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Original Article

ALPINE2: Efficacy and safety of 14-day vs 28-day inhaled aztreonam for *Pa* eradication in children with cystic fibrosis

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ABSTRACT

Background: Antibiotic eradication therapies recommended for newly isolated *Pseudomonas aeruginosa* (*Pa*) in people with cystic fibrosis (pwCF) can be burdensome. ALPINE2 compared the efficacy and safety of a shortened 14-day course of aztreonam for inhalation solution (AZLI) with 28-day AZLI in paediatric pwCF.

Methods: ALPINE2 (a double-blind, phase 3b study) included children aged 3 months to <18 years with CF and new-onset *Pa* infection. Participants were randomized to receive 75 mg AZLI three times daily for either 28 or 14 days followed by 14 days' matched placebo. The primary endpoint was rate of primary *Pa* eradication (no *Pa* detected during the 4 weeks post AZLI treatment). Non-inferiority was achieved if the lower 95% CI bound of the treatment difference between the two arms was above −20%. Secondary endpoints included assessments of *Pa* recurrence during 108 weeks of follow-up after primary eradication. Safety endpoints included treatment-emergent adverse events (TEAEs).

Results: In total, 149 participants were randomized (14-day AZLI, $n = 74$; 28-day AZLI, $n = 75$) and 142 (95.3%) completed treatment. Median age: 6.0 years (range: 0.3–17.0). Baseline characteristics were similar between treatment arms. Primary *Pa* eradication rates: 14-day AZLI, 55.9%; 28-day AZLI, 63.4%; treatment difference (CI), −8.0% (−24.6, 8.6%). *Pa* recurrence rates at follow-up end: 14-day AZLI, 54.1% ($n = 20/37$); 28-day AZLI, 41.9% ($n = 18/43$). TEAEs were similar between treatment arms. No new safety signals were observed.

Conclusions: Non-inferiority of 14-day AZLI versus 28-day AZLI was not demonstrated. Both courses were well tolerated, further supporting AZLI short-term safety in paediatric and adolescent pwCF.

ClinicalTrials.gov: NCT03219164

Abbreviations: AZLI, aztreonam lysine for inhalation solution; CF, cystic fibrosis; CI, confidence interval; FEV_1, forced expiratory volume in the first second of expiration; IgG, immunoglobulin G; IQR, interquartile range; *Pa*, *Pseudomonas aeruginosa*;

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1. Introduction

Pseudomonas aeruginosa (*Pa*) is an important pathogen in people with cystic fibrosis (pwCF), causing a significant symptom burden [1,2]. Chronic *Pa* infection in pwCF has been associated with accelerated lung function decline and structural lung disease, and is a predictor of reduced life expectancy [2–5]. The median age of first pulmonary infection with *Pa* in paediatric pwCF has been shown to be approximately 30 months, as detected by bronchoalveolar lavage [6]. New growths of *Pa* should be eradicated to prevent chronic infection, and the early use of antibiotic eradication therapies to target *Pa* is recommended as standard practice [2,7,8].

Many *Pa* eradication studies have been conducted, utilizing a variety of treatment regimens including inhaled, inhaled plus oral, and inhaled plus intravenous antibiotics. Similar rates of *Pa* eradication have been reported, despite differing study designs, eradication definitions and follow-up times [9,10]. Aztreonam lysine for inhalation solution (AZLI) is a lyophilized formulation of the monobactam antibiotic aztreonam approved for the treatment of chronic *Pa* infection in pwCF aged 6 years and above [11]. Colistimethate sodium (colistin), levofloxacin and tobramycin are inhaled antibiotics also approved for the treatment of chronic *Pa* infection in pwCF [12]. Currently, however, no inhaled antibiotic is approved specifically for the treatment of new-onset *Pa* infection in pwCF in Europe and the USA [12].

