Fosfomycin/Tobramycin for Inhalation in Patients with Cystic Fibrosis with *Pseudomonas* Airway Infection

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Rationale: Fosfomycin/tobramycin for inhalation (FTI), a unique, broad-spectrum antibiotic combination, may have therapeutic potential for patients with cystic fibrosis (CF).

Objectives: To evaluate safety and efficacy of FTI (160/40 mg or 80/20 mg), administered twice daily for 28 days versus placebo, in patients greater than or equal to 18 years of age, with CF, chronic *Pseudomonas aeruginosa* (PA) airway infection, and FEV₁ greater than or equal to 25% and less than or equal to 75% predicted.

Methods: This double-blind, placebo-controlled, multicenter study assessed whether FTI/placebo maintained FEV₁ % predicted improvements achieved following a 28-day, open-label, run-in course of aztreonam for inhalation solution (AZLI).

Measurements and Main Results: A total of 119 patients were randomized to FTI (160/40 mg; n = 41; 80/20 mg; n = 38) or placebo (n = 40). Mean age was 32 years and mean FEV₁ was 49% predicted at screening. Relative improvements in FEV₁ % predicted achieved by the AZLI run-in were maintained in FTI groups compared with placebo (160/40 mg vs. placebo: 6.2% treatment difference favoring FTI, *P* = 0.002 [primary endpoint]; 80/20 mg vs. placebo: 7.5% treatment difference favoring FTI, *P* < 0.001). The treatment effect on mean PA sputum density was statistically significant for the FTI 80/20 mg group versus placebo (−1.04 log₁₀ PA colony-forming units/g sputum difference, favoring FTI; *P* = 0.01). Adverse events, primarily cough, were consistent with CF disease. Respiratory events, including dyspnea and wheezing, were less common with FTI 80/20 mg than FTI 160/40 mg. No clinically significant differences between groups were reported for laboratory values.

Conclusions: FTI maintained the substantial improvements in FEV₁ % predicted achieved during the AZLI run-in and was well tolerated. FTI is a promising antipseudomonal therapy for patients with CF.

Keywords: *Pseudomonas aeruginosa*; fosfomycin; tobramycin; inhaled antibiotics; FTI

(Received in original form May 27, 2011; accepted in final form October 16, 2011)

*A complete list of study group members is provided at the end of the manuscript.

Supported by Gilead Sciences, Inc., with funding from Food and Drug Administration grant 1R01FD003016-01 and National Institutes of Health General Clinical Research Center grants M01 RR00188 and M01 RR10733.

Author Contributions: Conception and design, acquisition of data, or analysis and interpretation of data: all authors; drafting the article or revising it critically for important intellectual content: all authors; final approval of the version to be published: all authors.

This study was presented at the 2010 North American Cystic Fibrosis Conference in Baltimore, MD.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 185, Iss. 2, pp 171–178, Jan 15, 2012

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Originally Published in Press as DOI: 10.1164/rccm.201105-0924OC on November 17, 2011

Internet address: www.atsjournals.org

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Chronic *Pseudomonas aeruginosa* (PA) airway infection in adult patients with cystic fibrosis (CF) is associated with more rapid decline in lung function, and increased morbidity and mortality. The need for additional broad spectrum antibiotics to treat PA and other pathogens prompted the development of fosfomycin/tobramycin for inhalation (FTI), a 4:1 (wt:wt) combination of fosfomycin (a phosphonic acid antibiotic with activity against gram-positive, gram-negative, and anaerobic bacteria) and tobramycin (an aminoglycoside with gram-negative activity).

What This Study Adds to the Field

In this double-blind, placebo-controlled trial, FTI treatment was well tolerated and maintained the substantial improvements in FEV₁ % predicted achieved following a run-in period with another inhaled antibiotic (aztreonam for inhalation solution), while lung function in the placebo group decreased toward pretreatment levels. Continuous alternating therapy with several antibiotics is an appealing treatment option to improve lung function and respiratory symptoms to suppress PA and other pathogens. However, this type of treatment regimen has not been tested in well-controlled clinical trials with patients with CF. FTI could be a promising candidate to evaluate in such a trial.

Pseudomonas aeruginosa (PA) is the predominant pathogen found in the airways of adult patients with cystic fibrosis (CF). Chronic infection with PA is associated with a more rapid decline in lung function, and increased morbidity and mortality (1–3). Improved treatment has contributed to the increase in median age of patients with CF (2), and led to new clinical challenges. Data from the United States (U.S.) CF Foundation and other recent studies have demonstrated that the pattern of pulmonary bacterial colonization is changing in patients with CF (2, 4). Extensive antibiotic use has delayed development of chronic PA infection, but led to increasing antibiotic resistance among PA isolates; comparing data from 1995 and 2008 revealed a significant increase in the prevalence of aminoglycoside resistance (4). In addition, isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing (4, 5). There has also been wider recognition that other bacterial species, including anaerobic bacteria, are present in the lungs of patients with CF, although the role of these bacteria in disease pathogenesis

and progression is not well understood (6, 7). The changing microbiological profile in the airways of patients with CF has led to a need for additional therapies.

