Original Article

Inhaled aztreonam for chronic *Burkholderia* infection in cystic fibrosis: A placebo-controlled trial ☆,☆☆,★

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Abstract

Background: Individuals with *Burkholderia* spp. infection have historically been excluded from efficacy trials of inhaled antibiotics, including aztreonam for inhalation solution (AZLI).
Methods: A double-blind, placebo-controlled, 24-week trial of continuous AZLI/placebo treatment was undertaken in individuals with cystic fibrosis (CF) and chronic *Burkholderia* spp. infection. All subjects also received usual medical care (determined by their physicians). Additional antibiotic use was not restricted.
Results: Baseline FEV_1% predicted values ranged from 15.8% to 114.6%. No significant treatment differences (AZLI vs. placebo) were observed at week 24 for any endpoints, including FEV_1% predicted, number of respiratory exacerbations requiring systemic/inhaled antibiotics, or hospitalizations. Continuous AZLI administration was well tolerated. *Burkholderia* spp. susceptibility to antibiotics commonly used in CF therapy showed little change.
Conclusions: 24-weeks of continuous AZLI treatment did not significantly improve lung function in CF subjects with chronic *Burkholderia* spp. infection. Non-study antibiotic use may have confounded any potential AZLI effects.
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Keywords: Antipseudomonal; *Cepacia* complex; Inhaled antibiotics; *Pseudomonas aeruginosa*; Respiratory exacerbations

1. Introduction

Cystic fibrosis (CF) is characterized by a robust inflammatory response to chronic respiratory tract infection, leading to progressive airway obstruction and loss of lung function. *Burkholderia gladioli* and species in the *Burkholderia cepacia* complex are amongst the many opportunistic bacterial pathogens that cause respiratory infections in individuals with CF [1]. The prevalence of *B. cepacia* complex infection in individuals with CF is approximately 3% in North America; however, it varies significantly between treatment centers [1–3]. *Burkholderia*

infection is associated with higher rates of morbidity and mortality in individuals with CF, including more frequent and difficult-to-treat exacerbations, weight loss, and accelerated lung function loss [4,5]. Nevertheless, individuals with *Burkholderia* infection have historically been excluded from efficacy trials of inhaled antibiotics and currently there is no consensus regarding treatment of *Burkholderia* infection in individuals with CF [5,6].

Aztreonam for inhalation solution (AZLI; Cayston®; Gilead Sciences Inc.) is a monobactam antibiotic used to treat individuals with CF and *Pseudomonas aeruginosa* infection. During the AZLI Phase 3 clinical trials, anecdotal reports emerged of positive responses by subjects with chronic *Burkholderia* infection (Gilead Sciences, data on file). These reports, together with the demonstrated in vitro activity of AZLI against *Burkholderia* spp. [7–9], prompted consideration of AZLI as a possible therapy for treating *Burkholderia* infection. To this end, a double-blind, placebo-controlled, 24-week trial of continuous AZLI/placebo treatment was undertaken in individuals with CF and chronic *Burkholderia* infection [10–13]. Results from this prospective multicenter trial also provided a unique opportunity to obtain a contemporary picture of the clinical and microbiological characteristics of individuals with CF and chronic *Burkholderia* infection.

2. Methods

2.1. Study design

This Phase 3, double-blind, randomized, placebo-controlled, 2-part study was conducted at 35 sites (US: 34; Canada: 1; Feb 2010–Sept 2011). After screening, eligible subjects were stratified by region (due to differences in treatment-center approaches); and randomly assigned (1:1) to 24 weeks of continuous treatment with AZLI 75 mg or placebo, administered 3 times daily (weeks 0–24; comparative period); followed by a 24-week extension period of open-label AZLI treatment for all subjects (weeks 24–48); and a 4-week follow-up period (weeks 48–52). Subjects and investigators remained blinded to randomized treatment-assignments during the extension/follow-up periods. Subjects continued to receive their usual medical care, determined by their physicians, with no restrictions on additional antibiotic use.

Spirometry was performed according to American Thoracic Society guidelines at each visit (every 4 weeks). Forced expiratory volume in 1 s (FEV_1)% predicted was calculated with Hankinson's (adult subjects) and Wang's (pediatric) equations [14,15]. A medical reviewer, blinded to treatment-arm assignment, confirmed hospitalizations for respiratory exacerbations and antibiotic use during the study or in the 2 previous years.

AZLI (75 mg aztreonam, 52.5 mg lysine monohydrate) and placebo (5 mg lactose, 7.3 mg NaCl), reconstituted in 0.17% NaCl, were administered with the PARI Investigational eFlow® Nebulizer System (PARI Respiratory Equipment; Midlothian, VA, USA); ≥4 h separated the doses. AZLI/placebo was administered in the clinic at the first visit of each period and treatment-induced bronchospasm was monitored. Treatment

adherence was assessed by counting returned used/unused AZLI/placebo vials.

