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Original Article

Open label study of inhaled aztreonam for *Pseudomonas* eradication in children with cystic fibrosis: The ALPINE study ☆

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Abstract

Background: Consensus guidelines recommend early treatment to eradicate newly acquired *Pseudomonas aeruginosa* (*Pa*) infection in cystic fibrosis (CF) patients although there is no single preferred regimen. Aztreonam for inhalation solution (AZLI) significantly reduces sputum *Pa* density in CF patients with chronic *Pa* infection and has been well tolerated in the pediatric population. This single-arm, open-label Aztreonam Lysine for *Pseudomonas* Infection Eradication (ALPINE) study was conducted to evaluate the safety and efficacy of a 28-day treatment course of AZLI to eradicate newly acquired *Pa* infection in pediatric CF patients.

Methods: CF patients (3 months to <18 years) with new onset *Pa* infection were treated with AZLI 75 mg 3 times daily for 28 days. New onset *Pa* infection was defined as first lifetime *Pa*-positive respiratory tract culture (throat swab, sputum) or *Pa*-positive culture after a ≥2-year history of *Pa*-negative cultures (≥2 cultures/year). Sputum or throat swab cultures were collected at study entry (baseline) and at weeks 4 (end of treatment), 8, 16, and 28. Primary endpoint was the percentage of patients with cultures negative for *Pa* at all post-treatment time points.

Results: A total of 105 pediatric CF patients enrolled (3 months to <2 years, n = 24; 2 to <6 years, n = 25; 6 to <18 years, n = 56). Of the 101 patients who completed treatment, 89.1% (n = 90) were free of *Pa* at the end of treatment and 75.2% (n = 76) were free of *Pa* 4 weeks after the end of treatment. Of the 79 patients evaluable for the primary endpoint, 58.2% were free of *Pa* at all post-treatment time points.

Conclusions: AZLI was effective and well tolerated in eradicating *Pa* from newly infected pediatric patients with CF. These eradication rates are consistent with success rates reported in the literature for various antibiotic regimens, including other inhaled antibiotics studied for eradication.

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Keywords: Aerosolized antibiotics; Antipseudomonal; *P. aeruginosa*; Pediatric; Respiratory infections

1. Introduction

Patients with cystic fibrosis (CF) are susceptible to pulmonary infections with pathogenic microorganisms and acquisition of

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Pseudomonas aeruginosa (*Pa*) can significantly alter their clinical course [1–3]. Aggressive treatment of initial *Pa* infection delays progression to chronic infection, which is associated with increased morbidity and mortality in children with CF [2,3]. End-of-treatment eradication rates of 74–100% have been observed for various antibiotic regimens [3–18]. Eradication at initial *Pa* detection is recommended by consensus treatment guidelines and is now a treatment strategy used by most CF centers [19,20].

Aztreonam for inhalation solution (AZLI; Cayston®; Gilead Sciences), a lyophilized formulation of the monobactam antibiotic aztreonam, improves respiratory symptoms, delays time to need for additional antibiotics, reduces sputum *Pa* density in CF patients with chronic infection, and is well tolerated in the pediatric population (6–17 years of age) [21–24]. These findings provided the rationale for the Aztreonam Lysine for *Pseudomonas* Infection Eradication (ALPINE) study, which evaluated the safety and efficacy of a 28-day course of AZLI to eradicate new onset *Pa* infection in children with CF throughout a 24-week follow-up period.

2. Methods

2.1. Study design

This open-label, multicenter study was conducted at 46 CF centers (Europe: 23; US: 23; Oct 2011–May 2013). Eligible patients received 28 days of AZLI 75 mg three times daily, administered via the PARI Investigational eFlow® Nebulizer System, with the SmartMask® Baby for patients <2 years of age, the SmartMask Kids for patients 2 to <6 years, or the nebulizer mouthpiece for patients ≥6 years. Using these age criteria as guidelines, investigators determined the best method of administering AZLI. A short-acting bronchodilator was administered approximately 1 h before AZLI. Study visits included screening, baseline (day 1), week 4 (day 28), and weeks 8, 16, and 28 (during the 24-week follow-up period).

Study-related microbiology assessments were performed at a central laboratory. *Pa* culture results from local laboratories were not monitored except to assess study entry criteria and define infection history. Patients receiving additional (non-study) anti-pseudomonal antibiotics during the study were withdrawn from study assessments (discontinued), but followed until ongoing adverse events resolved.

