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Original Article

Aztreonam for inhalation solution (AZLI) in patients with cystic fibrosis, mild lung impairment, and *P. aeruginosa*☆

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Abstract

Background: Previous aztreonam for inhalation solution (AZLI) studies included patients with cystic fibrosis, *Pseudomonas aeruginosa* (*PA*) airway infection, and forced expiratory volume in 1 s (FEV_1) 25% to 75% predicted. This double-blind, multicenter, randomized, placebo-controlled trial enrolled patients (≥6 years) with FEV_1 >75% predicted.
Methods: AZLI 75 mg (n=76) or placebo (n=81) was administered 3-times daily for 28 days with a 14-day follow-up.
Results: Day 28 treatment effects were 1.8 points for CFQ-R-Respiratory Symptoms Scale (95%CI: −2.8, 6.4; p=0.443; primary endpoint); −1.2 for $\log_{10}$ sputum *PA* colony-forming units (p=0.016; favoring AZLI), and 2.7% for relative FEV_1% predicted (p=0.021; favoring AZLI). Treatment effects favoring AZLI were larger for patients with baseline FEV_1 <90% predicted compared to ≥90% predicted. AZLI was well-tolerated.
Conclusions: Effects on respiratory symptoms were modest; however, FEV_1 improvements and bacterial density reductions support a possible role for AZLI in these relatively healthy patients. ClinicalTrials.gov identifier: NCT00712166.
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Keywords: Aztreonam; Cystic fibrosis; Respiratory symptoms; Inhaled antibiotics; Pseudomonas

1. Introduction

Cystic fibrosis (CF) is an autosomal-recessive disease characterized by thick pulmonary secretions and chronic airway infection with difficult to treat pathogens, the most significant of which is *Pseudomonas aeruginosa* (*PA*) [1]. The prevalence of infection increases with age; in 2008, 39.2% of US patients <18 years and 73.6% of patients ≥18 years of age were *PA*-positive [2]. Chronic airway infection is associated with progressive loss of lung function, which is the primary cause of death for CF patients [2,3].

Alternate-month, suppressive therapy with inhaled antibiotics is standard of care for CF patients infected with *PA* [4,5]. Evidence for this practice came from studies of patients with

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moderately to severely impaired lung function. Currently available inhaled antibiotics in the US are approved for patients with CF and FEV_1 25%–75% predicted, as these were the entry criteria for registration trials. Since the goal of such therapy is to preserve lung function and patients with high baseline lung function have a greater risk of steeper decline in lung function, CF patients with *PA* airway infection and normal to mildly compromised lung function should also benefit [6]. The prevalence of patients with mildly compromised lung function is increasing; in 2008, 56.6% of CF patients <18 years of age had FEV_1 >90% predicted [2].

This study evaluated an inhaled antipseudomonal antibiotic, aztreonam for inhalation solution (AZLI; Cayston®, Gilead Sciences, Inc., Foster City CA). AZLI is indicated to improve respiratory symptoms in CF patients with *PA* [7]. Previous AZLI studies included patients with moderately to severely impaired lung function (FEV_1 25%–75% predicted) and showed significant improvements relative to placebo on the Cystic Fibrosis Questionnaire—Revised (CFQ-R) Respiratory Symptoms Scale (RSS), time-to-need for intravenous (IV) or additional inhaled antipseudomonal antibiotics, forced expiratory volume in 1 s (FEV_1), and $\log_{10}$ *PA* colony forming units (CFUs) in sputum [8–11]. The current study repeats a previous study design (AIR-CF1), extending the efficacy and safety evaluation of AZLI to include patients with CF, *PA* airway infection, and milder impairment of lung function (FEV_1 >75% predicted) [9,12].

2. Materials and methods

2.1. Study design

This double-blind, multicenter, multinational, randomized, placebo-controlled trial was conducted from June 2008 to June 2009 at 40 CF centers (US: 34; Canada: 1; Australia: 5). Eligibility was assessed at screening, ≤14 days before baseline (Day 0). Patients were randomly assigned (1:1) to a 28-day course of AZLI or placebo, administered three times daily (TID), with doses separated by ≥4 h. Randomization was stratified by age (6–13, 14–17, ≥18 years) and geographic region (North America, Australia; randomization code generated by Gilead Sciences). Additional study visits were at Day 14 (mid-treatment), Day 28 (end-of-treatment), and Day 42 (follow-up).

A physical examination was performed at screening. Post-bronchodilator spirometry was performed according to American Thoracic Society guidelines at each study visit [13]. FEV_1% predicted was calculated using the Knudson equation [14].

AZLI (75 mg aztreonam, 52.5 mg lysine monohydrate) and placebo (5 mg lactose, 7.3 mg NaCl) were diluted in 0.17% saline (1 mL) and self-administered with the investigational eFlow® electronic nebulizer (PARI GmbH, Starnberg, Germany). Patients self-administered a short acting bronchodilator before each study drug dose.