The study was conducted in accordance with the principles of the Declaration of Helsinki (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), ICH guidelines, or with the laws and regulations of the study countries, whichever afforded the greater protection to the study subject. Institutional review boards/independent ethics committees approved the study for each site. Subjects (or parents, guardians, or legally authorized representatives) provided written informed consent (assent) prior to any study-related procedures.

2.2. Study population

Eligible subjects (≥6 years of age) had documented CF and chronic infection with *Burkholderia* spp., defined at baseline for subjects with sputum or bronchoalveolar lavage samples as: 1) a culture positive for *Burkholderia* spp. within 6 months before baseline, 2) ≥50% of cultures (collected ≥1 month apart) over the prior 12 months positive for *Burkholderia* spp. (≥2 positive cultures), and 3) ≥1 culture (obtained at any time) confirmed to be a *Burkholderia* spp. by the CF Foundation *B. cepacia* Research Laboratory and Repository (BcRLR; University of Michigan, Ann Arbor, MI). For subjects with only oropharyngeal culture results available for the previous 12 months, chronic infection with *Burkholderia* spp. was confirmed by meeting the above criteria and: 1) ≥1 prior lower respiratory tract culture(s) (expectorated, induced sputum, or bronchoalveolar lavage, obtained at any time) positive for *Burkholderia* spp., confirmed at the BcRLR, and 2) concordance of the species isolated from prior lower respiratory tract culture(s) and recent oropharyngeal cultures (prior 12 months).

Additional study methods and inclusion/exclusion criteria are presented in an online appendix.

2.3. Study endpoints

The primary efficacy endpoint was mean relative change from baseline FEV_1% predicted, measured by AUC_{ave} (mean area under the curve corrected for baseline and adjusted by number of days on study) through week 24. Key secondary endpoints included: number of respiratory exacerbations requiring IV, oral, and/or inhaled antibiotics; number of respiratory hospitalizations; AUC_{ave} through week 24 for CF Questionnaire-Revised (CFQ-R [16]) Respiratory Symptoms scores; and time to respiratory exacerbation requiring IV, oral, and/or inhaled antibiotics. Expectorated sputum/oropharyngeal swab samples were collected for microbiology assessments (weeks 0, 4, 12, 24, 28, 36, 48, 52) [17,18]. Identification of *Burkholderia* spp. and related species was performed as described [19–23]. Adverse events were assessed throughout the study.

2.4. Statistical analyses

Analyses included subjects receiving ≥1 dose of AZLI/ placebo. Missing data were not imputed. A sample size of 50 subjects per treatment group was estimated as providing ≥80%

power to detect an 8.5% difference between groups in mean AUC_{ave} of relative change from baseline in $FEV_1\%$ predicted through week 24 (2-sided, 0.05-level), assuming a common standard deviation (SD) of 15. The primary analysis used a parametric analysis of covariance (ANCOVA) model (2-sided, 0.05-level); baseline $FEV_1\%$ predicted was a covariate. Analysis methods for key secondary and other endpoints are described in Table 2. A family alpha spending rule controlled the Type 1 error rate (α = 0.05), with the primary endpoint analysis serving as gatekeeper, and key secondary endpoints tested sequentially (α = 0.05) based upon the closed testing procedure [24].

3. Results

3.1. Disposition

Of 102 screened subjects, 101 were randomized and 100 were treated with AZLI (n = 48) or placebo (n = 52) during the comparative period. Overall, 84.0% of subjects (n = 84/100) completed the comparative period and continued into the AZLI

extension period, and 90.0% of these subjects (n = 76/84) completed the extension period (Online Fig. 1). Seven subjects (all treated with AZLI during comparative (n = 5) or extension (n = 2) periods) discontinued from the study due to an adverse event(s); most were respiratory events. The majority of subjects took $\geq 80\%$ of expected AZLI/placebo doses during the comparative and extension periods.

3.2. Demographics and baseline characteristics

Demographic and baseline characteristics were well balanced between treatment arms, except that mean (SD) baseline $FEV_1\%$ predicted was higher in the AZLI arm (60.7 [21.7]) than that in the placebo arm (52.6 [23.7]; Table 1). Overall, baseline $FEV_1\%$ predicted values ranged from 15.8% to 114.6%. Most subjects were ≥ 18 years old (83.0%; n = 83/100). Most subjects had a positive culture for *Burkholderia* spp. at baseline (94.8%; n = 91/96), although the proportion able to provide a sputum sample of sufficient quantity for *Burkholderia* spp. sputum density analysis was much lower (61.5%; n = 59/96).

Table 1
Subject demographics and baseline characteristics.