The ALPINE open-label study (ClinicalTrials.gov: NCT01375049) demonstrated that a 28-day course of AZLI (28-day AZLI) 75 mg three times daily in paediatric pwCF who had newly acquired *Pa* infection resulted in eradication rates similar to those reported for other inhaled antibiotics (75.2% at 4 weeks after treatment completion) [13]. Antibiotic regimens for *Pa* eradication and other CF treatment tasks are associated with a substantial burden for paediatric pwCF, and can require both parents and children spending over an hour a day on this [14]. This burden likely contributes to the high number of skipped doses observed in clinical trials and real-world studies of inhaled antibiotics, with reported treatment adherence ranging from 36 to 92% (47–92% for AZLI) [15–17]. Inhaled antibiotic treatment courses that have shorter durations could result in improved adherence to treatment for pwCF.

To investigate whether equivalent *Pa* eradication could be achieved with a shorter eradication course, the ALPINE2 study aimed to compare the efficacy and safety of a 14-day course of AZLI (14-day AZLI) with 28-day AZLI in paediatric and adolescent pwCF with new-onset *Pa* respiratory tract infection.

2. Methods

2.1. Participants

Participants eligible for this study were aged 3 months to <18 years at screening; had a diagnosis of CF as determined by the 2008 CF Consensus Conference criteria [18,19] (a sweat chloride level ≥60 mEq/L determined by quantitative pilocarpine iontophoresis, or a genotype with two identifiable mutations consistent with CF, or an abnormal nasal transepithelial potential difference and one or more clinical features consistent with CF); and had a local laboratory-documented new onset of *Pa*-positive respiratory tract culture in the 30 days prior to screening, defined as either a first lifetime *Pa*-positive culture or the first after being free from *Pa* for at least 2 years (minimum two cultures per year). The study excluded pwCF who had used intravenous or inhaled antipseudomonal antibiotics in the 2 years before screening or had used oral antipseudomonal antibiotics for a respiratory event in the 30 days before screening. Full inclusion and exclusion criteria are detailed in the **Supplementary Materials**.

This study was conducted in accordance with the principles of the Declaration of Helsinki, and the International Conference on Harmonisation guidelines and Good Clinical Practice principles.

This study was approved by institutional review boards or independent ethics committees for each site, and parents and/or guardians provided written informed consent before any study-related procedures.

2.2. Study design

ALPINE2 was a randomized, double-blind, multicentre phase 3b study (ClinicalTrial.gov: NCT03219164; EudraCT: 2016–002749–42) conducted at 53 centres in 12 countries between 28 November 2017 and 23 September 2021. Eligible pwCF were enrolled and randomized (1:1) to receive 75 mg AZLI three times daily for either 28 days or 14 days followed by 14 days of placebo-to-match. Randomization was achieved via an interactive web response system and pwCF were stratified by age group (3 months to <2 years old, 2 to <6 years old, 6 to <18 years old).

The study design outlining the study treatment phase, primary *Pa* eradication phase and the follow-up period is shown in Fig. 1. Respiratory samples were collected from pwCF at each visit and samples were cultured for *Pa* at the local and central laboratory. If a participant could not spontaneously expectorate sputum, lower respiratory tract samples were collected according to the local site standard of care. If lower respiratory tract sample collection was not possible, then oropharyngeal swabs were permitted. Blood samples were taken at baseline to test for the presence of anti-*Pa* immunoglobulin G (IgG) antibodies (negative, borderline, positive; methodology in the **Supplementary Materials**), and mucoid phenotyping of the respiratory samples (mucoid, nonmucoid) was performed at the central laboratory. pwCF with *Pa* recurrence after study treatment were retreated with a standard of care antipseudomonal antibiotic regimen (full list in the **Supplementary Materials**).

The ALPINE 2 study took place during the COVID-19 pandemic. During this time, the planned on-site study visits were scheduled only if it was considered safe and appropriate to do so based on upon recommendations from the local health authority and the clinical judgement of local investigators. Virtual visits were arranged when it was not deemed safe to continue with on-site visits.