A 28-day “on”/28-day “off” dosing schedule is used for inhaled antibiotics that are currently approved in the United States for treating patients with CF with airway *PA* (8, 9). This schedule was first piloted in the early 1990s, and was designed to reduce the development of bacterial resistance and limit the cumulative toxicity of aminoglycoside antibiotics such as tobramycin (10–12). However, in recent years, the rationale for the “off” period is being questioned, given the increasingly common use of two or more inhaled antibiotics in rotation, especially for patients who are more symptomatic and have more severe disease (13). This is particularly true for the expanding population of adults with CF, who, on average, have more severe and symptomatic disease. Additional inhaled antibiotics with good efficacy in adults will be needed to maximize the benefits of monthly alternating therapy. Further, while use of the aerosolized formulation (tobramycin inhalation solution [TIS]) results in a lower systemic exposure than parenteral tobramycin (8, 14), continuing increase in the median life expectancy of patients with CF has raised a potential concern about the cumulative toxicity from all routes of aminoglycoside administration.

Fosfomycin/tobramycin for inhalation (FTI) is being developed as an antibiotic therapy, tailored to help address the current antibiotic needs of patients with CF (15). FTI is a 4:1 (wt:wt) combination of fosfomycin and tobramycin. Fosfomycin is a phosphonic acid antibiotic active against gram-negative, gram-positive, and anaerobic bacteria, with good activity against *S. aureus* (both methicillin-sensitive [MSSA] and MRSA) (16–19). Fosfomycin inhibits bacterial cell wall synthesis. Tobramycin is an aminoglycoside antibiotic active against gram-negative bacteria, particularly *PA*. Tobramycin inhibits protein synthesis (14). In a previous FTI study, bactericidal drug levels were achieved in sputum and systemic exposure was low (20, 21). The study described herein evaluated the safety and efficacy of a 28-day course of FTI versus

placebo, following a 28-day, open-label, run-in course of aztreonam for inhalation solution (AZLI).

METHODS

See online supplement for additional information for each METHODS section.

Study Design

This randomized, double-blind study enrolled patients at 33 U.S. CF centers (June 2008–January 2010). After screening, patients received a 28-day, open-label, run-in course of AZLI 75 mg (Cayston, Gilead Sciences, Inc., Foster City, CA) administered three times daily (9). The run-in period was designed to ensure that patients began the FTI/placebo period from a stable baseline and to test alternating therapy. At baseline (Day 0), patients were randomized (2:2:1:1) to 28 days of FTI 80/20 mg, FTI 160/40 mg, or volume-matched placebo, administered twice daily. Randomization was stratified by disease severity at screening ($FEV_1 \leq 50\%$ and $FEV_1 > 50\%$ predicted). Patients were assessed midtreatment (Day 14), at treatment end (Day 28), and at two follow-up visits (Day 42, Day 56).

Patients administered their own treatments: AZLI and FTI/placebo were administered with an investigational eFlow Nebulizer System (PARI Respiratory Equipment, Midlothian, VA). A short-acting bronchodilator was administered before each dose. Patients continued any other prescribed bronchodilator therapy.

This study was conducted in compliance with the Declaration of Helsinki, the International Conference on Harmonization guidelines, and Good Clinical Practice principles. Institutional Review Boards approved the study for each site. Patients or their legal representatives provided written informed consent prior to any study procedures.

Study Population

Eligible patients (≥ 18 yr of age) had documented CF, $FEV_1 \geq 25\%$, and $FEV_1 \leq 75\%$ predicted at screening, and could perform reproducible pulmonary function tests. *PA* was present in expectorated sputum or throat swab culture at screening, or in two cultures obtained within the previous year (one within the previous 3 mo). Presence of *S. aureus* was not required for enrollment.

Figure 1. Disposition of the patients with cystic fibrosis.

