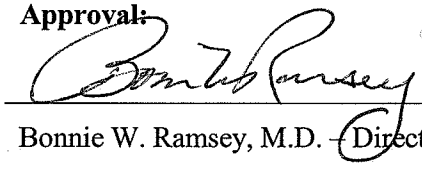
Effectiveness and Safety of Intermittent Antimicrobial Therapy for the Treatment of New Onset *Pseudomonas aeruginosa* Airway Infection in Young Patients with Cystic Fibrosis

Sponsor:	Name:	Bonnie W. Ramsey, M.D.
	Telephone:	(206) 987-5725
	Fax:	(206) 987-7504
	E-mail:	bonnie.ramsey@seattlechildrens.org
Co-Principal Investigators:	Name:	Miriam M. Treggiari, M.D.
	Telephone:	(206) 987-7544
	Fax:	(206) 987-7504
	E-mail:	miriam.treggiari@seattlechildrens.org
	Name:	George Retsch-Bogart, M.D.
	Telephone:	(919) 966-1055
	Fax:	(919) 966-6179
	E-mail:	gzrb@med.unc.edu
Medical Monitor:	Name:	Christopher Goss, M.D.
	Pager:	(800) 341-0961
	Fax:	(206) 987-7506
	E-mail:	christopher.goss@seattlechildrens.org
Coordinating Center:	Cystic Fibrosis Therapeutics Development Network Coordinating Center 1100 Olive Way Suite 500, MPW5-4 Seattle, WA 98101	
	Telephone:	206-987-5725
	Fax:	206-987-7505

Funding Agencies:	Cystic Fibrosis Foundation Therapeutics, Inc. 6931 Arlington Road Bethesda, MD 20814
	National Heart, Lung, and Blood Institute Building 31, Room 5A52 31 Center Drive, MSC 2486 Bethesda, MD 20892
	National Institute of Diabetes & Digestive & Kidney Diseases Building 31, Room 9A04 31 Center Drive, MSC 2560 Bethesda, MD 20892
Original Issue Date:	June 30, 2004
Amendment #1 Date:	November 15, 2004
Amendment #2 Date:	
IND #	55,438

Approval:



Bonnie W. Ramsey, M.D. – Director CF TDN Coordinating Center

1/06/06

Date

Effectiveness and Safety of Intermittent Antimicrobial Therapy for the Treatment of New Onset *Pseudomonas aeruginosa* Airway Infection in Young Patients with Cystic Fibrosis

Signature of Agreement for Protocol # EPIC-001

I have read this protocol and agree to conduct the study as outlined herein, in accordance with Good Clinical Practices (GCPs) and the Declaration of Helsinki, and complying with the obligations and requirements of clinical Investigators and all other requirements listed in 21CFR part 312.

_____

Print Name and Title

_____ _____

Principal Investigator Signature *Date*

Site # _____

Site Name _____

Address _____

_____

_____

_____

_____

Phone Number _____

This confidential information about an investigational drug is provided for the exclusive use of investigators of this drug and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information. These restrictions on disclosure will apply equally to all future oral or written information, supplied to you by the Cystic Fibrosis Foundation, which is designated as "privileged" or "confidential".

Table of Contents

LIST OF ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAL	Bronchoalveolar lavage
CBC	Complete blood count
CF	Cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFFT	Cystic Fibrosis Foundation Therapeutics, Inc.
CFR	Code of Federal Regulations
CFS	Cystic Fibrosis Services
CHRMC	Children's Hospital and Regional Medical Center (Seattle, WA)
CRF	Case report form
CRP	C reactive protein
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	Forced expiratory flow between 25 and 75 percent of vital capacity
FEV_1	Forced expiratory volume over one second
FVC	Forced vital capacity
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed consent form
IRB	Institutional Review Board
MIC	Minimal inhibitory concentration
NCCLS	National Commercial Clinical Laboratory Standards
NHLBI	National Heart, Lung, and Blood Institute
OP	Oropharyngeal
Pa	*Pseudomonas aeruginosa*
PE	Pulmonary exacerbation
PI	Principal Investigator
SAE	Serious adverse event
SOP	Standard Operating Procedure
TDN	Therapeutics Development Network
TDN-CC	Therapeutics Development Network- Coordinating Center
TOBI®	Registered trademark of Chiron Corporation tobramycin solution
TSI	Tobramycin solution for inhalation
Tx	Treatment
VRA	Visual Reinforcement Audiology

DEFINITIONS

CULTURE-BASED TREATMENT - therapy (tobramycin solution for inhalation and ciprofloxacin/placebo) administered only when quarterly respiratory cultures are positive for *Pa*.

CYCLED TREATMENT - therapy (tobramycin solution for inhalation and ciprofloxacin/placebo) administered in quarterly cycles.

Day 0 - the date the participant begins the first course of study treatment (tobramycin solution for inhalation and ciprofloxacin/placebo).

Enrollment – the date the informed consent form for the study was signed by the participant's parent/guardian.

New Onset of *Pseudomonas aeruginosa* (*Pa*) – Children > 15 months of age: *Pa* isolated for the first time from respiratory cultures (OP, sputum, or lower respiratory tract) or as *Pa* recovered after at least a two-year history of *Pa* negative respiratory cultures (≥ 1 culture/ year). **Children 12 – 15 months of age**: at least one lifetime *Pa* positive respiratory culture since birth or CF diagnosis.

Participant - the person enrolled in this study. Due to the age range of participants in this trial, this term may also refer to the participant's parent/guardian as appropriate.

Pulmonary Exacerbation – As defined in Appendix II.

PROTOCOL SYNOPSIS

Effectiveness and Safety of Intermittent Antimicrobial Therapy for the Treatment of New Onset *Pseudomonas aeruginosa* Airway Infection in Young Patients with Cystic Fibrosis

Study Design:

This is a multicenter, randomized clinical trial assessing the clinical and microbiologic efficacy and safety of treatment with antimicrobial therapy at the time of new onset of *Pa* positive oropharyngeal, sputum or lower respiratory tract culture in young children with Cystic Fibrosis (CF). Approximately three hundred participants will be randomized to one of two early anti-pseudomonal treatment algorithms (CYCLED or CULTURE-BASED THERAPY) (APPENDIX I). All participants will receive an initial course of anti-pseudomonal antibiotic therapy consisting of 28 days of tobramycin solution for inhalation and a 14-day course of oral ciprofloxacin/placebo regardless of randomization assignment. If respiratory cultures sampled after three weeks of the first anti-pseudomonal cycle remain *Pa* positive, participants will receive an additional 28-day course of TSI (Figure 1). During the remainder of the study, participants will be treated based on their randomization assignment as follows:

CYCLED THERAPY (n=150):

a. GROUP I (n=75): TSI and oral placebo for six consecutive quarterly cycles.

b. GROUP II (n=75): combination therapy of TSI and oral ciprofloxacin for six consecutive quarterly cycles.

CULTURE-BASED THERAPY (n=150):

a. GROUP I (n=75): TSI and oral placebo administered only when quarterly respiratory cultures are found positive for *Pa*.

b. GROUP II (n=75): combination therapy of TSI and oral ciprofloxacin administered only when quarterly respiratory cultures are found positive for *Pa*.

Sample Size:

Approximately 300 participants recruited from approximately 60 CF clinical care centers will be enrolled.

Drug and Dose:

Inhaled Therapy:

Each course of antimicrobial therapy will consist of commercially available 300 mg tobramycin solution for inhalation (TOBI®) twice daily for 28 days administered by a PARI LC jet nebulizer via a facemask or mouthpiece.

Oral Therapy:

Oral ciprofloxacin or identically matched oral placebo will be administered concomitantly with tobramycin solution for inhalation. Each 14-day course will consist of twice daily oral ciprofloxacin (15-20 mg/kg/dose, up to a maximum of 750 mg/dose), or matched oral placebo.

Inclusion Criteria:

1. Male or female ≥ 1 year and ≤ 12 years of age at enrollment.
2. Diagnosis of CF based upon the criteria established by the 1997 CF Consensus Conference[1]: (i) Sweat chloride > 60 mEq/L by quantitative pilocarpine iontophoresis; or (ii) genotype with two identifiable mutations consistent with CF; or (iii) an abnormal nasal transepithelial potential difference **and** (iv) one or more clinical features consistent with CF.
3. Participants > 15 months of age: documented new onset of positive oropharyngeal, sputum or lower respiratory tract culture for *Pa* within six months prior to the Baseline Visit, defined as either: a) first lifetime documented *Pa* positive culture; or b) *Pa* recovered after at least a two-year history of *Pa* negative respiratory cultures (≥ 1 culture/ year).
4. Participants 12 – 15 months of age: at least one documented positive oropharyngeal, sputum or lower respiratory tract culture for *Pa* since birth or CF diagnosis.
5. Clinically stable with no evidence of any significant respiratory symptoms and/or physical or chest radiograph findings at screening that would require administration of intravenous anti-pseudomonal antibiotics, oxygen supplementation, and/or hospitalization.
6. Signed informed consent by parent or legal guardian and applicable participant assent.

Exclusion Criteria:

1. History of aminoglycoside hypersensitivity or adverse reaction to inhaled aminoglycoside.
2. History of hypersensitivity or adverse reaction to ciprofloxacin or other fluoroquinolone.
3. History of persistent, unresolved hearing loss documented by audiometric testing on at least two occasions and not associated with middle ear disease or an abnormal tympanogram.
4. Abnormal renal function at the Baseline Visit (defined as serum creatinine greater than 1.5 times the upper limit of normal for age).
5. Abnormal liver function tests at the Baseline Visit (defined as ALT and/or AST greater than 2 times the upper limit of normal range).
6. Administration of any investigational drug within 30 days prior to the Baseline Visit.
7. Administration of loop diuretics, phenytoin, warfarin, theophylline or other methyl-xanthines ≤ 30 days prior to the Baseline Visit.
8. Administration of more than one course (at least 10 continuous days of therapy) of intravenous anti-pseudomonal antibiotics or more than one course (at least 28 continuous days of therapy) of inhaled anti-pseudomonal antibiotics within two years prior to the Baseline Visit. Intravenous or inhaled anti-pseudomonal antibiotics must be completed >30 days prior to the Baseline Visit.
9. Chronic macrolide use (more than 90 day duration) within 3 months prior to the Baseline Visit.
10. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.

Objectives:

Primary Objectives

Clinical Efficacy Primary Objective: To compare CYCLED THERAPY to CULTURE-BASED THERAPY on time to first pulmonary exacerbation requiring intravenous antibiotics and/or hospitalization during an 18-month study period.

Microbiologic Efficacy Primary Objective: To compare CYCLED THERAPY to CULTURE-BASED THERAPY on the proportion of *Pa* positive isolates from respiratory cultures during an 18-month study period.

Secondary Objectives

Clinical Secondary Objective: To compare additional indices of clinical efficacy including proportion of participants experiencing a pulmonary exacerbation, average number of pulmonary exacerbations, linear growth, weight gain, and hospitalization days between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY. In participants able to reproducibly perform spirometry, FEV_1, FVC, and $FEF_{25\%-75\%}$ will also be compared.

Microbiologic Secondary Objectives: To compare the microbiologic profile of *Pa* isolates from respiratory cultures, including changes in antibiotic susceptibility patterns and the presence of isolates demonstrating mucoidy from baseline to the end of the study between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY. The emergence of inherently aminoglycoside-resistant non-pseudomonal organisms will also be monitored and compared.

Safety Secondary Objectives: To compare safety profiles between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY.

Serology and Inflammatory Objectives: To compare the serologic response against selected *Pa* surface and secretory antigens between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY. Changes in inflammatory markers will also be compared between the groups.

Ciprofloxacin Comparison Objectives: To evaluate the combined effect of oral ciprofloxacin and tobramycin solution for inhalation therapy versus tobramycin monotherapy for each of the outcome measures listed in the primary and secondary endpoints.

1. BACKGROUND

The primary cause of morbidity and mortality in patients with cystic fibrosis (CF) is progressive obstructive lung disease associated with chronic *Pseudomonas aeruginosa* (*Pa*) endobronchial infection and the associated intense neutrophilic inflammatory response[2]. Bacterial infection and robust neutrophilic airway inflammation begin far earlier than previously understood, often prior to the onset of symptoms[3-7]. The prevalence of *Pa* infection increases with age, with positive respiratory tract cultures reported for up to 20-30% of infants, 30-40% of children 2-10 years of age, ~60% of adolescents, and ~80% of adults with CF[8]. Chronic *Pa* endobronchial infection is characterized by high concentrations of alginate-producing mucoidy *Pa* variants that may form biofilms rendering the organisms highly resistant to antibiotics *in vivo*[9, 10]. Once established, chronic *Pa* endobronchial infection is virtually impossible to eradicate. *Pa* infection is clearly associated with poorer clinical outcomes, including more rapid decline in pulmonary function and higher mortality rates[11-17]. Early age of *Pa* acquisition also adversely affects pulmonary disease and survival[12, 18].

Pa isolates from recently-infected young CF patients possess distinctive characteristics suggesting a "window of opportunity" for early intervention with anti-pseudomonal antibiotics in an attempt to delay or prevent chronic *Pa* infection. Early *Pa* isolates are generally non-mucoid in phenotype[7], highly antibiotic sensitive, and present at relatively low density compared to isolates from patients with established *Pa* infection[5, 6]. There is thus a growing interest in treatment with anti-pseudomonal antibiotics at the time of initial isolation of *Pa* from respiratory cultures.

