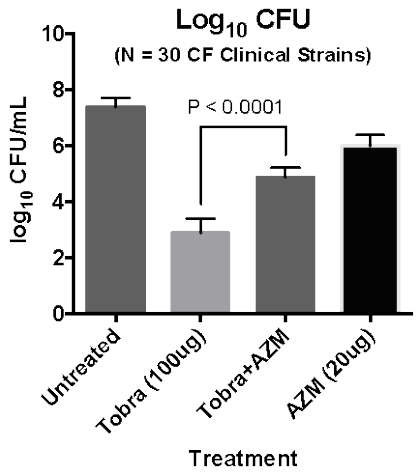
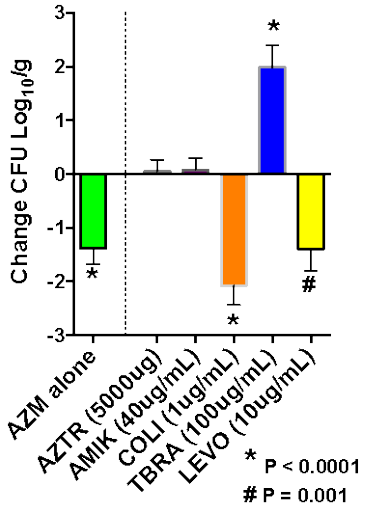


Figure 3b

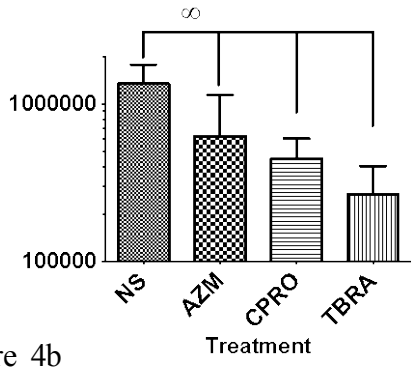


***In-vivo* Animal Model Data**

As part of a research project sponsored by the U.S. Department of Defense, we tested the ability of early azithromycin treatment, with or without subsequent additional antibiotics, to reduce *Pa* infection in cutaneous thermal injury (i.e., burn wound)[17]. *Pa* grows as a biofilm in the presence of exuberant neutrophilic inflammation in both burn wound infections and CF lung disease[21-23]. Tobramycin alone effectively reduced *Pa* infection at the wound site and systemic spread to the lung (Figure 4a). As predicted, azithromycin had additive antibacterial effects when combined with subsequent ciprofloxacin treatment. However, counter to our hypothesis, we found that the burden of *Pa* infection both at the wound site and systemically (lung) greatly increased when azithromycin treatment was followed by tobramycin (Figure 4b). Infection with combined azithromycin and tobramycin was no better than normal saline-treated control.

Figure 4a

Wound Site



Lung

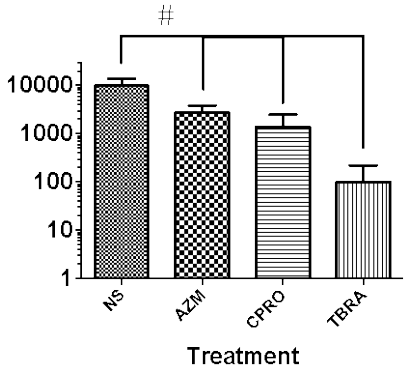
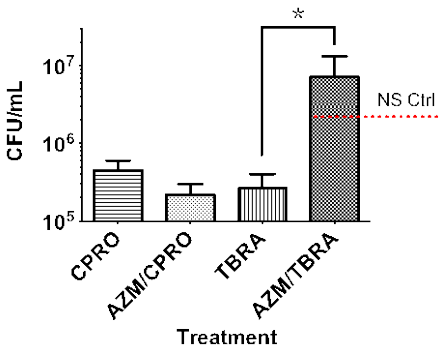
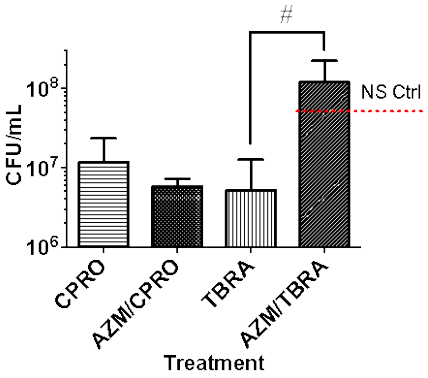


Figure 4b

Wound Site

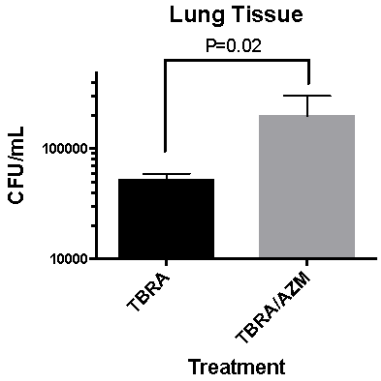


Lung



We then conducted follow up studies using a fibrin-based plug model to retain a CF clinical strain of *Pa* within the airways of mice overexpressing the epithelial sodium channel (ENaC). The transgenic ENaC mice, despite normal *CFTR,* model airway dehydration and chronic neutrophilic bronchitis present in human CF patients. In this experiment, animals were treated with azithromycin and/or tobramycin together and at the same time for 48 hours prior to tissue specimen collection (Figure 4c). We observed that azithromycin reduced the ability of systemic tobramycin to kill *Pa* in the lung of these animals (unpublished).

Figure 4c



Clinical Trials Data

We have analyzed the concomitant use of azithromycin in two CF clinical trials testing inhaled antibiotic therapy in CF subjects with chronic *Pa* airway infection. One is the active comparator study of inhaled tobramycin and inhaled aztreonam published in 2012[7]. The other is the AIR-CF2 study comparing twice daily vs. three times daily inhaled aztreonam lysine, following a 4-week run-in period with inhaled tobramycin[24]. In both studies, we observe that subjects reporting concomitant use of chronic oral azithromycin have little improvement in clinical outcome measures while receiving inhaled tobramycin. Subjects without azithromycin use appear to respond similarly to inhaled tobramycin or inhaled aztreonam lysine. Azithromycin use does not appear to significantly alter the response to inhaled aztreonam lysine in these trials. This pattern of selective drug interaction between azithromycin and an inhaled aminoglycoside is consistent across multiple related outcome measures. In these studies, the mean age of subjects is approximately 25 years, mean FEV_1 is approximately 55% predicted, and over 70% of enrolled subjects reported azithromycin as a concomitant medication.

Active Comparator Trial

Subjects randomized to inhaled tobramycin show a significant different change in FEV_1% over one cycle (Figure 5a) and three cycles (Figure 5b) of inhaled antibiotics based on azithromycin use. Change in FEV_1% in subjects randomized to inhaled aztreonam lysine was not significantly affected by azithromycin use. Users of azithromycin failed to reach a clinically significant change in self-reported quality of life over four weeks of inhaled tobramycin based on the CFQ-R respiratory symptom scale (Figure 5c) and experienced greater need for additional intravenous or inhaled antibiotic therapy (Figure 5d). Full analyses of azithromycin use in this study have been published[19].

Figure 5a

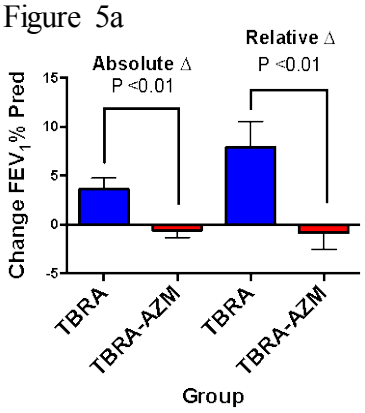


Figure 5b

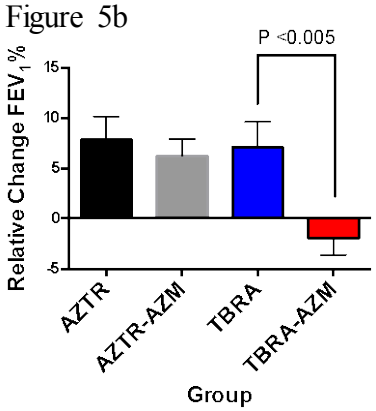


Figure 5c

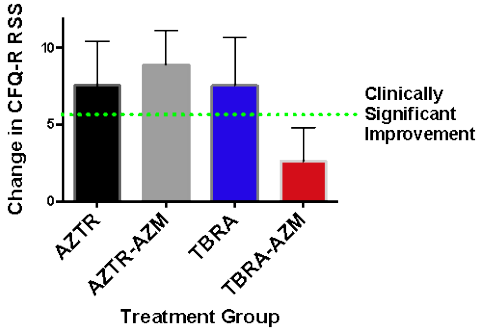
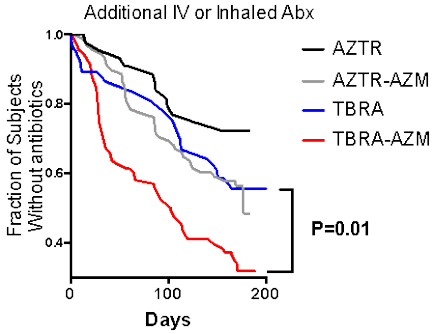