2.3. Study outcomes

The primary endpoint assessed the proportion of pwCF with primary *Pa* eradication at 4 weeks following the last AZLI dose. Primary *Pa* eradication was defined as having all *Pa*-negative cultures during the primary *Pa* eradication phase (14-day AZLI: week 4 and week 6; 28-day AZLI: weeks 4, 6 and 8) as per central laboratory results. Subgroup analyses evaluated primary eradication by age group, sex, *Pa* infection history (first or recurrent infection), anti-*Pa* IgG antibodies at baseline and *Pa* culture result at baseline

pwCF, people with cystic fibrosis; SD, standard deviation; TEAE, treatment-emergent adverse event; TNS, tobramycin nebulizer solution.

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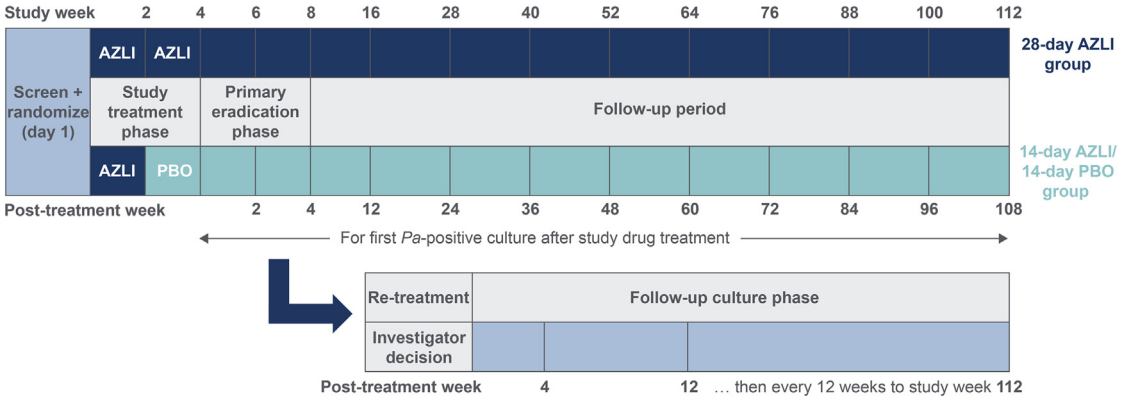

Fig. 1. Study design. Primary eradication was defined as no *Pa*-positive culture in week 4 and week 6 for the 14-day AZLI group and weeks 4, 6 and 8 for the 28-day AZLI group. pwCF with *Pa* recurrence after study drug treatment were retreated with a standard of care antipseudomonal antibiotic regimen at the discretion of the investigator, including 28-day AZLI, inhaled tobramycin 300 mg twice daily for 28 days, and any intravenous antibiotic regimen.
AZLI, aztreonam for inhalation solution; *Pa*, *Pseudomonas aeruginosa*; PBO, placebo; pwCF, people with cystic fibrosis.

per central laboratory, and in pwCF without mucoid *Pa* phenotype at baseline.

Secondary endpoints were: 1) median time from primary *Pa* eradication to *Pa* recurrence during the 108-week follow-up period; 2) median time to *Pa* eradication in a subset of the ALPINE2 population that matched the criteria of the efficacy analysis population in the tobramycin nebulizer solution (TNS) ELITE study [20]; and 3) the proportion of pwCF with primary *Pa* eradication in the 14-day AZLI group compared with historical pooled data for *Pa* eradication in pwCF treated with 28-day TNS 300 mg twice daily [21,22]. Recurrence was defined as a *Pa*-positive culture result from either the local or central laboratory after primary *Pa* eradication.

The exploratory endpoints were: 1) changes in lung function from baseline (forced expiratory volume in the first second of expiration [FEV_1]% predicted) among pwCF aged ≥6 years who could reliably perform spirometry; 2) the proportion of pwCF with *Pa* recurrence during the follow-up period (at study weeks 28, 52 and 112); and 3) the use of additional anti-*Pa* antibiotics during the study.

The safety endpoints were treatment-emergent adverse events (TEAEs), laboratory abnormalities, vital signs and physical examinations. TEAEs were defined as any adverse event that began on or after the day of the first dose of the study drug up to the last dose of the study drug plus 30 days, or that led to premature discontinuation of study treatment.