Most published studies of early intervention demonstrate a microbiologic effect of anti-pseudomonal therapy at the time of early *Pa* infection in terms of eradication of *Pa* from upper[19-22] and lower[13, 23] respiratory cultures. The observed differences in the frequency and duration of *Pa* eradication among published reports may be attributed to the wide variation in study design including the age range of the patient population, the site of respiratory tract cultures, the duration of *Pa* infection prior to treatment, the study endpoints, and the treatment regimen. In two Danish studies, treatment with oral ciprofloxacin and inhaled colistin at the time of first acquisition of *Pa* and again each time *Pa* was isolated from monthly upper airway cultures reduced the proportion of patients with chronic *Pa* infection at the end the 2-3 year study period[19, 20]. These authors also reported significant improvements in lung function and survival compared to historical controls.

Two studies found that prolonged treatment at the time of first *Pa* isolation with inhaled tobramycin resulted in nearly 100% *Pa* eradication[21, 22] that was maintained for up to one year[21]. In contrast, Munck et al. treated first isolation of *Pa* in young children with IV antibiotics followed by inhaled colistin and reported only transient *Pa* eradication in all subjects[24]. The majority of these subjects were re-colonized with a new *Pa* genotype after a mean duration of 8 months.

Two studies evaluating early *Pa* treatment utilizing lower airway (broncho-alveolar lavage (BAL) fluid) culture also found an acute but transient microbiologic effect[13, 23]. Australian investigators found that aggressive treatment with IV antibiotics and inhaled colistin at the time *Pa* was first isolated from BAL fluid resulted in lower airway eradication for ≥ 12 months in only 6/24 patients[13]. In a randomized, placebo controlled trial of inhaled tobramycin (300 mg twice daily for 28 days) following isolation of *Pa* from BAL fluid, Gibson et. al. observed eradication of both mucoid and non-mucoid *Pa* from both BAL and OP cultures in all 8 patients randomized to tobramycin therapy compared to 1/13 placebo treated patients[23]. Recurrent *Pa* infection with the same genotype occurred in oropharyngeal cultures from 2 patients by one month off treatment.

Many of these trials have been limited by small sample sizes, lack of controls, and minimal evaluation of safety. There has been no randomized, controlled trial to evaluate clinical efficacy and safety, including drug toxicities and emergence of resistant *Pa* or other pathogens. Nonetheless, given the compelling rationale for early aggressive intervention, a growing number of U.S. CF centers are treating with anti-pseudomonal antibiotics at first isolation of *Pa* from respiratory cultures. In 2002, antibiotic therapy was prescribed for 80% of recently *Pa* colonized patients: 25% received oral ciprofloxacin, 50% received inhaled tobramycin, 20% received intravenous anti-pseudomonal antibiotics, 15% received other antibiotics, and 20% received no therapy (not mutually exclusive)[8]. Based on this report, current "standard of care" in the U.S. appears to favor anti-pseudomonal therapy at the time of first isolation of *Pa*.

2. STUDY RATIONALE

Many important questions regarding treatment of initial *Pa* infection in patients with CF remain unanswered. The goal for early intervention with *Pa* acquisition is to prescribe the least invasive and safest treatment regimen for the shortest duration necessary to achieve both microbiologic and clinical benefits. No published trials exist comparing different anti-pseudomonal treatment regimens, so the optimal regimen is unknown. Which treatment regimen has the greatest antimicrobial efficacy? Which regimen has the best long-term safety profile? What is the risk of developing antibiotic resistance among *Pa* isolates associated with different treatment regimens? What is the effect of different treatment regimens on emergence of *Pa* mucoidy and change in genotype? What is the risk of selection of other resistant Gram-negative pathogens (e.g., *B. cepacia, S. maltophilia, A. xylosoxidans*) among the different regimens? Finally, which early intervention treatment regimen has the most beneficial impact on clinical outcomes?

To attempt to answer some of these questions, approximately 300 young children with CF ranging in age from 1 to 12 years will be enrolled at approximately 60 clinical centers nationwide to participate in an 18-month clinical trial. Children will be eligible to participate at the new onset of *Pa* infection. The 18-month clinical trial will investigate children assigned to two different antimicrobial treatment regimens: (1) culture-based therapy, and (2) cycled antibiotic therapy, i.e. treatment provided in quarterly cycles regardless of microbiology results. Tobramycin solution for inhalation (TSI) and oral ciprofloxacin will be the two antibiotics used in this study based on the rationale provided below. The results from this trial will provide valuable information regarding the microbiologic and clinical efficacy, as well as safety, of these two early intervention treatment strategies.

2.1. Rationale for Study Medication Selection and Dose

2.1.1. Tobramycin solution for inhalation (TSI)

Inhaled antibiotics, in particular aminoglycosides, can deliver high antibiotic concentration directly to the site of infection in the lower airways with limited systemic absorption. Tobramycin solution for inhalation (TOBI®) is the only inhaled antibiotic approved for use by the U.S. Food and Drug Administration (FDA). In two combined Phase III clinical trials of TSI in patients with CF 6 years of age or older, the *Pa* sputum density was reduced -1.6 $\log_{10}$ CFU/g after 28 days (1 on-drug cycle), and -1.0 $\log_{10}$ CFU/g after 6 months (3 on-drug cycles)[25]. For the youngest group, ages 6-12 years, the *Pa* sputum density was reduced –2.7 $\log_{10}$ CFU/g in the tobramycin group (n=45) compared to +0.3 $\log_{10}$ CFU/g in the placebo group (n=45) at Day 28 and -0.6 $\log_{10}$ CFU/g in the tobramycin group and +0.1 $\log_{10}$ CFU/g in the placebo group after six months. These data suggest that the younger patients have at least as great a microbiological response as the total population. No ototoxicity or nephrotoxicity was observed in these 6-month trials[25].

In children with CF <6 years of age, a single 300mg dose of TSI resulted in safe serum tobramycin levels as well as therapeutic lower airway concentrations[26]. A randomized controlled trial of TSI (300 mg twice daily for 28 days) conducted among children < 6 years of age was terminated prematurely due to evidence of a significant microbiological effect[23]. Twenty-one children were randomized (8 active; 13 placebo) and underwent BAL at baseline and on Day 28. No *Pa* was detected on Day 28 in 8 of 8 active group patients compared to 1 of 13 placebo group patients. The antimicrobial effect of a 28-day cycle of TSI was sustained 28 days following completion of therapy, at which time *Pa* was isolated from oropharyngeal cultures in 2 of 8 active group patients compared to 9 of 12 placebo group patients. The small sample and short duration of this study precluded any demonstration of clinical benefit.

Appropriate dosage depends on the potential for sputum binding and inactivation of the aminoglycoside[27], on the efficiency of the aerosol delivery system, and on the particle size necessary to deposit drug in the lower respiratory tract. Therefore, the considerations underlying the choice of tobramycin dose were based on the *in vitro* measurements of sputum activity of tobramycin, assumptions about the physical properties of the nebulizer, and the concentration of antimicrobial agent needed to inhibit strains of *Pa* inhabiting the airway of patients with CF. Aminoglycosides are the drug class for which there is the largest amount of information on sputum-antibiotic interactions in CF patients. From *in vitro* studies[27] it has been shown that a dose 10-25 times the MIC of *Pa* is required to ensure killing in the presence of purulent sputum. The recommended dosage of preservative-free inhaled tobramycin for adults and children ≥ 6 years of age is 300 mg. This dose was demonstrated to attain sputum levels adequate to overcome potential sputum antagonism[28]. The

recommended treatment regimen for chronically Pa infected patients ≥ 6 years of age is 28 days on therapy followed by 28 days off therapy to minimize emergence of resistance and reduce drug exposure[25]. Dose adjustments for age or weight are not required[26]. In the ongoing study in newly colonized patients < 6 years of age, preliminary data suggest that the effect of a 28-day cycle persists at least for 56 days in over half of the patients. These findings are further supported by an additional cohort of newly colonized patients receiving a 56-day treatment course, indicating persistent *Pa* negativity in 9 of 10 patients sampled 112 days after discontinuation of treatment. A consensus panel of experts also recommended quarterly therapy for the current study in this young population to reduce drug exposure. With a much lower bacterial burden than older, chronically colonized patients, it was felt that less frequent treatment was appropriate.

Therefore, the standard regimen of 300mg TSI given twice daily for 28 days followed by 56 days off therapy (quarterly administration) was selected as appropriate for the treatment of *Pa* in this study population. Because the 56-day treatment course was very effective in eradicating *Pa*, and since some patients did not demonstrate eradication after a 28-day treatment, participants in the current protocol who remain *Pa* positive after the first treatment cycle will also receive a second 28-day treatment.

2.1.2. Oral ciprofloxacin

Ciprofloxacin, an oral fluoroquinolone, is a broad spectrum antimicrobial agent with excellent *in vitro* activity against *Pa* strains from CF patients[29] and synergy with aminoglycosides[30] including tobramycin[31]. It has been widely used in patients with CF for two decades and several early studies demonstrated efficacy comparable to intravenous antibiotics as measured by improved lung function[32, 33]. Ciprofloxacin, usually administrated in previous studies at a dose between 15 and 20 mg/kg/dose given every 12 hours, has excellent GI absorption and penetration into respiratory secretions in CF patients[34, 35]. The emergence of *Pa* resistance to ciprofloxacin[36] appears minimized with short courses (≤14 days) of therapy[32, 37]. Though not approved by the U.S. FDA for use in pediatric patients with CF because of concerns of the emergence of histologic changes in articular cartilage of juvenile dogs receiving quinolones[38], ciprofloxacin has been studied[33, 39, 40] and widely prescribed[41, 42] in CF patients <18 years of age. Bayer has received FDA approval for use of ciprofloxacin in pediatric patients with chronic urinary tract infections (Steve Kowalsky, personal communication). There has been no documented evidence of drug-related arthropathy in >3000 courses of ciprofloxacin in pediatric patients[43]. Based upon these data, many clinicians have advocated a combination of an inhaled antibiotic with an oral fluoroquinolone for initial *Pa* eradication. There are several presumed advantages of this approach. First, ciprofloxacin is distributed systemically, delivering drug to reservoirs of infection in the sinuses or distal to mucus plugs in the lower airways[39] that might not be reached by an inhaled agent. Second, ciprofloxacin is a bactericidal agent that has documented *in vitro* synergy with tobramycin[30, 31]. Third, it is well tolerated and easily administered.

A recently published meta analysis described the pharmacokinetic profile of oral and intravenous ciprofloxacin in 150 pediatric patients ages 0.3-17 years, including 28 with CF. The study showed that although ciprofloxacin clearance was altered in CF patients, ciprofloxacin dose in pediatric patients may be calculated solely based on body weight in routine clinical use[35]. The doses and duration of ciprofloxacin administration in previous studies involving young children with CF were between 15 and 20 mg/kg/dose given every 12 hours, for a duration of two to three weeks[33, 37, 39-41]. On average, the most frequently used dose of ciprofloxacin in children is twice daily 15-20 mg/kg/dose up to a maximum of 750 mg/dose, for a two-week course[42]. Based on recommendations from a consulting group of infectious disease and pharmacology specialists, this regimen was selected for the present study.

3. STUDY DESIGN

This study is a multicenter, randomized clinical trial assessing the clinical and microbiologic efficacy and safety of treatment with antimicrobial therapy at the new onset of *Pa* positive oropharyngeal, sputum or lower respiratory tract culture in young children with CF. Approximately three hundred participants will be randomized to one of two early anti-pseudomonal treatment algorithms (CYCLED or CULTURE-BASED THERAPY) (APPENDIX I). All participants will receive an initial course of anti-pseudomonal antibiotic therapy consisting of 28 days of TSI and a 14-day course of oral ciprofloxacin/placebo regardless of randomization assignment. If respiratory cultures sampled after three weeks of the first anti-pseudomonal cycle remain *Pa* positive participants will receive an additional 28-day course of TSI (Figure 1). During the remainder of the

study, participants randomized to CYCLED THERAPY (n=150) will receive treatment administered in quarterly cycles and participants randomized to CULTURE-BASED THERAPY (n=150) will receive a course of treatment only when quarterly respiratory cultures are positive for *Pa*. Tobramycin solution for inhalation and oral ciprofloxacin/placebo will be administered according to the following regimens:

CYCLED THERAPY (n=150):

a. GROUP I (n=75): TSI and oral placebo for six consecutive quarterly cycles.

b. GROUP II (n=75): combination therapy of TSI and oral ciprofloxacin for six consecutive quarterly cycles.

CULTURE-BASED THERAPY (n=150):

a. GROUP I (n=75): TSI and oral placebo administered only when quarterly respiratory cultures are found positive for *Pa*.

b. GROUP II (n=75): combination therapy of TSI and oral ciprofloxacin administered only when quarterly respiratory cultures are found positive for *Pa*.

Participants that meet study eligibility criteria will be assigned to one of the study regimens.

Figure 1. Schedule of treatment regimens according to assigned study group.

TREATMENT REGIMENS (six quarters – 18 months)						
	Initial therapy First quarter			**Maintenance therapy** Subsequent five quarters		
	Treatment	Duration	Timing	Treatment	Duration	Timing
CYCLED therapy	TSI	28 days	Repeat 28 day course if culture still *Pa* positive	TSI	28 days	Every quarter
	Oral ciprofloxacin/ placebo	14 days	No repeat cipro/placebo	Oral ciprofloxacin/ placebo	14 days	
CULTURE-BASED therapy	TSI	28 days	Repeat 28 day course if culture still *Pa* positive	TSI	28 days	*Pa* positive quarters
	Oral ciprofloxacin/ placebo	14 days	No repeat cipro/placebo	Oral ciprofloxacin/ placebo	14 days	

4. STUDY OBJECTIVES

4.1 Primary Objectives

Clinical Efficacy Primary Objective: To compare CYCLED THERAPY to CULTURE-BASED THERAPY on time to first pulmonary exacerbation requiring intravenous antibiotics and/or hospitalization during an 18-month study period.