		AZLI (n = 48)	Placebo (n = 52)	Total (n = 100)
Age, years	Mean (SD)	28.0 (10.3)	24.7 (10.0)	26.3 (10.2)
	Min., max.	7.0, 53.0	6.0, 57.0	6.0, 57.0
Age group	≥ 18 years; n (%)	42 (87.5)	41 (78.8)	83 (83.0)
	>12 to <18 years; n (%)	3 (6.3)	8 (15.4)	11 (11.0)
	≥ 6 to ≤ 12 years; n (%)	3 (6.3)	3 (5.8)	6 (6.0)
Gender	Male; n (%)	26 (54.2)	35 (67.3)	61 (61.0)
BMI, kg/m^2	Mean (SD)	21.9 (4.5)	20.7 (3.2)	21.3 (3.9)
CFTR genotype	F508 del. homozygote; n (%)	27 (56.3)	26 (50.0)	53 (53.0)
	F508 del. heterozygote; n (%)	9 (18.8)	10 (19.2)	19 (19.0)
	Unidentified or other; n (%)	5 (10.4)	6 (11.5)	11 (11.0)
	Missing; n (%)	7 (14.6)	10 (19.2)	17 (17.0)
CF-related diabetes; n (%)		15 (31.3)	19 (36.5)	34 (34.0)
$FEV_1\%$ predicted	Mean (SD)	60.7 (21.7)	52.6 (23.7)	56.5 (23.0)
	Min., max.	17.0, 108.5	15.8, 114.6	15.8, 114.6
CFQ-R RSS scores [a]	Mean (SD)	58.3 (21.4)	59.0 (17.6)	58.6 (19.4)
Baseline non-study medications; n (%)				
Azithromycin		30 (62.5)	31 (59.6)	61 (61.0)
Dornase alfa		36 (75.0)	37 (71.2)	73 (73.0)
Hypertonic saline		24 (50.0)	21 (40.4)	45 (45.0)
Any antibiotic; any route of administration		39 (81.3)	41 (78.8)	80 (80.0)
Any inhaled antibiotic		5 (10.4)	12 (23.1)	17 (17.0)
Subjects hospitalized in previous 2 years; n (%)		36 (75.0)	44 (84.6)	80 (80.0)
No. hospitalizations in previous 2 years		121	187	308
Subjects using antibiotics in previous 2 years; n (%)		48 (100.0)	50 (96.2)	98 (98.0)
No. courses in previous 2 years; mean (SD)		22.8 (15.5)	26.0 (18.5)	24.5 (17.1)
Positive culture for *Burkholderia* spp.; [b] n (%)		44 (95.7)	47 (94.0)	91 (94.8)
Highest aztreonam MIC for *Burkholderia* spp.				
4–8 μg/mL		6	8	14
16–128 μg/mL		20	17	37
≥ 256 μg/mL		18	22	40
Subjects with *Burkholderia* spp. and CFU/g sputum data		28	29	57
Log$_{10}$ CFU/g sputum; mean (SD)		6.85 (1.82)	7.07 (1.47)	6.96 (1.64)

Note: Characteristics were collected at screening or baseline (week 0; the beginning of the comparative period).
BMI = body mass index; CFQ-R = Cystic Fibrosis Questionnaire-Revised; CFTR = cystic fibrosis transmembrane conductance regulator gene; CFU = colony forming units; Del. = deletion; FEV_1 = forced expiratory volume in 1 s; Max. = maximum; MIC = minimum inhibitory concentration; Min. = minimum; RSS = Respiratory Symptoms domain scores; SD = standard deviation.
[a] CRQ-R RSS scores available for 98 subjects (AZLI: 46; placebo: 52).
[b] Data available for 46 (AZLI), 50 (placebo), and 96 (total) subjects.

Burkholderia spp. isolates recovered in baseline cultures demonstrated high minimum inhibitory concentrations (MICs) for aztreonam. The MICs at which 50% and 90% of isolates were inhibited (MIC_{50} and MIC_{90}) for aztreonam were 64 and 512 μg/mL, for the AZLI arm respectively, and 128 and 1024 μg/mL, for the placebo arm respectively. At week 24, the

baseline for subjects entering the extension period, characteristics did not differ substantially from those of week 0, although the difference between treatment groups in FEV_1% predicted values had increased. The mean (SD) FEV_1% predicted was 62.2 (20.9) for subjects receiving AZLI during both the comparative and extension periods (AZLI/AZLI) and was 50.4 (24.5) for subjects

Table 2
Selected efficacy data for the comparative period (weeks 0 to 24).