2.2. Patients

Eligible patients were clinically stable, 3 months to <18 years of age, with documented CF, $FEV_1 \geq 80\%$ predicted at screening (for patients ≥6 years), and newly detected *Pa* respiratory tract infection within 30 days of screening (expectorated sputum or throat swab; cultured at local laboratories), defined as either first lifetime documented culture positive for *Pa* or first positive culture after ≥2 year history of ≥2 negative cultures/year.

Key exclusion criteria included the use of intravenous or inhaled anti-pseudomonal antibiotics (within 2 years before screening); oral anti-pseudomonal antibiotics (30 days before screening); hospitalization for pulmonary-related illness (28 days before screening); and other conditions potentially interfering with study participation/safety, in the opinion of the Investigators.

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), International Conference on Harmonisation guidelines, good clinical practice principles, and detailed guidelines in line with those principles. The study was approved by Institutional Review Boards or Independent Ethics Committees for each site. Patients and/or parents/guardians provided written informed consent before any study-related procedures.

2.3. Measures

The primary endpoint was the proportion of patients with cultures negative for *Pa* at all visits throughout the 24-week follow-up period. Subgroup analyses evaluated age, gender, *Pa* infection history (first lifetime/recurrence), baseline culture results (*Pa* negative/positive), and baseline anti-pseudomonal antibodies (negative/positive).

Secondary endpoints included proportion of patients with cultures negative for *Pa* at each follow-up visit, additional anti-pseudomonal antibiotic use, and for patients ≥6 years, changes from baseline in FEV_1 % predicted and Cystic Fibrosis Questionnaire-Revised Respiratory Symptoms Scale (CFQ-R-RSS) scores [25].

Other endpoints of interest included isolation of other respiratory pathogens, changes in minimum inhibitory concentration (MIC) of aztreonam for *Pa*, and *Pa*-specific serum antibodies (enzyme immunoassay, Mediagnost, Reutlingen Germany). Aztreonam plasma concentrations were measured for patients <6 years old. The genetic relatedness of *Pa* strains isolated at baseline and during follow-up for individual patients was compared by pulsed-field gel electrophoresis (PFGE) [26].

Safety endpoints included monitoring adverse events, airway reactivity (study drug-induced bronchospasm), vital signs, hospitalizations, and clinical laboratory analyses (biochemistry; hematology).

2.4. Statistics

The safety analysis set included all AZLI-treated patients. The primary efficacy evaluable set included patients completing the 28-day course of AZLI without receiving additional anti-pseudomonal antibiotics, who either completed all follow-up visits through week 28 with cultures negative for *Pa* at every visit without the use of additional anti-pseudomonal antibiotics (successful treatment), or had a study-related culture positive for *Pa* at any follow-up visit (treatment failure). Patients who used additional anti-pseudomonal antibiotics during the follow-up period without study-related cultures positive for *Pa* had been discontinued from the study and were not included in the primary efficacy evaluable set. These patients were included in a sensitivity analysis set and were considered treatment failures. The sensitivity analysis resulted in a decrease in the percentage of patients who met the primary eradication endpoint due to the

inclusion of these additional treatment failures in the denominator of the analysis.

Observed changes in FEV_1 % predicted (Wang pediatric equations [27]), summarized by patients who did/did not meet the sensitivity analysis endpoint, are reported. CFQ-R analyses used mixed-effect model repeated measure (MMRM) methods, including baseline value and visit in the model. Missing baseline data were not imputed.

Assuming an eradication rate of 0.8 over the 24-week follow-up period, a sample size of 60 evaluable patients was considered sufficient to estimate the proportion of patients with cultures negative for *Pa* to within 0.1, using a 95% confidence interval (CI). Assuming a non-evaluable rate of ~45%, 105 patients were to be enrolled.

3. Results

3.1. Disposition and demographics

Overall, 105 patients received AZLI treatment (Europe: 49; US: 56), with 101 completing the 4-week treatment course (Table 1). Reasons for discontinuing treatment were adverse event (n = 2), dosing non-compliance (n = 1), and withdrawal of consent (n =1). The most common reason for study discontinuation was meeting protocol-specified criteria (n = 45: culture-positive for *Pa* [n = 30]; the use of additional anti-pseudomonal antibiotics without being culture-positive [n = 15]).

Overall, 22.9% of patients were 3 months to <2 years, 23.8% were 2 to <6 years, and 53.3% were 6 to <18 years of age (Table 2). Most patients were white (94.3%; n = 99/105) and 55.2% (n = 58) were female. The positive culture documented within 30 days before screening was the first lifetime *Pa* infection for 70.5% of patients (n = 74/105). At baseline, cultures (performed at a central laboratory) were positive for *Pa* in 44.1% of patients (n = 45/102), using expectorated sputum specimens (63.6% patients; n = 14/22) or throat swabs (38.8%; n = 31/80). For patients ≥6 years old (n = 56), median FEV_1 was 95.4% predicted (range: 71.1–135.4) at baseline and median CFQ-R-RSS scores were 75.0 (range: 33.3–100). Most patients (96.2%; n = 101/105) used ≥90% of expected AZLI vials.