Figure 5d



AIR-CF2 Clinical Trial

During the 4-week run-in period with inhaled tobramycin, subjects with concomitant azithromycin use had lesser improvement in FEV_1 (Figures 6a and 6b). Immediately after inhaled tobramycin use, subjects received inhaled aztreonam lysine. Users of azithromycin had significantly greater improvement in FEV_1 with the non-aminoglycoside, aztreonam, than during the run-in period with tobramycin. Subjects without azithromycin use had approximately 4% relative increase in FEV_1 (liters) during both the tobramycin and subsequent aztreonam lysine periods of this study. Self-reported quality of life, again based on the change in CFQR-RSS, was also similarly impacted by azithromycin use (Figure 6c).

Figure 6a

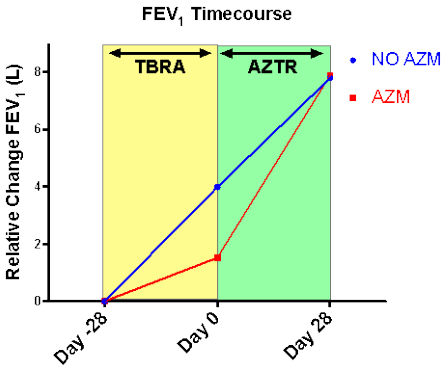


Figure 6b

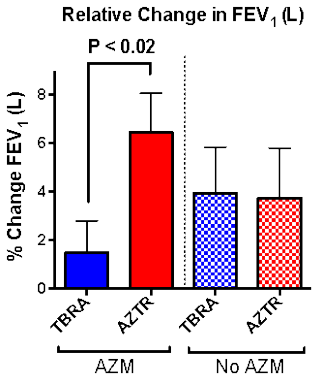
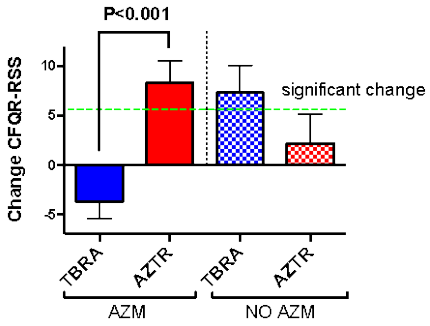


Figure 6c



2 STUDY RATIONALE

If our hypotheses are appropriately tested and proven correct, then current practice recommendations for tens of thousands of patients with CF worldwide may be altered. Based on the CF registry data, we estimate there are >10,000 patients in the US currently prescribed azithromycin and inhaled tobramycin. Over a lifetime, over 50% of patients will meet the clinical criteria to be prescribed these two medications as chronic therapy. Additional concerns extend to the use of intravenous (IV) tobramycin, which is selected twice as often as any other anti-pseudomonal drug for CF pulmonary exacerbations[25]. Impact of azithromycin on IV aminoglycosides is beyond the scope of this project but demonstrate broader implications for our research. There are sizable financial impacts related to the cost of these medications and outcomes related to decline in lung function (e.g., pulmonary exacerbations, quality of life, and survival).

Taken together, the data summarized above provide strong evidence that a drug-drug interaction may be present in current CF care recommendations. However, there is obvious risk for confounding by indication with azithromycin use when analyzing clinical data retrospectively. Reported baseline characteristics are similar between users and non-users of azithromycin in these data, but multiple recognized and unrecognized variables that may contribute to pulmonary decline are not accounted for. Therefore, a prospective study to both control for such confounders and intentionally monitor drug use is needed.

Our primary and secondary outcomes focus on relative change in FEV_1 (liters) and self-reported, disease related quality of life. Additional information, such as comparison with alternative inhaled antibiotics or the effect on pulmonary exacerbations, is viewed as important but would

require a much larger study. The planned design will allow us to test the primary hypothesis of an adverse drug interaction between azithromycin and inhaled tobramycin. It may be that both agents, when used without the other, are beneficial but that the combination is disadvantageous. If our theory is proven through this study, then these data can be used to justify and design additional research to address other important and related questions.

2.1 Risk / Benefit Assessment

No experimental or new medications are being introduced to subjects during this study. Removal of chronic azithromycin may decrease lung function or increase the rate of pulmonary exacerbation, based on prior studies of azithromycin in similar patients[10,26]. Our preliminary data does not support this concern and suggest that removing azithromycin may be protective in the majority of subjects also using inhaled tobramycin. This includes better clinical outcomes in lung function (FEV_1), patient reported quality of life, and time to need additional antibiotics[19]. However, a withdrawal study of either medication has never been reported and our preliminary data on lung function may not fully predict the risk of pulmonary exacerbation. Subjects experiencing a pulmonary exacerbation will be treated but will not be removed from the study.

Identifying an undesirable drug-drug interaction pertinent to over 50% of US patients with CF has clear and important safety and efficacy implications. Given that both of these medications benefit from strong support in US consensus guidelines, answering this question is a high priority.

Recent reports raise concern about potential proarrhythmic cardiotoxicity with oral azithromycin treatment in non-CF populations[27]. Patients with CF account for a significant portion of chronic azithromycin use in the US and cardiac arrhythmias have not been linked to this therapy. Similarly, the relative risk of cardiovascular death attributable to azithromycin was reported as <1 per 100,000 antibiotic courses in low risk patients[27]. There are approximately 30,000 CF patients in the entire US, and a repeat study found no increased risk in younger, lower risk adult populations[28]. However, this study provides an opportunity to analyze electrocardiograms (ECGs), focusing on any effect on QTc interval, in a group of CF subjects both on and off of azithromycin.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this study is to determine if azithromycin impairs the previously-recognized clinical benefits of inhaled tobramycin by comparing changes in pulmonary function as measured by FEV_1 between subjects randomized to azithromycin versus placebo.

3.2 Secondary Objectives

The secondary objectives for the randomized period are to:

- Compare changes in patient-reported quality of life between subjects randomized to azithromycin versus placebo

The secondary exploratory objectives for the randomized period are to:

- Compare the safety profile between subjects randomized to azithromycin versus placebo.

- Compare changes in additional spirometry measures, bacterial density, and weight between subjects randomized to azithromycin versus placebo
- Compare rates of pulmonary exacerbations, hospitalizations, and acute antibiotic usage between subjects randomized to azithromycin versus placebo

The objectives for those continuing in the open-label extension:

- Among those randomized to azithromycin and remaining on azithromycin, obtain additional data to determine the long term effect of azithromycin on clinical outcomes
- Among those randomized to placebo and switching to azithromycin, compare differences in clinical outcomes observed during the randomized and open-label periods

4 STUDY DESIGN

4.1 Study Overview

This is a prospective, randomized, placebo-controlled, double-blinded study of azithromycin in subjects with chronic *Pa* airway infection using inhaled tobramycin. Subjects who have received two (2) or more cycles of inhaled tobramycin within the 24 weeks prior to enrollment will be recruited into the study. This study will investigate whether azithromycin is associated with poorer clinical and microbiologic outcomes as compared to placebo during concurrent administration of inhaled tobramycin.