2.4. Statistics

The primary endpoint was reported for the Evaluable Analysis Set, which included all pwCF who completed AZLI treatment with ≥75% adherence and did not use other anti-*Pa* antibiotics while receiving AZLI. Non-inferiority of 14-day AZLI versus 28-day AZLI was assessed for the primary endpoint. The treatment difference (and 95% confidence intervals [CI]) between 14-day AZLI and 28-day AZLI were adjusted for age group as a stratification factor. Non-inferiority of 14-day AZLI was claimed if the lower bound of the 95% CI of the treatment difference in the proportion of pwCF with *Pa* eradication was above the non-inferiority margin of −20%.

It was determined that a sample size of 130 participants (65 pwCF per treatment group) would provide 75% power to show non-inferiority of 14-day AZLI versus 28-day AZLI. This was based on the primary eradication rate of 28-day AZLI in the ALPINE study (75.2% [13]). Assuming a non-evaluability proportion of 5–7%, up to 140 pwCF were required to be enrolled to obtain 130 evaluable pwCF for the primary endpoint. The treatment difference and 95% CIs were adjusted for age as a stratification factor for the primary analysis and the subgroup analyses. The analysis of the primary endpoint was repeated on the Per Protocol Analysis Set (defined in the **Supplementary Materials**). The TNS ELITE Study Matching Analysis Set consisted of pwCF from the Evaluable Analysis Set who also met the criteria for the efficacy analysis population in the ELITE study [20]. These criteria notably excluded pwCF with positive anti-*Pa* IgG antibodies at baseline (full criteria are listed in the **Supplementary Materials**).

For the evaluation of the primary endpoint, in the case of a missing central laboratory *Pa* culture result, the result from the local laboratory at the same study visit was used. If the local laboratory *Pa* culture result was also missing, then the *Pa* culture result from the next scheduled study visit was used.

The Safety Analysis Set included all pwCF who received at least one dose of AZLI.

3. Results

3.1. Participant demographics

Baseline demographics, CF disease characteristics and *Pa* infection characteristics were largely similar between treatment arms (Table 1). However, *Pa* mucoid phenotype was more common in the 14-day AZLI group than the 28-day AZLI group (21.6%, $n = 8/37$ versus 8.1%, $n = 3/37$), whereas a positive anti-*Pa* IgG antibody result was less common (14-day AZLI: 18.9%, $n = 10/53$ versus 28-day AZLI: 31.9%, $n = 19/60$). The median (range) age of the study population was 6.0 (0.3–17.0) years. At baseline, most respiratory samples were from oropharyngeal swabs, and the proportion of *Pa*-positive samples was similar between those from sputum and oropharyngeal swabs (**Table S1**).

3.2. Participant disposition

In total, 149 participants were randomized and received treatment (14-day AZLI [$n = 74$] or 28-day AZLI [$n = 75$]) (**Fig. S1**). The proportion of participants who completed study treatment was high (95.3%, $n = 142/149$) and similar between both arms. The reasons for discontinuing treatment were adverse events ($n = 2$), withdrawal of consent ($n = 2$), loss to follow-up ($n = 1$), protocol violation ($n = 1$) and nonadherence to study drug ($n = 1$) (**Fig. S1**).

In agreement with the Paediatric Committee of the European Medicines Agency, the study was terminated early on 23 September 2021 due to the COVID-19 pandemic and the risks of exposure through continuing study visits in this vulnerable participant population. At the time of termination, 93.3% ($n = 139/149$) of ran-

Table 1
Baseline participant demographics, CF disease characteristics and *Pa* infection characteristics.

