Empire-CF study: A phase 2 clinical trial of leukotriene A4 hydrolase inhibitor acebilustat in adult subjects with cystic fibrosis

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EMPIRE-CF STUDY: A PHASE 2 CLINICAL TRIAL OF LEUKOTRIENE A4 HYDROLASE INHIBITOR ACEBILUSTAT IN ADULT SUBJECTS WITH CYSTIC FIBROSIS

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Abstract (249 words)

Background: Cystic fibrosis (CF) is characterized by neutrophilic inflammation in the airways. Leukotriene B4 (LTB_4) is a neutrophil chemoattractant and has been implicated in CF pathogenesis. Acebilustat, a novel, synthetic, small-molecule leukotriene A4 hydrolase inhibitor, reduces LTB_4 production. We report findings from a randomized placebo-controlled trial of acebilustat in adult subjects with mild-to-moderate lung disease.

Methods: Subjects were randomized (1:1:1) to once-daily acebilustat 50 mg, 100 mg or placebo for 48 weeks, concomitantly with their current therapeutic regimen. Subjects were stratified by use of concomitant CF transmembrane conductance regulator (CFTR) modulators, baseline percent predicted forced expiratory volume in 1 second ($ppFEV_1$) 50-75 and >75, and number of pulmonary exacerbations in the past year (1 or >1). Primary endpoints were the change from baseline in $ppFEV_1$ and safety. Secondary endpoints included the rate of pulmonary exacerbations.

Results: Overall, 199 subjects were randomized and dosed (acebilustat 50 mg, n=67; acebilustat 100 mg, n=66; placebo, n=66). Baseline demographics and disease profile were well balanced among treatment groups. Acebilustat had no statistically significant effect on the primary endpoint of change in $ppFEV_1$ at week 48 or the secondary endpoint pulmonary exacerbations. There was a trend towards reduced pulmonary exacerbations in subjects receiving acebilustat in pre-specified populations with $ppFEV_1$>75 (35% rate reduction) and those on concomitant CFTR modulator therapy (20% rate reduction). Acebilustat was well tolerated.

Conclusions: Acebilustat did not improve lung function. A trend towards reduced pulmonary exacerbations in subjects with an earlier stage of lung disease suggests a potential effect in this population.

Highlights

- First evaluation of acebilustat in a large, 48-week clinical trial
- Acebilustat was safe and well tolerated
- No significant benefit for spirometry or pulmonary exacerbations
- A beneficial trend in exacerbations for subjects with higher lung function and/or CFTR modulator use

1. Introduction

Dysfunction of the CF transmembrane conductance regulator (CFTR) gene results in airway dehydration, abnormal mucus, and defective host defense leading to a cycle of airway obstruction, inflammation, infection, and bronchiectasis resulting in morbidity and mortality [1]. Inflammation, sustained or triggered by infections, is a prominent feature of pulmonary exacerbations and drives airway damage. Airway inflammation in CF is dominated by excessive influx and activation of neutrophils, which contribute to airway damage and progressive lung disease through multiple mechanisms [2]. Inflammation and elevated markers of neutrophil activity such as neutrophil elastase levels are observed as early as 3 months of age in children with CF and correlate with airway damage and pulmonary exacerbations [3-5].

In high doses, the nonsteroidal anti-inflammatory drug ibuprofen attenuates lung function decline and improves survival in children with CF [6-9]. Among other potential mechanisms, this beneficial effect is thought to occur by modulation of leukotriene B4 (LTB_4) [10, 11], a chemoattractant and activator of neutrophils that is also released by neutrophils [12]. LTB_4 levels are elevated in CF airways and correlate with reduced pulmonary function [13, 14]. Despite evidence of efficacy, high dose ibuprofen is infrequently used to treat inflammation in CF due to concerns of gastrointestinal and renal toxicity and the need for pharmacokinetic-based dosing [15, 16]. CFTR modulator therapies provide acute improvements in lung function, as indicated by forced expiratory volume in 1 second (FEV_1), and reduce long-term rates of decline of lung function [17, 18]. However, subjects receiving these therapies continue to experience pulmonary exacerbations and lung function decline persists. CFTR modulators do not improve markers of pulmonary inflammation to normal levels [19, 20].

Acebilustat is a novel, synthetic small-molecule inhibitor of the enzyme leukotriene A_4 hydrolase (LTA_4H), which catalyzes LTB_4 synthesis. In a placebo-controlled two-week phase 1 trial conducted in subjects with CF, acebilustat was well tolerated, reduced LTB_4 activity in

blood, and improved inflammatory biomarkers in the sputum [21]. In this phase 2 trial, we evaluated the efficacy and safety of acebilustat in adult subjects with mild-to-moderate CF lung disease.