3.2. Efficacy

Of 79 patients in the primary efficacy evaluable set (Table 1), 46 patients (58.2%; 95% CI: 47.4%, 69.1%) remained culture-negative for *Pa* throughout the 24-week follow-up period (Table 3). Subgroups with notably lower percentages of patients meeting the primary endpoint included patients *Pa* culture-positive at baseline (36.7% met endpoint; n = 11/30) and patients with anti-pseudomonal antibodies at baseline (35.7% met endpoint; n = 5/14; Table 3). A higher percentage of patients experiencing their first lifetime *Pa* infection met the primary endpoint (62.5%; n = 35/56) compared to those with recurrent infection (47.8%; n = 11/23). In an exploratory analysis of outcomes by region, a smaller proportion of European patients in the primary efficacy analysis set met the primary efficacy endpoint, compared with US patients (46%; n = 18/39 vs. 70%; n = 28/40). However, when baseline *Pa*-culture positivity was examined, a higher percentage of European patients in the sensitivity analysis set were *Pa*-culture positive at baseline,

Table 1
Patient disposition and derivation of efficacy analysis populations.

Patient disposition	
Screened,[a] n	109
Enrolled and treated, n	105
AZLI treatment status[b]	
Completed 4 weeks of AZLI treatment, n (%)	101 (96.2)
Discontinued from AZLI treatment, n (%)	4 (3.8)
Adverse event	2 (1.9)
Non-compliance with dosing	1 (1.0)
Withdrew consent	1 (1.0)
Study completion status[b]	
Completed study, n (%)	55 (52.4)
Discontinued from study (per protocol or other criteria), n (%)	50 (47.6)
Protocol-specified criteria for withdrawal: positive[c] *P. aeruginosa* culture	30 (28.6)
Positive culture with the use of additional anti-pseudomonal antibiotics	26
Positive culture without the use of additional anti-pseudomonal antibiotics	4
Protocol-specified criteria for withdrawal: the use of additional anti-pseudomonal antibiotics without positive[c] *P. aeruginosa* culture	15 (14.3)
During 4-week treatment period	1
During follow-up period (weeks 4 through 28)	14
Adverse event	2 (1.9)
Withdrew consent	2 (1.9)
Lost to follow-up	1 (1.0)

Derivation of efficacy analysis populations	n
Enrolled and treated	105
Did not complete treatment[b]	(4)
Completed 28 days of AZLI treatment	101
Missing *P. aeruginosa* culture data during follow-up period[d]	(3)
Sensitivity analysis set	98
Received additional anti-pseudomonal antibiotics without evidence of positive *P. aeruginosa* culture[c,e]	(19)
Efficacy evaluable set for primary endpoint	79

[a] Reasons for failing screening included not meeting all inclusion criteria (n = 1), meeting 1 or more of the exclusion criteria (n = 3), and/or withdrawal of consent (n = 1).

[b] Reasons for discontinuing AZLI treatment differed from reasons for discontinuing the study for 3 patients: 1 patient withdrew from treatment due to an adverse event and withdrew from the study due to protocol-specified criteria (the use of additional antibiotics without positive *P. aeruginosa* culture during the 4-week treatment period); 1 patient withdrew from treatment due to non-compliance with dosing and withdrew consent for the study, and 1 patient withdrew consent for treatment and withdrew from the study due to protocol-specified criteria (the use of additional antibiotics without positive *P. aeruginosa* culture during follow-up period). The fourth patient who discontinued AZLI treatment discontinued from the study due to an adverse event.

[c] Based on central laboratory *P. aeruginosa* culture results.

[d] Two of the 3 patients with missing *P. aeruginosa* culture data completed the study and 1 discontinued from the study due to withdrawal of consent.

[e] The 19 patients who were included in the sensitivity analysis set but excluded from the efficacy evaluable set due to receipt of additional anti-pseudomonal antibiotics without a positive *P. aeruginosa* culture were recorded as discontinuing from the study due to: protocol-specified criteria: 15 (the use of additional anti-pseudomonal antibiotics without a positive *P. aeruginosa* culture); completed study: 3 (although they did use additional anti-pseudomonal antibiotics), and adverse event: 1.

Table 2
Baseline characteristics.