Microbiologic Efficacy Primary Objective: To compare CYCLED THERAPY to CULTURE-BASED THERAPY on the proportion of *Pa* positive isolates from respiratory cultures during an 18-month study period.

4.2. Secondary Objectives

Clinical Secondary Objective: To compare additional indices of clinical efficacy including proportion of participants experiencing a pulmonary exacerbation, average number of pulmonary exacerbations, linear growth, weight gain, and hospitalization days between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY. In participants able to reproducibly perform spirometry, FEV_1, FVC, and $FEF_{25\%-75\%}$ will also be compared.

Microbiologic Secondary Objectives: To compare the microbiologic profile of *Pa* isolates from respiratory cultures, including changes in antibiotic susceptibility patterns and the presence of mucoid isolates from baseline to the end of the study between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY. The emergence of inherently aminoglycoside-resistant non-pseudomonal organisms will also be monitored and compared.

Safety Secondary Objectives: To compare safety profiles between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY.

Serology and Inflammatory Objectives: To compare the serologic response against selected *Pa* surface and secretory antigens between participants randomized to CULTURE-BASED THERAPY and participants randomized to CYCLED THERAPY. Changes in inflammatory markers will also be compared between the groups.

Ciprofloxacin Comparison Objectives: To evaluate the combined effect of oral ciprofloxacin and tobramycin solution for inhalation therapy versus tobramycin monotherapy for each of the outcome measures listed in the primary and secondary endpoints.

5. MEASUREMENTS, EVALUATIONS AND ANALYTICAL METHODS

5.1. Primary Endpoints

Primary Clinical Efficacy Endpoint: Time to first pulmonary exacerbation (as defined in Appendix II) requiring intravenous anti-pseudomonal therapy or hospital admission within the 18-month study period.

Primary Microbiologic Efficacy Endpoint: The proportion of *Pa* positive cultures among all cultures obtained after randomization.

5.2. Secondary Endpoints

Secondary Clinical Endpoints: Proportion of participants experiencing a pulmonary exacerbation requiring hospitalization or intravenous antibiotics during the 18-month study period, average number of pulmonary exacerbations requiring hospitalization or intravenous antibiotics during the 18-month study period, changes in linear growth, changes in weight gain, and average number of hospitalization days during the 18-month study period. In addition, both the proportion and average number of participants during the 18-month period experiencing any pulmonary exacerbation defined per criteria in Appendix II will be compared. In participants able to reproducibly perform spirometry, changes in FEV_1, FVC, and $FEF_{25\%-75\%}$ will also be collected.

Secondary Microbiologic Endpoints: The proportion of *Pa* isolated for which the MIC of tobramycin is ≥ 16 μg/mL or the MIC of ciprofloxacin is ≥ 2 μg/mL during the 18-month study period, changes in minimal inhibitory concentrations of other antibiotics against *Pa*, the presence and changes in pattern of mucoid *Pa* isolates identified by colony morphology, and emergence of inherently aminoglycoside-resistant non-pseudomonal organisms (e.g.*B. cepacia, S. maltophilia, A. xylosoxidans*).

Secondary Safety Endpoints: Adverse events, including changes in renal function (as measured by serum creatinine), hearing acuity, articular-skeletal symptoms, hematology (CBC with differential), and liver function (as measured by AST, ALT, and GGT).

Secondary Serology and Inflammatory Endpoints: Changes and patterns in anti-pseudomonal antibody titers against *Pa* surface and secretory antigens such as Exotoxin A and in inflammatory markers in blood (white blood count with differential, and C reactive protein).

6. PARTICIPANT SELECTION

6.1. Study Population

Approximately 300 male and female participants ≥ 1 year and ≤ 12 years of age with a diagnosis of CF who have documented new onset (*see Inclusion Criteria #3 and #4*) of positive oropharyngeal, sputum or lower respiratory tract culture for *Pa* will be eligible for participation. Approximately 60 CF clinical care centers will contribute participants to this trial.

6.2. Inclusion Criteria

1. Male or female ≥ 1 year and ≤ 12 years of age at enrollment.
2. Diagnosis of CF based upon the criteria established by the 1997 CF Consensus Conference [1]: (i) Sweat chloride > 60 mEq/L by quantitative pilocarpine iontophoresis; or (ii) genotype with two identifiable mutations consistent with CF; or (iii) an abnormal nasal transepithelial potential difference **and** (iv) one or more clinical features consistent with CF.
3. Participants > 15 months of age: documented new onset of positive oropharyngeal, sputum or lower respiratory tract culture for *Pa* within six months prior to the Baseline Visit, defined as either: a) first lifetime documented *Pa* positive culture; or b) *Pa* recovered after at least a two-year history of *Pa* negative respiratory cultures (≥ 1 culture/ year).
4. Participants 12 – 15 months of age: at least one positive oropharyngeal, sputum or lower respiratory tract culture for *Pa* since birth or CF diagnosis.
5. Clinically stable with no evidence of any significant respiratory symptoms and/or physical or chest radiograph findings at screening that would require administration of intravenous anti-pseudomonal antibiotics, oxygen supplementation, and/or hospitalization.
6. Signed informed consent by parent or legal guardian and applicable participant assent.

6.3. Exclusion Criteria

1. History of aminoglycoside hypersensitivity or adverse reaction to inhaled aminoglycoside.
2. History of hypersensitivity or adverse reaction to ciprofloxacin or other fluoroquinolone.
3. History of persistent, unresolved hearing loss documented by audiometric testing on at least two occasions and not associated with middle ear disease or an abnormal tympanogram.
4. Abnormal renal function at the Baseline Visit (defined as serum creatinine greater than 1.5 times the upper limit of normal for age).
5. Abnormal liver function tests at the Baseline Visit (defined as ALT and/or AST greater than 2 times the upper limit of normal range).
6. Administration of any investigational drug within 30 days prior to the Baseline Visit.
7. Administration of loop diuretics, phenytoin, warfarin, theophylline or other methyl-xanthines ≤ 30 days prior to the Baseline Visit.
8. Administration of more than one course (at least 10 continuous days of therapy) of intravenous anti-pseudomonal antibiotics or more than one course (at least 28 continuous days of therapy) of inhaled anti-pseudomonal antibiotics within two years prior to the Baseline Visit. Intravenous or inhaled anti-pseudomonal antibiotics must be completed >30 days prior to the Baseline Visit.
9. Chronic macrolide use (more than 90 day duration) within 3 months prior to the Baseline Visit.
10. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.

7. INVESTIGATIONAL PLAN

7.1. Overview of Assessments

A summary of the study visits is provided in Appendix III.

Following the informed consent process and enrollment in the study, medical history will be reviewed, height and weight will be measured, and a complete physical examination, including a joint exam, will be performed. A respiratory specimen for a baseline culture and blood for research assays and clinical safety assays will be obtained. Female participants who have experienced onset of menarche will have either a urine or serum pregnancy test performed. Spirometry will be performed on age-appropriate participants. A chest radiograph will also be obtained. Audiometry will be performed at selected study sites. A diary will be sent home with the participant to document treatment adherence, changes in concomitant medications, and adverse medical events including occurrence of acute intercurrent respiratory illnesses.

Participants who meet all eligibility criteria will be randomized and the first course of treatment will be prescribed. Participants must begin study treatment within 14 days of randomization. Participants will have a second visit scheduled 21 days (±2 days) after initiation of the first 28-day cycle of TSI. The participant's interim medical history, including diary entries, will be reviewed and a respiratory specimen will be collected at this visit. Participants will be advised to discontinue TSI for 24 hours prior to respiratory specimen collection. The specimen will be cultured at the site microbiology laboratory. If the culture is *Pa* negative, TSI will be discontinued after the first 28 days of therapy are completed. If the Visit 2 culture is positive for *Pa*, the participant will continue on monotherapy of TSI for an additional 28-day course. These participants must be off TSI a minimum of 28 days prior to beginning any protocol required Visit 3 treatment.

Subsequently, participants will have quarterly study visits. These visits can occur in conjunction with routine clinic visits. These visits will be scheduled to occur approximately two weeks prior to initiation of a treatment cycle to ensure availability of respiratory culture results prior to prescribing treatment. At each visit, participants will undergo a complete physical exam and a respiratory specimen will be collected for culture. A structured joint examination will be performed at the Baseline Visit, Visit 4, Visit 6, and the End of Study Visit. Interim medical history, including diary entries, will be reviewed. Blood samples will be obtained for monitoring safety and research assays approximately every 24 weeks. Female participants, who have experienced onset of menarche, will have either a urine or serum pregnancy test performed. Spirometry will be performed on age-appropriate participants. A chest radiograph will be obtained at the End of Study Visit. Audiometry will be repeated at WEEK 46 and the End of Study Visit at selected sites.

Participants will be contacted between study visits (see Appendix III) by study staff to ensure delivery of study drugs, to assess compliance with study drug regimens, and to monitor for adverse events including occurrence of acute intercurrent respiratory illnesses.

7.2. Duration of Study

Participants will receive a maximum of seven (range 1 to 7) 28-day treatment courses of tobramycin solution for inhalation over an 18-month period and a maximum of six (range 1 to 6) 14-day courses of ciprofloxacin or placebo. Once participants have been assigned to a treatment group, participants and treating physicians should adhere to the assigned regimen for the 18-month study period whenever medically feasible. Except for the initial two study visits, participants will be seen for the study on a quarterly basis. These visits can occur in conjunction with routine clinic visits. The proposed enrollment period is 2.5 years (120 participants annually) at approximately 60 CF Care Centers. The projected total study duration is 4 years.

7.3. Treatment of a Pulmonary Exacerbation

During the study period, participants may receive appropriate intravenous antibiotic therapy for a pulmonary exacerbation as defined in Appendix II. Study drug should not be discontinued unless the investigator feels that stopping study drug is indicated for the well-being of the patient. If study drug is discontinued due to an acute pulmonary exacerbation meeting the definition in Appendix II, the participant should not complete that cycle of therapy, but should restart a new treatment regimen (per protocol) at the beginning of the next quarterly cycle. A pulmonary exacerbation or episodes of hospitalization do not represent conditions requiring

withdrawal of participants from the study unless deemed medically necessary by the Principal Investigator at the clinical site.

In the event that study drug is temporarily discontinued for treatment of a pulmonary exacerbation, the CF TDN Coordinating Center Medical Monitor must be notified within 1 working day of the reason for discontinuation.

7.4. Study Treatment Discontinuation

Study drug may be temporarily discontinued at the discretion of the Principal Investigator as medically required. Permanent discontinuation of study drug administration during the clinical trial will occur only when the Principal Investigator deems it is clinically indicated, e.g. pregnancy or adverse events related to study drug administration.

If oral ciprofloxacin/placebo therapy is either temporarily or permanently discontinued, treatment with tobramycin solution for inhalation may be continued. However, due to safety concerns, if tobramycin solution for inhalation therapy needs to be discontinued, ciprofloxacin/placebo therapy must also be terminated.

If study drugs are permanently discontinued, the participant should be encouraged to remain in the study and to complete all study visits and procedures. However, in the event of participant withdrawal from the study, all procedures for the End of Study Visit should be completed. (see Sections 9.8 and 12)

In the event that study drug is temporarily or permanently discontinued, the CF TDN Coordinating Center Medical Monitor must be notified within 1 working day of the reason for discontinuation.

7.5. Randomization

A central randomization system utilizing an Interactive Voice Response System (IVRS) will assign participants to the four different study arms. Randomization will be performed with a permuted block design and will allocate participants 1:1:1:1 among each of the four treatment groups (cycled treatment with oral ciprofloxacin; cycled treatment with oral placebo; culture-based treatment with oral ciprofloxacin; culture-based treatment with oral placebo).

7.6. Blinding

Participants and study personnel will be blinded to treatment assignment of oral ciprofloxacin/oral placebo, but will not be blinded to treatment arm assignment or administration of tobramycin solution for inhalation.

7.7. Measures to Minimize Bias

To minimize bias, participants will be randomized to the CYCLED or CULTURE-BASED treatment arms. Although the study treatment regimens are unblinded, the primary microbiologic and clinical endpoints are objective in nature, also minimizing bias. The microbiologic endpoints are based upon culture results. Microbiology laboratory personnel will be blinded to treatment assignment. Clinical endpoints include the following objective measures: administration of IV antibiotics and/or hospitalization in response to a pulmonary exacerbation as defined in Appendix II. Linear growth is also included as an important secondary outcome measure in this age group. Height/length is an easily measured, reproducible parameter available at all clinical sites and may be a useful measure of overall health status.

7.8. Assignment of Participant Identification Numbers

All participants who complete the informed consent process will be assigned an identification number. Numbers will be assigned in consecutive order within each clinical site, starting with the number 001. This number will be preceded by the site identification number and followed by the participant's initials. Participants will be referenced by their study identification number. After the results of all screening tests have been obtained and it has been determined that the participant meets all Inclusion/Exclusion Criteria, the participant will be entered into the treatment phase of the study. Participants who fail screening will not be included in the study, but will retain their identification number.

7.9. Replacement of Participants

Participants who fail screening or who are randomized but not dosed and who withdraw prior to completing Visit 2 (Week 3) will be replaced. Study participants presenting at the Baseline Visit with new onset of a pulmonary exacerbation requiring intravenous antibiotics or hospital admission will be considered screening failures, but may be re-enrolled the following quarter if they meet the study eligibility criteria. Re-enrolled participants will receive a new study number and have a new Baseline Visit.