2. Methods

2.1. Trial design, participants and assessments [22]

EMPIRE-CF (Evaluation of Modulation of Pulmonary Inflammatory Response in CF) was a randomized, double-blind, placebo-controlled phase 2 study conducted from October 30, 2015, to May 16, 2018. The study enrolled subjects aged 18–30 years diagnosed with CF of any CFTR genotype, percent predicted forced expiratory volume in 1 second ($ppFEV_1$) ≥50 at screening, and ≥1 pulmonary exacerbation as clinically determined by the investigator in the 12 months before screening. Potential subjects with unstable or deteriorating respiratory disease within 14 days before screening or between screening and first dosing were excluded. Full inclusion and exclusion criteria are listed in the online supplement.

Subjects meeting the inclusion criteria and none of the exclusion criteria were stratified by baseline $ppFEV_1$ (50–75 or >75), number of pulmonary exacerbations in the 12 months preceding screening (1 or >1), and the use of CFTR-modulator therapy (none or ivacaftor or ivacaftor plus lumacaftor) before they were randomized 1:1:1 to receive 50 mg acebilustat, 100 mg acebilustat, or placebo once daily for 48 weeks. All subjects provided written informed consent before undergoing study procedures. Following the baseline visit, subjects had research visits at Weeks 4, 6, 8, 12, 16, 24, 32, 40, 48 and 52. Details of trial assessments have been previously published [22]. Overall trial safety was monitored approximately every 8 weeks by an independent data safety monitoring committee comprising of members of the Cystic Fibrosis Foundation Data Safety Monitoring Board. The clinicaltrials.gov identifier is NCT02443688.

2.2. Statistical analysis

The details of the statistical methods, including the prespecified subgroups, are provided in the online supplement and have been published [22]. The primary efficacy analysis of change from baseline to Week 48 in ppFEV_1 was performed on the full analysis population (FAP) consisting of all treated subjects based on their randomized treatment group and on analysis of variance (ANOVA). The primary analysis was repeated according to the actual treatment received for those subjects in the FAP who met all inclusion/exclusion criteria, completed the Week 48 visit, received ≥80% of assigned treatment doses by Week 48 and did not start a CFTR modulator during the study (per-protocol population [PP]). The primary analysis was based on the average of the Week 48 change from baseline in pp FEV_1 for the two acebilustat doses compared with placebo. If the primary analysis (aggregate acebilustat effect versus placebo) reached the 0.05 level of significance (1-sided), the individual acebilustat doses were to be compared to the placebo arm using Dunnett's procedure at the 0.05 (2-sided) alpha level. No other adjustments for multiplicity were used and other statistical analyses were viewed as supportive. Predefined subgroups included stratifications by lung function (FEV_1 ≤75 and >75), CFTR modulator use, and the number of pulmonary exacerbations in the 12 months before screening (1 or >1).

Protocol-defined pulmonary exacerbations were determined as treatment with oral, inhaled, or IV antibiotic(s) for ≥4 of symptoms/signs listed per the modified Fuchs criteria [23] The secondary analysis of time to first protocol-defined pulmonary exacerbation was analyzed using a Cox proportional hazards model. The number of protocol-defined pulmonary exacerbations was annualized (a year is defined by 52 weeks) and was analyzed using a negative binomial regression model. Other pulmonary exacerbation endpoints were summarized using descriptive statistics. Adverse events (AEs) were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 18.0 and analyzed in the safety population, which consisted of all treated subjects based on their actual treatment. Other secondary and

exploratory outcome measures included changes nutritional status measured by body mass index, quality of life measured by the respiratory domain of the cystic fibrosis questionnaire (revised), and biomarkers of inflammation and infection.

3. Results

3.1. Patient population

From 21 October 2015 to 16 May 2018, 284 subjects were screened, 200 subjects were enrolled, and 199 subjects received (the FAP population) at least one dose of study drug (acebilustat 50 mg, n=67; acebilustat 100 mg, n=66; placebo, n=66) (Fig. 1). One subject in the placebo group was randomized but discontinued before receiving study drug. Overall, 32 subjects (16%) discontinued study participation before the Week 48 visit, including 21 (15.8%) from the acebilustat treatment groups and 11 (16.7%) from the placebo group. The most common reasons for discontinuation were withdrawal of informed consent (5%) and noncompliance with study drug (3%). The PP analysis included 162 subjects (acebilustat 50 mg, n=54; acebilustat 100 mg, n=54; placebo, n=54). Mean compliance with study drug assessed by pill counting was 96% in the acebilustat 50 mg group, 93% in the acebilustat 100 mg group, and 96% in the placebo group.