	AZLI (n = 48)	Placebo (n = 52)	p-Value	Treatment difference (95% CI)
Adj. mean (SE) for AUC_{ave} of relative change from baseline at week 24 for:				
FEV_1% predicted [a]	0.16 (1.50)	−0.75 (1.43)	0.663 [b]	0.91 (−3.24, 5.06)
CFQ-R RSS score [c]	2.97 (1.70)	2.79 (1.58)	0.939 [b]	0.18 (−4.43, 4.78)
Adj. mean (SE) for actual change from baseline at week 24 for:				
BMI [d] kg/m^2	0.34 (0.16)	0.21 (0.15)	0.531 [e]	0.14 (−0.29, 0.56)
Log_{10} *Burkholderia* spp. CFU/g sputum [f]	1.41 (0.58)	0.48 (0.50)	0.232 [b]	0.93 (−0.62, 2.48)
Other efficacy comparisons	AZLI (n = 48)	Placebo (n = 52)	p-Value	
Subjects with IV, oral, and/or inhaled antibiotic use for any respiratory indication; [g, h] n (%)	39 (81.3)	49 (94.2)	0.06	
Subjects with IV, oral, and/or inhaled antibiotic use for respiratory exacerbation; [h, i] n (%)	29 (60.4)	38 (73.1)	0.206	
Time to antibiotic use for respiratory exacerbation; [i, j, k] estimated median days (95% CI)	75.0 (49.0, 117.0)	51.0 (35.0, 95.0)	0.266	
Number of unique days of IV, oral, and/or inhaled antibiotic use for any respiratory indication; [g, l] mean (SD)	70.3 (59.5)	91.7 (60.7)	0.071	
Cumulative days of IV, oral, and/or inhaled antibiotic use for any respiratory indication (antibiotic burden) [l, m]	111.3 (101.2)	148.4 (117.2)	0.105	
Number of unique days of IV, oral, and/or inhaled antibiotic use for respiratory exacerbations; [a, i, l] mean (SD)	26.1 (31.3)	38.2 (38.3)	0.094	
Total number of IV, oral, and/or inhaled antibiotic courses for respiratory exacerbation [i, n]	54	73	0.416	
IV, oral, and/or inhaled antibiotic course rate per subject year for respiratory exacerbation [i]	2.69	3.13	NA	
Subjects hospitalized at least once for respiratory exacerbation; [h, i] n (%)	17 (35.4)	21 (40.4)	0.682	
Number of days hospitalized for respiratory exacerabations; [i] mean (SD)	15.3 (11.4)	20.4 (24.8)	NA	
Total number of respiratory hospitalizations [i, n]	25	32	0.807	
Respiratory hospitalization rate per subject-year [i]	1.24	1.37	NA	
Percent of days of missed school/work; [l, o] mean (SD)	1.9 (3.3)	4.7 (7.6)	0.284	

Adj. = adjusted; AUC_{ave} = calculated area under the curve corrected for baseline and adjusted by the number of days on study; BMI = body mass index; CFQ-R = Cystic Fibrosis Questionnaire-Revised; CI = confidence interval; CFU = colony forming units; FEV_1 = forced expiratory volume in 1 s; IV = intravenous; RSS = Respiratory Symptoms Scale scores; SD = standard deviation; SE = standard error.

[a] n = 47 (AZLI), 52 (placebo).

[b] p-Value for treatment difference was based on an analysis of covariance (ANCOVA) model with baseline value as a covariate.

[c] n = 45 (AZLI), 52 (placebo).

[d] n = 39 (AZLI), 45 (placebo).

[e] p-Value was based on a mixed-effect model repeated measure (MMRM) model that included terms for treatment, visit, baseline, and treatment × visit interaction.

[f] n = 15 (AZLI), 20 (placebo).

[g] Includes maintenance therapies for chronic lung infection and treatments for respiratory exacerbations; days of antibiotic use included unique days (e.g., receiving 3 antibiotics on 1 day counted as 1 unique day of antibiotic use).

[h] Compared with Fisher's exact test.

[i] Antibiotics used for respiratory exacerbations and hospitalizations for respiratory exacerbations were determined by a blinded medical reviewer. Only antibiotics that started on or after the first dose date were included. A single antibiotic course may represent the use of multiple antibiotics. Days of antibiotic use included unique days.

[j] Median estimated with Kaplan–Meier methods.

[k] p-Value was based on log-rank test.

[l] Compared with Wilcoxon rank sum test.

[m] Includes maintenance therapies for chronic lung infection and treatments for respiratory exacerbations; cumulative days of antibiotic use included all antibiotics and all days (e.g., receiving 3 antibiotics on 1 day counted as 3 days of antibiotic use).

[n] Compared with a negative binomial regression model.

[o] n = 32 (AZLI), 40 (placebo).

receiving placebo during the comparative period and AZLI during the extension period (placebo/AZLI).

3.3. Efficacy

The primary efficacy endpoint, adjusted mean (SE) AUC_{ave} of relative change from baseline in $FEV_1\%$ predicted at week 24, was not significantly different for subjects treated with AZLI (0.16 [1.50]) or placebo (−0.75 [1.43]), with a treatment difference of 0.91 (95% confidence interval [CI]: −3.24, 5.06; p = 0.66; Table 2). When subgroups were examined, AUC_{ave} was numerically higher for AZLI-treated subjects than for placebo-treated subjects for some subgroups, but no statistically significant (p ≤ 0.05) treatment differences were observed (Online Fig. 2). Adjusted mean (SE) AUC_{ave} of relative change from baseline CFQ-R Respiratory Symptom scores at week 24 were also similar for the AZLI- and placebo-treated subjects (p = 0.939; Table 2). Numerical improvements after AZLI treatment were observed for some secondary efficacy endpoints, including incidences of respiratory exacerbations and hospitalizations; however, AZLI vs. placebo treatment differences were not statistically significant.