7.10. Missing Microbiology Results

In the event that respiratory specimen culture results from the CFF TDN Core Microbiology Laboratory are unavailable, participants will be asked to provide another specimen. Replacement specimens will be submitted to the CFF TDN Core Microbiology Laboratory for culture.

8. STUDY PROCEDURES AND GUIDELINES

Prior to conducting any study-related activities, written informed consent must be obtained and signed and dated by the participant's parent/guardian. Participant assent will be obtained as required by individual study sites.

8.1. Medical History

The participant's lifetime medical history should be reviewed at the Baseline Visit via medical records and participant interview. All pertinent medical events and all surgical procedures should be noted. Interim medical history should note medical events that occurred between the previous study visit and the current study visit, including intercurrent respiratory illnesses. Site culture results from interim respiratory specimens obtained for clinical care will also be recorded.

8.1.1 Concomitant and Prior Medications

All medications taken within the 30 days prior to the Baseline Visit, including prescription drugs, over-the-counter medications, herbal medications, and other vitamin supplements must be recorded. All prior anti-pseudomonal antibiotics and any prior chronic macrolide use (more than 90 day duration) must also be recorded.

All participants should be maintained on the same medications throughout the entire study period, as medically feasible. During the study period, the only therapies not permitted for chronic use (defined as >30 days) are inhaled antibiotics (including those administered via Sinuneb® or similar devices for nasal administration), oral ciprofloxacin, macrolides, theophylline and other methylxanthines, phenytoin, warfarin, and loop diuretics. Participants are not allowed to receive other investigational drugs during the 18-month study period. If any changes in concomitant medications are required due to adverse events (i.e. illness, laboratory abnormalities, surgical procedures, etc.), the reason(s) for the change(s) must be recorded on the participant's CRF.

Treatment of Pulmonary Exacerbations: Regardless of randomization assignment, participants may receive short-term (< 30 days) administration of anti-pseudomonal antibiotics for signs and symptoms of a pulmonary exacerbation (APPENDIX II) (see also Section 7.4 regarding discontinuation of study drug).

8.2. Clinical Assessments

8.2.1. Physical Examination

A complete physical examination will be performed by a physician or other medically qualified sub-investigator at all visits, excluding Visit 2 (Week 3). Vital signs will also be recorded. A structured joint examination (as defined in the Study Manual) will be performed at the Baseline Visit, Visit 4 (Week 22), Visit 6 (Week 46), and the End of Study Visit. New abnormal physical exam findings must be documented, treated appropriately, and be followed by an investigator until satisfactory resolution or stabilization.

8.2.2 Height/Length and Weight

Height/Length and Weight measurements will be obtained at all visits except Visit 2 (Week 3).

Height/Length

Recumbent length should be measured on all participants approximately ≤ 18 months of age. Height should be measured on all participants approximately > 18 months of age. At the time a participant transitions from recumbent to standing height, the participant will be measured in both positions to ascertain the difference. Consistent equipment for each type of measurement (standing or recumbent) should be used.

Weight

Participants who are approximately ≤ 18 months of age should be weighed without any clothing or diapers. Participants who are approximately > 18 months of age can be weighed in street clothing, with a dry diaper (if applicable), and no shoes. Weight may be measured using either a standing or sitting scale (as appropriate) and should be obtained using consistent equipment for each type of measurement (standing or sitting).

8.2.3 Spirometry

Spirometry will be performed at each visit except Visit 2 (Week 3) by all participants 4 years of age and older who are able to reproducibly perform spirometry. Only sites that routinely perform spirometry on pre-school age patients should attempt spirometry on participants 4-5 years of age. Procedures will be performed in accordance with the 1994 American Thoracic Society recommendations for the performance and interpretation of tests[44], and include modified end of test criteria for children[45]. The same spirometry equipment should be used for the duration of the study whenever possible.

In participants 4-5 years of age, the above guidelines will be used with the following modified criteria: 1) an artifact-free exhalation flow-volume loop, 2) selection of the best FVC and FEV_1 effort, 3) acceptance of efforts with premature termination of exhalation, and 4) acceptance of the best effort in spite of non-reproducibility[46].

The Principal Investigator or other medically-qualified sub-investigator will review all spirometry for acceptability. Results will be recorded for all participants with acceptable maneuvers. Copies of spirometry loops for each participant will also be collected and interpreted centrally at the TDN-CC. Spirometry readings obtained while the participant was in stable health and obtained ≤ 1 month prior to the Baseline Visit will be accepted for the Baseline Visit provided the participant has not had an acute illness since the date of the test.

8.2.4 Audiometry

At a subset of approximately 30 sites, participants enrolled in the study will be evaluated for potential ototoxicity related to inhaled aminoglycoside therapy. A licensed audiologist with experience testing young children will perform visual reinforcement audiometry (VRA) using sound field testing for participants approximately 12 to 30 months old, play audiometry with earphones for participants approximately ages 30 months to 5 years (developmental age), and standard audiometry for those approximately 5 to 12 years of age. The appropriate specific testing mechanism(s) for each participant will be left to the discretion of the audiologist. Participants with PE tubes should be excluded from testing. Audiometric responses will be measured from 500 to 6000 Hz for VRA and 500 to 8000 Hz for play and standard audiometry, using screening levels of 25 and 20 dBHL at each test frequency, for VRA and play/standard audiometry, respectively. If the participant does not respond at the screening level for any frequency, a complete threshold audiogram will be obtained. Abnormal hearing will be defined as an auditory threshold >25 dBHL for VRA at any frequency and >20 dBHL for play or standard audiometry at any frequency in either ear. Those with abnormal thresholds will return for a confirmatory audiometric evaluation four weeks later. Children with abnormal audiologic findings will have a tympanometry performed, and if both are abnormal, will be referred to the PI or medically-qualified sub-investigator for an ear examination. Audiometric evaluations will be performed at the Baseline Visit, at Visit 6 (Week 46), and at the End of Study Visit. Audiometry performed ≤ 3 months prior to the Baseline Visit will be accepted for the Baseline Visit provided that since the date of the test the participant has not used Aminoglycosides and has not had middle ear infection or other health problems which could affect hearing.

8.2.5 Chest radiograph

A 2-view (posterior/anterior and lateral) chest radiograph will be obtained at the Baseline Visit. Chest radiographs obtained while the participant was in stable health and obtained ≤ 3 months prior to the Baseline Visit will be accepted for the Baseline Visit provided the participant has not had an acute illness since the date of the radiograph. A second chest radiograph will be obtained at the End of Study Visit, Week 70. Chest radiographs will be reviewed at the site by the Principal Investigator or medically-qualified sub-investigator to detect the onset of new lobar infiltrates, atelectasis, or other abnormalities that may indicate a pulmonary exacerbation (Appendix II) requiring intravenous antibiotics or hospitalization or precluding patient enrollment (see Inclusion criteria #5).

8.3. Clinical Laboratory Assessments / Evaluations

The PI or medically-qualified sub-investigator will review all laboratory assessments and evaluations for clinical significance. The review will be documented by signature and dated. Safety labs (Hematology and Chemistry) will be obtained at the Baseline Visit, Visit 4 (Week 22), Visit 6 (Week 46), and End of Study Visit.

8.3.1. Hematology

Blood will be obtained and sent to each site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count and differential, and platelet count) and serum C-reactive protein (CRP) for assessment of systemic evidence of infection and inflammation.

8.3.2. Blood Chemistry Profile

Blood will be obtained and sent to each site's clinical chemistry lab for determination of creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) values.

8.3.3. Pregnancy Test

Female participants who are post-menarche will be advised to avoid pregnancy and will have either a urine or serum pregnancy test performed. Pregnancy testing will be repeated at all quarterly visits. Participants will be advised to notify site study staff immediately if they suspect they may be pregnant. Participants who become pregnant should immediately discontinue all study medications, but will be requested to perform all remaining study procedures not contraindicated during the pregnancy. Participants who test positive for pregnancy at the Baseline Visit will be considered screening failures and will be withdrawn from the study (see Exclusion Criteria #10).

8.4 Research Laboratory Assessments

8.4.1 Bacteriology

Oropharyngeal (OP) specimens will be obtained at all study visits. Specimens will be collected with a cotton-tipped swab from the posterior oropharyngeal wall and tonsillar pillars. Participants will be encouraged to cough prior to collection of the OP specimen. The OP specimen obtained at Visit 2 (Week 3) must be collected no sooner than 24 hours after the last dose of TSI. The Visit 2 OP specimen will be cultured at the site clinical laboratory for identification of *Pa* and *Pa* isolates from this culture should be shipped to the CFF TDN Core Microbiology Laboratory at Children's Hospital and Regional Medical Center (CHRMC) in Seattle, WA. Oropharyngeal specimens collected at all other study visits will be shipped to the CFF TDN Core Microbiology Laboratory at Children's Hospital and Regional Medical Center (CHRMC) in Seattle.

If a participant produces an expectorated sputum specimen at a study visit, it should also be shipped to the CHRMC Microbiology Laboratory for culture. Expectorated sputum specimens collected at Visit 2 (Week 3) will be cultured at the site clinical laboratory. If the results from the Visit 2 expectorated sputum specimen are used to determine treatment per protocol, the *Pa* isolate from this culture should be shipped to the CHRMC Microbiology Laboratory. Expectorated sputum specimens may not replace oropharyngeal specimens.

Specimens will be sent on wet ice by overnight express shipment to the CHRMC Laboratory. Specimens must be received within 2 calendar days of collection. It has been demonstrated that bacteria will survive 48-72 hours on wet ice without a significant effect on bacterial density of *Pa* (unpublished data). Bacterial culture

techniques will be performed according to CFF TDN Core Microbiology Lab standard procedures[47]. Minimal inhibitory concentrations (MICs) for *Pa* will be determined by standard NCCLS methods. All *Pa* isolates will be assessed for mucoid phenotype. Bacterial isolates will be archived for use in future studies.

8.4.2 Pa Serology

A serum sample for serology assessment will be obtained at the Baseline Visit, Visit 4 (Week 22), Visit 6 (Week 46), and at the End of Study Visit. The blood sample will be centrifuged, serum extracted, and the specimen labeled and stored at the site at -70°C. The NHLBI laboratory of the Pulmonary and Critical Care Division in Bethesda, MD, will perform serological assays directed against selected *Pa* surface and secretory antigens such as Exotoxin A[7] and Exoenzyme S[48, 49]. All serum samples collected during the study will be batched and shipped frozen on dry ice upon request.

8.4.3. Serum Aliquot for Specimen Banking (at selected sites)

An aliquot of serum will be obtained from participants at selected sites at the Baseline Visit, Visit 4 (Week 22), Visit 6 (Week 46), and at the End of Study Visit. Separate informed consent regarding this process will be obtained from each study participant at these sites. The blood sample will be centrifuged and serum extracted. Serum will be labeled and stored at the site at -70°C. All serum samples collected during the study will be batched and shipped frozen on dry ice at the end of the study or upon request and may be used for ancillary CF studies approved by the CFF.

8.5 Participant Diary

A diary will be sent home with the participant at the Baseline Visit to document study treatment administration, changes in concomitant medications, and adverse events including intercurrent respiratory illnesses. Participant diaries will be reviewed at all subsequent study visits.

8.6 Participant Follow-up

Study staff will contact participants after each study visit to review the treatment plan, monitor participant status, and evaluate adherence (see section 9, Evaluations by Visit). Participants will also be contacted as necessary by CF Services Pharmacy staff to dispense study medication and confirm receipt.

9. EVALUATIONS BY VISIT

9.1. Visit 1, Baseline

- Informed Consent
- Medical history review
  - History of CF, diagnosis method/date, treatment history
  - Concomitant medication review
- Physical examination, including vital signs and structured joint examination
- Height/Length
- Weight
- Spirometry (in age-appropriate participants)
- Respiratory specimen(s) for culture (send to Core Microbiology Laboratory)
- Clinical laboratory tests:
  - Chemistry (serum creatinine, AST, ALT, and GGT)
  - CBC with differential (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count)
  - C-reactive protein
- Research laboratory specimens:
  - Serum for *Pa* Serology (all sites)
  - Serum for ancillary CF studies (selected sites)

- Pregnancy test, if applicable
- Chest radiograph (Posterior/Anterior and Lateral)
- Audiometry (at selected sites)
- Medication Administration Instructions
- Participant diary review

9.1.1. Participant Contact following Visit 1, BASELINE VISIT

Confirm Eligibility within 3 (± 2) days of the Baseline Visit. After confirming eligibility:

- Randomize participant
- Contact participant to notify participant of eligibility and review treatment plan
- Prescribe study drug

Within 14 (±2) days of start of treatment :

- Contact participant to check status, treatment adherence, and to schedule Visit 2

Note: Day 0 is defined as the date the participant begins study treatment (inhaled tobramycin and ciprofloxacin/placebo). Timing of subsequent visits reflects number of weeks from Day 0.