Characteristic		AZLI (N = 105)
Age, years; mean (SD)		6.26 (4.74)
Age; range		3 months–16 years
Age group; n (%)	3 months to <2 years	24 (22.9)
	2 to <6 years	25 (23.8)
	6 to <18 years	56 (53.3)
Gender; n (%)	Female	58 (55.2)
	Male	47 (44.8)
Race; n (%)	Asian, or Black or African Heritage	2 (1.9)
	White	99 (94.3)
	Other or not provided	4 (3.8)
Ethnicity; n (%)	Hispanic	5 (4.8)
	Not Hispanic	93 (88.6)
	Not permitted	7 (6.7)
BMI, kg/m^2; median (range)		16.4 (12.0, 30.0)
BMI, z scores, mean (SD)		0.5 (1.3)
Received *P. aeruginosa* vaccination, yes; n (%)		1 (1.0)
Infection history; n (%)	First *P. aeruginosa* infection	74 (70.5)
	Recurrence of *P. aeruginosa* infection	31 (29.5)
	1 infection	16 (51.6)
	3 infections	5 (16.1)
	4 infections	5 (16.1)
	≥5 infections	5 (16.1)
P. aeruginosa baseline culture result;[a,b] n (%)	Negative	57 (55.9)
	Positive	45 (44.1)
	Non-mucoid	40 (88.9)
	Mucoid	5 (11.1)
	Highest aztreonam MIC ≤2 μg/mL	7 (15.6)
	Highest aztreonam MIC 4–8 μg/mL	38 (84.4)
MIC of aztreonam for all *P. aeruginosa* isolates;[c] μg/mL	MIC_{50}	4
	MIC_{90}	8
Log_{10} *P. aeruginosa* CFU/g sputum;[d] mean (SD)		4.6 (1.2)
Antibodies to *P. aeruginosa* at baseline;[a,e] n (%)	Negative	64 (62.7)
	Borderline	20 (19.6)
	Positive	18 (17.6)
Baseline medications; n (%)	Azithromycin	12 (11.4)
	Dornase alfa	60 (57.1)
	Hypertonic saline	35 (33.3)

[a] Data available for 102 patients.
[b] *P. aeruginosa* positive culture within 30 days before screening was required for study entry.
[c] 56 isolates from 45 patients with *P. aeruginosa* cultures positive at baseline; the concentration inhibiting growth of 50% of the isolates was the MIC_{50} and the concentration inhibiting growth of 90% of the isolates was the MIC_{90}.
[d] Data available for 9 patients.
[e] Serum samples were diluted serially (eg, 1:2, 1:4, 1:8) and tested for the presence of IgG antibodies to *P. aeruginosa* (enzyme immunoassay; Mediagnost, Reutlingen, Germany); samples with no antibodies detectable at dilutions through 1:256 (ie, titer < 500) were considered negative, titers ≥500 to <1250 were considered borderline, and titers ≥1250 to <10,000 were considered positive. Three antigens (alkaline protease, elastase, and endotoxin A) were tested for each patient and the highest titer for each patient was used to categorize the presence/absence of antibodies.

compared with US patients (49%; n = 23/47 vs. 35%; n = 19/54). This difference in *Pa*-culture positivity could be confounding the primary efficacy results by region, since overall, fewer patients with a positive *Pa*-culture at baseline met the primary eradication endpoint (Table 3).

The sensitivity efficacy analysis set included 19 additional patients without study-related cultures positive for *Pa*, but who used additional anti-pseudomonal antibiotics during the follow-up period, presumably due to disease progression or local microbiology laboratory results (Table 1); 46.9% (n = 46/98) of these patients met the primary efficacy endpoint (Table 3).

Of the 101 patients who completed 4 weeks of AZLI treatment, 89.1% had cultures negative for *Pa* at week 4, and 75.2%, 63.4%, and 47.5% were culture-negative at weeks 8, 16, and 28, respectively (Table 4). In subgroup analyses, the percentages of patients with cultures negative for *Pa* at week 8, 16, or 28 were significantly higher for patients who were culture-negative at baseline compared with patients who were culture-positive (Table 4). No significant differences were observed by age group or baseline anti-pseudomonal antibodies (negative/positive).

In an exploratory analysis, *Pa* culture-negative rates were assessed for a subset modeled after analyses from an eradication study of tobramycin inhalation solution (TIS) [14]. This subset included patients without detectable anti-pseudomonal antibodies at baseline who completed 4 weeks of AZLI treatment (n = 62; Online Table 3). Forty-nine of these 62 patients (79.0%) were

Table 3
Efficacy results.