This study was conducted in compliance with the protocol, the Declaration of Helsinki, the International Conference on Harmonisation guideline for Good Clinical Practices, all applicable US Food and Drug Administration regulations, Canadian Health Products and Food Branch regulations, Australian Therapeutic Goods Administration regulations, and all other applicable local laws and regulations. Institutional review boards or ethics committees approved the study for each site. Patients and/or their legal representatives/guardians provided informed consent, or assent as applicable, before any study-related procedures, including screening procedures, were performed.

2.2. Study population

Eligible patients were ≥6 years of age, with documented CF and FEV_1 >75% predicted at screening. *PA* was present in expectorated sputum or throat swab culture samples at screening or documented in 2 samples within previous 12 months (1 positive sample obtained ≤3 months before screening). Eligible patients had experienced ≥2 of the following chronic and/or intermittent CF symptoms for ≥28 days before baseline with no worsening of symptoms within 7 days before baseline: chest congestion, daily cough, productive cough, wheezing, trouble breathing, or nocturnal wakening due to coughing. Eligible patients did not require immediate antipseudomonal antibiotic treatment of an impending exacerbation; investigators considered them to be in a stable condition. Patients were able to perform reproducible pulmonary function tests.

Exclusion criteria included known hypersensitivity to monobactam antibiotics, inability to tolerate short-acting bronchodilators, lung transplantation history, or previous enrollment in an AZLI trial. Changes in baseline medications (azithromycin, hypertonic saline, or dornase alpha) or administration of any antipseudomonal antibiotic within 28 days before screening were excluded, as were changes in antimicrobial, bronchodilator, or corticosteroid medications or physiotherapy technique or schedule within 7 days before screening. Patients had a chest radiograph within 180 days before screening, with no significant acute findings (e.g., lobar infiltrate and atelectasis, pneumothorax, or pleural effusion). Females of child-bearing potential had a negative urine pregnancy test at screening, were practicing an acceptable method of birth control, and were not lactating. Patients who had received any investigational drug or device within 28 days (or 6-half lives) before screening were excluded, as were patients with any serious or active medical or psychiatric illness, which in the opinion of the investigator, would interfere with patient treatment, assessment, or compliance with the protocol.

2.3. Study endpoints

The primary efficacy endpoint was change from baseline at Day 28 on the CFQ-R RSS [11]. CFQ-R domains and scales are scored from 0 to 100, with higher scores indicating fewer symptoms, better functioning, or higher health-related quality of life. Secondary endpoints were: change from baseline at Days 14 and 42 on the CFQ-R RSS, change from baseline at Day 28 on the CFQ-R Physical Functioning Scale, use of additional

antipseudomonal antibiotics, proportion of patients hospitalized, and change from baseline at Day 28 for $\log_{10}$ *PA* CFUs in sputum and FEV_1% predicted. Additional efficacy endpoints included changes in other pulmonary function measures, weight, body mass index (BMI), other CFQ-R scores, and number of hospitalization days or missed school/work days.

Safety endpoints included adverse events, acute airway reactivity, vital signs, serum chemistry, and hematology.

Microbiological endpoints included the disappearance or appearance of respiratory pathogens and the change in minimum inhibitory concentration (MIC) of aztreonam for *PA*. The aztreonam concentrations required to inhibit the growth of 50% or 90% of *PA* isolates were reported as MIC_{50} or MIC_{90} values, respectively.

2.4. Statistical methods

Analyses included all randomly-assigned patients receiving ≥ 1 dose of study drug. Statistical tests were 2-tailed superiority analyses ($\alpha = 0.05$). Spirometry changes were calculated as relative values, calculated as a percentage of the baseline measurement. Microbiology assessments did not include data from all patients; some patients were not able to expectorate sputum samples, and some were *PA*-positive but CFUs were not reported.

Based on the results from a previous AZLI study, a sample size of 140 patients provided ≥ 90% power to detect a 10-point difference between groups in mean change from baseline at Day 28 on the CFQ-R RSS, using a 2-sided 0.05-level test and assuming a standard deviation of 17.5 [9].

For the primary efficacy analysis, treatment effect was assessed by a parametric analysis of covariance (ANCOVA); treatment and age group were fixed effects and baseline CFQ-R RSS score was a covariate. Analyses of other continuous variables used similar ANCOVA models, with respective baseline values as covariates. Exploratory analyses were performed for patients in mild ($FEV_1 > 75$ to < 90% predicted) and normal ($FEV_1 \geq 90$% predicted) lung function categories as established by the Cystic Fibrosis Foundation (CFF), except the CFF "mild" category extends to 70% predicted [2].

3. Results

A total of 192 patients were screened and 157 patients received study-drug treatment (Fig. 1). Most patients used ≥ 80% of study-drug doses (AZLI: 85.5% patients; placebo: 92.6%). One patient (AZLI) discontinued from the study and 6 patients discontinued treatment but remained in the study, discontinuing treatment was associated with pulmonary exacerbation for 3 patients (AZLI: 2; placebo: 1) and with the adverse event of FEV_1 decreased for 1 patient (AZLI).