TABLE 1. PATIENT DEMOGRAPHICS AND CHARACTERISTICS

Characteristic	Placebo (N = 40)	FTI 80/20 mg (N = 38)	FTI 160/40 mg (N = 41)	All Patients (N = 119)
Age, yr, mean (SD)	31 (8.8)	35 (10.9)	31 (10.2)	32 (10.1)
Male, n (%)	27 (68)	21 (55)	20 (49)	68 (57)
Race, n (%)				
White	38 (95)	38 (100)	39 (95)	115 (97)
Black or African heritage	1 (3)	0 (0)	2 (5)	3 (3)
Asian	1 (3)	0 (0)	0 (0)	1 (1)
CFTR genotype, n (%)				
Homozygous for $\Delta F508$	15 (38)	14 (37)	26 (63)	55 (46)
Heterozygous for $\Delta F508$	14 (35)	17 (45)	6 (15)	37 (31)
Unidentified, other, or missing	11 (28)	7 (18)	9 (22)	27 (23)
BMI, kg/m ² , mean (SD)	22 (3.5)	22 (3.2)	21 (2.8)	22 (3.1)
FEV ₁ , % predicted, mean (SD)	48 (13.6)	50 (13.4)	48 (14.6)	49 (13.8)
FEV ₁ $\leq$ 50% predicted, n (%)	22 (55)	19 (50)	21 (51)	62 (52)
Log ₁₀ PA CFU/g sputum, mean (SD)	6.7 (1.54)	6.3 (1.65)	6.2 (2.35)	6.4 (1.88)
Multi-drug resistant PA*,†, n (%)	12 (34)	9 (27)	15 (40)	36 (30)
Tested positive for MRSA at/before baseline‡, n (%)	10 (25)	9 (24)	10 (24)	29 (24)
Tested positive for MSSA at/before baseline‡, n (%)	7 (18)	13 (34)	14 (34)	34 (29)
Concomitant medications (recorded at screening), n (%)				
Pancreatic enzyme supplements§	33 (83)	33 (87)	38 (93)	104 (87)
Dornase alfa	32 (80)	34 (90)	31 (76)	97 (82)
Azithromycin	28 (70)	23 (61)	33 (81)	84 (71)
Hypertonic saline	20 (50)	17 (44)	21 (51)	58 (49)
Inhaled antibiotic use within previous 12 mo, n (%)				
TOBI/TIS	23 (58)	27 (71)	25 (61)	75 (63)
AZLI	7 (18)	9 (24)	9 (22)	25 (21)
MIC of tobramycin for all PA isolates, μ g/ml				
MIC ₅₀	4	2	8	4
MIC ₉₀	64	64	64	64
MIC of fosfomycin for all PA isolates, μ g/ml				
MIC ₅₀	64	64	128	64
MIC ₉₀	512	512	2048	1024
MIC of fosfomycin:tobramycin for all PA isolates , μ g/ml				
MIC ₅₀	8	8	16	16
MIC ₉₀	64	32	64	64
Number of PA isolates tested for MIC values†	67	57	66	190

Definition of abbreviations: BMI = body mass index; CFTR = cystic fibrosis transmembrane conductor; CFU = colony-forming units; FEV₁ = forced expiratory volume in 1 s; FTI = fosfomycin/tobramycin for inhalation; MIC = minimum inhibitory concentration; MRSA = methicillin-resistant *Staphylococcus aureus*; PA = *Pseudomonas aeruginosa*; TOBI, TIS = tobramycin inhalation solution.

Data were collected at screening unless noted, except weight and PA CFU/g values are presented for Day -28.

* Resistance was defined using CF Foundation guidelines: resistance to all antibiotics tested in two of the three drug classes (aminoglycosides, β -lactams, and quinolones; 2).

† Isolates were obtained from 35 (placebo), 34 (FTI 80/20), 38 (FTI 160/40), and 107 (all) patients.

‡ At screening (Day -42), Day -28, and/or baseline (Day 0).

§ Includes nortase (n = 1) and pancrelipase (n = 103).

^{||} Fosfomycin:tobramycin (4:1, w/w); MIC values given as the concentration of fosfomycin in the mixture.

Exclusion criteria included administration of intravenous, oral, or inhaled antipseudomonal antibiotics or changes in azithromycin regimen within 14 days prior to screening, or changes in antimicrobial, bronchodilator, corticosteroid, hypertonic saline, or dornase alfa medications, or physiotherapy technique or schedule within 7 days. Need for additional antipseudomonal antibiotic therapy or additional fosfomycin, tobramycin, or aztreonam during the study resulted in discontinuation from the study.

Efficacy, Safety, and Microbiological Measures

The relative change from baseline (Day 0) in FEV₁ % predicted at Day 28 for FTI 160/40 mg versus placebo was the primary efficacy endpoint. The corresponding change for FTI 80/20 mg versus placebo was a secondary endpoint. Other secondary endpoints included the following: change from baseline at Day 28 in other lung-function parameters, Cystic Fibrosis Questionnaire-Revised (CFQ-R) Scales (22), and comparisons between treatment groups for hospitalizations, missed school/work days, and nonstudy antipseudomonal antibiotic use. The minimal clinically important difference on the CFQ-R Respiratory Symptoms Scale (RSS) was previously reported as 4 points (23).

Spirometry was performed at every visit (24). FEV₁ % predicted was calculated using Hankinson equations (25). Physical examinations were performed at screening, Day 28, and Day 56. Adverse events, airway reactivity, and clinical laboratory values were monitored.