Demographic/characteristic	14-day AZLI/14-day PBO(n = 74)	28-day AZLI(n = 75)	Total(N = 149)
Age, years			
Median (range)	7.5 (0.3–17.0)	6.0 (0.3–17.0)	6.0 (0.3–17.0)
Mean (SD)	7.3 (5.34)	6.5 (4.91)	6.9 (5.13)
Age group, n (%)			
3 months to <2 years	15 (20.3)	15 (20.0)	30 (20.1)
2 years to <6 years	20 (27.0)	22 (29.3)	42 (28.2)
6 years to <18 years	39 (52.7)	38 (50.7)	77 (51.7)
Sex at birth, n (%)			
Male	39 (52.7)	42 (56.0)	81 (54.4)
Female	35 (47.3)	33 (44.0)	68 (45.6)
Race, n (%)			
American Indian or Alaska Native	1 (1.4)	0	1 (0.7)
Asian	1 (1.4)	1 (1.3)	2 (1.3)
Black or African-American	1 (1.4)	0	1 (0.7)
White	69 (93.2)	73 (97.3)	142 (95.3)
Other	2 (2.7)	1 (1.3)	3 (2.0)
Ethnicity, n (%)			
Not Hispanic or Latino	66 (89.2)	65 (86.7)	131 (87.9)
Hispanic or Latino	8 (10.8)	9 (12.0)	17 (11.4)
Not permitted[a]	0	1 (1.3)	1 (0.7)
CF genotype (ΔF508), n (%)			
Heterozygous	34 (45.9)	32 (42.7)	66 (44.3)
Homozygous	29 (39.2)	33 (44.0)	62 (41.6)
Other	10 (13.5)	10 (13.3)	21 (13.4)
Missing	1 (1.4)	0	1 (0.7)
FEV_1% predicted,[b] mean (SD)	97.6 (12.8)	96.6 (10.8)	97.1 (11.8)
Pa infection history, n (%)			
First *Pa* infection	53 (71.6)	56 (74.7)	109 (73.2)
Recurrent *Pa* infection	21 (28.4)	19 (25.3)	40 (26.8)
Anti-*Pa* IgG antibodies, n (%)	n = 53	n = 60	n = 113
Negative	29 (54.7)	28 (46.7)	57 (50.4)
Borderline	14 (26.4)	13 (21.7)	27 (23.9)
Positive	10 (18.9)	19 (31.7)	29 (25.7)
Pa phenotype,[c] n (%)	n = 37	n = 37	n = 74
Mucoid	8 (21.6)	3 (8.1)	11 (14.9)
Nonmucoid	29 (78.4)	34 (91.9)	63 (85.1)
Pa culture result per central laboratory, n (%)	n = 67	n = 71	n = 138
Negative	29 (43.3)	34 (47.9)	63 (45.7)
Positive	38 (56.7)	37 (52.1)	75 (54.3)

AZLI, aztreonam for inhalation solution; CF, cystic fibrosis; FEV_1, expiratory volume in the first second of expiration; IgG, immunoglobulin G; *Pa, Pseudomonas aeruginosa*; PBO, placebo; SD, standard deviation.
[a] Collection of ethnicity information was not allowed by some local regulations.
[b] Only recorded for participants aged ≥6 years who could reliably perform spirometry.
[c] *Pa* phenotyping was performed at the central laboratory.

domized participants had completed the primary *Pa* eradication phase and were eligible for inclusion in the Evaluable Analysis Set. This number of participants was sufficient to analyse the primary endpoint as planned. Additionally, 74.1% (n = 103/139) of evaluable participants had completed the 108-week follow-up period at the time of study termination. The proportion of patients with at least one missing *Pa* culture result due to COVID-19 during the study was 49.6% (n = 69/139).

3.3. Efficacy

Primary *Pa* eradication occurred in 55.9% (n = 38/68) of participants in the 14-day AZLI treatment group versus 63.4% (n = 45/71) in the 28-day AZLI group. The treatment difference (95% CI) was −8.0% (−24.6, 8.6%). Given that the lower bound of the 95% CI was not above the −20% non-inferiority margin, 14-day AZLI failed the non-inferiority assessment versus 28-day AZLI (Fig. 2 and **Fig. S2**). The sensitivity analysis in the Per Protocol Analysis Set and the subgroup analyses supported the conclusion of the primary analysis (**Table S2**).