9.2. Visit 2 (Week 3) Day 21 (± 2 days) from Day 0

- Interim medical history review (participant report and diary review)
  - Concomitant medications
  - Adverse Events
- Medication adherence (review diary, count unused medications)
- Respiratory specimen(s) for *Pa* culture (send to **site** microbiology laboratory, collect isolate if *Pa* +)

9.2.1. Participant Contact following VISIT 2

Within 3 (± 2) days of Visit 2:

- Contact participant to provide culture result and review treatment plan and medication administration instructions (as necessary)
- Prescribe study drug if culture is positive for *Pa*

If participant requires treatment (*Pa* positive culture):

- Contact participant within 14 (± 2) days of start of treatment (approximately mid-treatment) to monitor participant status and adherence to treatment regimen

→ *Note*: *participants requiring treatment must be off TSI a minimum of 28 days prior to beginning any protocol required **Visit 3** study treatment.*

All participants (mid-quarter follow-up call):

- Contact participant within 6 (± 1) weeks of Visit 2 to monitor participant status and adherence to protocol

9.3. Visit 3 (Week 10, ± 2 weeks)

- Interim medical history review (participant report and diary review)
  - Concomitant medications
  - Adverse Events
- Medication adherence (review diary, count unused medications)

- Physical examination, including vital signs
- Height/Length
- Weight
- Spirometry (in age-appropriate participants)
- Respiratory specimen(s) for culture (send to Core Microbiology Laboratory)
- Pregnancy test, if applicable
- Review Medication Administration Instructions (as necessary)

9.3.1. Participant Contact following VISIT 3

Within 7 (± 2) days of Visit 3:

- Contact participant to provide culture result and review treatment plan
- Prescribe study drug according to protocol

If participant requires treatment (per protocol):

- Contact participant within 14 (±2) days of start of treatment to monitor participant status and adherence to treatment regimen

All participants (mid-quarter follow-up call):

- Contact participant within 6 (± 1) weeks of Visit 3 to monitor participant status and adherence to protocol

9.4. Visit 4 (Week 22 ± 2 weeks)

- Interim medical history (participant report and diary review)
  - Concomitant medications
  - Adverse Events
- Medication adherence (review diary, count unused medications)
- Physical examination, including vital signs and structured joint examination
- Height/Length
- Weight
- Spirometry (in age-appropriate participants)
- Respiratory specimen(s) for culture (send to Core Microbiology Laboratory)
- Clinical laboratory tests:
  - Chemistry (serum creatinine, AST, ALT, and GGT)
  - CBC with differential (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count)
  - C-reactive protein
- Research laboratory specimens:
  - Serum for *Pa* Serology (all sites)
  - Serum for ancillary CF studies (selected sites)
- Pregnancy test, if applicable
- Review Medication Administration Instructions (as necessary)

9.4.1. Participant Contact following VISIT 4

Within 7 (±2) days of Visit 4:

- Contact participant to provide culture result and review treatment plan
- Prescribe study drug according to protocol

If participant requires treatment (per protocol):

- Contact participant within 14 (±2) days start of treatment (approximately mid-treatment) to monitor participant status and adherence to treatment regimen

All participants (mid-quarter follow-up call):

- Contact participant within 6 (±1) weeks of Visit 4 to monitor participant status and adherence to protocol

9.5. Visit 5 (Week 34 ± 2 weeks)

- Interim medical history review (participant report and diary review)
  - Concomitant medications
  - Adverse Events
- Medication adherence (review diary, count unused medications)
- Physical examination, including vital signs
- Height/Length
- Weight
- Spirometry (in age-appropriate participants)
- Respiratory specimen(s) for culture (send to Core Microbiology Laboratory)
- Pregnancy test, if applicable
- Review Medication Administration Instructions (as necessary)

9.5.1. Participant Contact following VISIT 5 (Week 36)

Within 7 (±2) days of Visit 5:

- Contact participant to provide culture result and review treatment plan
- Prescribe study drug according to protocol

If participant requires treatment (per protocol):

- Contact participant within 14 (±2) days of start of treatment (approximately mid-treatment) to monitor participant status and adherence to treatment regimen

All participants (mid-quarter follow-up call):

- Contact participant within 6 (±1) weeks of Visit 5 to monitor participant status and adherence to protocol

9.6. Visit 6 (Week 46 ± 2 weeks)

- Interim medical history review (participant report and diary review)
  - Concomitant medications
  - Adverse Events
- Medication adherence (review diary, count unused medications)
- Physical examination, including vital signs and structured joint examination
- Height/Length
- Weight
- Spirometry (in age-appropriate participants)
- Respiratory specimen(s) for culture (send to Core Microbiology Laboratory)
- Clinical laboratory tests:
  - Chemistry (serum creatinine, AST, ALT, and GGT)

- CBC with differential (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count)
- C-reactive protein

- Research laboratory specimens:
  - Serum for *Pa* Serology (all sites)
  - Serum for ancillary CF studies (selected sites)
- Pregnancy test, if applicable
- Audiometry (at selected sites)
- Review Medication Administration Instructions (as necessary)

9.6.1. Participant Contact following VISIT 6

Within 7 (±2) days of Visit 6:

- Contact participant to provide culture result and review treatment plan
- Prescribe study drug according to protocol

If participant requires treatment (per protocol):

- Contact participant within 14 (±2) days of start of treatment (approximately mid-treatment) to monitor participant status and adherence to treatment regimen

All participants (mid-quarter follow-up call):

- Contact participant within 6 (±1) weeks of Visit 6 to monitor participant status and adherence to protocol

9.7. Visit 7 (Week 58 ± 2 weeks)

- Interim medical history review (participant report and diary review)
  - Concomitant medications
  - Adverse Events
- Medication adherence (review diary, count unused medications)
- Physical examination, including vital signs
- Height/Length
- Weight
- Spirometry (in age-appropriate participants)
- Respiratory specimen(s) for culture (send to Core Microbiology Laboratory)
- Pregnancy test, if applicable
- Review Medication Administration Instructions (as necessary)

9.7.1. Participant Contact following VISIT 7

Within 7 (±2) days of Visit 7:

- Contact participant to provide culture result and review treatment plan
- Prescribe study drug according to protocol

If participant requires treatment (per protocol):

- Contact participant within 14 (±2) days of start of treatment (approximately mid-treatment) to monitor participant status and adherence to treatment regimen

All participants (mid-quarter follow-up call):

- Contact participant within 6 (±1) weeks of Visit 7 to monitor participant status and adherence to protocol

9.8. End of Study Visit (Week 70 ± 2 weeks, or within 2 weeks of participant withdrawal)

- Interim medical history review (participant report and diary review)
  - Concomitant medications
  - Adverse Events; ongoing Adverse Events must be followed (see Sec. 11.2)
- Medication adherence (review diary, count unused medications)
- Physical examination, including vital signs and structured joint examination
- Height/Length
- Weight
- Spirometry (in age-appropriate participants)
- Respiratory specimen(s) for culture (send to Core Microbiology Laboratory)
- Clinical laboratory tests:
  - Chemistry (serum creatinine, AST, ALT, and GGT)
  - CBC with differential (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count)
  - C-reactive protein
- Chest radiograph (Posterior/Anterior and lateral)
- Research laboratory specimens:
  - Serum for *Pa* Serology (all sites)
  - Serum for future ancillary studies (selected sites)
- Pregnancy test, if applicable
- Audiometry (at selected sites)

10. TREATMENTS

10.1 Product Information – Tobramycin Solution for Inhalation

Tobramycin solution for inhalation, 300mg/5ml (TOBI®), will be provided by Chiron Corporation to the CF Services Pharmacy.

10.1.1. Packaging and Labeling

Each box of tobramycin solution for inhalation dispensed to a participant in this study will contain a prescription label with instructions for use and storage. The boxes of tobramycin solution for inhalation will be labeled with the FDA warning statement for investigational agents.

Each box of tobramycin solution for inhalation contains 56 doses corresponding to a 28-day supply.

10.1.2. Study Drug Dispensing

Study sites will fax study drug prescription forms to the CF Services Pharmacy. A supply of tobramycin solution for inhalation will be dispensed directly to each participant. CF Services Pharmacy will contact participants to arrange medication delivery. Receipt of study drug will be verified and notification provided to the study site.

PARI will provide PARI-Proneb® Ultra compressors and PARI LC Plus® reusable nebulizers and associated drug administration supplies required for this study.

10.1.3. Storage of tobramycin solution for inhalation

Tobramycin solution for inhalation should be stored at 2-8°C. Families will be instructed to store tobramycin solution for inhalation in the refrigerator.

10.1.4. Dosage/Dosage Regimen

Participants will be randomized to receive 28 days of tobramycin solution for inhalation either for six consecutive quarterly cycles (CYCLED THERAPY) or 28 days of tobramycin solution for inhalation when respiratory cultures results are positive for *Pa* (CULTURE-BASED THERAPY). Participants must begin treatment within 14 days of randomization. With the exception of the first cycle, each quarterly cycle of antimicrobial therapy will consist of commercially available 300 mg tobramycin solution for inhalation twice daily for 28 days followed by 56 days off therapy. For the first cycle only, a second identical 28-day course of therapy will be repeated (for a total of 56 days of consecutive therapy) if respiratory cultures sampled at treatment day 21 (± 2 days) of the first 28-day cycle remain *Pa* positive. Participants will be advised to discontinue TSI for 24 hours prior to the Visit 2 culture. Tobramycin solution for inhalation will be administered via PARI-Proneb® Ultra compressor and the PARI LC Plus® reusable nebulizer via a facemask or mouthpiece.

10.2 Product Information – Oral ciprofloxacin and matching placebo

Ciprofloxacin hydrochloride tablets (250mg, 500mg, and 750mg) and ciprofloxacin 10% oral suspension (100mg/ml) and matching placebo will be provided by Bayer Corporation to CF Services Pharmacy.

10.2.1. Packaging and Labeling

All ciprofloxacin study drug products will be dispensed to study subjects in amber plastic pharmacy bottles that meet USP requirements. A prescription label will be attached to each bottle of blinded ciprofloxacin or placebo prior to dispensing to provide families with directions for use and storage. Each bottle of oral ciprofloxacin will be labeled with the required FDA investigational agent warning statement.

10.2.2. Study Drug Dispensing

Study sites will fax study drug prescription forms to the CF Services Pharmacy. A supply of ciprofloxacin or placebo will be dispensed directly to each participant based on his/her treatment assignment. CF Services Pharmacy will contact participants directly to arrange medication delivery. Receipt of study drug will be verified and notification provided to the study site.

10.2.3. Storage of ciprofloxacin

Ciprofloxacin active drug and placebo are stored at room temperature.

10.2.4. Dosage/Dosage Regimen

Within the two treatment algorithms (CYCLED and CULTURE-BASED THERAPY), participants will be randomized in a double-blind fashion to receive a 14-day course of oral ciprofloxacin or an identically matched oral placebo starting at the beginning of each quarterly cycle. Participants must begin treatment within 14 days of randomization. Each ciprofloxacin course will consist of twice daily oral ciprofloxacin (15-20 mg/kg/dose, up to a maximum of 750 mg/dose), or matched oral placebo. Suspension must be prescribed to participants weighing < 14kgs. Participants weighing ≥ 14kgs may be prescribed either suspension or tablet(s) based on participant preference.

The table below illustrates the dose of drug per kilogram and the respective formulation:

Weight	Suspension dose	Tablet dose
5-7 kg	100 mg/ 1 ml	*
8-10 kg	150 mg/ 1.5 ml	*
11-13 kg	200 mg/ 2 ml	*
14-17 kg	250 mg/ 2.5 ml	250mg
18-24 kg	375 mg/ 3.75 ml**	**
25-37 kg	500 mg/ 5 ml	500 mg
> 38 kg	750 mg/ 7.5ml	750 mg

*Suspension only

**Participants may take either 375mg suspension BID or 250mg tablets TID

10.3. Blinding of Study Medication

All tobramycin solution for inhalation will be dispensed as open-label active drug. Oral ciprofloxacin/placebo will be dispensed in a blinded fashion. Placebo suspension will be taste-masked to preserve blinding.

10.4. Study Medication Accountability

Drug accountability records will be kept centrally at the CF Services Pharmacy for the ciprofloxacin/placebo and tobramycin solution for inhalation.

10.5. Adherence Monitoring

Monitoring for patient adherence to prescribed treatment regimen (CYCLED and CULTURE-BASED treatment arms) will include a review at each visit after the Baseline Visit of participant diary entries. In addition, families will bring all unused study medications and all empty bottles/vials to each visit after the Baseline Visit. Study site personnel will document the amount of remaining ciprofloxacin/placebo, remaining tobramycin solution for inhalation, and the number of empty bottles/vials at each of these visits. Unused ciprofloxacin/placebo, tobramycin solution for inhalation, and all empty bottles/vials will be destroyed at the study site based on site SOPs for investigational drug destruction.

10.6. Procedures for Unblinding (only applies to oral ciprofloxacin/placebo)

Breaking the blind for a single participant should only be done when the participant's physician requires knowledge of the treatment assignment in order to determine appropriate care. The investigator must contact the medical monitor to request unblinding.

11. ASSESSMENT OF SAFETY

11.1. Safety Parameters

Safety outcomes including renal function, liver function, hearing acuity, and changes in articular- skeletal symptoms will be evaluated to assess study drug toxicity. These outcomes will be compared between the four study treatment combinations.

11.2. Definitions

Adverse Event: An Adverse Event (AE) is any untoward medical occurrence not consistent with baseline medical history in a study participant. AEs may include new symptoms or the worsening of existing symptoms. A causal relationship with the study treatment is not necessary. Therefore, an AE can be any unfavorable and unintended sign, symptom, or disease whether or not considered related to the study treatment.

Note: For purposes of this study, abnormal laboratory values will not be considered adverse events unless deemed clinically significant by the Investigator. All abnormal laboratory values will be recorded in the database and appropriate analyses presented in the final study report.

Serious Adverse Event: A Serious Adverse Event (SAE) is defined as an AE that: results in death, is life-threatening, requires subject hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. Other important medical events may also be considered SAEs when they jeopardize the subject or when they require medical or surgical intervention to prevent one of the outcomes listed above. The Investigator should judge the seriousness of an AE independent of the relationship to study drug or expectancy of this AE in the population studied.

Unanticipated / Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information.