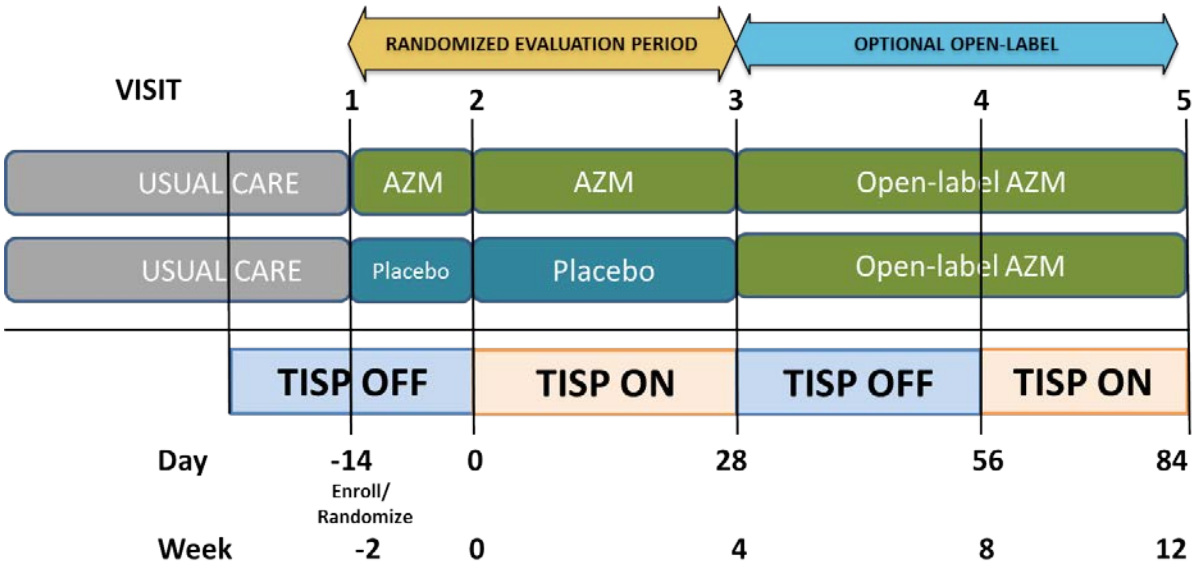
Approximately 120 eligible subjects will be enrolled and randomized to either azithromycin or placebo at Visit 1, approximately 14 days prior to the start day of their next planned 28-day nebulized solution or dry powder tobramycin (TISP) cycle. Both tobramycin inhalation solution (TIS) and tobramycin inhalation powder (TIP) will be allowed, based on clinical prescription. Tobramycin solution should be nebulized with an approved nebulizer and air compressor.

Subjects will be randomized in a 1:1 fashion to azithromycin (500 mg three times per week) or matched placebo. Randomization will be stratified by FEV_1% predicted (25%-50%, >50%-75%, >75%), chronic oral azithromycin use for the past 30 days, inhaled tobramycin formulation (TIS or TIP), and site. Between Visit 1 (Day -14) and Visit 2 (Day 0), a two-week run-in period will be used to begin administration of either azithromycin or placebo. Among those randomized to azithromycin, the 2-week run-in period will be used to initiate azithromycin and standardize uptake prior to the start of the next TISP cycle. Among those randomized to placebo, the run-in period will be used as a standardized washout prior to the start of the next TISP cycle. Subjects will begin TISP at Visit 2 in addition to their continued dosing of randomized study drug treatment (azithromycin or placebo). Subjects will continue both TISP and study drug dosing for 28 days (up until Visit 3), which corresponds with the end of a clinically prescribed 4-week cycle of TISP.

Subjects completing the study through Visit 3 will be offered participation and consent to an 8-week open-label extension period during which azithromycin will be provided. During the open-label period, subjects will be instructed to remain off of TISP for the first 4-week period, followed by a 4-week period of TISP.

The total duration of the study is expected to be approximately 33.5 months: 30 months for subject recruitment and 3.5 months for the final subject enrolled to complete the study (Figure 7).

Figure 7. Study Schematic



A follow up safety visit is not necessary for this study as it does not expose subjects to new or investigational products.

5 CRITERIA FOR EVALUATION

5.1 Primary Endpoint

The primary endpoint in the study is the difference between the azithromycin and placebo treatment groups in the relative change in FEV_1 (liters) from randomization (Visit 1) to Visit 3.

5.2 Secondary Endpoints

Randomized Period

Secondary Endpoints:

- Difference between the azithromycin and placebo treatment groups in the relative change in FEV_1(liters) during administration of TISP from Visit 2 to Visit 3.
- Difference between the azithromycin and placebo treatment groups in the change from Visit 1 to Visit 3 in the Cystic Fibrosis Respiratory Symptom Diary (CFRSD) - Chronic Respiratory Infection Symptom Score (CRISS©)
- Difference between the azithromycin and placebo treatment groups in the change from Visit 1 to Visit 3 in the scales derived from the CFQ-R

Exploratory Secondary Endpoints:

- Difference in adverse event rates and rates of abnormal QTc parameters from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups
- Difference in the proportion of subjects hospitalized from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups Difference in the proportion of subjects

prescribed acute oral, inhaled and IV antibiotics from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups
- Difference in the proportion of subjects experiencing a protocol-defined pulmonary exacerbation requiring antibiotic therapy from Visit 1 through Visit 3 between the azithromycin and placebo treatment groups
- Difference between the azithromycin and placebo treatment groups in the absolute change from Visit 1 to Visit 3 and Visit 2 to Visit 3 in FEV_1 (liters and % predicted), absolute and relative change in FVC (liters and % predicted), and absolute and relative change in $FEF_{25-75\%}$
- Difference between the azithromycin and placebo treatment groups in the change from Visit 1 to Visit 3 in weight
- Difference in the change from Visit 1 to Visit 3 in sputum *Pa* bacterial density between the azithromycin and placebo treatment groups

Open-Label Period
- Among those randomized to azithromycin and remaining on azithromycin, longitudinal changes and/or rates in all clinical outcomes across the randomized and open-label periods
- Among those randomized to placebo and switching to azithromycin, differences in all clinical outcomes between the randomized and open-label periods

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of CF who meet all of the inclusion and none of the exclusion criteria will be eligible for enrollment in this study.

6.2 Inclusion Criteria

Inclusion Criteria for Randomized Period:

1. Male or female ≥ 12 years of age at Visit 1
2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and one or more of the following criteria:
   - Sweat chloride equal to or greater than 60 mEq/L by quantitative pilocarpine iontophoresis test (QPIT)
   - Two mutations in the CFTR gene believed to be disease causing in the opinion of the site investigator
   - Abnormal nasal potential difference (change in NPD in response to a low chloride solution and isoproterenol of less polarizing than -7 mV)
3. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative
4. Two or more respiratory cultures (sputum, OP swab, BAL sample) growing *Pa* collected within the 12 months prior to randomization, at least 1 month apart, with at least one collected in the 6 months prior to randomization
5. FEV_1% predicted between 25-100% at Visit 1

6. Off TISP and other inhaled anti-pseudomonal antibiotics for at least two weeks at randomization
7. Willingness to begin the next course of TISP at the scheduled Visit 2
8. Use of at least 2 cycles of TISP within 24 weeks prior to Visit 1
9. Ability to swallow capsules (similar in size to pancreatic enzyme capsules)
10. Most recent LFTs less than 4x ULN. Must be obtained within 12 months prior to Visit 1
11. Current azithromycin use at Visit 1 or prior chronic azithromycin use defined as four or more consecutive weeks. Subjects do not have to be using chronic oral azithromycin at Visit 1
12. Stable clinical status with no significant change in medication or airway clearance routine for four weeks prior to Visit 1, as determined by site investigator

Inclusion Criteria for Open-Label Period:

1. Completion of the randomized, placebo-controlled period (up through Visit 3)
2. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative
3. Ability for subject to comply with the requirements of the study
4. Willingness to begin the next course of TISP at the scheduled Visit 4
5. Clinically stable at Visit 3 as assessed by the site investigator

6.3 Exclusion Criteria

Exclusion Criteria for Randomized Period:

1. Weight < 40 kg at Visit 1
2. For women of child-bearing potential:
   a. positive pregnancy test at Visit 1 or
   b. lactating or
   c. unwilling to practice a medically acceptable form of contraception (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal during the study
3. Inability to perform reproducible spirometry
4. Inability or unwillingness to cycle off TISP for one 4-week period
5. Respiratory culture with *Burkholderia cepacia* complex species within the 24 months prior to Visit 1
6. Respiratory culture with nontuberculous mycobacteria (NTM) within 18 months prior to Visit 1
7. Use of IV or oral anti-pseudomonal antibiotics within four weeks prior to Visit 1 (other than azithromycin)
8. Use of an investigational therapy within four weeks prior to Visit 1
9. History of unresolved, abnormal neutropenia (ANC $\leq$ 1000 cells/mm^3)
10. Current use of systemic corticosteroids equivalent to a daily dose more than 10 mg prednisone at Visit 1

11. Current use of nelfinavir, warfarin, haloperidol, or methadone at Visit 1
12. Initiation of approved CFTR modulators less than 30 days prior to Visit 1
13. ECG abnormalities identified at Visit 1 that require prompt, further medical evaluation or QTc interval >480 msec for males and >486 msec for females at Visit 1
14. Any other condition that, in the opinion of the site investigator/designee, would preclude informed consent or assent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

Exclusion Criteria for Open-Label Period:

1. Weight < 40 kg at Visit 3
2. For women of child bearing potential: unwilling to practice a medically acceptable form of contraception (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal during the study
3. Identified respiratory culture positive for NTM or *Burkholderia cepacia* complex species during randomized study period
4. Use of an investigational therapy at Visit 3
5. Current use of systemic corticosteroids equivalent to a daily dose more than 10 mg prednisone
6. Current use of nelfinavir, warfarin, haloperidol, or methadone
7. Any other condition that, in the opinion of the site investigator/designee, would preclude informed consent or assent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

6.4 Study Specific Tolerance for Inclusion/Exclusion Criteria

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

6.5 Screen Fail Criteria

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

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

For both the randomized and open-label periods, standard therapy for CF is allowed except for treatments noted in the conditionally allowed and prohibited medications sections below.