Demographic characteristics were well balanced between treatment arms (Table 1). The majority of patients were 6–17 years of age (56.7%). Most patients were receiving dornase alfa (81.5%) and pancreatic enzymes (88.5%) at baseline. Patients had received a mean of 2.9 courses of TIS in the previous year; 65.0% of patients had received ≥ 1 course.

3.1. Efficacy

Adjusted mean change at Day 28 from baseline CFQ-R RSS scores was 3.22 for AZLI-treated and 1.41 for placebo-treated patients (Table 2A; Fig. 2). The treatment effect (1.80; 95% confidence interval [CI]: −2.83, 6.44) was not statistically significant ($p = 0.443$). Thus this study did not meet the primary efficacy endpoint. Subgroup analyses were performed for a pre-selected group of variables. Larger treatment effects were observed in favor of AZLI in female and adult patients, although these treatment effects were not statistically significant (Table 2A).

Statistically significant treatment effects favoring AZLI were observed for several secondary efficacy endpoints: change from baseline at Day 28 for adjusted mean $\log_{10}$ *PA* CFUs in sputum (AZLI: −1.4; placebo: −0.14; $p = 0.016$) and adjusted mean relative change in FEV_1% predicted (AZLI: 0.29%; placebo: −2.5%; $p = 0.021$; Table 2A; Fig. 2). Amongst other efficacy endpoints, significant treatment effects favoring AZLI were

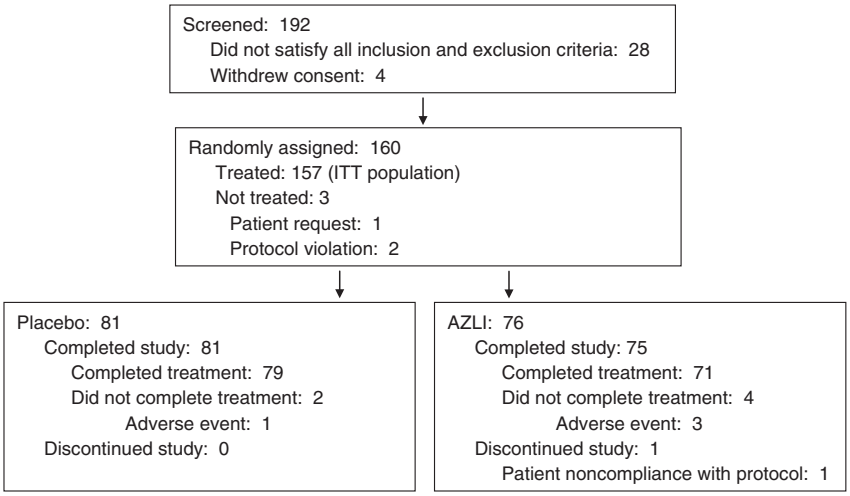

Fig. 1. Patient disposition.

Table 1
Baseline demographic and clinical characteristics.

	Placebo N=81	AZLI N=76	Total N=157
Region; n (%)			
North America	75 (92.6)	72 (94.7)	147 (93.6)
Canada	2 (2.5)	0	2 (1.3)
United States	73 (90.1)	72 (94.7)	145 (92.4)
Australia	6 (7.4)	4 (5.3)	10 (6.4)
Age, years; mean (SD)	18.9 (9.1)	19.5 (9.1)	19.2 (9.1)
Age group; n (%)			
6 to 13 years	25 (30.9)	22 (28.9)	47 (29.9)
14 to 17 years	22 (27.2)	20 (26.3)	42 (26.8)
≥18 years	34 (42.0)	34 (44.7)	68 (43.3)
6 to 17 years	47 (58.0)	42 (55.3)	89 (56.7)
Male gender; n (%)	44 (54.3)	46 (60.5)	90 (57.3)
White race; n (%)	77 (95.1)	74 (97.4)	151 (96.2)
Weight, kg; mean (SD)	53.5 (14.6)	57.9 (17.8)	55.6 (16.3)
Body mass index, kg/m^2; mean (SD)	20.7 (3.0)	21.7 (4.2)	21.2 (3.7)
CFTR genotype[a]; n (%)			
Homozygous for Δ508	40 (57.1)	39 (54.2)	79 (55.6)
Heterozygous for Δ508	26 (37.1)	23 (31.9)	49 (34.5)
Unidentified	2 (2.9)	9 (12.5)	11 (7.7)
Other	2 (2.9)	1 (1.4)	3 (2.1)
TIS courses in previous year; mean (SD)	3.0 (2.7)	2.9 (2.5)	2.9 (2.6)
Use of medications at baseline; n (%)			
Azithromycin	48 (59.3)	41 (53.9)	89 (56.7)
Dornase alfa	65 (80.2)	63 (82.9)	128 (81.5)
Pancrelipase	69 (85.2)	70 (92.1)	139 (88.5)
Salbutamol	54 (66.7)	48 (63.2)	102 (65.0)
Baseline values; mean (SD)			
FEV_1% predicted	94.7 (12.9)	95.5 (12.7)	95.1 (12.8)
FEV_1 (L)	2.9 (0.8)	3.1 (0.9)	3.0 (0.9)
Log_{10} *PA* CFUs in sputum[b]	4.4 (3.1)	4.8 (2.6)	4.6 (2.9)
CFQ-R Respiratory Symptoms Score	73.6 (17.9)	70.3 (18.5)	72.0 (18.2)
MIC of aztreonam for all *PA* isolates at baseline			
MIC_{50}; μg/mL	≤1	≤1	≤1
MIC_{90}; μg/mL	16	8	8
Minimum MIC; μg/mL	≤1	≤1	≤1
Maximum MIC; μg/mL	128	128	128
No. isolates tested[c]	104	104	208
Highest MIC[c]; n (%)			
≤8 μg/mL	53 (84.1)	58 (90.6)	111 (87.4)
>8 μg/mL	10 (15.9)	6 (9.4)	16 (12.6)