11.3. Adverse Event Recording / Documentation

11.3.1 AE Assessment

The Investigator or designee 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. For each AE, the start and resolution dates, the severity, whether it meets the definition of an SAE (see Section 11.2), the relationship of the event to the study drug, the action taken regarding study drug, and the outcome of the event should be

noted. Data should be transcribed from the source documents to the case report forms as per the case report form instructions.

AE Severity Grading

Severity	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom, but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention / therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse event as it occurred. This does not refer to an event that hypothetically might have caused death if it were more severe.

11.3.2 AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following guidelines:

Relationship to Study 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.

11.4 Serious Adverse Event Reporting

All serious adverse events (as defined in Section 11.2 above), whether judged related or not to study medication, will be reported to the Sponsor (or designated Medical Monitor) by telephone, e-mail, or facsimile within 24 hours of the Investigator becoming aware of such SAEs. This preliminary notification will be followed by completion of the SAE case report form detailing the circumstances of the event.

11.4.1 Sponsor Responsibilities

The Sponsor (or designated Medical Monitor) will assess the SAE with the Investigator and determine whether the SAE is reportable to the IRB/IEC and other investigator or regulatory agencies as specified in the applicable regulations. The Sponsor is responsible for filing all reportable SAEs with the FDA or other regulatory agencies per applicable regulatory requirements. The Sponsor will provide SAE report forms, contact information, and instructions for completing SAE forms in the Study Manual.

11.4.2 Investigator Responsibilities

The Investigator is responsible for reporting all SAEs to the Sponsor or designated Medical Monitor **within 24 hours** of becoming aware of the event and for completing all required documentation regarding the event in accordance with applicable regulatory requirements. The SAE report should not be delayed in order to obtain additional information about the SAE. Any additional information, if collected, can be reported to the Medical Monitor as a follow-up to the initial report. Additional updates from the Investigator may be necessary as more information becomes available on the SAE, and all SAEs will be followed until the acute event has resolved, even if the subject discontinues study participation prior to the resolution. The Investigator is responsible for reporting SAEs to his/her IRB/IEC as per his/her institution's policy.

12. WITHDRAWAL CRITERIA

Participants may be withdrawn from the study for any of the following reasons:

- At the participant's or parent's request
- At the discretion of the Investigator, if deemed appropriate, for any reason
- At the discretion of the Sponsor, and/or funding agencies if deemed appropriate, for any reason
- A serious adverse event related to the administration of study drug

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. If a participant is withdrawn from the study because of an adverse event, the participant will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized. The Principal Investigator should make every effort to ensure that any participant withdrawn from the study completes a Study Withdrawal Visit. The Investigator must record the reason for the early termination.

13. STUDY TERMINATION

The trial or parts of the trial (e.g. a treatment arm) may be discontinued at any time during the study at the recommendation of the Sponsor, and/or funding agencies, and/or at the recommendation of the Data Safety Monitoring Board (DSMB) in consultation with the Sponsor.

If the study is terminated for any reason, the Investigator will promptly inform the participants and provide appropriate therapy and follow-up.

The Investigator will return study documentation and unused study devices as instructed by the Sponsor. The Investigator will also provide a written statement to the IRB and, if required, to the FDA and other appropriate regulatory authorities concerning the termination.

14. DATA SAFETY MONITORING

Safety will be monitored on an ongoing basis throughout the trial by a Data and Safety Monitoring Board (DSMB) in accordance with a DSMB Charter approved by the committee prior to study initiation. The DSMB will be appointed by the NHLBI. The DSMB will conduct interim reviews at fixed times during the study as outlined in the DSMB charter. The DSMB will review safety data, enrollment data, protocol violations, and overall study progress in reports generated for the interim reviews. Additionally, Serious Adverse Events related to study drug will be forwarded to the DSMB chair on an ongoing basis as outlined in the Charter. All SAEs and AEs will be summarized at the interim reviews. The DSMB will decide, based on the committee members' judgment, what constitutes a significant safety concern that would potentially lead to stopping the trial early. Participant accrual will be monitored throughout the trial by the DSMB. If accrual is lower than expected, the DSMB will evaluate the reasons and determine if it is due to causes that cannot be

corrected, such as a lower than expected *Pa* conversion rate. Issues regarding feasibility of the study will be evaluated by the DSMB who will make a recommendation to the Sponsor and funding agencies regarding continuation of the study.

15. DATA COLLECTION, RETENTION AND MONITORING

15.1. Data Collection Instruments

Study personnel at each site will enter data from source documents corresponding to a participant's visit onto the CRF when the information corresponding to that visit is available. Study participants will not be identified by name on any study documents to be collected by the Sponsor or authorized designee, but will be identified by a site number, identification number and participant initials.

All clinical information requested in this protocol will be entered on the CRFs. CRF corrections will be made without obliterating the original entry.

The Principal Investigator is responsible for all information collected on participants enrolled in this study. All data collected will be reviewed by the Investigator throughout the course of the study for completeness and accuracy. The CRF for each participant will be signed by the Investigator and dated as verification of the accuracy of the recorded data.

A copy of the CRF will remain at the Investigator's site at the completion of the study.

15.2. Data Management Procedures

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

15.3. Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the clinical investigators and clinical monitors for resolution. The study database will be updated in accordance with the resolved query reports. All changes to the study database will be documented.

15.4. Archival of Data

At all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access. Databases will be backed up by the database administrator in conjunction with any updates or changes to the database. Tape or cartridge backup copies will be maintained at an off-site safe storage location. When the structure of the database is changed, a permanent archive of the database will be made to protect against loss of data in the changeover. When each backup is made, the media will be checked for usability and the integrity of the database will be verified.

At critical junctures of the protocol (e.g., production of interim reports and final reports), a permanent archive of the database will be made. Archived versions of the database will be saved for at least three years after the end of the study. Copies of each critical database will be sent to the sponsor.

15.5. Availability and Retention of Investigational Records

The Investigator must make study data accessible to the Sponsor or authorized designee and appropriate regulatory authorities upon request. A file for each participant must be maintained that includes the signed Informed Consent and, if required, Assent Form and copies of all source documentation related to that participant. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

15.6. Monitoring

The Sponsor or authorized designee will monitor this study in accordance with current Good Clinical Practices (GCPs). By signing this protocol, the Investigator grants permission to the Sponsor or authorized designee, and appropriate regulatory authorities to conduct on-site monitoring of all appropriate study documentation.

15.7. Participant Confidentiality

In order to maintain participant confidentiality, only a site number, identification number, and participant initials will identify all study participants in reports and on specimens.

16. STATISTICAL METHODS AND CONSIDERATIONS

Prior to the final analysis of data from this study, a detailed statistical analysis plan will be created documenting all analyses and statistical tests that will be performed. The statistical analysis plan will indicate any modifications to the proposed analyses described below.

16.1. Data Sets Analyzed

A modified intent-to-treat (ITT) population will be defined as all randomized participants who received at least one dose of study drug and have completed at least one scheduled post-baseline visit or phone call.. Whenever possible, analyses will be conducted using the modified ITT population. Participants who are randomized but do not actually receive any amount of study drug will not be included in ITT analyses. However, participants who withdraw early will be included in the analyses whenever possible. In the event that an endpoint measure is not available for a participant, this participant may need to be excluded from all analyses including that endpoint.

16.2. Analysis of Primary Endpoints

The primary analyses will compare participants assigned to the CYCLED therapy (n=150) to participants assigned to CULTURE-BASED therapy (n=150). Additional secondary analyses will be performed to compare participants who were assigned to oral ciprofloxacin (regardless of their inhaled regimen; n=150) to participants assigned to oral placebo (n=150), as well as investigate differences between groups defined by the interaction between the inhaled and oral regimens.

Primary Clinical Endpoint: Relative risk of exacerbations resulting in administration of IV antibiotics and/or hospitalization will be estimated using a proportional hazards regression model. The censored failure time will be taken as time to exacerbation or the end of follow-up; the failure will be the occurrence of exacerbation. Time will be calculated as days since first dose of study treatment. The baseline hazard will be stratified by age group. The significance of the treatment group variable will be tested by the likelihood ratio test at a 0.05 level of significance. Secondary analyses of the primary clinical endpoint will include adding additional covariates to the model for gender, season, and region.

Primary Microbiologic Endpoint: Proportions of *Pa*-positive respiratory cultures among all respiratory cultures taken during the 18 months of the follow-up will be compared between treatment groups using generalized estimating equation methods. The response will be binary (positive vs. negative culture). Each patient will contribute up to seven *Pa* cultures into the analysis. The GEE model will use the logit link and include the treatment assignment covariate. Independence structure will be used as the working correlation matrix in the GEE. The estimated treatment effect will be interpreted as the marginal odds ratio of positive respiratory cultures during the 18 months and tested by the Wald test. Secondary analyses of the primary microbiologic endpoint will include adding additional covariates to the model for age, gender, season, and region.

16.3. Analysis of Secondary Endpoints

The secondary analyses will compare participants assigned to CYCLED therapy (n=150) to participants assigned to CULTURE-BASED therapy (n=150). Additional secondary analyses will be performed to compare participants who were assigned to oral ciprofloxacin, regardless of their inhaled regimen, (n=150) to participants assigned to oral placebo (n=150), as well as investigate differences between groups defined by the interaction between the inhaled and oral regimens.

Secondary Clinical Endpoints: Height and weight will be compared between treatment groups using a linear regression model adjusting for baseline values of height/weight, age, and gender. F-tests will be used to test the hypothesis of equal means in the treatment groups at the end of follow-up given the baseline. FVC, $FEF_{25\%-75\%}$, and FEV_1 values will be compared between treatment groups using a linear regression model adjusting for their respective baseline values, as well as age, gender, and height. F-tests will be used to test the hypothesis of equal means of FEV_1 (or FVC or $FEF_{25\%-75\%}$) at the end of follow-up given the baseline.

Proportions of participants experiencing pulmonary exacerbation requiring intravenous antibiotics and/or hospitalization at any time during the study will be compared between treatment groups using a logistic regression model adjusted for age, gender, and season of entry into the study. The season will be constructed as a categorical covariate with four levels: January-March, April-June, July-September, and October-December. Likelihood ratio tests will be used to test the hypothesis of equal probabilities of exacerbation in the treatment groups during the follow-up. Pulmonary exacerbations, including those not requiring intravenous antibiotics or hospitalization, will be analyzed in a similar manner. Number of hospitalizations and/or exacerbations will be compared between treatment groups using a Poisson loglinear regression model. The Poisson rates will be adjusted for age, gender, and season of entry. Likelihood ratio tests will be used to test the hypothesis of equal frequencies of exacerbation/hospitalization in the treatment groups during the follow-up. Number of inpatient days will be compared between treatment groups using a linear regression model adjusted for age, gender, and season of entry. F-tests will be used to test the hypothesis of equal means in the treatment groups at the end of follow-up.

Secondary Microbiologic Endpoints: Minimal inhibitory concentrations (MICs) at each time point and changes from baseline will be descriptively summarized for each group. The proportion of participants in each treatment group with tobramycin resistant *Pa* isolates and ciprofloxacin resistant *Pa* isolates at each time point, as well as the presence of isolates demonstrating mucoidy will be summarized. Continuous outcomes will be compared between treatment arms by linear regression adjusting for age, sex, and month of entry into the study. Binary outcomes will be compared between treatment arms by logistic regression adjusting for age, sex, and month of entry into the study.

Secondary Safety Endpoints: Safety profiles will be compared between treatment groups, including adverse events such as abnormal renal function, loss of hearing acuity, articular-skeletal symptoms, and abnormal liver function. Adverse event rates will be summarized by body system and Medra classification term. They will also be tabulated by intensity, seriousness, duration, treatment, and their relationship to treatment. Safety data specified above will be summarized by treatment group using descriptive statistics. Univariate comparisons between groups will be performed using two-sample t-tests or non-parametric Wilcoxon statistics for continuous variables and chi-squared statistics for Fisher's exact test for categorical or ordinal variables.

Secondary Serology and Inflammatory Endpoints: Changes in inflammatory markers from baseline will be compared between groups. Descriptive statistics and/or graphical methods will be used to examine changes in each parameter. Exploratory analyses of differences between groups will use t-tests or non-parametric methods as appropriate. Graphical displays and descriptive statistics will be used to evaluate within and between group differences in the magnitude of the Exotoxin A and other serologic responses as a function of time. Exploratory analyses using longitudinal data methods such as generalized estimating equations will describe Exotoxin A titers as functions of time, and will allow testing for differences between treatment groups. Adjustments for other relevant covariates will be made in these analyses.

16.4. Sample Size and Power

By design, the primary study endpoint for which the study is powered is the clinical efficacy endpoint, time to first exacerbation requiring intraveneous antibiotics and/or hospitalization. The primary analysis will compare the more aggressive treatment group, CYCLED therapy, to the less aggressive treatment group, CULTURE-BASED therapy.

To determine a reasonable relative risk size for which to power this clinical efficacy endpoint, we estimated the annual exacerbation event rate using data on over 40,000 person-years represented in the CFF National Patient Registry during 1985–2000. All patients born in 1985 or later and who were between the ages of 1 and 12 were classified into three mutually exclusive groups at each calendar year: (i) patients with no positive *Pa* culture since birth; (ii) patients who had the first positive culture during that year, and (iii) patients with at least one positive culture in previous years. Based on the percentages of patients experiencing at least one exacerbation related event during a calendar year, we estimated that the annual incidence rate of exacerbations was 0.17 in Group (i), 0.36 in Group (ii), and 0.37 in Group (iii). Note that these rates were consistent across age categories within each Group. Hence, an aggressive early antibiotic therapy, if effective, could potentially reduce the risk of exacerbation by as much as 0.5 when comparing Group (ii) to Group (i).