Ongoing chronic treatment (>30 days prior to Visit 1) with inhaled dornase alpha, hypertonic saline, short and long acting bronchodilators, FDA-approved CFTR modulators, high dose ibuprofen, low-dose inhaled or systemic steroids and airway clearance is allowed.

7.2 Conditionally Allowed Medications and Treatments

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 (azithromycin or placebo) or open-label azithromycin **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) or open-label azithromycin **must** be temporarily stopped during use of these medications. After acute treatment is completed, subjects should re-start study drug or open-label azithromycin on the next scheduled treatment day.
- Ongoing chronic treatment (>30 days prior to Visit 1) with antidepressant medications citalopram and escitalopram is allowed without affecting study participation if:
    - The subject has been using both chronic oral azithromycin AND citalopram (or escitalopram) consistently for greater than 30 days at Visit 1

      AND
    - The Visit 1 ECG demonstrates QTc within the normal limits as defined by the protocol

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").

Acute use of macrolide antibiotics may be required for certain infections and is allowed but also requires temporarily stopping the study drug or open-label azithromycin. The study drug or open-label azithromycin should be restarted on the next scheduled day following completion of the prescribed macrolide antibiotic.

Treatment for pulmonary exacerbations 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 and initiate or increase the dose of acute systemic corticosteroids (defined as less than 10 days) only in the presence of increased symptoms suggesting a pulmonary exacerbation.

7.3 Prohibited Medications and Treatments

For both the randomized and open-label periods, the following medications are prohibited (for the randomized period, between Visit 1 through Visit 3; for the open-label period, between Visit 3 and Visit 5).

- Investigational therapies
- Chronic inhaled antibiotics other than cycled TISP (e.g., Cayston®, colistin). Chronic treatment is defined as inhaled antibiotics prescribed as a maintenance therapy and not used temporarily to treat a pulmonary exacerbation.
- Increase in chronic systemic corticosteroid dose beyond prednisone equivalent of 10 mg per day, or initiation of chronic systemic (oral or intravenous) corticosteroids at any dose. Chronic use is defined as greater than 10 days continuous use.
- New or increase in ibuprofen dose of greater than 800 mg total dose per 24 hours for more than 5 days
- Nelfinavir, warfarin, haloperidol, or methadone

7.4 Change in Medications or Therapies

Subjects should receive all medication as clinically necessary, regardless of what impact this may have on the study.

Maintaining a stable therapeutic regimen between Visit 1 and Visit 5 is desirable to minimize the risk of confounding. Unless otherwise medically indicated, subjects not using these therapies should not be started on them after Visit 1 or at any time during the trial and subjects that have been using them chronically should be strongly encouraged to continue them throughout the entire study period (through Visit 5).

8 STUDY TREATMENTS

8.1 Randomization

At Visit 1, eligible subjects will be enrolled and randomized to study drug. Study personnel at the investigative site will use the Medidata® Rave and Balance™ systems to randomize each subject. An adaptive randomization (dynamic allocation based on minimization[31]) will be employed. Subjects will be randomly assigned in a 1:1 ratio to azithromycin (500 mg three times per week) or matched placebo. Randomization will be stratified by FEV_1 % predicted (25%-50%, >50%-75%, >75%), chronic oral azithromycin use for the past 30 days, inhaled tobramycin formulation (TIS or TIP), and site. The dynamic allocation algorithm seeks to optimize randomization balance by minimizing a weighted average of the marginal imbalance[32] 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[33] to include a chance of allocation to a treatment arm other than the arm that optimizes balance. After data collection at Visit 1, subjects will be randomized to one of the two treatment groups. To randomize a new subject, study personnel at the investigative site will complete the electronic CRF in the Medidata Rave system required to provide the stratification information and the subject will then be assigned to a treatment group.

8.2 Blinding

Study treatment (azithromycin or placebo) will be assigned in a double-blinded fashion. Subjects and site research staff will not know the treatment assigned, with the exception of the dispensing site pharmacist or designee and designated TDNCC personnel.

The following study procedures will be in place to ensure double-blind administration of 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 designated TDNCC study personnel
- Test and control treatments will be manufactured to be identical in appearance and taste-matched
- Packaging and labeling of test and control treatments will be identical

The study blind will be broken upon completion of the clinical study and after the study database has been locked and study results released. The site investigators will be provided with each subject's treatment assignment following completion of data analysis.

8.3 Unblinding Procedures

During the study, the blind may be broken for individual subjects in emergencies when knowledge of the subject'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. Individual SAEs that are unexpected, deemed related to study drug, and confirmed by the Medical Monitor will be forwarded to the DSMB Chair within 7 business days. Additional information or data summaries may also be requested by the DSMB at this time.

If the blind is broken and the subject is on study drug, a Safety Report will be sent to all participating investigators. Emergency unblinding procedures are provided in the Study Manual. Refer to protocol section 11.2.1 Serious Adverse Experience Reporting.

8.4 Formulation of Test and Control Products

8.4.1 Formulation of Test Product

Azithromycin 500 mg capsules will be provided. Over-encapsulated azithromycin tablets capsules will be provided during the randomized period of the study.

During the open-label extension period, commercially-available azithromycin 500 mg capsules (without over-encapsulation) will be used.

8.4.2 Formulation of Control Product

Placebo capsules using the same materials used for azithromycin over-encapsulation will be provided. The placebo will be appearance-matched.

8.4.3 Packaging and Labeling

Capsules for the randomized period of the study will be provided in labeled bottles. A six-week supply plus one extra (19) of capsules will be included in each bottle. One bottle will be supplied at Visit 1.

If the subject enrolls in the open-label period, commercially-available azithromycin will be provided by the site pharmacy.

8.5 Supply of Study Drug to the Site

Study drug for the randomized period will be shipped to each site pharmacy. The initial study drug shipment will be shipped after site approval to enroll (i.e., all required regulatory documentation has been received by the TDNCC and a contract has been executed). Subsequent study drug shipments will be made as needed.

For the open-label period, sites will obtain their own supplies of commercially-available azithromycin capsules.

8.5.1 Dosage/Dosage Regimen

Randomized study drug (azithromycin or placebo) and open-label azithromycin will be taken by mouth three times weekly during the following study periods:

- Treatment V1-V2: azithromycin or placebo without TISP: 14 days
- Treatment V2-V3: azithromycin or placebo with TISP: 28 days

Subjects participating in the optional open-label extension:

- Treatment V3-V4: azithromycin without TISP: 28 days
- Treatment V4-V5: azithromycin with TISP 28 days

8.5.2 Study Drug Preparation and Dispensing

Appropriately trained site personnel will be responsible for dispensing study drug (includes the randomized period and open-label period). Study drug shall be dispensed and labeled in accordance with federal and local state Board of Pharmacy regulations. Capsules will be provided in labeled bottles from the study pharmacy with instructions to take one capsule three times weekly.

If the subject enrolls in the open-label period, an 8-week supply plus one extra (25) of commercially-available azithromycin tablets will be dispensed at Visit 3.

8.5.3 Administration Instructions

Study drug (azithromycin or placebo and open-label azithromycin) should be taken by mouth with food and pancreatic enzymes (if clinically prescribed).

It is recommended that the first dose be taken at the Visit 1 to ensure that the subject is able to swallow the capsule.

8.5.4 Storage

Study drug (azithromycin or placebo and open-label azithromycin) should be stored by the study site at controlled room temperature, 15 to 30 ºC (59 to 86 ºF) in a secure area under restricted access. 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.

Subjects will be instructed to store study drug (azithromycin or placebo and open-label azithromycin) in original packaging at room temperature.