[a] No. of patients with data=70 (placebo), 72 (AZLI), and 142 (total).
[b] No. of patients with data=43 (placebo), 47 (AZLI), and 90 (total).
[c] No. of patients with data=63 (placebo), 64 (AZLI), and 127 (total) CFTR=cystic fibrosis transmembrane conductance regulator; CFQ-R=Cystic Fibrosis Questionnaire—Revised; CFUs=colony forming units; FEV_1=forced expiratory volume in 1 s; MIC=minimum inhibitory concentration; *PA*=*P. aeruginosa.*

observed for relative mean change from baseline FEV_1 (L) at Day 28 and CFQ-R Social Functioning scores (Table 2B). Use of oral, IV, or additional inhaled antibiotics was similar for the AZLI (n=19) and placebo (n=21) groups during the entire study, with most use occurring during the follow-up period for both treatment groups (AZLI: 15; placebo: 17).

This study enrolled patients with mild disease severity (FEV_1>75% predicted). Using the CFF's established lung function categories, the disease severity category was subdivided into "mild" (FEV_1>75 to <90% predicted at baseline, 39.5% patients, n=62/157), and "normal" (FEV_1≥90% predicted at baseline, 60.5% patients, n=95/157), and patient characteristics and efficacy responses were characterized in an exploratory analysis. Patients with mild lung function impairment (FEV_1>75 to <90% predicted) at baseline were older (mean [SD] age=20.7 [8.8] vs. 18.2 [9.1] years), with lower baseline scores on the CFQ-R RSS (67.4 [19.7] vs. 75.2 [16.5]) and higher baseline log_{10} *PA* sputum CFUs (5.30 [2.50] vs. 4.00 [3.01]), compared to patients with normal lung function (baseline FEV_1≥90% predicted). AZLI vs. placebo treatment effects for scores on the CFQ-R RSS were larger at Day 28 for patients with baseline FEV_1<90% predicted than for patients with baseline FEV_1≥90% predicted (Table 2A). Likewise, AZLI vs. placebo treatment effects for mean relative change in FEV_1% predicted were larger at Day 28 for patients with baseline FEV_1<90% predicted (4.8% treatment effect; p=0.032) than for patients with baseline FEV_1≥90% predicted (1.4% treatment effect, p=0.302).

Table 2A
Efficacy results: primary and secondary endpoints.