The median (IQR) time to *Pa* recurrence following *Pa* eradication was 82.9 weeks in the 14-day AZLI group (49.3–NA) and was not achieved in the 28-day AZLI group because fewer than 50% of participants had *Pa* recurrence (Fig. 3). A numerically greater proportion of participants had *Pa* recurrence at the end of follow-up, 108 weeks post-treatment, in the 14-day AZLI group compared with the 28-day AZLI group (54.1%, n = 20/37 versus 41.9%, n = 18/43). *Pa* recurrence at study weeks 28 and 52 is shown in **Table S3**.

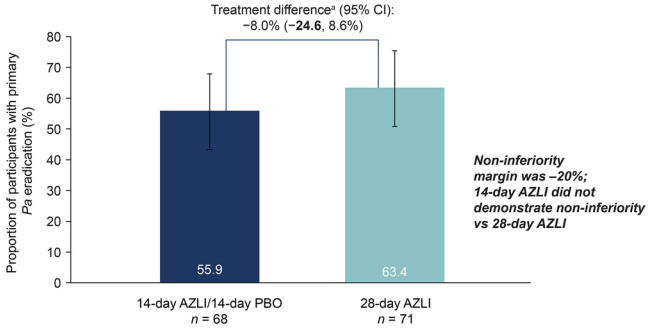

Fig. 2. Primary *Pa* eradication in the 14-day and 28-day AZLI treatment groups.
AZLI, aztreonam for inhalation solution; CI, confidence interval; *Pa*, *Pseudomonas aeruginosa*; PBO, placebo.
[a]The treatment difference and 95% CI calculated were adjusted for age group as a stratification factor.

Table 2
Summary of TEAEs.

Participants with any TEAEs,[a] n (%)	14-day AZLI/14-day PBO(n = 74)	28-day AZLI (n = 75)
TEAE[b]	50 (67.6)	46 (61.3)
Non-productive cough	12 (16.2)	11 (14.7)
Pyrexia	4 (5.4)	6 (8.0)
Upper respiratory tract infection	4 (5.4)	4 (5.3)
Diarrhoea	5 (6.8)	1 (1.3)
Influenza	1 (1.4)	5 (6.7)
Rhinitis	5 (6.8)	0
Productive cough	2 (2.7)	3 (4.0)
Rhinorrohea	2 (2.7)	3 (4.0)
TEAE related to study drug	13 (17.6)	8 (10.7)
Grade ≥3 TEAE	2 (2.7)	2 (2.7)
Grade ≥3 TEAE related to study drug[c]	0	1 (1.3)
Serious TEAE[d]	5 (6.8)	4 (5.3)
Serious TEAE related to study drug[c]	0	1 (1.3)
TEAE leading to premature discontinuation of study drug	1 (1.4)	1 (1.3)

AZLI, aztreonam for inhalation solution; PBO, placebo; TEAE, treatment-emergent adverse event.
[a] TEAEs were defined as any adverse event that began on or after the day of the first dose of the study drug up to the last dose of the study drug plus 30 days, or that lead to premature discontinuation of study treatment.
[b] Only the TEAEs that were in the top five most common TEAEs in either treatment arm are shown.
[c] All serious TEAEs resulted in hospitalization.
[d] One serious TEAE (grade 3 bronchial obstruction) in the 28-day AZLI group was assessed by the investigator as being related to the study drug. Grading was determined according to the Common Terminology Criteria for Adverse Events Version 4.03.

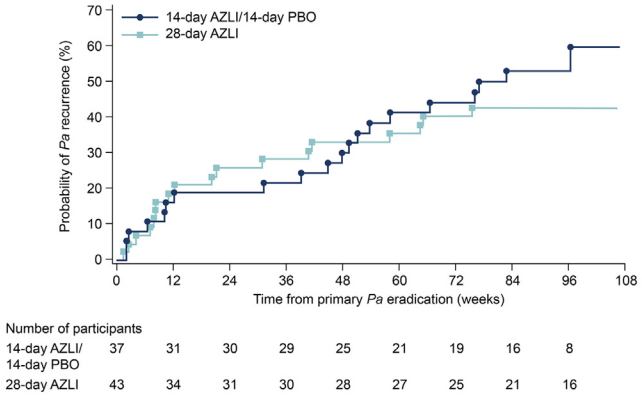

Fig. 3. Time from primary *Pa* eradication to first *Pa* recurrence in the 14-day AZLI and 28-day AZLI treatment groups.
AZLI, aztreonam for inhalation solution; *Pa Pseudomonas aeruginosa*; PBO, placebo.