Based on the annual exacerbation incidence rates estimated in the registry, with a sample size of 300 patients (150 in each of the CYCLED therapy and CULTURE-BASED therapy arms), we have powered our 18-month study to be able to provide 80% power to detect a relative risk of approximately 0.6 or lower.

The primary microbiologic analysis of the proportion of *Pa* positive cultures in the 18-month study period will also compare the CYCLED therapy arm to the CULTURE-BASED therapy arm. For this primary objective, a sample size of 300 (150 in each of the CYCLED therapy and CULTURE-BASED therapy arms) also provides 80% power to detect an odds ratio 0.6 or lower for *Pa*-positive respiratory cultures within the 18-month study period between treatment groups.

Enrollment: Based on the CFF Patient Registry, about 550 total CF patients of ages 1–12 years experience the first *Pa* positive respiratory culture each year. The 60 largest CF centers have about 300 eligible patients each year. During the 2.5-year enrollment period, we plan to recruit about 40% of eligible participants from approximately 60 large CF centers.

Estimated annual number of eligible patients among 60 U.S. CF centers in 2000 based on the CFF Registry

Age Group	Number with first *Pa* positive oropharyngeal culture	Number previously *Pa* negative	% Annual rate of first *Pa* positive oropharyngeal culture
1-3	104	569	18.4
4-6	77	573	13.4
7-9	62	482	12.8
10-13	71	485	14.7
Total	314	2109	14.9

17. ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

17.1. Informed Consent Form

The TDN-CC must review and approve each site's study-specific Informed Consent Form(s) (ICF), including Assent Forms, if required, prior to site submission for IRB approval. Changes requested by site IRBs must also be reviewed and approved by the TDN-CC. This process also includes consents regarding the banking of data and/or specimens. An IRB-approved copy of site Consent Forms and applicable Assents will be forwarded to the TDN-CC for the study file.

The ICF will document the study-specific information the Investigator provides to the participant and the participant's agreement to participate. Among other things, the Investigator will fully explain in layman's terms the nature of the study, along with the aims, methods, anticipated benefits, potential risks and discomforts, and any monetary compensation participation may entail. The ICF must be signed and dated by all applicable parties before any study-related procedures are performed. The original and any amended signed and dated ICFs must be retained in the participant's file at the study site; and a copy must be given to the participant.

Information about the Health Insurance Portability & Accountability Act (HIPAA) that protects the participant's individually identifiable health information (protected health information) and authorization (or agreement) in order for researchers to be able to use or disclose the participant's protected health information for research purposes will be provided to each participant in accordance with site specific institutional requirements.

17.2. Institutional Review Boards and Ethics Committees

Prior to the initiation of the trial, an institutional review board (IRB) must review and give approval on the suitability of the Protocol, and on the methods and materials to be used in obtaining informed consent. The Protocol must be reviewed for scientific and ethical considerations.

In the event that IRB requires changes in the Protocol, the Sponsor shall be advised in advance. The Sponsor must approve all modifications to the Protocol in advance. The Principal Investigator shall not modify the

study described in the Protocol once finalized and after approval by the IRB without the prior written approval of Sponsor. IRB approval should be obtained in writing, and should clearly identify the trial, the date of the review and approval, and the documents that were reviewed and approved, including informed consent and applicable assent.

17.3. Quality Control and Quality Assurance

Audits at selected study sites will be conducted by the Sponsor or authorized designee. Study site audits will include review of regulatory documents, including study drug and device accountability records and medical source documents. Data and information will be disclosed to the regulatory authorities in full compliance with applicable regulations.

17.4. Investigator Responsibilities

By signing the FDA Form 1572 the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or authorized designee), except when to protect the safety, rights or welfare of subjects/participants.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor/Medical Monitor 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 and are listed on the appropriate study documents.
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 authorized 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. To promptly report to the IRB and the Sponsor or authorized designee all changes in the research activity and all unanticipated problems involving risks to subjects/participants or others (to include amendments and IND safety reports).
9. Not make any changes in the research study without IRB approval, except when necessary to eliminate hazards to the subjects/participants.
10. To comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

17.5. Publications and Other Rights

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.

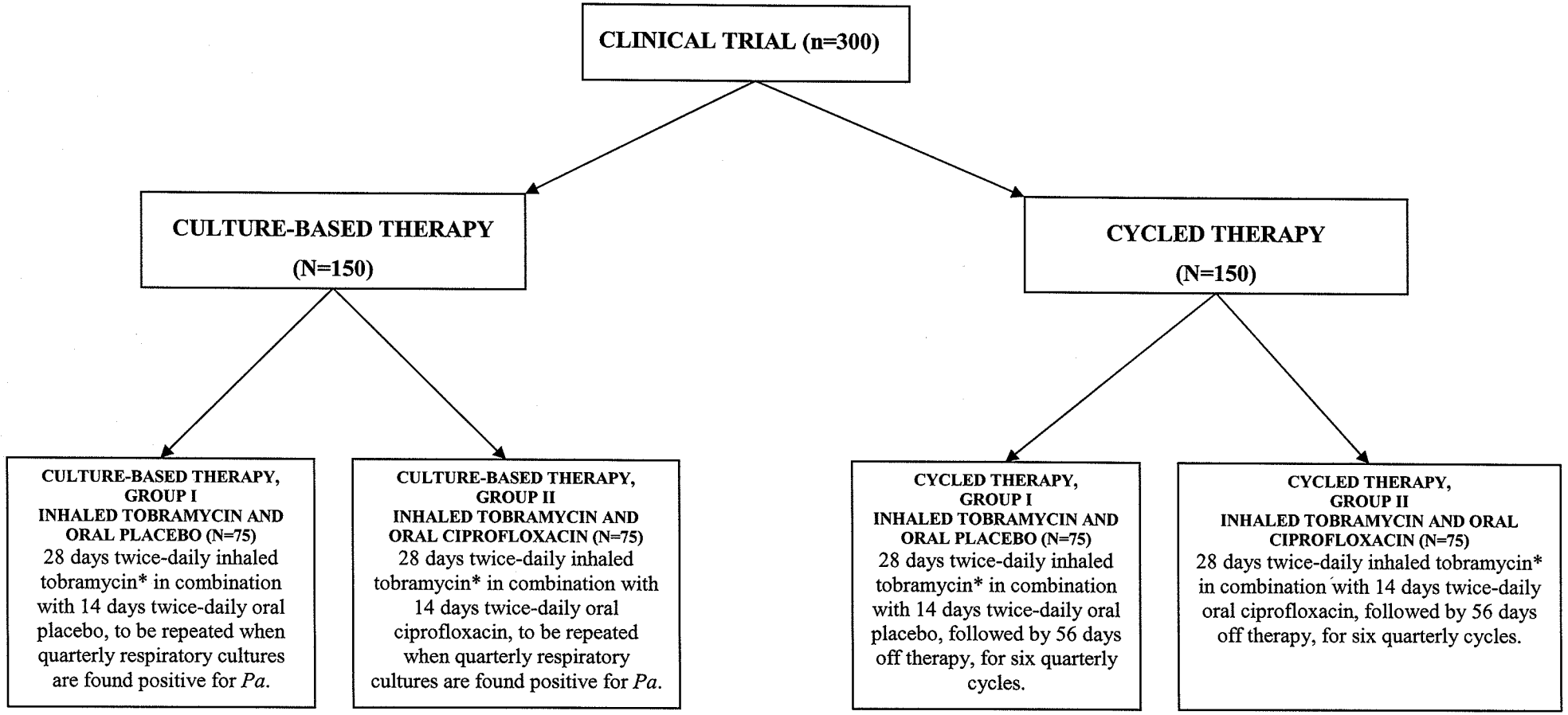
18. REFERENCES

1. Rosenstein, B.J. and G.R. Cutting, *The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. [see comments.].* Journal of Pediatrics, 1998. **132**(4): p. 589-95.
2. Cantin, A., *Cystic fibrosis lung inflammation: early, sustained, and severe.* American Journal of Respiratory & Critical Care Medicine, 1995. **151**(4): p. 939-41.
3. Armstrong, D.S., et al., *Bronchoalveolar lavage or oropharyngeal cultures to identify lower respiratory pathogens in infants with cystic fibrosis. [see comments.].* Pediatric Pulmonology, 1996. **21**(5): p. 267-75.
4. Balough, K., et al., *The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis.* Pediatric Pulmonology, 1995. **20**(2): p. 63-70.
5. Dakin, C.J., et al., *Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis.* Am J Respir Crit Care Med, 2002. **165**(7): p. 904-10.
6. Rosenfeld, M., et al., *Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis.* Pediatric Pulmonology, 2001. **32**(5): p. 356-66.
7. Burns, J.L., et al., *Longitudinal assessment of Pseudomonas aeruginosa in young children with cystic fibrosis.* Journal of Infectious Diseases, 2001. **183**(3): p. 444-452.
8. *Cystic Fibrosis Foundation. Patient Registry 2002 Annual Data Report.* 2003, Cystic FIbrosis Foundation: Bethesda, MD.
9. Singh, P.K., et al., *Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms.* Nature, 2000. **407**(6805): p. 762-4.
10. Worlitzsch, D., et al., *Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients.* J Clin Invest, 2002. **109**(3): p. 317-25.
11. Pamukcu, A., A. Bush, and R. Buchdahl, *Effects of pseudomonas aeruginosa colonization on lung function and anthropometric variables in children with cystic fibrosis.* Pediatr Pulmonol, 1995. **19**(1): p. 10-5.
12. Emerson, J., et al., *Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis.* Pediatr Pulmonol, 2002. **34**(2): p. 91-100.
13. Nixon, G.M., et al., *Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis.* Journal of Pediatrics, 2001. **138**(5): p. 699-704.
14. Henry, R.L., C.M. Mellis, and L. Petrovic, *Mucoid Pseudomonas aeruginosa is a marker of poor survival in cystic fibrosis.* Pediatr Pulmonol, 1992. **12**(3): p. 158-61.
15. Parad, R.B., et al., *Pulmonary outcome in cystic fibrosis is influenced primarily by mucoid Pseudomonas aeruginosa infection and immune status and only modestly by genotype.* Infection & Immunity, 1999. **67**(9): p. 4744-50.
16. Schaedel, C., et al., *Predictors of deterioration of lung function in cystic fibrosis.* Pediatr Pulmonol, 2002. **33**(6): p. 483-91.
17. Demko, C.A., P.J. Byard, and P.B. Davis, *Gender differences in cystic fibrosis: Pseudomonas aeruginosa infection.* J Clin Epidemiol, 1995. **48**(8): p. 1041-9.
18. Aebi, C., et al., *The age at onset of chronic Pseudomonas aeruginosa colonization in cystic fibrosis--prognostic significance.* Eur J Pediatr, 1995. **154**(9 Suppl 4): p. S69-73.
19. Frederiksen, B., C. Koch, and N. Hoiby, *Antibiotic treatment of initial colonization with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis.* Pediatric Pulmonology, 1997. **23**(5): p. 330-5.
20. Valerius, N.H., C. Koch, and N. Hoiby, *Prevention of chronic Pseudomonas aeruginosa colonisation in cystic fibrosis by early treatment.* Lancet, 1991. **338**(8769): p. 725-6.
21. Wiesemann, H.G., et al., *Placebo-controlled, double-blind, randomized study of aerosolized tobramycin for early treatment of Pseudomonas aeruginosa colonization in cystic fibrosis.* Pediatr Pulmonol, 1998. **25**(2): p. 88-92.
22. Ratjen, F., G. Doring, and W.H. Nikolaizik, *Effect of inhaled tobramycin on early Pseudomonas aeruginosa colonisation in patients with cystic fibrosis.* Lancet, 2001. **358**(9286): p. 983-4.