Patient group	Total no. in group	Met primary[a] efficacy endpoint; n (%)	95% CI (%)
Efficacy evaluable set[b]	79	46 (58.2)	47.4 to 69.1
Subgroups			
Age			
3 months to <2 years	19	11 (57.9)	35.7 to 80.1
2 years to <6 years	18	10 (55.6)	32.6 to 78.5
6 years to <18 years	42	25 (59.5)	44.7 to 74.4
Gender			
Female	42	26 (61.9)	47.2 to 76.6
Male	37	20 (54.1)	38.0 to 70.1
P. aeruginosa infection history			
First infection	56	35 (62.5)	49.8 to 75.2
Recurrent infection	23	11 (47.8)	27.4 to 68.2
P. aeruginosa baseline culture result[c]			
Negative	47	34 (72.3)	59.6 to 85.1
Positive	30	11 (36.7)	19.4 to 53.9
Antibodies to *P. aeruginosa* [d]			
Antibody negative	51	34 (66.7)	53.7 to 79.6
Antibody borderline	12	6 (50.0)	21.7 to 78.3
Antibody positive	14	5 (35.7)	10.6 to 60.8
Sensitivity analysis set[e]	98	46 (46.9)	37.1 to 56.8

[a] Patients with cultures that were negative for *P. aeruginosa* at every visit from weeks 4 through 28 and did not use additional anti-pseudomonal antibiotics were considered to have met the primary endpoint.

[b] The evaluable analysis set (see Table 1) included patients who completed the 28-day course of AZLI, did not receive additional anti-pseudomonal antibiotics during the treatment period, and either completed the study follow-up period through week 28 with *P. aeruginosa*-negative cultures at every visit and without the use additional anti-pseudomonal antibiotics or had evidence of a positive *P. aeruginosa* culture during the follow-up period.

[c] Data available for 77 patients.

[d] Method and titers used to categorize responses are described in footnote e of Table 2.

[e] The sensitivity analysis set differed from the evaluable analysis set (see Table 1) by including patients who used additional anti-pseudomonal antibiotics during the follow-up period without study-related cultures positive for *Pa*; these patients were censored from the primary analysis and considered treatment failures in the sensitivity analysis.

culture-negative for *Pa* at week 8 (4 weeks post-treatment; Online Table 4). For these 49 patients, 85.7%, and 71.4% were culture-negative for *Pa* at weeks 16 and 28, respectively.

Patients ≥6 years old in the sensitivity analysis set who met the primary endpoint (n = 25), had FEV_1% predicted remain near baseline until week 16, with a 2.5% mean actual decrease from

Table 4
Patients who were Culture-Negative for *P. aeruginosa* by Visit.

		N	Patients culture-negative for *P. aeruginosa*; n (%)			
			Week 4 (EOT)	Week 8	Week 16	Week 28
All patients completing the 28-day treatment period		101	90 (89.1)	76 (75.2)	64 (63.4)	48 (47.5)
Subgroups						
Age	3 months to <2 years	23	20 (87.0)	17 (73.9)	14 (60.9)	12 (52.2)
	2 to <6 years	24	23 (95.8)	19 (79.2)	15 (62.5)	10 (41.7)
	6 to <18 years	54	47 (87.0)	40 (74.1)	35 (64.8)	26 (48.1)
P. aeruginosa infection history	First	71	63 (88.7)	55 (77.5)	47 (66.2)	37 (52.1)
	Recurrent	30	27 (90.0)	21 (70.0)	17 (56.7)	11 (36.7)
P. aeruginosa culture at baseline[a]	Positive	42	36 (85.7)	23 (54.8)	18 (42.9)	11 (26.2)
	Negative	56	52 (92.9)	50 (89.3)	43 (76.8)	35 (62.5)
				p < 0.001	p = 0.005	p = 0.004
P. aeruginosa phenotype at baseline[b]	Mucoid	3	3 (100)	3 (100)	1 (33.3)	1 (33.3)
	Non-mucoid	39	33 (84.6)	20 (51.3)	17 (43.6)	10 (25.6)
Antibodies to *P. aeruginosa* at baseline[a,c]	Negative	62	57 (91.9)	49 (79.0)	42 (67.7)	35 (56.5)
	Borderline	19	17 (89.5)	13 (68.4)	11 (57.9)	6 (31.6)
	Positive	17	15 (88.2)	12 (70.6)	9 (52.9)	5 (29.4)

P-values noted for descriptive comparisons between subgroups where significant, using Fisher's exact test.
EOT = end of treatment.

[a] Baseline data missing for 3 patients.

[b] *P. aeruginosa* phenotype determined for 42 positive baseline cultures.