In total, 32.2% (n = 48/149) of all randomized participants matched the TNS ELITE study criteria (14-day AZLI, n = 23; 28-day AZLI, n = 25) (**Fig. S3**). In this analysis, the median (IQR) times from primary *Pa* eradication to *Pa* recurrence in the 14-day and 28-day AZLI groups were 82.9 weeks (49.3–NA) and 65.0 weeks (21.1–NA), respectively (**Fig. S4**). The primary *Pa* eradication rate in the 14-day AZLI group (55.9%, n = 38/68) was descriptively compared with pooled data from two studies [21,22] of participants with new-onset *Pa* infection who received 28-day TNS 300 mg twice daily (77.0%, n = 24/31).

With regard to the lung function exploratory endpoint, at baseline, the mean (standard deviation) FEV_1% predicted values for pwCF in the Evaluable Analysis Set were 97.2% (13.1) for the AZLI 14-day group (n = 35) and 96.9% (11.1) for the AZLI 28-day group (n = 33). These values remained stable for both groups during the study (**Fig. S5**). The proportion of participants who used additional anti-*Pa* antibiotics is shown in **Table S4**.

3.4. Safety

The frequency of TEAEs was similar between treatment arms; TEAEs occurred in 67.6% (n = 50/74) and 61.3% (n = 46/75) of participants in the 14-day and 28-day AZLI treatment groups, respectively (Table 2). When stratified by age group, the incidence of TEAEs was largely similar between the 14-day and 28-day AZLI treatment groups and was slightly higher in participants aged <6 years than those aged 6 to <18 years (**Table S5**). The most common type of TEAE in both the 14-day and 28-day AZLI groups was non-productive (dry) cough (n = 12/74 [16.2%] and n = 11/75 [14.7%], respectively) (Table 2). The most commonly reported TEAEs by age group are shown in **Table S6**. The incidences of grade ≥3 and serious TEAEs were low (14-day AZLI: n = 2 [2.7%] and n = 5 [6.8%]; 28-day AZLI: n = 2 [2.7%] and 4 [5.3%]) and one participant in each AZLI group (0.7%) had a TEAE that led to premature discontinuation of the study drug (Table 2 and **Table S7**). There were no grade ≥3 laboratory abnormalities or clinically significant findings related to vital signs or physical examinations.

4. Discussion

ALPINE2 was the first study to evaluate the efficacy and safety of a shortened 14-day AZLI course compared with a 28-day AZLI course in paediatric and adolescent pwCF with new-onset *Pa* infection. This study aimed to reduce the treatment burden associated with the *Pa* eradication protocols. Although AZLI was well tolerated in both treatment arms, non-inferiority of the shortened 14-day AZLI protocol was not shown versus 28-day AZLI for primary *Pa* eradication.

In ALPINE2, the 28-day AZLI group had a primary *Pa* eradication rate of 63.4% (n = 45/71) compared with 75.2% (n = 76/101) in the previous ALPINE study [13]. Comparing efficacy across studies is challenging owing to differences in participant populations and study designs. Nonetheless, the difference in the observed eradication rates between these studies may be related to the higher proportion of pwCF with anti-*Pa* antibodies (borderline or positive) at baseline in ALPINE2 versus ALPINE (49.6%, n = 56/113 versus 37.3%, n = 38/102). pwCF who truly had a lifetime first *Pa* infection or had been *Pa*-free for 2 years should have had very low or undetectable anti-*Pa* antibody levels, given that a *Pa*-negative antibody test is highly predictive of a 'never' or 'intermittent' *Pa* infection status rather than chronic infection [23]. A higher proportion of pwCF with *Pa* antibodies at baseline may therefore reflect more pwCF with established *Pa* infection. Although the study was termi-

nated early due to the COVID-19 pandemic, it was determined that this decision did not have an impact on the analysis of primary *Pa* eradication in the ALPINE2 study.