Microbiological measures included change in log₁₀ colony-forming units (CFU)/g sputum for PA, MSSA, and MRSA; change in minimum inhibitory concentration (MIC) values; and disappearance/appearance of respiratory pathogens.

Statistical Analyses

Safety and efficacy analyses included randomized patients who received FTI/placebo, unless otherwise noted. Placebo groups were pooled for analyses presented herein. Forty subjects per group provided at least 80% power to detect an 8% difference in relative change from baseline at Day 28 in FEV₁ % predicted between FTI 160/40 mg and placebo, using a two-sided, 0.05-level test, assuming a common standard deviation (SD) of 12. The primary analysis used a parametric analysis of variance (ANOVA) model to compare FTI 160/40 mg and placebo, controlling for treatment group and disease severity at screening. If the primary analysis was significant, a similar analysis was used to compare FTI 80/20 mg and placebo. Proportion of patients requiring nonstudy drug antipseudomonal antibiotics was analyzed using the Cochran-Mantel-Haenszel procedure (26), stratified by disease severity. Proportion of patients hospitalized and proportion of patients who missed school/work days were analyzed using Fisher's exact test. Number of hospitalization days was analyzed using the Wilcoxon test. In some analyses of change from Day 0, a last observation carried forward

Figure 2. Changes in FEV₁ % predicted, CFQ-R Respiratory Symptoms Scores, and *Pseudomonas aeruginosa* density in sputum. For each panel, observed mean (SE) change is presented during the open-label AZLI run-in period for the 119 patients randomized to FTI/placebo at baseline (Day 0). Imputed mean (SE) change is presented during the FTI/placebo period; data were imputed using the last observation carried forward method. *P* values are from ANOVA models (see Table 2), comparing change from Day 0 to Day 28; only significant values (*P* < 0.05) are shown. Relative change in FEV₁ % predicted at Day 0 was calculated as the percentage change from the value at Day -28, and at Days 14 and 28 was calculated as the percentage change from the value at Day 0. For each panel in the figure, change values at Day 14 and Day 28 were added to the Day 0 value for each group prior to being plotted, to allow the data to be displayed in continuous lines.

imputation approach was used. For subjects with data present at Day 0 and missing one or more subsequent values, missing data was imputed using data from the last available observation.

RESULTS

Of 162 patients screened, 135 were enrolled, 121 completed the 28-day AZLI run-in period (Figure 1), and 119 initiated FTI/placebo therapy. Efficacy and safety analyses are reported herein for the 119 patients treated with FTI/placebo, unless otherwise noted. A total of 103 patients completed FTI/placebo treatment (87%) and 99 patients completed the study (83%). Fewer study discontinuations were observed in the FTI 80/20 mg group (7.9%; *n* = 3/38) than in the FTI 160/40 mg (22%; *n* = 9/41) or placebo (20%; *n* = 8/40) groups. Relative treatment adherence was high; mean (SD) use was 97% (8.6) of vials among all subjects during FTI/placebo treatment period.

Patient Characteristics

Demographic and baseline characteristics were generally balanced between treatment arms; differences were observed between treatment groups for CFTR genotypes and sex (Table 1). At screening, mean age was 32 years and mean FEV₁ was 49% predicted. Concomitant medications included dornase alfa (82%), azithromycin (71%), and hypertonic saline (49%).

Efficacy

After the 28-day AZLI run-in course, mean improvement in FEV₁ % predicted across all patients who received AZLI was 7.0% (Figure 2). The course of FTI maintained this improvement in lung function; in contrast, lung function in placebo-treated patients declined toward pre-AZLI levels (FTI 160/40 mg group: Day 0 = 1.92 L, Day 28 = 1.95 L, change = 0 ml; FTI 80/20 mg group: Day 0 = 1.93 L, Day 28 = 2.01 L, change = 13 ml; placebo: Day 0 = 1.96 L, Day 28 = 1.87 L, change = -136 ml. Note that results at all visits are shown in Tables 1 and 2 (see online supplement). These differences were significant (FTI 160/40 mg vs. placebo: *P* < 0.001; FTI 80/20 mg vs. placebo: *P* < 0.001). The primary efficacy endpoint of the study was met (Table 2); the FTI 160/40 mg versus placebo treatment effect at Day 28 was 6.2% (favoring FTI; 95% confidence interval [CI]: 2.4, 10.1; *P* = 0.002). The FTI 80/20 mg versus placebo treatment effect at Day 28 was 7.5% (favoring FTI; 95% CI: 3.6, 11.5; *P* < 0.001). Significant treatment effects favoring FTI over placebo were also observed at Day 28 for other lung-function parameters (Table 2).

Mean score on the CFQ-R RSS for all patients who received AZLI improved 4.8 points at the end of the AZLI run-in period (Figure 2). At Day 28, maintenance of this treatment effect was better for both FTI groups than for the placebo group; however, the treatment effects were not significant (FTI 80/20 mg vs. placebo: *P* = 0.10; FTI 160/40 mg vs. placebo: *P* = 0.36; Table 2).