8.6 Dose Modifications

8.6.1 Due to Toxicity

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

- If a study subject receiving study drug three times weekly (TIW) experiences drug-related toxicity, the dosing INTERVAL of the study drug will be extended to two times weekly (BIW; e.g., Monday and Thursday)
- If a study subject does not tolerate receiving study drug BIW, the subject should be permanently discontinued from study drug due to intolerance.

Dose can return to TIW at the discretion of the site investigator.

8.6.2 Due to Treatment with Antibiotics – Temporary Discontinuation of Study Drug

Study drug should be temporarily discontinued for subjects prescribed macrolide antibiotics. Study drug may be temporarily discontinued at the discretion of the treating clinician during acute treatment with other medications (e.g., additional antibiotics or drugs believed to increase QT prolongation).

Regardless of study drug use, subjects should be encouraged to complete all study visits.

8.6.3 Missed Doses

Missed doses may be made up as long as they are not taken within 24 hours of the next scheduled dose.

8.7 Study Drug Accountability

Study drug (azithromycin or placebo and open-label azithromycin) dispensed to subjects will be counted as noted in the Schedule of Events. Study drug distribution will be recorded, including study drug identifier (randomized period), date, quantity, and subject ID.

8.8 Measures of Treatment Compliance

Subject daily diaries recording study drug and TISP use will be reviewed and the number of doses taken will be recorded throughout the study. Subjects will be asked to bring study drug bottles and the diary to each study visit and the number of study drug capsules remaining will be compared with the number ideally taken. Subjects will be questioned at each visit about missed or extra doses of study drug, open-label azithromycin, and/or TISP. Treatment compliance for

study drug will be based on the number of doses reported taken by the subject, with consideration of drug returns.

8.9 Standard of Care Treatment with TISP

Subjects will not be allowed to change from inhaled tobramycin solution to inhaled tobramycin powder (i.e., TOBI® Podhaler) or vice versa while enrolled in the study. Either tobramycin delivery mechanism will be allowed but subjects must be stable on one delivery mechanism, either solution or dry powder, for one or more on/off cycles (i.e., 8 weeks) prior to Visit 1.

8.9.1 Administration Instructions

If the subject is using inhaled tobramycin solution, inhaled tobramycin will be administered as per the manufacturer's instructions (e.g., 300 mg in 4 or 5 mL solution, nebulized and inhaled twice daily using a device approved by the drug manufacturer). Tobramycin solution should be nebulized with an approved nebulizer and air compressor without mixing or diluting with other solutions, according to manufacturer instructions.

If the subject is using inhaled tobramycin powder, the TOBI Podhaler will be administered as per the manufacturer's instructions (e.g., 4 dry powder capsules inhaled twice daily using the supplied device).

Ideally, tobramycin should be inhaled twice daily following airway clearance. Airway clearance includes any inhaled bronchodilators, mucolytics (e.g., DNase, hypertonic saline), and mechanical clearance devices (e.g., Vest, acapella, hand percussion). If airway clearance is not clinically recommended at least twice daily, then tobramycin should be inhaled following the single airway clearance session and again each day without airway clearance.

Tobramycin should be stored refrigerated per manufacturer's instructions.

9 STUDY PROCEDURES AND GUIDELINES

The procedures described below will be performed at the visits noted in the Schedule of Events (Appendix 1) and in Section 10.

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 subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented as noted in the Schedule of Events. Dose, route, unit, frequency of administration, indication for administration and dates of medication will be captured.

9.1.2 Demographics and CFF Registry ID

Demographic information (date of birth, sex, race) will be recorded at Visit1. CF Registry ID number will be recorded for participating CF subjects who provide optional consent.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded. Previous use of azithromycin and inhaled tobramycin will also be collected.

9.1.4 CF Diagnosis

Diagnosis date and sweat chloride test, CF genotype, and transepithelial nasal potential difference test result will be recorded as available.

9.1.5 Physical Examination

A complete or abbreviated physical examination will be performed by a licensed professional (MD, NP, RN, PA) as noted in the Schedule of Events. The abbreviated exam includes respiratory, cardiovascular, and abdominal assessments.

After the initial exam at Visit 1, new clinically significant, abnormal physical exam findings must be documented as AEs.

9.1.6 Weight and Height

Weight will be measured on the same scale and recorded as noted in the Schedule of Events. Adults and children may remain in clothes (without shoes). A standing height will be measured and recorded as noted in the Schedule of Events.

Note that accurate height measurements will significantly improve the data quality, as this is incorporated into equations used to derive the FEV_1% of predicted based on a reference population.

9.1.7 Vital Signs

Resting (minimum of 5 minutes) measurements of body temperature, blood pressure, pulse and respirations will be performed and recorded as noted in the Schedule of Events.

9.1.8 Spirometry

Spirometry will be performed at as noted in the Schedule of Events and in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests.

Subjects should be encouraged to perform the same airway clearance routine and inhaled bronchodilators prior to each study visit. Additional instructions for study personnel will be provided in the Study Manual.

9.1.9 Respiratory Culture

If the patient does not have sufficient culture history to assess eligibility, a respiratory culture may be collected prior to randomization. If the respiratory culture is positive, the remaining Visit 1 procedures and randomization will be conducted at a subsequent visit.

9.1.10 Subject Questionnaire: CFRSD-CRISS

The subjects will be given a CF specific symptom diary called the CFRSD-CRISS as noted in the Schedule of Events. This diary includes 8 questions and takes less than 5 minutes to complete. The CFRSD-CRISS should be administered at the beginning of each visit.

9.1.11 Subject Questionnaire: Cystic Fibrosis Questionnaire-Revised (CFQ-R)

The CFQ-R is a patient reported quality of life instrument, regarding symptoms and mood over the preceding 2 weeks. The age appropriate version of the CFQ-R will be completed at the beginning of each visit as noted in the Schedule of Events by the subject. Completion should take less than 5 minutes.

9.1.12 Subject Diary

Subjects will be requested to complete a daily diary to document daily dose of study drug treatments and TISP treatments, and to record changes in concomitant medications and symptoms through the end of the study. The diary will be dispensed and reviewed as noted in the Schedule of Events.

9.1.13 Electrocardiogram (ECG)

Standard 12-lead ECG tracing will be obtained as noted in the Schedule of Events. ECG is only needed for an acute visit if the subject has a cardiovascular-related AE or will no longer be participating in the study.

Testing will be conducted and reviewed at the site by trained personnel with experience interpreting electrocardiograms. Any test results of clinical concern, should be reported to the Medical Monitor 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.

Abnormal results at the time of follow-up testing are defined as a QTc >500 msec, or an increase in QTc of ≥60 msec. It is recommended that testing be repeated after 10 minutes for any ECGs that meet the protocol definition for abnormal. If test results are confirmed, subjects will be instructed to stop study drug or open-label azithromycin and the site investigator will continue to observe the subject for clinical signs of arrhythmia (specifically torsades de pointes).

The ECG tracing will be collected for additional safety review.

9.1.14 Signs and Symptoms Evaluation

When a subject initiates a new IV, oral or inhaled antibiotic, the presence of specific signs and symptoms will be assessed and documented. These data will be used to determine the incidence of a protocol defined pulmonary exacerbation based on the definition in Appendix 2.

9.1.15 Adverse Events

Information regarding occurrence of AEs will be captured throughout each subject's study participation, starting after the first dose of study drug and ending once the subject has

terminated from the study. Duration (start and stop dates), grade, seriousness, outcome, treatment and relation to study participation will be recorded on the CRF.

9.2 Clinical Laboratory Measurements

9.2.1 Pregnancy Test

Urine or blood (approximately 2 cc) will be collected from females who are of childbearing potential for a pregnancy test as noted in the Schedule of Events and tested by the site according to site standard procedures.

9.2.2 Sputum Collection for Microbiological and Host Response Analysis

Expectorated sputum will be collected from subjects who are able to produce sputum as noted in the Schedule of Events. The samples collected will be stored frozen at -70 to -80ºC at the site and batch shipped as instructed by the Therapeutics Development Network Coordinating Center (TDNCC). Instructions for specimen collection, processing, storage and shipping of samples will be provided in the Study Laboratory Manual. Processed aliquots will be banked for future analysis.

10 EVALUATIONS BY VISIT

Randomized Period

10.1 Visit 1 – Day -14

*A respiratory culture may be obtained prior to randomization to confirm eligibility criteria #4. If a respiratory culture is needed, the remaining Visit 1 procedures should be completed after confirming positive *Pa* culture results.