In the descriptive comparison of *Pa* eradication rates between the 14-day AZLI group and historical pooled data from two studies involving pwCF with new-onset *Pa* infection treated with 28-day TNS [21,22], the observed eradication rate for 14-day AZLI was numerically lower compared with the pooled TNS studies. However, the limitations of using this pooled historical data should be considered. The median age of the ALPINE2 population was 6.0 years compared with 3.7–4.0 years in one of the two pooled studies (Gibson et al.) [21]. In the study by Gibson et al., the proportion of pwCF with an observed antimicrobial treatment effect was larger than that demonstrated in ALPINE and ALPINE2. However, this may have been due to the *Pa* infection characteristics in younger pwCF, such as lower *Pa* density, and a lower mucoid phenotype prevalence, which enabled participants to have an improved response to TNS treatment. Additionally, the sample size in the Gibson et al. study was small ($n = 8$) owing to the premature termination of that study by the data monitoring committee. Finally, differences in respiratory sample collection methods between ALPINE2 and the pooled studies may have influenced the observed difference in *Pa* eradication rate.

For *Pa* recurrence, the median (IQR) time was 82.9 weeks (49.3–NA) in the 14-day AZLI group and was not reached for the 28-day group because fewer than 50% of pwCF had recurrence. Although the recurrence rate up to week 112 was lower in the 28-day AZLI group than the 14-day AZLI group, this was not statistically significant. pwCF from the ALPINE2 study population were also matched according to the criteria for the TNS ELITE study, which only included pwCF without anti-*Pa* antibodies at baseline, thereby increasing the certainty that these participants had a first *Pa* isolation. In this analysis, the 14-day AZLI group achieved a longer median time to *Pa* recurrence than the 28-day AZLI group, but by week 112 the proportion of pwCF with recurrence was similar between the two groups. Conclusions should be drawn cautiously from this comparison, especially owing to the substantial reduction in participant numbers after matching to the TNS ELITE study criteria.

With regard to safety, both AZLI courses were well tolerated, as demonstrated by the high proportion of participants who completed treatment, and no new safety signals were observed. The frequency and type of TEAEs were similar in both AZLI treatment arms but TEAEs were slightly more frequent in pwCF aged <6 years than in those aged ≥ 6 years. Fewer than 3% of pwCF reported a grade ≥ 3 TEAE.

5. Conclusions

Treatment with 14-day AZLI did not demonstrate non-inferiority compared with 28-day AZLI for the primary *Pa* eradication rate. Therefore, these results do not provide sufficient evidence to support the use of 14-day AZLI for this purpose. This study further supports the short-term safety profile of AZLI in paediatric and adolescent pwCF given that both AZLI treatment courses were well tolerated, and no new safety signals were observed, including in pwCF aged ≤ 6 years.

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Author contributions

HT, **MA**, **MB**, **TRW** and **BF** contributed to methodology. **OG** contributed to formal analysis. All authors contributed to writing - reviewing and editing. All authors approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work.

Data availability statement

Anonymized individual patient data will be shared upon request for research purposes dependent upon the nature of the request, the merit of the proposed research, the availability of the data, and its intended use. The full data sharing policy for Gilead Sciences, Inc. can be found at https://www.gilead.com/about/ethics-and-code-of-conduct/policies.

Declaration of Competing Interest

SAM has received advisory fees from Vertex Pharmaceuticals, Inc. **FS** has received speaking and consulting fees from Gilead Sciences, Inc. and Vertex Pharmaceuticals, Inc. **MA**, **OG**, **TRW** and **BF** are employees and shareholders of Gilead Sciences, Inc. **MB** is a former employee and shareholder of Gilead Sciences, Inc. **MS** has received speaking fees from Vertex Pharmaceuticals, Inc. All other authors declare that they have nothing to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2023.06.008.

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