A majority of subjects received IV, oral, or inhaled antibiotics other than AZLI for respiratory exacerbations during the comparative (AZLI: 60.4%; placebo: 73.1%) and extension (AZLI/AZLI: 79.5%; placebo/AZLI: 75.6%) study periods (Table 2; Online Table 1). The incidence of non-AZLI antibiotic use during the comparative period was greater when antibiotics prescribed for all respiratory indications (i.e., maintenance therapy and/or acute exacerbation treatments) were included (AZLI: 81.3%; placebo: 94.2%). In exploratory analyses, no significant AZLI vs. placebo treatment differences were observed in the primary efficacy endpoint, after adjusting for number of courses of IV/oral/inhaled antibiotics used for respiratory exacerbations during the comparative period. In similar analyses, no differences were found when adjusting for either number of unique days on additional antibiotics or total antibiotic-days (antibiotic burden). The largest improvements in relative change in $FEV_1\%$ predicted occurred after the first 28-days of AZLI treatment during the comparative period (AZLI group) or the extension period (placebo/AZLI group; Online Fig. 3).

3.4. Safety

During the 24 week comparative period, the incidence of the most commonly observed adverse events was generally comparable between treatment arms, although wheezing (AZLI: 20.8%; placebo: 5.8%) and chills (AZLI: 12.5%; placebo: 3.8%) were reported for more AZLI-treated than placebo-treated subjects when considered by incidence (Online Table 2) or after adjusting for study duration (Online Table 3). The wheezing observed among AZLI-treated subjects appeared to be associated with underlying disease (asthma or prior history of wheezing), was associated with a viral infection, or was one of multiple symptoms reported for acute pulmonary exacerbation. The onsets of the events of wheezing were distributed across the comparative period and most events resolved on study. One subject discontinued from the study due to wheezing and several other respiratory events; the subject also experienced a concomitant exacerbation of CF. The chills observed among AZLI-treated subjects were one of multiple symptoms reported for a viral infection or a pulmonary exacerbation. The onsets of the events of chills were distributed across the comparative period and all events resolved on study. Eleven AZLI-treated (22.9%) and 10 placebo-treated (19.2%) subjects experienced severe adverse events; the majority was respiratory events and all were considered by the investigators as unrelated to treatment. One life-threatening adverse event (respiratory failure) and 2 deaths (progressive CF lung disease [baseline FEV1% predicted: 16.95]; and acute hypercarbic respiratory failure [baseline $FEV_1\%$ predicted: 21.08]) were reported during the comparative period; all occurred in the AZLI-treatment group and were considered unrelated to treatment. Treatment-related adverse events were reported for 9 AZLI-treated (18.8%) and 3 placebo-treated (5.8%) subjects, with respiratory events reported for all 9 AZLI-treated and none of the placebo-treated subjects.

Table 3
Isolation of individual *Burkholderia* species.

Burkholderia species	Isolation during comparative period (weeks 0 to 24)		MIC range for aztreonam at baseline (all isolates)		
	AZLI (n = 48) n (%)	Placebo (n = 52) n (%)	MIC range (µg/mL)	No. subjects [a]	No. isolates
B. cenocepacia	20 (41.7)	22 (42.3)	8 to >2048	40	73
B multivorans	17 (35.4)	16 (30.8)	2 to >2048	27	49
B. gladioli	3 (6.3)	5 (9.6)	16 to 128	8	14
B. dolosa	3 (6.3)	3 (5.8)	4 to 1024	6	10
B. vietnamiensis	2 (4.2)	1 (1.9)	2 to 64	3	6
Burkholderia spp. (indeterminate)	1 (2.1)	2 (3.8)	4 to 1024	3	6
B. ambifaria	1 (2.1)	1 (1.9)	32 to 2048	2	6
B. metallica	1 (2.1)	0 (0)	32	1	2
B. cepacia	0 (0)	1 (1.9)	1024	1	1
Burkholderia spp. absent	0 (0)	1 (1.9)	–	5	–

[a] Data available for 96 of 100 subjects.

Table 4
Isolation of respiratory pathogens.

Pathogen	Isolation of respiratory pathogens during the comparative period (weeks 0 to 24)						
	AZLI (n = 48)				Placebo (n = 52)		
	With isolation at baseline[a] (Week 0)		With first isolation during comparative period[b]	With isolation anytime during comparative period[c]	With isolation at baseline[a] (Week 0)		With first isolation during comparative period[b]	With isolation anytime during comparative period[c]
	Yes, n	No, n	n (%)	n (%)	Yes, n	No, n	n (%)	n (%)
P. aeruginosa	16	30	5 (16.7)	21 (45.7)	13	37	9 (24.3)	22 (44.0)
MSSA	10	36	7 (19.4)	17 (37.0)	10	40	10 (25.0)	20 (40.0)
MRSA	13	33	2 (6.1)	15 (32.6)	18	32	4 (12.5)	22 (44.0)
Aspergillus spp.	2	44	6 (13.6)	8 (17.4)	6	44	2 (4.5)	8 (16.0)
Stenotrophomonas maltophilia	1	45	3 (6.7)	4 (8.7)	4	46	2 (4.3)	6 (12.0)
Achromobacter spp.	0	46	1 (2.2)	1 (2.2)	1	49	2 (4.1)	3 (6.0)

MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *S. aureus*.