	Placebo (N=81)		AZLI (N=76)		Treatment effect[e]	P-value[f]
	Adjusted mean change from baseline to Day 28 (SE)					
	No.		No.			
Primary efficacy endpoint[a]						
CFQ-R Respiratory Symptoms Score	81	1.4 (1.6)	75	3.2 (1.7)	1.8	0.443
Subgroup analyses[a]						
Region						
North America	75	2.0 (1.7)	71	3.5 (1.8)	1.4	0.562
Australia	6	−4.0 (5.4)	4	−0.88 (6.7)	3.2	0.725
Age group						
6–13 years	25	0.85 (3.4)	22	1.3 (3.6)	0.45	0.927
14–17 years	21	3.4 (2.7)	19	1.3 (2.8)	−2.1	0.593
≥18 years	34	0.44 (2.4)	34	5.1 (2.4)	4.7	0.183
6–17 years	46	1.8 (2.2)	41	1.5 (2.4)	−0.23	0.942
Gender						
Male	44	3.7 (2.3)	45	3.7 (2.3)	−0.04	0.990
Female	37	−1.2 (2.4)	30	2.7 (2.6)	3.9	0.273
CFQ-R version						
Child	26	−3.0 (8.7)	22	−2.6 (9.4)	0.45	0.927
Adolescent/adult	55	1.8 (1.8)	53	4.0 (1.9)	2.3	0.385
Highest aztreonam MIC for *PA*[b]						
≤8 μg/mL	53	0.83 (2.3)	57	3.1 (2.1)	2.2	0.463
>8 μg/mL	10	−2.1 (4.1)	6	10.5 (5.3)	12.6	0.103
FEV_1 at baseline[c]						
<90% predicted	33	2.7 (2.7)	29	9.5 (2.7)	6.7	0.072
≥90% predicted	48	0.55 (2.1)	46	−0.81 (2.2)	−1.4	0.658
Secondary efficacy endpoints						
CFQ-R Respiratory Symptoms Score (baseline to Day 14)[a]	81	0.28 (1.6)	75	3.7 (1.6)	3.4	0.133
CFQ-R Respiratory Symptoms Score (baseline to Day 42)[a]	81	2.9 (1.7)	75	3.0 (1.7)	0.10	0.965
CFQ-R Physical Functioning Score (baseline to Day 28)[a]	80	−0.69 (1.5)	76	1.8 (1.6)	2.5	0.256
Log_{10} *PA* CFUs in sputum (baseline to Day 28)	31	−0.14 (0.36)	37	−1.4 (0.36)	−1.2	0.016
Relative change in FEV_1% predicted (baseline to Day 28)[a]	81	−2.5 (0.82)	76	0.29 (0.85)	2.7	0.021
	n (%); Baseline to End of Study					P-value[g]
Use of Oral, IV, or Additional Inhaled Antipseudomonal Antibiotics	21 (25.9)		19 (25.0)			>0.999
Number of Patients Hospitalized[d]	3 (3.7)		8 (10.5)			0.122

CFQ-R=Cystic Fibrosis Questionnaire—Revised; CFUs=colony forming units; FEV_1=forced expiratory volume in 1 s; IV=intravenous; MIC=minimum inhibitory concentration; *PA*=*P. aeruginosa.*

[a] Missing postbaseline data were imputed with the last observation carried forward (LOCF) method.
[b] The parenteral breakpoint for aztreonam is 8 μg/mL.
[c] Exploratory analysis.
[d] Two additional patients (AZLI: 1, placebo: 1) were hospitalized off-study, each on Day 55, for pulmonary exacerbation.
[e] AZLI — placebo.
[f] ANCOVA analyses. ANCOVA models included treatment, age group, and baseline values. Models for age subgroups included treatment and baseline values.
[g] Fisher's Exact Test.

3.2. Safety

The majority of patients experienced one or more adverse events during the study (AZLI: 77.6%, n=59/76; placebo: 76.5%, n=62/81). Adverse events were mild to moderate in severity for most of these patients (AZLI: 91.5%, n=54/59; placebo: 95.2%, n=59/62). Cough and productive cough were the most commonly reported adverse events (Table 3). The incidence of the most commonly reported adverse events was similar for both treatment groups, except abdominal pain was reported for significantly fewer AZLI-treated patients. The most commonly reported adverse events considered by investigators to be treatment-related were cough (AZLI: 9.2%; placebo: 4.9%), productive cough (3.9%; 3.7%), respiratory tract congestion (3.9%; 0%), and pulmonary function test decreased (0%; 3.7%). Serious adverse events were reported for 11.8% (n=9/76) of AZLI-treated and 3.7% (n=3/81) of placebo-treated patients (p=0.073). These 12 patients experienced 20 serious adverse events; all were associated with hospitalizations and none were considered by investigators as treatment-related. The 12 on-study hospitalizations of 11 patients were associated with pulmonary exacerbation (AZLI: 2; placebo: 2); bronchitis, bronchiectasis, or bronchopneumonia (AZLI: 3), gastrointestinal conditions (AZLI: 2; placebo: 1), fever (placebo: 1), and influenza (AZLI: 1). No patients experienced an acute decrease in FEV_1 of ≥15% after dosing at clinic visits and there were no reports of anaphylaxis. There were no significant differences between AZLI and placebo groups in clinical chemistry or