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Assign the subject a unique study number.
3. If there is not a recent respiratory culture available, obtain culture to confirm eligibility:
    - Review culture results.
    - If *Pa* positive and meets criteria #4, schedule subsequent visit to complete remaining Visit 1 procedures.
    - If *Pa* negative, record as Screen Fail.
4. Review and record previous use of azithromycin and inhaled tobramycin.
5. Record demographics and CFF Registry ID, if consent obtained.
6. Record medical history, including a history of CF diagnosis and date.
7. Record concomitant medications.
8. Administer PRO questionnaires (CFRSD-CRISS and CFQ-R).
9. Perform a complete physical examination.
10. Measure and record height and weight.
11. Obtain and record vital signs.

12. Perform and record spirometry.
13. Collect blood or urine for pregnancy test (if female of child-bearing potential).
14. Obtain resting ECG.
15. If able to expectorate sputum, collect expectorated sputum.
16. Confirm subject eligibility.
17. Randomize to study drug.
18. Dispense study drug.
19. Review study drug dose and schedule with subject.
20. Provide subject diary and instructions for completion.
21. Remind subject to NOT use inhaled tobramycin between this day and Visit 2.
22. Instruct subject to bring study drug bottle, any remaining capsules, and diary to Visit 2.
23. Schedule subject for Visit 2.

10.2 Visit 2 - Day 0 (visit window -2/+4 days)

1. Administer PRO questionnaires (CFRSD-CRISS and CFQ-R).
2. Review subject diary.
3. Perform pill count on returned study drug.
4. Record any AEs.
5. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that a Signs and Symptoms form was completed.
6. Perform an abbreviated physical examination.
7. Measure and record height and weight.
8. Obtain and record vital signs.
9. Perform and record spirometry.
10. If able to expectorate sputum, collect expectorated sputum.
11. Provide subject diary.
12. Review inhaled tobramycin schedule and remind subject to take TISP between Visits 2 and 3.
13. Instruct subject to bring study drug bottle, any remaining capsules, and diary to Visit 3.
14. Schedule subject for Visit 3. Visit 3 should coincide with completion of approximately 4 weeks use of inhaled tobramycin combined with study drug.

End of Randomized Period and Start of Open-Label Period

10.3 Visit 3 – Day 28 (visit window -2/+4 days), Week 4

1. Administer PRO questionnaires (CFRSD-CRISS and CFQ-R).
2. Review subject diary.
3. Perform pill count on returned study drug.

4. Record TISP use.
5. Record any AEs.
6. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that a Signs and Symptoms form was completed.
7. Perform abbreviated physical examination.
8. Measure and record height and weight.
9. Obtain and record vital signs.
10. Perform and record spirometry.
11. Obtain resting ECG.
12. If able to expectorate sputum, collect expectorated sputum.

Visit 3: Additional procedures for subjects enrolling in the open-label extension

13. Assess subject eligibility for open-label extension
14. Review the open-label extension period of the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
15. Dispense open-label azithromycin.
16. Provide subject diary.
17. Remind subject to NOT use inhaled tobramycin between this day and Visit 4.
18. Instruct to bring the azithromycin bottle, any remaining tablets, and diary to Visit 4.
19. Schedule subject for Visit 4.

Open-Label Period

10.4 Visit 4 – Day 56 (visit window +/- 4 days), Open-Label Week 4

1. Administer PRO questionnaires (CFRSD-CRISS and CFQ-R).
2. Review subject diary.
3. Perform pill count on returned azithromycin.
4. Record any AEs.
5. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that a Signs and Symptoms form was completed.
6. Perform abbreviated physical examination.
7. Measure and record height and weight.
8. Obtain and record vital signs.
9. Perform and record spirometry.
10. If able to expectorate sputum, collect expectorated sputum.

11. Provide subject diary.
12. Instruct subject to bring the azithromycin bottle, any remaining tablets, and diary to Visit 5.
13. Review inhaled tobramycin schedule and remind subject to take TISP between Visits 4 and 5.
14. Schedule subject for Visit 5.

10.5 Visit 5 - Day 84 (visit window +/- 4 days), Open-Label Week 8

1. Administer PRO questionnaires (CFRSD-CRISS and CFQ-R
2. Review subject diary.
3. Perform pill count on returned azithromycin.
4. Record TISP use.
5. Record any AEs.
6. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that a Signs and Symptoms form was completed.
7. Perform abbreviated physical examination.
8. Measure and record height and weight.
9. Obtain and record vital signs.
10. Perform and record spirometry.
11. Obtain resting ECG.
12. If able to expectorate sputum, collect expectorated sputum.
13. Instruct subject that study participation is complete and to use medications according to instructions from clinical care providers.

10.6 Unscheduled* or Early Discontinuation of Study Drug or Study Participation Visit

*An Unscheduled Visit may be conducted as a study visit when, in the opinion of the site investigator, safety follow up is required as a result of a possible study-related procedure or study drug-related event.

1. Administer PRO questionnaires (CFRSD-CRISS and CFQ-R).
2. Review subject diary.
3. Perform pill count on returned study drug (if during randomized period) or azithromycin (if during open-label period).
4. Record any AEs.
5. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that a Signs and Symptoms form was completed.
6. Perform complete physical examination.

7. Measure and record height and weight.
8. Obtain and record vital signs.
9. Perform and record spirometry.
10. Obtain resting ECG, if appropriate (see footnote [b] in Table for Schedule of Events).
11. If able to expectorate sputum, collect expectorated sputum.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An 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 or of greater severity or frequency than expected based on the information in the Prescribing or Product Information.

The site investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the subject CRF.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, as modified for CF, 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 Reference Binder. 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 (azithromycin or placebo for the randomized period, azithromycin for the open-label period) 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.

11.2 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 subject or require intervention to prevent one of the outcomes listed.

11.2.1 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 TDNCC within one business day of the site learning of the event. Sites will send the SAE report by either:

- Email (scanned copy) to: cfsaesfacsys@seattlechildrens.org
- TDNCC SAE Fax: (206) 985-3278

The site will notify the Medical Monitor of additional information or follow-up to an initial SAE Report as soon as relevant information is available. The Medical Monitor may request additional information related to the SAE. 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.

11.3 Medical Monitoring

The TDNCC Medical Monitoring Group should be contacted directly at this number to report medical concerns or questions regarding safety.

- (800) 341-0961

The TDNCC Medical Monitoring Group will serve as the independent Medical Monitors for this study. AEs and SAEs will be collected and organized by the TDNCC and provided to the TDNCC Medical Monitoring Group for review in a timely manner. The TDNCC Medical Monitoring Group will communicate with the independent DSMB at a predetermined schedule, and additionally as warranted.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Permanent Discontinuation of Study Treatment

A subject may be permanently discontinued from study treatment at any time if the subject, the investigator, or the Sponsor-Investigator feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for early permanent discontinuation of study treatment:

- Subject or subject's legal representative decision
- Adverse event, including pulmonary exacerbation
- Protocol violation
- Death

If a subject is discontinued from treatment due to an AE, the subject will be followed and treated by the site investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who permanently discontinue study treatment early should come in for an early discontinuation of study treatment visit as soon as possible (see Appendix 1) and then should be encouraged to complete all remaining scheduled visits and procedures.

Refer to Section 8.6.2 for temporary discontinuation of study drug.

12.2 Early Withdrawal of Subjects from the Study

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. This may include subjects who withdraw from study

treatment early and who decline to continue to come in for remaining follow-up visits or it may include subjects who completed treatment and decline to come in for remaining follow up visits.

Reasonable attempts will be made by the investigator to provide a reason for early subject withdrawals. The reason for the subject's early withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw early from the study should be encouraged to come in for a final early study withdrawal visit (and the procedures to be followed would include those for an early discontinuation of study drug visit – see Appendix 1).

12.3 Replacement of Subjects

Subjects who withdraw from the study will not be replaced.

13 PROTOCOL VIOLATIONS

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

- Failure to meet eligibility criteria
- Use of a prohibited concomitant medication. This includes the use of conditionally allowed medications without meeting the criteria to use these medications (e.g. Temporarily stopping study drug or open-label azithromycin).
- Failure to obtain spirometry at Visits 1-3

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

When a protocol violation occurs, it will be discussed with the 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.

14 DATA SAFETY MONITORING

A Data Safety Monitoring Board (DSMB) will review comprehensive interim enrollment, safety, and efficacy data reports when approximately 50% and 75% of patients have completed the randomized period of the study. Regular reporting frequency to the DSMB is detailed in the DSMB charter. *A priori* interim stopping rules for futility and efficacy/harm with respect to the primary endpoint are outlined in the DSMB charter. SAEs will be monitored by the committee on an ongoing basis throughout the study. Further details are included in the DSMB charter.