Data are presented by decreasing overall prevalence in the AZLI treatment group.

[a] Number of subjects with or without pathogen at baseline (week 0); data were available for 46 AZLI-treated and 50 placebo-treated subjects.

[b] Number of subjects with pathogen isolated for the first time at least once during the comparative period. Percentages were calculated as the proportion of the number of subjects without the pathogen at the baseline visit.

[c] Number of subjects with pathogen isolated at any time during the comparative period, including baseline; data were available for 46 AZLI-treated and 50 placebo-treated subjects.

During the 24 week extension period, adverse event incidences were generally comparable to those observed during the comparative period (Online Table 2; Online Table 3). Two deaths were reported: respiratory failure related to CF lung disease (AZLI/AZLI; baseline $FEV_1\%$ predicted: 31.25) and sepsis (placebo/AZLI; baseline $FEV_1\%$ predicted: 62.48); both were considered unrelated to treatment.

Airway reactivity ($\geq 15\%$ drop in FEV_1 [L] 30 min after administering study medication) was not reported for any subjects at either the comparative or extension period baseline visits.

3.5. Microbiology

B. cenocepacia and *B multivorans* were the most commonly observed *Burkholderia* spp. and a range of MIC values for aztreonam were observed at baseline amongst the isolates of each species (Table 3). As expected for a chronic infection in individuals with CF, positive cultures for *Burkholderia* spp. were observed for >90% of subjects at each assessment during the comparative period; only 1 subject never tested positive for *Burkholderia* spp. (placebo group). Positive sputum cultures were also observed for *P. aeruginosa* (44.8% subjects; n = 43/96) and methicillin-resistant *Staphylococcus aureus* (MRSA; 38.6% subjects; n = 37/96; Table 4). Appearance of other respiratory pathogens during the comparative period was comparable between the AZLI and placebo treatment groups (Table 4).

Burkholderia spp. and *P. aeruginosa* susceptibility to antibiotics commonly used in CF therapy was examined across the study (Fig. 1). A 4-fold increase from baseline for the aztreonam MIC_{50} for *Burkholderia* spp. was observed in the AZLI, but not the placebo arm, at the end of the comparative period (week 24). At week 24, other changes from baseline MIC_{50} differed 2-fold or less between treatment arms. It was only possible to compare change in bacterial density ($\log_{10}$ colony forming units [CFU]/g sputum) for subjects with data at both baseline and week 24; this analysis included subjects who expectorated adequate sputum samples and/or subjects with smaller sputum samples or throat swabs who were confirmed negative for *Burkholderia* spp. at one or both time points. Adjusted mean (SE) change in *Burkholderia* spp. density from baseline to week 24 was 1.41 (0.58) for AZLI-treated (n = 15/39) and 0.48 (0.50) for placebo-treated (n = 20/46) subjects (p = 0.232). Adjusted mean (SE) change in *P. aeruginosa* density from baseline to week 24 was 0.00 (0.64) for AZLI-treated (n = 9/39) and 0.93 (0.58) for placebo-treated (n = 11/46) subjects (p = 0.307).