23. Gibson, R.L., et al., *Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis.* Am J Respir Crit Care Med, 2003. **167**(6): p. 841-9.
24. Munck, A., et al., *Genotypic characterization of Pseudomonas aeruginosa strains recovered from patients with cystic fibrosis after initial and subsequent colonization.* Pediatric Pulmonology, 2001. **32**(4): p. 288-92.
25. Ramsey, B.W., et al., *Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group.* New England Journal of Medicine, 1999. **340**(1): p. 23-30.
26. Rosenfeld, M., et al., *Serum and lower respiratory tract drug concentrations after tobramycin inhalation in young children with cystic fibrosis.* Journal of Pediatrics, 2001. **139**(4): p. 572-7.
27. Mendelman, P.M., et al., *Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum.* American Review of Respiratory Disease, 1985. **132**(4): p. 761-5.
28. Eisenberg, J., et al., *A comparison of peak sputum tobramycin concentration in patients with cystic fibrosis using jet and ultrasonic nebulizer systems. Aerosolized Tobramycin Study Group.* Chest, 1997. **111**(4): p. 955-62.
29. Klinger, J.D. and S.C. Aronoff, *In-vitro activity of ciprofloxacin and other antibacterial agents against Pseudomonas aeruginosa and Pseudomonas cepacia from cystic fibrosis patients.* J Antimicrob Chemother, 1985. **15**(6): p. 679-84.
30. Haller, I., *Comprehensive evaluation of ciprofloxacin-aminoglycoside combinations against Enterobacteriaceae and Pseudomonas aeruginosa strains.* Antimicrob Agents Chemother, 1985. **28**(5): p. 663-6.
31. Saiman, L., et al., *Antibiotic susceptibility of multiply resistant Pseudomonas aeruginosa isolated from patients with cystic fibrosis, including candidates for transplantation.* Clinical Infectious Diseases, 1996. **23**(3): p. 532-7.
32. Bosso, J.A., *Use of ciprofloxacin in cystic fibrosis patients.* American Journal of Medicine, 1989. **87**(5A): p. 123S-127S.
33. Church, D.A., et al., *Sequential ciprofloxacin therapy in pediatric cystic fibrosis: comparative study vs. ceftazidime/tobramycin in the treatment of acute pulmonary exacerbations. The Cystic Fibrosis Study Group.* Pediatric Infectious Disease Journal, 1997. **16**(1): p. 97-105; discussion 123-6.
34. Smith, M.J., et al., *Pharmacokinetics and sputum penetration of ciprofloxacin in patients with cystic fibrosis.* Antimicrob Agents Chemother, 1986. **30**(4): p. 614-6.
35. Rajagopalan, P. and M.R. Gastonguay, *Population pharmacokinetics of ciprofloxacin in pediatric patients.* J Clin Pharmacol, 2003. **43**(7): p. 698-710.
36. Ball, P., *Emergent resistance to ciprofloxacin amongst Pseudomonas aeruginosa and Staphylococcus aureus: clinical significance and therapeutic approaches.* Journal of Antimicrobial Chemotherapy, 1990. **26 Suppl F**: p. 165-79.
37. Schaad, U.B., et al., *Ciprofloxacin as anti-pseudomonal treatment in patients with cystic fibrosis.* Pediatric Infectious Disease Journal, 1997. **16**(1): p. 106-11; discussion 123-6.
38. Burkhardt, J.E., et al., *Histologic and histochemical changes in articular cartilages of immature beagle dogs dosed with difloxacin, a fluoroquinolone.* Veterinary Pathology, 1990. **27**(3): p. 162-70.
39. Rubio, T.T., *Ciprofloxacin in the treatment of Pseudomonas infection in children with cystic fibrosis.* Diagn Microbiol Infect Dis, 1990. **13**(2): p. 153-5.
40. Richard, D.A., et al., *Oral ciprofloxacin vs. intravenous ceftazidime plus tobramycin in pediatric cystic fibrosis patients: comparison of antipseudomonas efficacy and assessment of safety with ultrasonography and magnetic resonance imaging. Cystic Fibrosis Study Group.* Pediatr Infect Dis J, 1997. **16**(6): p. 572-8.
41. Alghasham, A.A. and M.C. Nahata, *Clinical use of fluoroquinolones in children.* Ann Pharmacother, 2000. **34**(3): p. 347-59; quiz 413-4.
42. Schaad, U.B., et al., *Panel discussion: antimicrobial therapy in pediatric cystic fibrosis patients.* Pediatric Infect Dis, 1997. **16**: p. 123-6.
43. Chysky, V., et al., *Safety of ciprofloxacin in children: worldwide clinical experience based on compassionate use. Emphasis on joint evaluation.* Infection, 1991. **19**(4): p. 289-96.
44. *Standardization of Spirometry, 1994 Update. American Thoracic Society.* Am J Respir Crit Care Med, 1995. **152**(3): p. 1107-36.

45. Desmond, K.J., et al., *Redefining end of test (EOT) criteria for pulmonary function testing in children.* Am J Respir Crit Care Med, 1997. **156**(2 Pt 1): p. 542-5.
46. Marostica, P.J., et al., *Spirometry in 3- to 6-year-old children with cystic fibrosis.* Am J Respir Crit Care Med, 2002. **166**(1): p. 67-71.
47. Burns, J.L., et al., *Microbiology of sputum from patients at cystic fibrosis centers in the United States.* Clinical Infectious Diseases, 1998. **27**(1): p. 158-63.
48. Banwart, B., et al., *Children with cystic fibrosis produce an immune response against exoenzyme S, a type III cytotoxin of Pseudomonas aeruginosa.* J Infect Dis, 2002. **185**(2): p. 269-70.
49. Moss, J., et al., *Sera from adult patients with cystic fibrosis contain antibodies to Pseudomonas aeruginosa type III apparatus.* Infect Immun, 2001. **69**(2): p. 1185-8.

APPENDIX I: OVERVIEW OF STUDY DESIGN



*Patients with culture positive for *Pa* at the end of 28 days of inhaled therapy will receive a second 28-day treatment course of inhaled tobramycin monotherapy for the first cycle only

APPENDIX II: DEFINITION OF PULMONARY EXACERBATION

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

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

Please note that the presence of a pulmonary exacerbation does not *mandate* therapy with anti-pseudomonal antibiotics. Rather, this definition is the *minimal* criterion for treatment with anti-pseudomonal antibiotics.

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

(1) Decrease in FEV_1 of ≥10% from best baseline within past 6 months, unresponsive to albuterol (in participants able to reproducibly perform spirometry)
(2) Oxygen saturation <90% on room air *or* ≥ 5% decline from previous baseline
(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 with duration criteria in the absence of major criteria)*

(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

Sign/Symptom Duration: *(Required with two minor signs/symptoms in absence of major criteria)*

- Duration of sign/symptoms ≥5 days

APPENDIX III: STUDY VISIT SCHEDULE

Note: Day 0 is defined as the first date of study treatment (TOBI and cipro/placebo). Timing of subsequent visits reflects number of weeks from Day 0.	Visit 1	Follow-up Contact[a]		Visit 2	Follow-up Contact[a]		
	Baseline	Baseline plus 3 (±2) days	Treatment day 14 (±2) days	Day 21 (± 2 days) from Day 0	Visit 2 plus 3 (±2) days	IF Pa + Tx start plus 14 (±2) days	Visit 2 plus 6 (±1) weeks
Informed Consent	X						
Medical History Review	X						
Interim Medical History[g]				X			
Provide Participant Diary	X						
Medication Adherence				X			
Physical Exam[i]	X						
Height/Length, Weight	X						
Spirometry[h]	X						
Respiratory Specimen[b]	X			X			
Clinical Laboratory Tests[c]	X						
Research Laboratory Specimen(s)[d]	X						
Pregnancy Test (if applicable)[e]	X						
Tx Instructions / review	X				X		
Chest Radiograph[f]	X						
Audiometry[j] (selected sites)	X						
Randomize participant		X					
Contact re: eligibility, review Tx plan		X					
Contact re: results, review Tx plan					X		
Prescribe drug per protocol		X			X		
Schedule Visit 2 (Tx plus 21(±2) days)			X				
Contact re: status and adherence			X			X	X

APPENDIX III: STUDY VISIT SCHEDULE (CONTINUED)

Note: Day 0 is defined as the first date of study treatment (TOBI and cipro/placebo). Timing of subsequent visits reflects number of weeks from Day 0.	Visit 3◆ Week 10 (± 2 weeks)	Visit 4◆ Week 22 (± 2 weeks)	Visit 5◆ Week 34 (± 2 weeks)	Visit 6◆ Week 46 (± 2 weeks)	Visit 7◆ Week 58 (± 2 weeks)	End of Study Visit Week 70 (± 2 weeks) or within 2 weeks of withdrawal
Informed Consent						
Medical History Review						
Interim Medical History[g]	X	X	X	X	X	X
Medication Adherence	X	X	X	X	X	X
Physical Exam[i]	X	X	X	X	X	X
Height/Length, Weight	X	X	X	X	X	X
Spirometry[h]	X	X	X	X	X	X
Respiratory Specimen[b]	X	X	X	X	X	X
Clinical Laboratory Tests[c]		X		X		X
Research Laboratory Specimen(s)[d]		X		X		X
Pregnancy Test (if applicable)[e]	X	X	X	X	X	X
Tx Instructions / review	X	X	X	X	X	
Chest Radiograph[f]						X
Audiometry[j] (selected sites)				X		X

◆Visit 3, 4, 5, 6, & 7 Follow-up Contact[a]	Visit date plus 7 (±2) days	If Tx Req'd Tx start date plus 14 (±2) days	Visit date plus 6 (± 1) weeks
Contact re: results, Tx plan	X		
Prescribe drug per protocol	X		
Contact re: status and adherence		X	X

Footnotes:

[a] Follow-up contact may be by phone, email, or in-person.

[b] Specimen for respiratory culture will be OP swab. Expectorated sputum specimens (if obtained) should be submitted **in addition to** the OP swab.

[c] Clinical lab tests: complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count), serum C-reactive protein, creatinine, AST, ALT, and GGT.

[d] Research lab specimens: serum sample for *Pa* serology. (Selected sites will also collect an aliqot of serum.)

[e] Pregnancy testing on female participants (after onset of menarche) may be done via either urine or serum.

[f] Chest radiograph will be two-view (posterior/anterior and lateral). Chest radiographs obtained while the participant was in stable health and obtained ≤ 3 months prior to the Baseline Visit will be accepted provided the participant has not had an acute illness since the date of the radiograph.

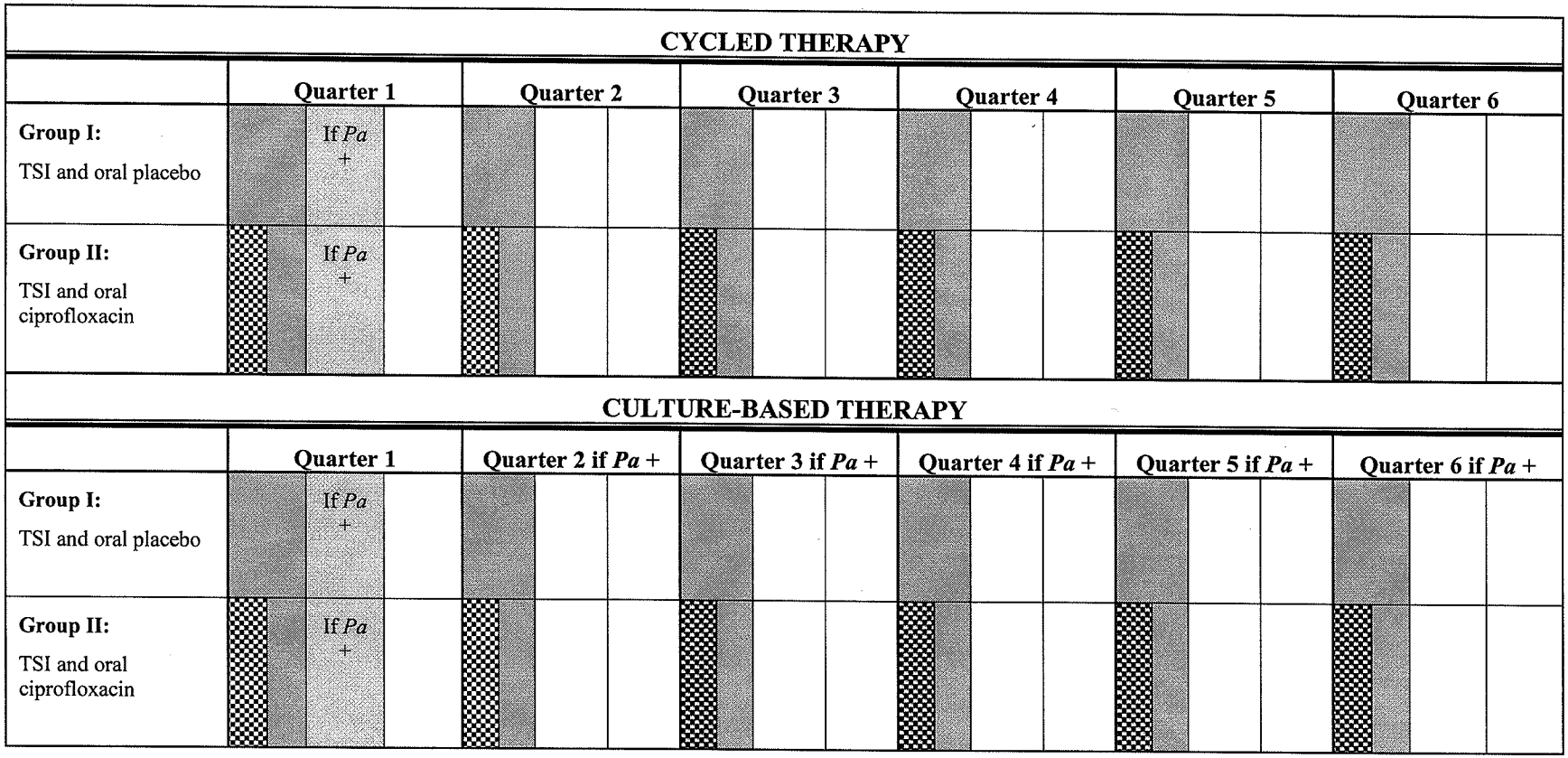
[g] Interim medical history will include concomitant medication review and adverse event review.

[h] Spirometry will be performed in age-appropriate participants. Spirometry readings obtained while the participant was in stable health and obtained ≤ 1 month prior to the Baseline Visit will be accepted for the Baseline Visit provided the participant has not had an acute illness since the date of the test.

[i] Physical exam will include vital signs. A structured joint examination will be performed at the Baseline Visit, Visit 4, Visit 6 and the End of Study Visit.

[j] Audiometry performed ≤ 3 months prior to the Baseline Visit will be accepted for the Baseline Visit provided that since the date of the test the participant has not used Aminoglycosides and has not had middle ear infection or other health problems which could affect hearing.

APPENDIX IV: TREATMENT REGIMEN BY QUARTER



Legend:

	TSI (300mg/kg/dose) x 28 days administered if Visit 2 (Week 3) *Pa* culture is positive
	TSI (300mg/kg/dose) x 28 days and oral placebo BID x 14 days
	TSI (300mg/kg/dose) x 28 days and oral ciprofloxacin (15-20mg/kg/dose) BID x 14 days

TSI = tobramycin solution for inhalation