Other antipseudomonal antibiotics (nonstudy drug) were used in 16% of the FTI 80/20 mg group (*n* = 6/38), 24% of the FTI 160/40 mg group (*n* = 10/41), and 33% of the placebo group (*n* = 13/40), but these differences were not significant (FTI 80/20 mg vs. placebo: *P* = 0.09; FTI 160/40 mg vs. placebo: *P* = 0.39; Table 3, see online supplement). Oral antibiotics alone were used by 3 patients (FTI 80/20 mg: 1, placebo: 2), intravenous or additional inhaled antibiotics were used by 23 patients (FTI 80/20 mg: 4, FTI 160/40 mg: 8, placebo: 11), intravenous or inhaled antibiotics in addition to oral antibiotics were used by 2 patients (FTI 80/20 mg: 1, FTI 160/40 mg: 1), and intranasal antibiotics were used by 1 patient (FTI 160/40 mg).

Eleven patients were hospitalized at least once (FTI 80/20 mg: 4, 11%; FTI 160/40 mg: 3, 7%; placebo: 4, 10%). No significant differences were found between treatment groups (FTI 80/20 mg vs. placebo: *P* = 1.00; FTI 160/40 mg vs. placebo: *P* = 0.71). Fewer patients missed school/work due to CF in the FTI 80/20 mg group (8%; *n* = 3/38) than in the FTI 160/40 mg (24%; *n* = 10/41) or the placebo (20%; *n* = 8/40) groups; however, these differences were not significant (FTI 80/20 mg vs. placebo: *P* = 0.19; FTI 160/40 mg vs. placebo: *P* = 0.79; Table 3, see online supplement).

Safety

Adverse events related to study drug (in the opinion of the site investigator) were reported for 38 patients (FTI 80/20 mg: 29%, *n* = 11/38; FTI 160/40 mg: 51%, *n* = 21/41; placebo: 15%, *n* = 6/40); all events were mild or moderate in severity. The most commonly reported drug-related events were cough (FTI 80/20 mg: 4 patients; FTI 160/40 mg: 10; placebo: 3) and wheezing (FTI 80/20 mg: 2; FTI 160/40 mg: 5; placebo: 0).

Serious adverse events were reported for 11 patients (FTI 80/20 mg: 4; FTI 160/40 mg: 3; placebo: 4); none of the 13

TABLE 2. EFFECTS OF FTI ON PRIMARY AND SELECTED EFFICACY ENDPOINTS DURING THE FTI/PLACEBO PERIOD*

Endpoint	Mean Change from Baseline (Day 0) to Day 28			Treatment Difference (95% CI); P Value	
	Placebo (N = 40)	FTI 80/20 mg (N = 38)	FTI 160/40 mg (N = 41)	FTI 80/20 mg vs. Placebo	FTI 160/40 mg vs. Placebo
FEV ₁ , % predicted (relative change), % [†]	−6.5	1.0	−0.3	7.5 (3.6, 11.5); P < 0.001	6.2 (2.4, 10.1); P = 0.002
FEV ₁ (L) (absolute change), ml [†]	−136	13	0	148 (70, 226); P < 0.001	136 (59, 212); P < 0.001
FVC (L) (relative change), % [†]	−5.6	−0.1	−1.3	5.5 (1.8, 9.1); P = 0.004	4.3 (0.7, 7.9); P = 0.02
FEF _{25–75%} (relative change), % [†]	−7.4	4.9	2.9	12.4 (4.2, 20.5); P = 0.003	10.3 (2.3, 18.3); P = 0.01
CFQ-R RSS [‡] , points	−7.8	−1.1	−4.1	6.7 (−1.3, 14.6); P = 0.10	3.7 (−4.2, 11.5); P = 0.36
Sputum PA density, log ₁₀ CFU/g; n	0.67; 31	−0.37; 24	0.39; 24	−1.04 (−1.83, −0.26); P = 0.01	−0.28 (−1.06, 0.50); P = 0.48
Sputum MRSA density, log ₁₀ CFU/g, (SD); n [‡]	−0.9 (3.17); 8	−2.2 (3.56); 7	−5.3 (2.25); 7	P = 0.33	P = 0.02
Sputum MSSA density, log ₁₀ CFU/g, (SD); n [‡]	1.7 (2.73); 7	−5.5 (1.55); 6	−2.0 (4.57); 8	P = 0.003	P = 0.07

Definition of abbreviations: CFQ-R RSS = Cystic Fibrosis Questionnaire-Revised Respiratory Symptoms Scale; CFU = colony-forming units; FEF_{25–75%} = forced expiratory volume from 25 to 75% of FVC; FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity; FTI = fosfomycin tobramycin for inhalation; MIC = minimum inhibitory concentration; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-sensitive *S. aureus*; PA = *P. aeruginosa*.