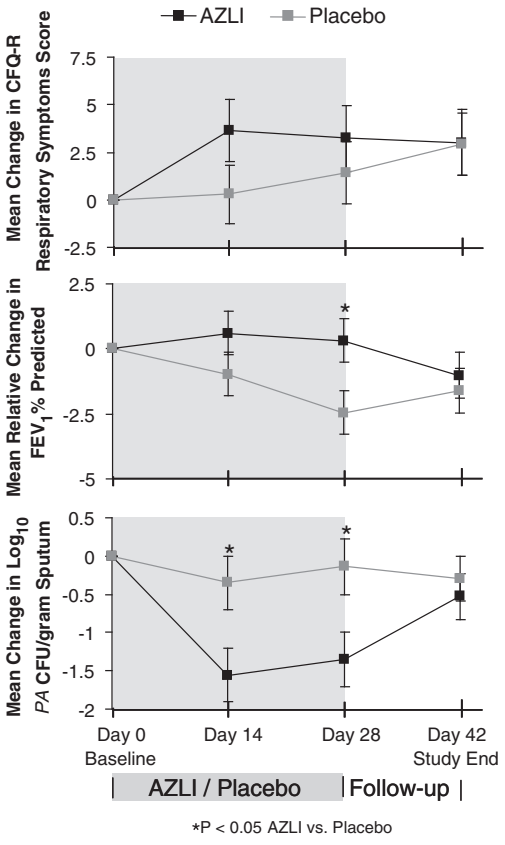

Fig. 2. Key efficacy endpoints. Mean changes in CFQ-R Respiratory Symptoms Scores (in points, on a scale of 0–100), mean relative changes in FEV_1% predicted (percentage change from baseline values), and changes in $\log_{10}$ *PA* CFU/g sputum are plotted across the study visits.

hematology results or in vital signs except shifts of white blood cell, neutrophil, and platelet counts above the normal reference ranges were observed for fewer AZLI-treated patients. There were no deaths in this study.

3.3. Microbiology

Sputum samples were positive for *PA* for 86.5% of AZLI-treated patients at baseline (n=64/74); this decreased to 66.2% of patients at Day 28 (n=49/74) and increased to near baseline values at Day 42 (80.0%, n=60/75). The prevalence of *PA*-positive placebo-treated patients showed little change (baseline: 84.0%, n=63/75; Day 28: 83.5%, n=66/79; Day 42: 82.7%, n=62/75).

For *PA* isolates from AZLI-treated patients, the MIC_{50} of aztreonam increased from baseline to Day 14 (≤1 μg/mL to 4 μg/mL) as did the MIC_{90} (8 μg/mL to 32 μg/mL); these increases were maintained at Days 28 and 42. For *PA* isolates from placebo-treated patients, the MIC_{50} (≤1 μg/mL) and MIC_{90} (16 μg/mL) of aztreonam remained unchanged during the study.

The proportion of AZLI-treated patients who had *PA* isolates with aztreonam MIC values >8 μg/mL (parenteral breakpoint) increased from 9.4% at baseline (n=6/64) to 29.4% at Day 14 (n=15/51), and then remained fairly constant (Day 28: 26.5%, n=13/49; Day 42: 25.0%, n=15/60). The proportion of placebo-treated patients who had *PA* isolates with aztreonam MIC values >8 μg/mL remained fairly constant (baseline: 15.9%, n=10/63; Day 14: 20.3%, n=14/69, Day 28: 13.6%, n=9/66; Day 42: 16.1%, n=10/62). The *PA* isolate that was least susceptible to aztreonam was determined at baseline and at Day 28 for each patient. A ≥4-fold increase in aztreonam MIC for the least susceptible *PA* isolate was observed in 21 patients (AZLI: 13; placebo: 8; Fig. 3). Of these 21 patients, 4 patients in each treatment group had ≥4-fold increases in MIC that crossed or persisted above the aztreonam parenteral breakpoint of 8 μg/mL. No relationship was observed between the magnitude of the highest MIC of aztreonam for *PA* isolates for each patient at baseline and mean changes in CFQ-R RSS scores, FEV_1% predicted, or $\log_{10}$ *PA* CFUs in sputum.

There was no evidence for persistent increases in the isolation of *Burkholderia* spp., *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Aspergillus* spp., or *Staphylococcus aureus*.

4. Discussion

Patients treated with AZLI showed a modest improvement in respiratory symptoms, as measured on the CFQ-R RSS. When compared with placebo, the treatment effect was not clinically or statistically significant, thus the study did not meet the primary efficacy endpoint. A significant treatment effect was observed at Day 28 for FEV_1% predicted, a secondary endpoint. This primarily resulted from a decrease from baseline at Day 28 for placebo-treated patients; a small improvement was observed for AZLI-treated patients.

On the CFQ-R RSS, larger AZLI vs. placebo treatment effects were observed for females compared with males, for adults compared with children, for patients with highest aztreonam MIC for *PA* >8 μg/mL compared with ≤8 μg/mL, and for patients with FEV_1<90% predicted at baseline compared with ≥90% predicted, but none of these differences were statistically significant. The larger treatment effects in females and adults are in agreement with more compromised lung function typically seen in these subgroups [3].

The FEV_1% predicted treatment effect observed in this study differed from the previously reported lack of significant lung function treatment effect observed for a subset of patients with baseline FEV_1 ≥75% predicted in the placebo-controlled AZLI Phase 2 study [15]. Lack of significant effects on lung function were also reported in the literature for a placebo-controlled study of tobramycin inhalation solution (TIS) in patients with mild lung function impairment [16]. Further, significant treatment effects were not observed for FEV_1% predicted or on the CFQ-R RSS in a recent hypertonic saline trial in patients with FEV_1>80% predicted [17]. The response of the placebo group in the current study also differed from responses observed in previous AZLI studies; in the current study both FEV_1% predicted and scores on the CFQ-R RSS improved in the placebo group during the 14-day follow-up period and in previous studies both remained unchanged or decreased [8,9]. The reason for this improvement in the placebo group is not

Table 2B
Efficacy results: additional endpoints.