[c] Method and titers used to categorize responses are described in footnote e of Table 2.

baseline at week 28 (Fig. 1). For patients not meeting the primary endpoint (n = 27), corresponding decreases in observed values were 4.2%, 5.1%, and 8.9%, at weeks 8, 16, and 28, respectively.

Mean changes in CFQ-R RSS for the patients in the sensitivity analysis set who met the primary eradication endpoint (n = 25) were numerically higher or similar to patients who did not meet the endpoint (n = 31), with mean changes above the minimum important difference score for stable patients (4.0 points [28]) at all but 1 time point (week 16 for patients who did not meet the primary endpoint; Online Fig. 1).

3.3. Plasma concentrations

For patients <6 years old (n = 49), median (range) aztreonam plasma concentrations were 405 (84–2550) ng/mL, 1 h after the first AZLI dose (day 1; n = 40) and 79 (1–741) ng/mL, immediately before the last dose (day 28; n = 43). Median (range) aztreonam plasma concentrations were comparable for patients 3 months to <2 years (n = 24; 355 [84–2550] and 92 [1–741] ng/mL at days 1 and 28, respectively; n = 21 with data) and patients 2 years to <6 years (n = 25; 466 [96–1880] and 43 [1–711] ng/mL at days 1 and 28, respectively; n = 19 and 22 with data).

3.4. Safety

Treatment emergent events included those occurring during or within 30 days after AZLI treatment. The most commonly reported treatment-emergent adverse events were cough (41.0%, n = 43/105), pyrexia (14.3%, n = 15/105), and rhinorrhea (9.5%, n = 10/105); these events were also the most common events during the follow-up period (Online Table 1). Cough (14.3%, n = 15/105) was the only treatment-related adverse event reported for >1 patient. Adverse event terms corresponding to decreased pulmonary function and shortness of breath were pooled; such events were experienced by 5 (4.8%) and 2 (1.9%) patients, respectively. For the majority of patients with serious adverse events during the study, such events were due to worsening CF symptoms that led to hospitalization. A ≥15% decrease in FEV_1 was observed 30 min after in-clinic AZLI treatment for 2 patients ≥6 years old (baseline: n = 1; week 4: n = 1).

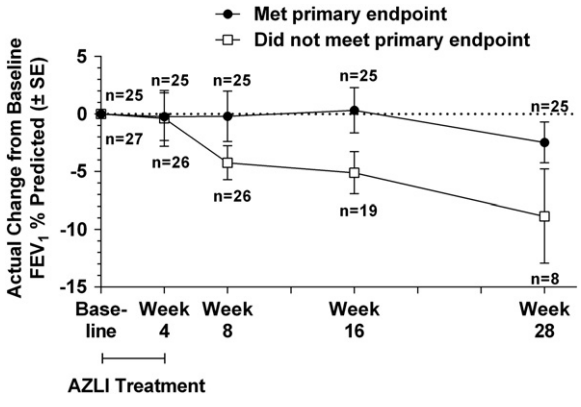

Fig. 1. Change in FEV_1 % predicted. Actual change from baseline FEV_1 % predicted for patients ≥6 years of age in the sensitivity analysis set (n = 52), who met (n = 25) or did not meet (n = 27) the primary study efficacy endpoint.

Two patients discontinued AZLI treatment due to adverse events; events were considered AZLI treatment-related for 1 patient (3 months of age; dyspnea, cough, agitation, and post-tussive vomiting, study day 1).

Overall, 16.2% (n = 17/105) of patients were hospitalized at least once during the study, with 10.5% (n = 11/105) hospitalized for respiratory events (identified by study medical monitor); most of these (n = 9/11) occurred during the follow-up period (Online Table 2).

3.5. Microbiology

The aztreonam MIC_{50} and MIC_{90} for all 56 baseline *Pa* isolates were 4 and 8 μg/mL, respectively, and remained unchanged (≤2-fold) for the 50 isolates obtained from later visits during the study. No baseline isolates had an aztreonam MIC >8 μg/mL (established parenteral susceptibility breakpoint); such isolates were observed for 2 patients each at weeks 4 and 8. The MIC_{50} and MIC_{90} of cefepime, ceftazidime, meropenem, piperacillin, piperacillin/tazobactam, ticarcillin/clavulanate, and tobramycin remained unchanged (≤2-fold) from baseline during the study. The MIC_{90} of amikacin increased 4-fold from baseline in week 4 *Pa* isolates (to 16 μg/mL; n = 11 isolates) and the MIC_{90} for ciprofloxacin increased 16-fold from baseline at week 16 (to 4 μg/mL; n = 14 isolates). No concerning changes were observed in the presence of other respiratory pathogens (*Achromobacter*, *Burkholderia*, or *Aspergillus* spp., *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, or methicillin-sensitive or methicillin-resistant *Staphylococcus aureus*).