4. Discussion

This study was the largest, randomized, placebo-controlled trial to evaluate the safety and efficacy of inhaled antibiotic therapy in individuals with CF and *Burkholderia* infection. Although these infections are associated with significant morbidity and mortality, a recent Cochrane Review identified no published randomized or quasi-randomized controlled trials of treatments for exacerbations of pulmonary symptoms in
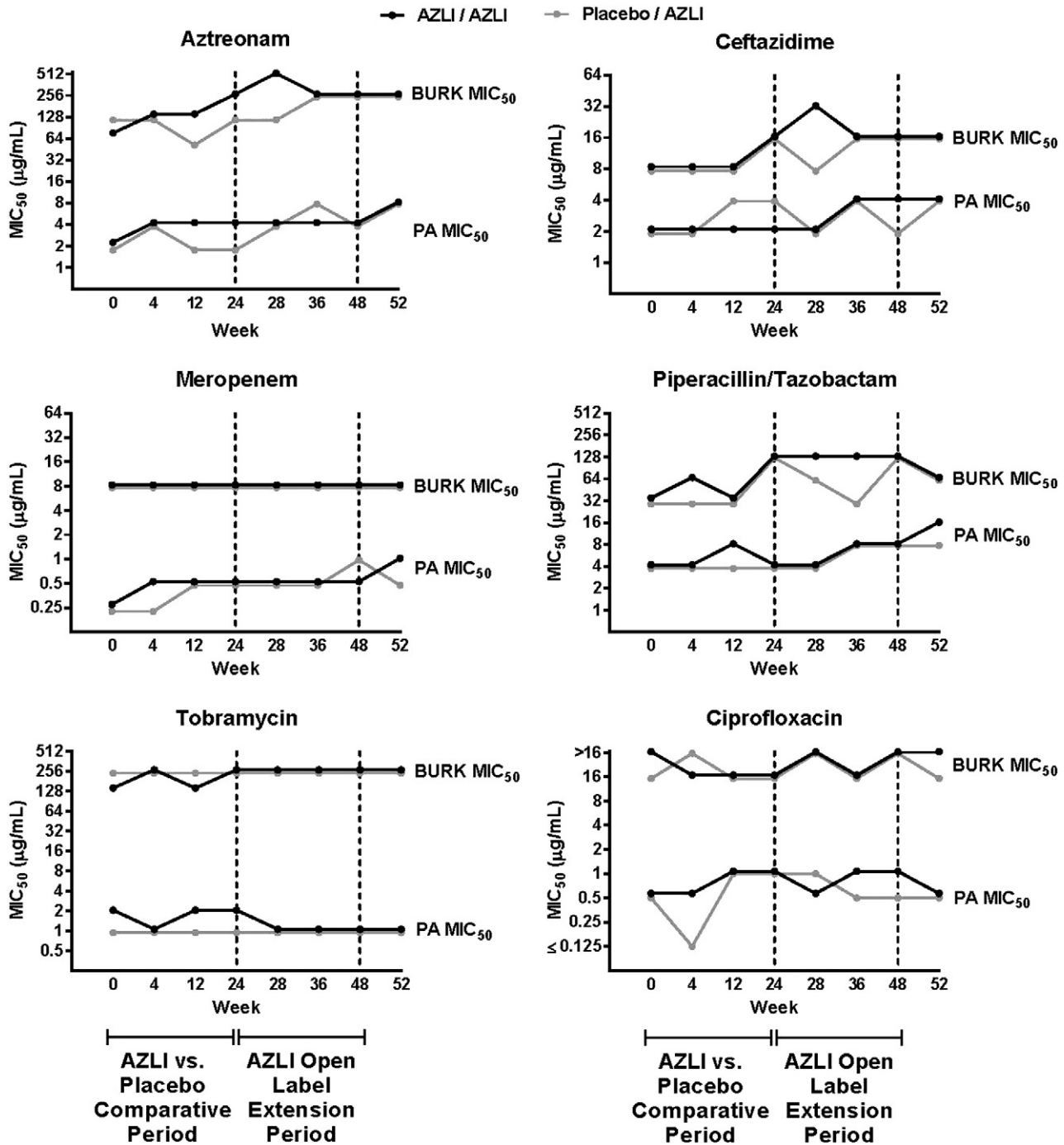
Fig. 1. MIC$_{50}$ values of selected antibiotics for *Burkholderia* spp. and *P. aeruginosa*. *Burkholderia* spp. (BURK) isolates were obtained from 44, 41, 40, and 35 AZLI-treated subjects and 47, 47, 42, and 40 placebo-treated subjects at weeks 0, 4, 12, and 24, respectively and from 35, 34, 33, 33, and 31 AZLI/AZLI-treated subjects and 39, 39, 36, 38, and 33 AZLI/placebo-treated subjects at weeks 24, 28, 36, 48, and 52, respectively. The number of isolates tested at any time point for any group ranged from 58 to 102. *P. aeruginosa* (PA) isolates were obtained from 16, 13, 18, and 10 AZLI-treated subjects and 13, 15, 11, and 12 placebo-treated subjects at weeks 0, 4, 12, and 24, respectively, and from 10, 11, 13, 11, and 13 AZLI/AZLI-treated subjects and 12, 8, 9, 6, and 9 AZLI/placebo-treated subjects at weeks 24, 28, 36, 48, and 52, respectively. The number of isolates tested at any time point for any group ranged from 14 to 37. MIC$_{50}$ = minimum inhibitory concentration at which 50% of isolates are inhibited.

individuals with CF and chronic *B. cepacia* complex infections [6]. The vast majority of published clinical data on *Burkholderia* infection in individuals with CF is comprised of uncontrolled, anecdotal, and/or single center experiences, and no consensus has emerged regarding treatment [5,6].

The *Burkholderia* spp. observed in this study are consistent with previous reports, with *B. cenocepacia* and *B. multivorans* as the most common species identified [26]. There were no enrollment restrictions on FEV$_1$% predicted and baseline lung function varied widely in the study population. FEV$_1$% predicted

at baseline (range: 15.8–114.6%) reflected significant heterogeneity amongst subjects in both severity of the CF phenotype [27] and lung disease stage.

In this study of chronic *Burkholderia*-infected individuals with CF who were receiving usual medical treatment, we observed no significant treatment difference between AZLI- and placebo-treated groups in FEV_1% predicted over the 6-month comparative treatment period. Similarly, no differences were observed between treatment groups with respect to acute respiratory exacerbations, hospitalizations for respiratory exacerbations, and additional antibiotic use either for respiratory exacerbations or for all respiratory indications. The largest increases in FEV_1% predicted occurred during the first 28 days of AZLI treatment, but such increases were not sustained across the subsequent months of continuous treatment. Exploratory analyses did not identify subgroups that showed a statistically significant differential treatment response to AZLI, including subgroups based on gender, age, baseline lung function, specific *Burkholderia* spp., antibiotic use, baseline aztreonam MIC for infecting organisms, or co-infection with *P. aeruginosa* or MRSA. It is not possible to determine whether these results are due to lack of treatment effect or to small numbers in these subgroups leading to underpowered analyses.