* Comparisons are from parametric ANOVA models. Models for pulmonary-function tests included terms for treatment group and disease severity at screening. Models for CFQ-R RSS included terms for treatment group and disease severity at screening as well as treatment and disease severity interaction. Models for log₁₀ PA CFU/g included terms for treatment group and PA CFU/g at screening.

[†] Missing data for FEV₁ and CFQ-R were imputed using the last observation carried forward method.

[‡] P values are from two-sided Wilcoxon tests.

events were considered drug related and 10 out of the 13 were respiratory-related events. The 12 hospitalizations of 11 patients were due to physician-defined pulmonary exacerbations (FTI 80/20 mg: 4; FTI 160/40 mg: 3; placebo: 3), gastrointestinal events (FTI 160/40 mg: 1; placebo: 1), and other events (behavioral health event; FTI 80/20 mg: 1). One hospitalization was given two primary causes.

Cough, productive cough, and dyspnea were the most commonly reported adverse events (Table 3). Fewer patients experienced adverse events in the FTI 80/20 mg group (53%; n = 20/38) than in the FTI 160/40 mg (85%; n = 35/41) and placebo (85%, n = 34/40) groups. Comparing the FTI 80/20 mg and placebo groups, the incidence of cough was substantially lower in the FTI 80/20 mg group (37%) than in the placebo group (53%), as was the incidence of productive cough (13% vs. 33%). In contrast, the incidence of wheezing was higher in the FTI 80/20 mg group (13%) than in the placebo group (5%), as was the incidence of nasal congestion (16% vs. 8%). Comparing the two FTI groups, the incidence of wheezing was substantially lower in the FTI 80/20 mg group (13%) than in the FTI 160/40 mg group (24%). Dyspnea, hemoptysis, and rales also occurred with lower incidence in the FTI 80/20 mg group than in the FTI 160/40 mg group. During the AZLI run-in period, the only

adverse event reported for ≥10% of patients was cough, reported for 21% of patients (n = 28/135) who received AZLI.

Three patients (FTI 160/40 mg: 2; placebo: 1) experienced bronchospasm (decrease of ≥15% in FEV₁ [L]) within 30 min of dosing at a clinic visit. Fourteen patients discontinued study drug due to adverse events (FTI 80/20 mg: 1; FTI 160/40 mg: 7; placebo: 6; Figure 1), with 11/14 patients discontinuing due to one or more respiratory events, most commonly cough (n = 8), dyspnea (n = 7), and productive cough (n = 6), and 3/14 patients discontinuing due to nonrespiratory events: 1 patient (FTI 80/20 mg) reporting bipolar disorder, 1 patient (FTI 160/40 mg) reporting ear pain, and 1 patient (placebo) reporting “confusional state.” Three patients discontinued study drug due to adverse events assessed as related to study drug: one patient (placebo) reporting dyspnea, one patient (FTI 160/40 mg) reporting cough, dyspnea, and throat tightness, and one patient (placebo) reporting “confusional state.”

There were no clinically significant differences in hematology or clinical chemistry parameters between treatment groups. Grade 3 elevations in blood glucose levels (>13.9–27.8 mmol/L) were reported for five patients (FTI 80/20 mg: 1; FTI 160/40 mg: 2; placebo: 2) and a grade 4 elevation (>27.8 mmol/L or acidosis) was reported for one patient (FTI 160/40 mg). A grade 3

TABLE 3. ADVERSE EVENTS REPORTED FOR ≥10% OF PATIENTS IN ANY TREATMENT GROUP DURING THE FTI/PLACEBO PERIOD

Event*	Incidence of Adverse Events, n (%)		
	Placebo (N = 40)	FTI 80/20 mg (N = 38)	FTI 160/40 mg (N = 41)
Any adverse event	34 (85)	20 (53)	35 (85)
Cough	21 (53)	14 (37)	19 (46)
Productive cough	13 (33)	5 (13)	12 (29)
Dyspnea	6 (15)	5 (13)	11 (27)
Pyrexia	8 (20)	5 (13)	6 (15)
Wheezing	2 (5)	5 (13)	10 (24)
Nasal congestion	3 (8)	6 (16)	6 (15)
Fatigue	4 (10)	4 (11)	6 (15)
Rhinorrhea	4 (10)	4 (11)	6 (15)
Hemoptysis	3 (8)	2 (5)	7 (17)
Rales	4 (10)	2 (5)	6 (15)

* Adverse events coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 11.1. Events are presented in order of decreasing incidence for all patients.

Definition of abbreviations: FTI = fosfomycin/tobramycin for inhalation.

elevation in γ -glutamyl transpeptidase (>5.0 – $20 \times$ upper limit of normal) was also noted for 1 patient (FTI 80/20 mg).