The percentage of patients with anti-pseudomonal antibodies was comparable across the study (baseline: 19.6% of patients with borderline titers and 17.6% positive; week 4: 21.6% and 13.4%; week 28: 18.3% and 14.0%). Antibody status changed from baseline to week 28 (or early termination visit) for 18 patients (negative to borderline or positive: 7; borderline or positive to negative: 11). The proportion of patients able to produce sputum did not change substantially across the study. Genetic relatedness (baseline vs. follow-up) was evaluated by PFGE for 59 *Pa* isolates from 20 patients [27]. The strain isolated during follow-up in 19 patients was identical to their original strain, while 2 post-treatment isolates differed from the baseline strain for 1 patient (Online Fig. 2).

4. Discussion

Consensus guidelines recommend early and aggressive treatment to eradicate recently acquired *Pa* in CF patients although no specific regimen is preferred [20]. Eradication rates of >75% over a variety of time points have been reported, however differences in study designs make direct comparisons difficult to perform [12,18].

In the current study, pediatric patients with CF and newly acquired *Pa* infection received a 28-day AZLI treatment course, and respiratory cultures were obtained over a 24 week follow-up period. In the primary efficacy evaluable set, 58.2% of the 79

patients remained culture-negative for *Pa* throughout the follow-up period. Of the 101 patients who completed 4 weeks of AZLI treatment, 89% and 75% were free of *Pa* at treatment end and 4 weeks post-treatment, respectively.

This was the first AZLI clinical trial enrolling CF patients with *Pa* who were <6 years of age. Eradication rates were comparable for the 3 age subgroups in this study (3 months to <2 years; 2 to <6 years, 6 to <18 years), and plasma aztreonam levels for patients <6 years were comparable to those reported previously for patients ≥6 years old [22,29]. AZLI was well tolerated with an adverse event profile consistent with the previously established clinical trial experience [21–24]. Cough and other respiratory events were the most common treatment-emergent adverse events. Pyrexia was also common (14.3%), consistent with the incidence of pyrexia (18%) observed in pediatric patients 6–17 years of age in previous AZLI studies [30]. There were minimal changes in *Pa* susceptibility to aztreonam or other antibiotics and concerning changes were not observed in the appearance of other respiratory pathogens. However, it is important to note that the majority of cultures were throat swabs. This was consistent with the low prevalence of sputum producers in the pediatric study population, which had relatively well-preserved lung function. Fastidious organisms, including fungal and mycobacterial pathogens, are less easily recoverable from throat swab specimens. Further, the sample size was too small to make firm statements on the occurrence of uncommon pathogens.

The open-label design could also be considered a limitation of the current study; however, the microbiological endpoint is objective, and knowledge of study treatment would not have affected the bacterial culture results. These objective culture results allow comparisons between different studies for corresponding time points in relation to the end of treatment. After 2 early placebo-controlled eradication studies, the second of which was stopped early due to evidence of efficacy [5,10], subsequent trials have been open-label without a placebo arm (note that although the EPIC study [31] was placebo-controlled for oral ciprofloxacin, all patients received open-label TIS). A randomized, blinded, placebo-controlled trial is difficult to recruit given the accepted need and current consensus guidelines recommendations for prompt *Pa* eradication treatment. Even postponing treatment by one month was recently considered unethical by most investigators in the European Cystic Fibrosis Society-Clinical Trials Network (ECFS-CTN), thereby limiting that alternative design option.

Results from ALPINE compare favorably to those reported in the "ELITE" study of TIS for *Pa* eradication [14]. Both studies reported *Pa* culture-negative rates of ~90% immediately after 4 weeks of treatment; however, selection criteria differed for the efficacy analysis study populations (Online Table 3). ELITE randomized 71.5% of enrolled patients (n = 88/123), with most exclusions (31/35) due to the presence of anti-pseudomonal antibodies at baseline. The "efficacy" population for assessing time to recurrence of *Pa* (primary endpoint) included randomized patients who were culture-negative for *Pa* 1 month after TIS treatment ended (n = 65/88; 73.9%). Patients with anti-pseudomonal antibodies detectable at baseline were not excluded from ALPINE analyses. Using a comparable subset of patients (without detectable anti-pseudomonal antibodies at baseline), 49/62 (79%) of ALPINE patients were culture-negative for *Pa* 4 weeks after AZLI treatment ended. The culture-negativity rates maintained by this selected group of ALPINE patients at weeks 16 and 28 (85.7%, and 71.4%, respectively) were comparable to those reported for ELITE at the corresponding time points (~82–86%; ~76–82%; Online Table 4).