In contrast to other efficacy trials of inhaled antibiotics in CF, this trial allowed use of systemic or other inhaled antibiotics for maintenance treatment of chronic respiratory infections and study subjects had substantial additional antibiotic exposure. In fact, higher percentages of subjects received antibiotics for acute respiratory exacerbations during the comparative period of this trial (AZLI: 60.4%, placebo: 73.1%) than in a recent AZLI vs. tobramycin inhalation solution (TIS) comparative trial for subjects with CF and *P. aeruginosa* infection (AZLI: 38.2%, TIS: 57.6%) [28]. Further, the incidence of antibiotic use for any respiratory indication in the comparative period of the current trial was even higher (AZLI: 81.3% of subjects, placebo: 94.2%). This lack of restrictions on ongoing or newly initiated antibiotic therapy presented a limitation to this study. It is possible that extensive antibiotic exposure had confounding effects on the efficacy results in this study, limiting the potential for incremental responses to AZLI treatment. Nevertheless, we believed this design was necessary given a priori concerns that CF care providers have regarding the risk of poor outcomes for individuals infected with *Burkholderia* spp.

There was no significant decrease in either *Burkholderia* spp. or *P. aeruginosa* sputum density when comparing AZLI and placebo treatment groups. This differs markedly from a previous AZLI Phase 3 trial, in which mean *P. aeruginosa* sputum density decreased 1.453 log_{10} CFU/g [25]. It is unclear what caused the lack of decrease in sputum density in the current study. Sputum density measurements may have been impacted by additional antibiotic use or by the number of subjects who could produce samples of sufficient quantity for bacterial density analysis; this changed from 63.0% (n = 29/46) to 51.4% (n = 19/37) of AZLI-treated and from 60.00% (n = 30/50) to 61.4% (n = 27/44) of placebo-treated subjects from baseline to the end of the comparative period.

It is difficult to draw on experience from the AZLI trials in CF patients with chronic *P. aeruginosa* infection because there are 2 variables in the current study that differ from previous studies: the presence of *Burkholderia* spp., an inherently more antibiotic-resistant organism, and a different treatment regimen, a continuous therapy instead of intermittent 28-day on/off treatment periods. Both of these factors would be expected to have influences on the complex microbiome of these CF patients with *Burkholderia* infection. The exact nature of these effects as well as the corresponding clinical outcomes is open to speculation and in need of further scientific study.

In this study we chose to use continuous, rather than intermittent administration of inhaled AZLI. This was based on consideration of: 1) high airway concentrations achieved by AZLI and lack of emergent resistance observed during 18 months of intermittent use for *P. aeruginosa* infection in subjects with CF [29], and 2) treatment benefits and current treatment practices: subjects receiving intermittent AZLI therapy for 18 months experienced recrudescence of symptoms during off-treatment periods [29], and individuals with CF and *Burkholderia* infections often receive continuous antibiotics as part of clinical care. We observed that continuous AZLI therapy for up to 48 weeks did not increase the prevalence of other respiratory pathogens, nor did it compromise *P. aeruginosa* susceptibility to AZLI or other commonly used antipseudomonal antibiotics. Continuous AZLI therapy was also well tolerated. No new safety signals were detected and the adverse event profile was consistent with previous AZLI clinical trial experience [25,28–31]. Four of the 100 subjects died during this 1 year study. Three deaths were caused by respiratory failure/advanced CF disease and the fourth by sepsis. This mortality rate was numerically higher than that observed in the general CF population (1.5 deaths were reported per 100 individuals in the 2010 CFF Registry database of 26,298 individuals [32]), and likely reflects the increased mortality in the population of individuals with CF and *Burkholderia* infection.

This study demonstrated that it was feasible to enroll a 100 patient study relatively quickly. Given the heterogeneity of the study population, subsequent studies could utilize narrower inclusion criteria in order to select a more homogenous study population and increase the chance of measuring a treatment benefit. Examples could include excluding patients with $FEV_1 > 75$% predicted or including only those with a history of frequent pulmonary exacerbations. However, stricter inclusion criteria will limit the availability of qualifying patients and could create challenges to enrolling an adequately powered study.

In conclusion, although 24-week continuous AZLI treatment did not demonstrate a significant improvement in lung function, this study has created a unique and comprehensive clinical and microbiological dataset describing individuals with CF and chronic *Burkholderia* infection. Further, individuals with CF and *Burkholderia* infection were willing and able to participate in this study without adverse consequences, which suggest that future trials could be conducted in this patient population. The results of the current trial will be informative in the design of such future studies.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2013.08.011.

Study investigators

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