Microbiology and Clinical Pharmacology

Mean $\log_{10}$ *PA* CFU/g sputum across all patients who received AZLI decreased by 0.71 during the AZLI run-in period (Figure 2). At Day 28, maintenance of this treatment effect was better for both FTI groups than for the placebo group; the treatment effect was statistically significant for the FTI 80/20 mg group (FTI 80/20 mg vs. placebo: -1.04 ; $P = 0.01$; FTI 160/40 mg vs. placebo: -0.28 ; $P = 0.48$; Table 2).

The MIC_{50} and MIC_{90} of fosfomycin, tobramycin, and fosfomycin:tobramycin (4:1) for all *PA* isolates remained unchanged ($\leq$ twofold increase or decrease) or decreased from baseline to Day 28, except for the MIC_{90} of fosfomycin, which increased for isolates from the FTI 80/20 mg group (Table 4). The MIC_{50} and MIC_{90} of amikacin, aztreonam, cefepime, ciprofloxacin, meropenem, piperacillin, piperacillin/tazobactam, and ticarcillin/clavulanic acid for all *PA* isolates remained unchanged ($\leq$ twofold increase or decrease) or decreased from baseline (Day 0) to Day 28.

With respect to *S. aureus*, 63 (53%) patients were positive at or before baseline (Day 0), including 34 (29%) patients with MSSA and 29 (24%) with MRSA. In 8 of 34 patients, MSSA was not cultured again from Day 28 to study end (Day 56), and in 3 of 29, MRSA was not cultured; these 11 patients were among the 46 *S. aureus*-positive patients receiving FTI. Among the small subset of patients with MRSA at baseline, mean $\log_{10}$ MRSA CFU/g decreased substantially in patients receiving FTI compared with those receiving placebo; the treatment difference was significant in the FTI 160/40 mg group ($P = 0.02$; Table 2).

Treatment-emergent *Achromobacter* species were isolated once after baseline (Day 0) in three patients (FTI: 2; placebo: 1). Most patients who tested negative for *Stenotrophomonas maltophilia* at baseline remained culture negative during the study (FTI 80/20 mg: 81%; FTI 160/40 mg: 92%; placebo: 91%). There was no change in the prevalence of *Aspergillus* species. Only one patient (placebo) had *Burkholderia* species present at any study visit.

Mean (SD) plasma tobramycin concentrations obtained 1 h after dosing were 86 (51.2) ng/ml for the FTI 80/20 mg group, 138 (117.7) ng/ml for the FTI 160/40 mg group, and 5 (18.3) ng/ml for the placebo group. Mean (SD) sputum tobramycin concentrations obtained 10 min after dosing were 137 (125.3) μ g/ml for the FTI 80/20 mg group, 264 (254.9) μ g/ml for the FTI 160/40 mg group, and 8 (34.9) μ g/ml for the placebo group.

Post-treatment Period

At study end (Day 56), FEV₁ % predicted and CFQ-R RSS values in the FTI groups had declined but remained above pre-AZLI levels and were numerically superior to placebo (Figure 1, see online supplement). *PA* sputum density for all treatment groups fluctuated in the post-treatment period, initially increasing toward pre-AZLI levels at Day 42 but decreasing at Day 56. However, both FTI groups remained numerically superior to placebo at the two follow-up visits.

DISCUSSION

This study met the primary study endpoint: after 28 days of treatment with FTI/placebo, the FTI 160/40 mg dosing group maintained the improvement in FEV₁ % predicted achieved by the

TABLE 4. MIC_{50} AND MIC_{90} OF FOSFOMYCIN, TOBRAMYCIN, AND FOSFOMYCIN:TOBRAMYCIN FOR ALL *PA* ISOLATES

Treatment	Visit	MIC_{50} (μ g/ml)	MIC_{90} (μ g/ml)	Number of <i>PA</i> Isolates
Fosfomycin				
Placebo (N = 40)	Day 0	64	512	64
	Day 14	64	1024	67
	Day 28	64	512	64
FTI 80/20 (N = 38)	Day 0	64	512	45
	Day 14	128	$>2,048$	45
	Day 28	64	$>2,048$	48
FTI 160/40 (N = 41)	Day 0	128	2,048	59
	Day 14	64	$>2,048$	60
	Day 28	128	$>2,048$	55
Tobramycin				
Placebo (N = 40)	Day 0	2	64	64
	Day 14	2	16	67
	Day 28	4	16	64
FTI 80/20 (N = 38)	Day 0	2	16	45
	Day 14	4	16	45
	Day 28	2	32	48
FTI 160/40 (N = 41)	Day 0	4	64	59
	Day 14	4	16	60
	Day 28	4	16	55
Fosfomycin:Tobramycin (4:1)*				
Placebo (N = 40)	Day 0	8	32	64
	Day 14	8	32	67
	Day 28	8	32	64
FTI 80/20 (N = 38)	Day 0	8	32	45
	Day 14	8	64	45
	Day 28	8	64	48
FTI 160/40 (N = 41)	Day 0	8	32	59
	Day 14	8	32	60
	Day 28	16	64	55

A change of greater than or equal to fourfold was considered an increase/decrease in MIC.