15 STATISTICAL METHODS AND CONSIDERATIONS

A detailed statistical analysis plan will be written that will describe all analyses 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).

15.1 Data Sets Analyzed

All analyses will be performed using a modified intent-to-treat (m-ITT) population, which is defined as all randomized subjects who received at least one dose of study drug. Subjects who are discontinued from study drug temporarily or permanently are encouraged to complete all remaining study visits and will remain in the analyses population according to ITT. The primary efficacy analyses will be repeated in the per-protocol population, which is defined as subjects having completed ≥80% of doses (azithromycin and or placebo) and who did not require the use of acute antibiotics or steroids. All tests will be two-sided and evaluated at a 0.05 level of significance.

15.2 Demographic and Baseline Characteristics

Baseline demographics and clinical characteristics recorded at Visit 1 will be summarized including (but not limited to) age, sex, CFTR genotype, race, height, weight, BMI, FEV_1% predicted, *Pa* bacterial density, delivery method of chronic tobramycin (i.e., dry powdered or inhaled), and use of other chronic medications (e.g., CFTR modulators, inhaled dornase alfa and hypertonic saline).

15.3 Analysis of Primary Endpoint

The primary endpoint will be the difference between the azithromycin and placebo treatment groups in the relative change from Visit 1 to Visit 3 in FEV_1 (liters). The primary endpoint will be compared between treatment groups using linear regression adjusted for randomization strata. Least squares means and the treatment effect for the relative change will be presented as well as corresponding 95% confidence interval and p-values. The p-values will be evaluated against a two-sided 0.05 level of significance.

Additional secondary analysis may be performed to adjust for potential baseline confounders including age, sex, and baseline FEV_1. Sensitivity analyses of the primary endpoint will be performed using the per-protocol population. Additional sensitivity analyses will be performed to address missing data. Specifically, the least favorable treatment arm imputation method will be used which imputes that missing value with the mean change from the treatment arm with the worst change in the observed case analysis. Further details regarding the handling of missing data will be provided in the statistical analysis plan.

15.4 Analysis of Secondary Endpoints

Randomized Period

Descriptive analyses and graphical displays will be used to summarize all secondary endpoints. Endpoints will be compared between the azithromycin and placebo treatment groups from Visit 1 through Visit 3.

All reported SAEs and AEs will be coded using MedDRA and grouped by body system. The incidence of AEs (including emergent abnormal QTc parameters) between the azithromycin and placebo treatment groups will be tabulated by seriousness, severity, and relationship to study drug. Rates of (S)AEs by System Organ Class (SOC) will also be summarized. Poisson regression modeling will be used to derive rate ratios and corresponding 95% confidence and to compare groups using a using a two-sided 0.05 level test. Histograms showing the frequency of the number of (S)AEs in each treatment group will be included.

The proportion of subjects permanently or temporarily discontinuing study drug will also 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 TISP and azithromycin /placebo therapies.

Continuous secondary endpoints including changes in sputum bacterial density, weight, patient-reported quality of life (as measured by scales derived from the CFQ-R and CFRSD-CRISS), and pulmonary function (FEV_1 % predicted, FVC, and $FEF_{25\%-75\%)}$ will be modeled similarly to the primary endpoint. Predicted values for spirometry measures at each visit will be calculated using the Global Lung Initiative reference equations.[30] Least squares means and the treatment effect for the change for each secondary endpoint from Visit 1 to Visit 3 will be presented as well as corresponding 95% confidence intervals and p-values. The relative change in FEV_1(liters) during administration of TISP from Visit 2 to Visit 3 will be analyzed in a similar manner.

Event rates for hospitalization, pulmonary exacerbations, intravenous antibiotic usage, inhaled antibiotic usage, and oral antibiotic usage from Visit 1 through Visit 3 will be descriptively summarized and differences in the proportions of subjects with an event between the treatment groups will be estimated with accompanying 95% confidence intervals. Event rates will be compared between treatment groups using Poisson regression.

Open-Label Period

Selected endpoints obtained during the randomized period will continue to be collected during the open-label period of the study. The change in lung function during the open-label period will be modeled among placebo subjects, and compared to both the change observed in this group during administration of TISP in the randomized trial and the change observed in the active group during administration of TISP in the randomized trial. Moreover, among those randomized to azithromycin, differences in the lung function between the randomized and open-label periods of TISP administration will be compared. Similarly, other endpoints measured in the open-label period will be used to estimate longer term changes in response to azithromycin in the group randomized to azithromycin. Data from the placebo subjects who entered into the open-label period will be used to compare results with those observed during the randomized period of the trial. Further details will be provided in the SAP that will be finalized prior to the first interim data analysis.

15.5 Interim Analysis

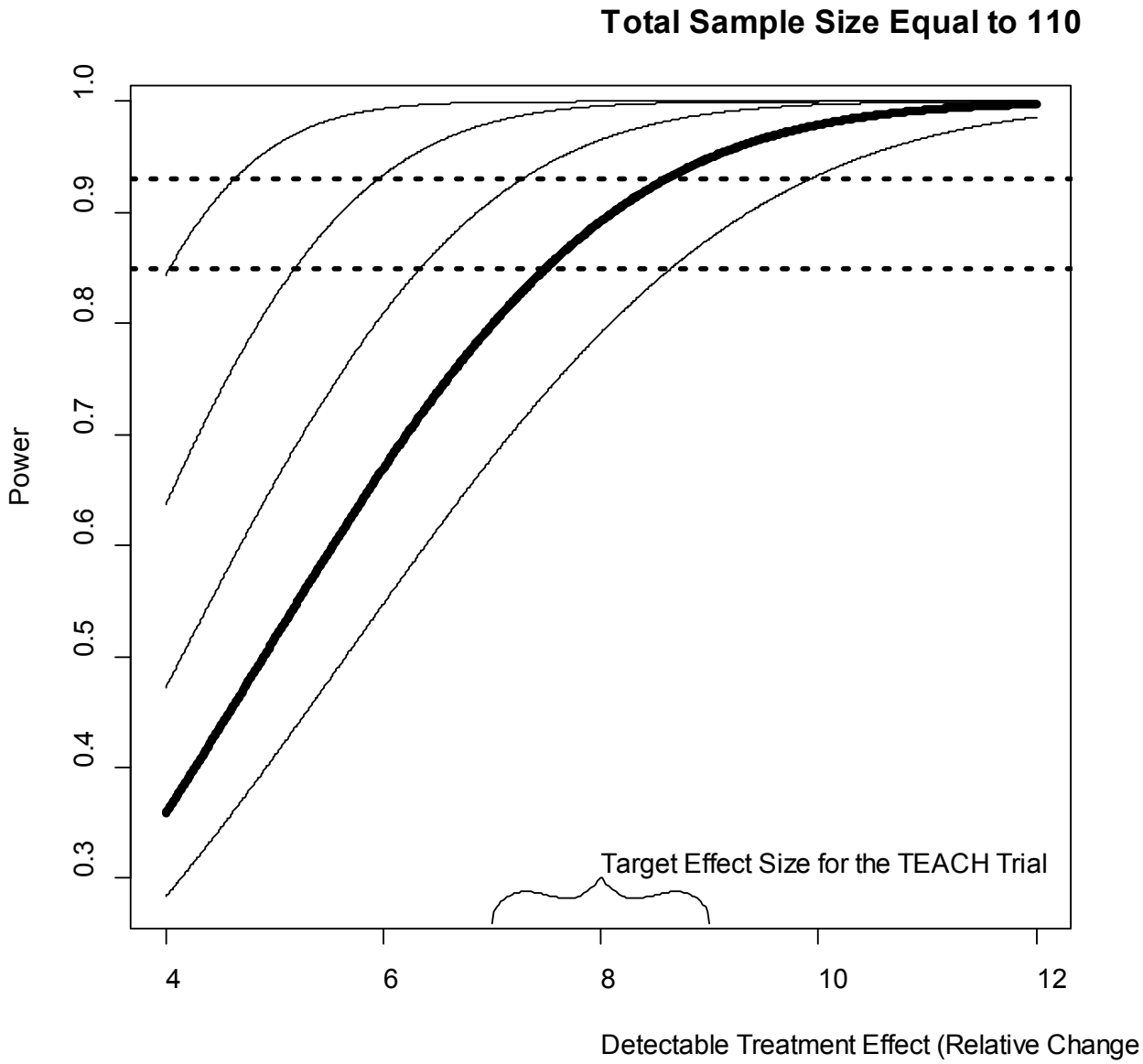
An independent data monitoring board (DSMB) will review comprehensive interim reports when approximately 50% and 75% of patients have completed the randomized period of the study. These interim reports will include an overview of enrollment, detailed summaries of SAEs, AEs, withdrawals, drug discontinuations, hospitalizations, protocol violations and other clinical safety endpoints. The primary endpoint and select secondary endpoints will be summarized as outlined in the statistical analysis plan. Should the DSMB consider stopping the trial based on the primary endpoint, they will be guided by *a priori* interim stopping rules for futility and efficacy/harm outlined in the DSMB charter. Regular reporting frequency to the DSMB is

detailed in the DSMB charter. SAEs will be monitored by the committee on an ongoing basis throughout the study.