Another eradication study, "EPIC," utilized a complex design with repeated courses of TIS-containing treatment regimens and patients receiving additional antibiotics for pulmonary exacerbations were allowed to continue on the study, making direct comparison to other studies more difficult [30]. The reported *Pa* culture-positive rates, 10 weeks after the initial treatment course, were 12% and 15% for the TIS + ciprofloxacin and TIS + placebo groups, respectively. Taken together, results from ALPINE, ELITE, and EPIC support a short treatment course (28 days) as effective for initial eradication of *Pa* for most CF patients.

One challenge of eradication studies was highlighted in results from the current study. All patients had cultures positive for *Pa* within 30 days of screening (at local laboratories); however, 56% of these patients were culture-negative for *Pa* at baseline. This patient subgroup demonstrated higher eradication rates over the course of the study. Several factors likely contributed to the lower number of culture-positive patients at baseline. The majority of the baseline specimens collected were throat swabs, which can have lower sensitivity than expectorated sputum for detecting *Pa*. The inability of pediatric CF patients with new onset *Pa* infection to produce sputum is not unexpected, as compared to adults with more advanced lung disease. Further, *Pa* may be isolated intermittently in CF patients with either new or chronic infection. It should be noted that a comparable percentage of patients were culture-positive for *Pa* (40%) at baseline in the EPIC study [31]. These factors highlight the inherent challenges in culturing bacteria from respiratory tract samples, since detection may vary with sampling technique. They also highlight the challenges in interpreting results from eradication trials, in which *Pa* is only detected intermittently and in which there are logistical factors associated with transport of specimens to a central laboratory that could potentially influence whether or not *Pa* is detected.

Study design issues in *Pa* eradication trials were addressed in a recent workshop, which noted that negative cultures for *Pa* should be considered to represent "apparent" eradication, due to limitations in sampling respiratory specimens [32]. Another recommendation was that the most appropriate primary endpoint should reflect negative cultures obtained during the immediate post-treatment timeframe (4–6 weeks), because it is biologically implausible to expect a single course of inhaled antibiotics to prevent long-term new acquisition of *Pa*. These study design issues should be considered in developing future *Pa* eradication protocols, and in evaluating results from previous studies.

In the current study, differences in spirometry results were observed for patients with versus without successful *Pa* eradication after AZLI treatment. Patients remaining culture-negative for *Pa* throughout the 24-week follow-up period also maintained mean baseline FEV_1 % predicted values, whereas patients with recurrence of cultures positive for *Pa*, or those who received anti-pseudomonal antibiotics in the follow-up period, experienced

decreases in mean FEV_1 % predicted. This observation of the impact of successful *Pa* eradication on pulmonary function supports the rationale of the consensus guideline recommendations and current clinical practice of aggressive antibiotic treatment for new onset *Pa* infection. Further, these data suggest it will be important for future studies to identify which patients are at risk for failing initial eradication therapies and determine optimal treatment strategies for this group.

In conclusion, 28 days of AZLI treatment in pediatric patients 3 months to <18 years of age is both effective and well tolerated in the treatment of early *Pa* pulmonary infection associated with CF. The observed eradication rates are consistent with the success rates reported in the literature for various antibiotic regimens, including those utilizing other inhaled antibiotics.

Conflict of interest statements

HAWMT: has received unconditional research grants from Gilead Sciences and Chiesi Pharmaceuticals; has received honoraria and travel expenses for lectures and participation in expert panels from Novartis, Gilead, Roche, Pharmaxis, Insmed, and Vertex; and has a patent together with Activaero licensed.

KDB: has served on an advisory board for Gilead Sciences.

JPC: has grant support from the NIH and the CFF; has contract research support from Gilead, Vertex, Kalibios, and N30; has received support from Vertex and Genentech to provide educational talks; and has received support for participation on scientific boards for Vertex, Gilead, and Insmed and grant review boards for the NIH and Gilead.

MF: has served on advisory boards for Gilead Sciences, Novartis and Vertex; and has received travel grants from Novartis and Vertex.

HGMA: has served on an advisory board for Gilead Sciences.

MB, AD, and SAL are employees and shareholders of Gilead Sciences.

CMO: has no conflicts to declare.

Role of the funding source

This study was sponsored by Gilead Sciences. HAWMT, KDB, MF, and CMO participated in study design. HAWMT, KDB, JPC, MF, HGMA, and CMO were clinical investigators for the study. SAL oversaw statistical analyses. MB wrote and edited the draft manuscript. All authors revised the manuscript and approved the final version for submission. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2014.06.003.

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