Definition of abbreviations: FTI = fosfomycin/tobramycin for inhalation; MIC = minimum inhibitory concentration; *PA* = *P. aeruginosa*.

* MIC values are presented as the concentration of fosfomycin in the fosfomycin:tobramycin (4:1; wt/wt) mixture.

28-day AZLI run-in period, in contrast to the placebo treatment group, where lung function declined toward pre-AZLI levels. FEV₁ % predicted was also maintained in the FTI 80/20 mg dosing group. The proportion of patients hospitalized and the number of hospitalization days did not differ significantly between treatment groups over the course of the study. Improvement in respiratory symptoms achieved by the AZLI run-in, as measured by the CFQ-R RSS, was better maintained in the FTI 80/20 mg treatment group than in the FTI 160/40 mg or placebo groups. This result is consistent with the higher incidence of respiratory adverse events in the FTI 160/40 mg group; wheezing, dyspnea, hemoptysis, and rales were reported with higher incidence in the FTI 160/40 mg group than in the FTI 80/20 mg group.

The activity of FTI against gram-positive bacteria is intriguing, since chronic *S. aureus* infection is of increasing concern in patients with CF. Data from 63 patients coinfecting with *S. aureus* and *PA* provided several interesting observations, including the inability to culture *S. aureus* from the end of the FTI treatment period to study end in 13 patients receiving FTI. However, interpretation of the *S. aureus* data was confounded by the small sample sizes. Further, investigators were informed by the central laboratory if a patient tested positive for MRSA at any visit during the study. This may have influenced the use of concomitant medications in patients with MRSA, which was not prohibited. Thus the impact of FTI on *S. aureus* airway infection will require further investigation, using a study specifically designed to assess these infections.

The amount of tobramycin in a single dose of FTI (20 mg or 40 mg) is substantially lower than that in the approved dose of TIS (300 mg). The dose of medication actually delivered and absorbed also depends on the efficiency of the nebulizer used. However, the lower doses of tobramycin in FTI resulted in substantially lower systemic exposures when compared with TIS: the average serum concentration of tobramycin observed 60 minutes after the first dose of FTI 80/20 mg in this study (86 ng/ml) was 11-fold lower than the reported average serum concentration of tobramycin 60 minutes after inhalation of a 300-mg dose of TIS (950 ng/ml) (8).

During development of TIS, the 300-mg tobramycin dosage was chosen to yield sputum concentrations that were high enough to overcome sputum antagonism, rather than on the basis of a dose-response trial (27). The low amount of tobramycin in a dose of FTI raises the question of whether the treatment effects observed in this FTI trial would be achieved with 20- or 40-mg doses of tobramycin alone. Reported trials of low-dose tobramycin (80 mg) using a less efficient nebulizer did not show acute changes in FEV₁ (27, 28). Further, compared with earlier studies (8), a much more attenuated FEV₁ response was observed in a recent study of 300 mg TIS; the study included a patient population that was similar to this FTI study (29). A number of studies have reported a synergistic effect of fosfomycin and other antibiotics; however, a recent review of all the available literature concluded that additional studies with more stringent definitions of synergy were required (30). There are several potential mechanisms that may underlie the efficacy observed for FTI in the current study: (1) fosfomycin may potentiate the uptake of tobramycin (31); (2) fosfomycin may decrease antagonism of tobramycin by sputum (32); and (3) fosfomycin's antibiotic activity may contribute to the treatment effects observed. These mechanisms may all play a role in the efficacy observed, and additional studies of this combination product are needed to address the contributions of the individual FTI components.

In conclusion, both doses of FTI maintained the substantial improvements in FEV₁ % predicted that had been achieved following the AZLI run-in, while lung function in placebo-treated patients decreased toward pre-AZLI levels. FTI was well tolerated; however, FTI 80/20 mg-treated patients

completed treatment more frequently and reported fewer respiratory adverse events, compared with FTI 160/40 mg, supporting the selection of FTI 80/20 mg for future trials. Continuous alternating therapy with several antibiotics is an appealing treatment option to improve lung function, decrease respiratory symptoms, and suppress *PA* and other pathogens in the airways of patients with CF. This treatment regimen has not been tested in well-controlled clinical trials. FTI could be a promising candidate to evaluate in such a trial.

Author disclosures are available with the text of this article at www.atsjournals.org.

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Acknowledgment: The authors thank the patients and their families as well as the research site coordinators for each study site. Medical writing assistance was provided by Kate Loughney, under the sponsorship of Gilead Sciences, Inc.

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