15.6 Sample Size

The primary endpoint is the difference between the azithromycin and placebo treatment groups in the relative change from Visit 1 to Visit 3 in FEV_1 (liters). Data from prior published studies of similar duration are available to estimate sample size and power for the study including a randomized, placebo-controlled trial of azithromycin in individuals with CF chronically infected with *Pa* and a randomized, placebo controlled trial of aztreonam lysine similarly among those with chronic *Pa*. These studies suggest a standard deviation of the relative change in FEV_1 ranging from 10 to 16. Based on these prior studies, we anticipate the relative change in FEV_1 from Visit 1 to Visit 3 to be -2% in the azithromycin arm and +6.85% in the placebo arm for an overall treatment effect of 8.65%. A sample size of 110 provides 85% power to detect a treatment effect of 7.5% or greater assuming a standard deviation of 13. 110 subjects provides 93% power to detect a treatment effect similar to what was observed in the preliminary data (8.65%) assuming a standard deviation of 13. It is noteworthy that the standard deviation from more recently completed studies CF studies have observed standard deviations in short term relative change in FEV (liters) ranging from 8-11, confirming our conservatism in the standard deviation estimate used for our sample size calculations. Assuming a standard deviation of 11, a sample size of 110 provides 85% power to detect a treatment effect of 6.3% or greater in favor of the placebo group. Figure 8 displays several scenarios for the statistical power we have to detect a range of differences in relative change in FEV_1 (L).

Figure 8. Power to detect a range of differences in relative change in FEV_1 (L) for total sample size of 110 (55 per group) assuming a two-sided 0.05 level t-test. Curves (in ascending order) correspond to standard deviations of 15, 13, 11, 9, and 7.



16 DATA COLLECTION, RETENTION AND CLINICAL MONITORING

16.1 Data Collection Instruments

The investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic CRF when the information corresponding to that visit is available. Subjects 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 subject number and initials.

If a correction is required for a CRF, the time and date stamps track the person entering or updating CRF data and create an electronic audit trail.

The investigator is responsible for all information collected on subjects 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. At the completion of the study, a copy of the CRF data will be provided to the site to be retained at the Investigator's site.

16.2 Data Management Procedures

TDNCC utilizes Medidata Solutions, Inc. (Medidata) Rave for their EDC studies. The Medidata Rave EDC system is designed to be US Code of Federal Regulations (CFR) 21 Part 11 compliant with a robust audit trail system and electronic signature capabilities. Study personnel at each site will enter data from a subject's visit onto electronic CRF screens via a web browser. Study subjects will not be identified by name in the study database or on any data capture screens, but will be identified by initials and a unique subject identification number. Only study personnel at the individual sites will be able to link the study ID to the subject's name. The Data Management group of the TDNCC 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.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, data validation checks will be applied 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.

16.4 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 Biostatistics and Clinical Data Management group of the TDNCC.

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 Technology group. Data is backed up regularly according to the Information Services group's procedures.

Note that there is an intention to make biospecimens and associated data available to investigators for future exploration. The biospecimens will be collected under IRB approval,

processed according to a rigorous 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 administered through CFFT. 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) secure 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 to the CFFT.

16.5 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 subject must be maintained that includes the signed informed consent, Health Insurance Portability and Accountability Act (HIPAA) authorization and assent form (if applicable) and copies of all source documentation related to that subject. 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, Essential Document and Study Reference Binders) must be kept secured for a period of two years following completion of the study. 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.

16.6 Monitoring

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, if needed. Monitoring visits may be conducted by representatives of the Sponsor according to the U.S. CFR 21 Part 312 to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP.

16.7 Subject Confidentiality

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

17 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 subject, 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).

17.1 Protocol Amendments

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

17.2 Institutional Review Boards

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. SAEs 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, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRBs 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 TDNCC 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 patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC 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 patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), 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 US Code of Federal Regulations 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 subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject and the original will be maintained with the subject's records.

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

17.4 Consent for Collection and Use of CFF Registry ID Number

To facilitate possible future evaluation of retrospective and prospective information from all patients who screen for this study, consent will also be sought to collect the subject's CFF Registry ID number at the screening visit. The CFF Registry collects data on all CF patients who consented to participate in the CFF Registry and who are followed at CFF-accredited care centers. The registry data includes information from clinical encounters, hospitalizations courses of antibiotics, and year-end surveys. Data also include microbiology results, spirometry results, CF genotype and other information. If specific consent is given to collect this number, the subject's CF Registry ID number will be recorded in the CRF.

17.5 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 study Sponsor and 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.

17.6 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 subjects.
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 subjects 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/subjects.
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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19 APPENDIX 1 SCHEDULE OF EVENTS

	Randomized, Placebo-controlled, Double Blind		Overlap	Open-Label, Follow-On		Unscheduled/ Early Term Visit [f]
	Visit 1* Day -14	**Visit 2 (Day 0) (-2 to +4 days)**	**Visit 3 Day 28 (-2 to +4 days)**	**Visit 4_{EXT} Day 56 (± 4 days)**	**Visit 5_{EXT} Day 84 (±4 days)**	
Informed Consent	X		X[e]			
Respiratory Culture (as needed)	X[h]					
Demographics and Medical History	X					
Complete CFQ-R and CFRSD-CRISS	X	X	X	X	X	X
Complete Physical Exam	X					X
Abbreviated Physical Exam		X	X	X	X	
Height	X	X	X	X	X	X
Weight	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X
Spirometry	X	X	X	X	X	X
Pregnancy Test at site (Urine or Serum) [a]	X					
ECG Testing	X		X		X	X [b]
Collect sputum for analysis and banking [c]	X	X	X	X	X	X
Randomize to azithromycin or placebo	X					
Dispense azithromycin/placebo dose and review dose schedule	X					
Dispense open-label azithromycin and review dose schedule			X[e]			
Provide Subject Diary	X	X	X[e]	X		

	RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE BLIND		OVERLAP	OPEN-LABEL, FOLLOW-ON		UNSCHEDULED/ EARLY TERM VISIT [f]
	VISIT 1* Day -14	**VISIT 2 (Day 0) (-2 to +4 days)**	**VISIT 3 Day 28 (-2 to +4 days)**	**VISIT 4_{EXT} Day 56 (± 4 days)**	**VISIT 5_{EXT} Day 84 (±4 days)**	
Instruct to hold use of inhaled tobramycin for off-cycle until the next study visit	**X**		**X[e]**			
Concomitant Medication Review	**X**	**X**	**X**	**X**	**X**	**X**
Adverse Events		**X**	**X**	**X**	**X**	**X**
Review Subject Diary and pill count		**X**	**X**	**X**	**X**	**X**
Record TISP use			**X**		**X**	**X**
Review inhaled tobramycin schedule		**X**		**X**		
Assess eligibility for Open-label			**X[e]**			
Signs and Symptoms Assessment [g]	**Start**	➔	**Stop Start[e]**	➔	**Stop**	

* If the patient does not have sufficient culture history to assess eligibility, a respiratory culture may be collected prior to randomization. If the respiratory culture is positive, the remaining Visit 1 procedures and randomization will be conducted at a subsequent visit.

[a] Only females of child-bearing potential

[b] ECG is only required at an Unscheduled visit (if the subject has a cardiovascular related adverse event) or at an Early Termination Visit (when the subject permanently discontinues study drug or will no longer be participating in the study)

[c] Expectorated sputum will be collected from subjects who are able to produce sputum

[e] Perform only if rolling over into Open-Label

[f] Early termination includes early permanent discontinuation of study drug or early study withdrawal. An Unscheduled Visit may be conducted as a study visit when, in the opinion of the site investigator, safety follow up is required as a result of a possible study-related procedure or study drug-related event.

[g] Assessment only when a new antibiotic is prescribed during the study

h If there is not a recent respiratory culture available, obtain culture to confirm eligibility.

20 APPENDIX 2 PROTOCOL DEFINED PULMONARY EXACERBATION DEFINITION

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 pulmonary exacerbation 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