Additional efficacy endpoints	Placebo (N=81)		AZLI (N=76)		Treatment effect[c]	P-value[d]
	Adjusted mean change (SE) from baseline to Day 28					
	No.		No.			
Relative change in FEV_1 (L)[a]	81	−2.5 (0.83)	76	0.36 (0.85)	2.7	0.021
Relative change in FVC (L)[a]	81	−1.4 (0.71)	76	−0.04 (0.73)	1.4	0.171
Relative change in FEF_{25-75}[a]	81	−2.9 (2.2)	76	2.6 (2.3)	5.5	0.079
Relative change in weight[a]	81	−1.9 (1.3)	76	1.9 (1.3)	3.0	0.111
Change in BMI[a] (kg/m^2)	81	−0.22 (0.24)	76	0.33 (0.24)	0.56	0.097
Change on CFQ-R Scales[a,b]						
Body image	80	1.1 (1.6)	76	3.7 (1.7)	2.6	0.259
Digestion	81	5.5 (1.9)	75	3.6 (1.9)	−1.9	0.470
Eating disturbances	81	−1.2 (1.5)	76	0.5 (1.5)	−1.7	0.409
Emotional functioning	80	3.7 (1.2)	76	3.3 (1.2)	−0.37	0.828
Health perceptions	54	−1.8 (2.0)	54	1.8 (2.0)	3.6	0.194
Role/school	55	1.2 (1.4)	53	0.29 (1.5)	−0.90	0.650
Social functioning	80	−2.6 (1.3)	75	1.9 (1.4)	4.5	0.020
Treatment burden	81	5.1 (1.6)	76	1.2 (1.6)	−3.9	0.075
Vitality	54	−2.2 (2.2)	54	0.38 (2.2)	2.6	0.402
Weight	55	2.6 (2.9)	53	4.3 (2.9)	1.7	0.667
	Mean (SD) Baseline to End of Study					P-value[e]
Number Hospitalization Days	81	0.4 (2.5)	76	0.9 (3.1)		0.100
Number Missed School/Work Days	81	1.2 (3.0)	76	1.6 (4.9)		0.573

BMI=body mass index; CFQ-R=Cystic Fibrosis Questionnaire—Revised; FEF_{25-75}=forced expiratory volume from 25% to 75% of FVC; FEV_1=forced expiratory volume in 1 s; FVC=forced vital capacity; *PA*=*P. aeruginosa.*

[a] Missing postbaseline data were imputed with the last observation carried forward (LOCF) method.
[b] CFQ-R Health Perceptions, Role/School, Vitality, and Weight scales have only an adolescent/adult version.
[c] AZLI — placebo.
[d] ANCOVA analyses. ANCOVA models included treatment, age group, and baseline values.
[e] Wilcoxon Rank Sum Test.

known; it was not explained by differential use of oral, IV, or additional inhaled antipseudomonal antibiotics during the follow-up period as use of such antibiotics was similar for the placebo and AZLI treatment groups.

AZLI was well tolerated, most adverse events were mild to moderate in severity and the most commonly reported adverse events were associated with respiratory symptoms.

In contrast to previously reported AZLI trials, including one of 18-month duration, there were slight increases in this study for

Table 3
Adverse events reported for ≥ 10% of patients in either treatment group.

Preferred term	Placebo (N=81) n (%)	AZLI (N=76) n (%)	P-value[a]
Cough	31 (38.3)	35 (46.1)	0.337
Productive cough	13 (16.0)	18 (23.7)	0.316
Nasal congestion	15 (18.5)	13 (17.1)	0.838
Headache	10 (12.3)	14 (18.4)	0.376
Oropharyngeal pain	11 (13.6)	12 (15.8)	0.822
Rhinorrhoea	12 (14.8)	8 (10.5)	0.479
Respiratory tract congestion	6 (7.4)	11 (14.5)	0.201
Fatigue	10 (12.3)	6 (7.9)	0.434
Pulmonary function test decreased	9 (11.1)	7 (9.2)	0.795
Diarrhea	9 (11.1)	3 (3.9)	0.133
Abdominal pain	10 (12.3)	1 (1.3)	0.010

[a] P-values are based on Fisher's Exact Test.

MIC_{50} and MIC_{90} of aztreonam for *PA* after AZLI treatment; these increases suggest an element of inducible resistance [8–10]. This interpretation is supported by the observation that the baseline susceptibility of *PA* isolates in this study was generally greater than in the previous studies and the observation that, for most patients in this study, MIC values for *PA* isolates at Day 28 remained below the aztreonam parenteral breakpoint. Patients in previous studies had poorer lung function and were, on average, older than the patients in the current study, and thus likely had a history of more extensive treatment of *PA* airway infections. High aztreonam MICs have not been predictive of lack of efficacy, presumably due to the high aztreonam concentration achieved in sputum.

Since the CF medical community recognizes the importance of preserving lung function in patients with chronic *PA* infection, it is important to explore why a drug like AZLI did not have a substantial treatment benefit on the CFQ-R RSS in this study. It seems unlikely that AZLI does not benefit this population; AZLI was effective in previous studies and there is no pathophysiologic reason to believe that an effective antibiotic would not benefit any CF population with chronic *PA* infection. It is possible that the sensitivity of the CFQ-RSS is not sufficient for patients with more modest symptoms at baseline (ceiling effect) or that the study may not have been adequately powered to detect a change. Sample size assumptions were based on observing a 10-point treatment difference

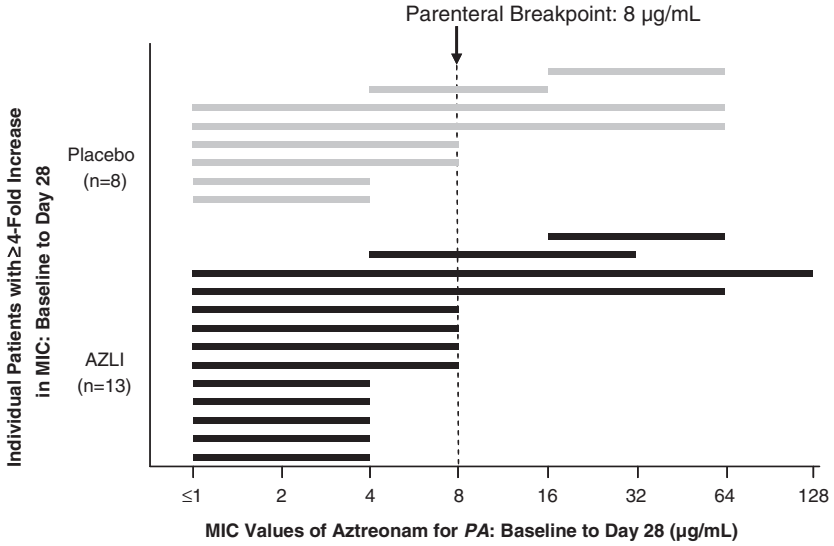

Fig. 3. Four-fold or greater changes in minimum inhibitory concentrations (MICs) of aztreonam for *P. aeruginosa* (*PA*). Each horizontal bar represents data from a single patient. The left and right ends of a bar denote the aztreonam MIC of the least susceptible *PA* isolate at baseline and Day 28, respectively. Data are shown for all patients with ≥4-fold increases in aztreonam MIC values from baseline to Day 28. The vertical dotted line represents the parenteral susceptibility breakpoint of aztreonam for *PA* (8 µg/mL). Patients with MIC data: AZLI (N=44), placebo (N=54).

on the CFQ-R RSS; however, the baseline CFQ-R RSS score was approximately 10 points higher than in previous AZLI studies [8–10]. This indicates that patients in this study had fewer symptoms at baseline, and coupled with their high baseline FEV_1% predicted values suggests that patients without measurable compromise of lung function will not have measurable improvement, in spite of chronic *PA* infection. This hypothesis is supported by analyses of the patient subset with FEV_1 >75 to <90% predicted; they had both measurable compromise and measured benefit.

This study demonstrated a significant reduction in sputum bacterial density and maintenance of FEV_1% predicted in AZLI-treated patients, compared with placebo. FEV_1 has been shown to decline in the placebo arm of other studies as well; further supporting that maintenance of FEV_1 should be a meaningful outcome even in a short term study. In spite of the general lack of demonstrated efficacy in patients with FEV_1 >75% predicted, prescribing guidelines do not specify a target level of lung compromise for azithromycin, hypertonic saline, or dornase alfa, but are limited in the US to patients with FEV_1 25% to 75% predicted for TIS and AZLI.

While this study of AZLI in CF patients with mild lung function impairment and airway *PA* did not meet its primary endpoint, much has been learned about this population. AZLI was well tolerated, FEV_1% predicted declined in placebo-treated patients even over the short course of this study, and *PA* bacterial density decreased with AZLI treatment. In exploratory analyses, patients with FEV_1 >75 to <90% predicted had measurable improvement in respiratory symptoms and statistically significant improvements in FEV_1% predicted, compared to patients with $FEV_1 \geq 90$% predicted. Further studies of antipseudomonal therapies such as AZLI are warranted in patients with mild loss of lung function. Studies in patients with FEV_1 >75 to <90% predicted may be able to use short-term clinical outcomes, but measuring responses in patients with $FEV_1 \geq 90$% predicted likely will require trial designs that use outcome measures that focus on the pathophysiology of the CF airway disease, or may need long-term studies measuring outcomes such as longitudinal decline in lung function.

Conflict of interest statement

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