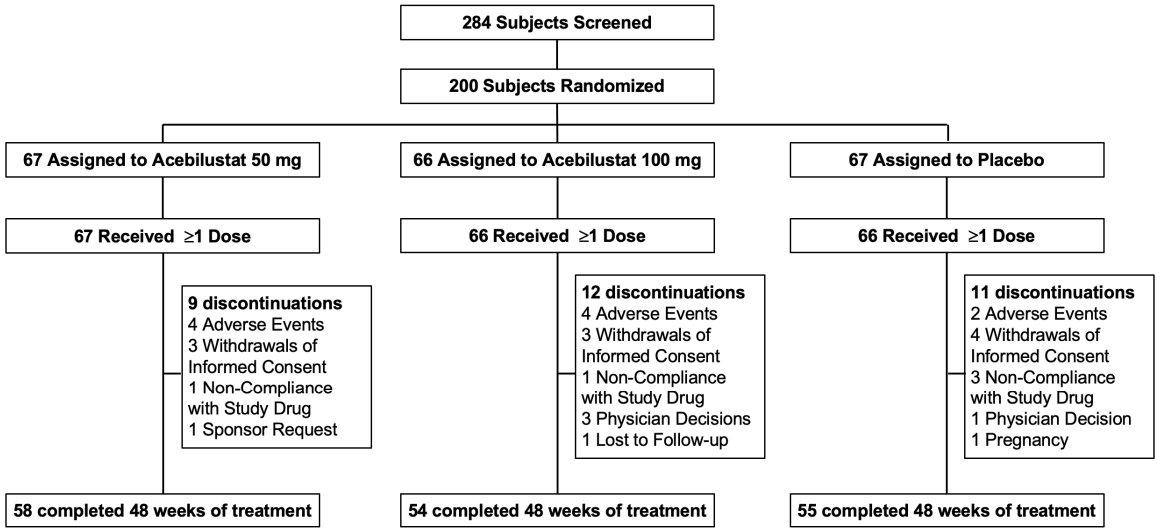


Figure. 1. Subject disposition.

Baseline characteristics are summarized in Table 1. Subjects enrolled were predominantly white and non-Hispanic with an equal proportion of men and women. Baseline characteristics were generally well balanced; the mean $ppFEV_1$ ranged from 69 to 72, and the mean number of exacerbations in the prior year ranged from 1.9 to 2.3 across the three arms. About one third of the subjects were using concomitant CFTR modulators.

Characteristic	Placebo (n = 66)	Acebilustat 50 mg (n = 67)	Acebilustat 100 mg (n = 66)
Men, n (%)	33 (50.0)	29 (43.3)	36 (54.5)
Age at screening, median (range), y	23 (18, 30)	24 (18, 30)	24 (18, 30)
$ppFEV_1$, mean (SD)	70.5 (16.5)	71.5 (14.4)	69.2 (14.9)
50–75, n (%)	42 (63.6)	42 (62.7)	44 (66.7)
>75, n (%)	24 (36.4)	25 (37.3)	22 (33.3)
Pulmonary exacerbations in prior year, mean (SD)	1.94 (1.4)	2.13 (1.5)	2.32 (1.9)
BMI, kg/m^2, mean (SD)	22.1 (3.1)	22.9 (3.1)	23.1 (3.2)
Concomitant medications, n (%)			
CFTR modulator	19 (28.8)	22 (32.8)	21 (31.8)
Dornase alfa	55 (83.3)	54 (80.6)	54 (81.8)
Azithromycin	31 (47.0)	37 (55.2)	42 (63.6)

Table 1. Baseline Demographics and Clinical Characteristics.

BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator, $ppFEV_1$, percentage of predicted forced expiratory volume in 1 second; SD, standard deviation; y, year

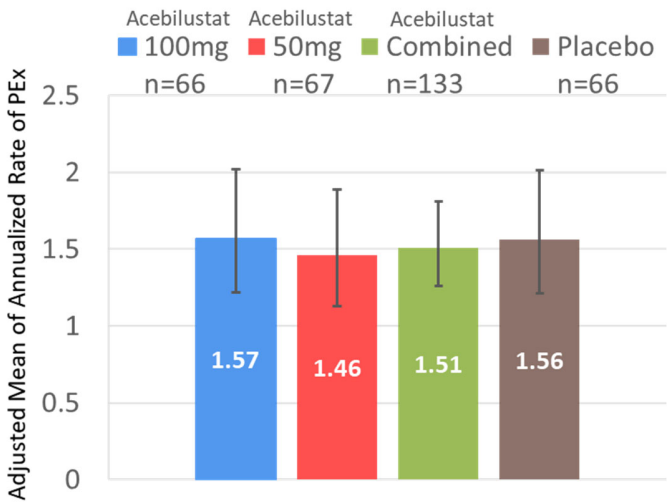
3.2. Efficacy

Adjusted mean absolute change (95% confidence interval [CI]) from baseline in $ppFEV_1$ in the FAP at Week 48 based on ANOVA model was −2.53 (−3.88, −1.18) percentage points in the combined acebilustat groups, −3.76 (−5.65, −1.86) in the 50 mg dose group, −1.30 (−3.22, −0.62) in the 100 mg dose group and −2.69 (−4.63, −0.75) in the placebo group; no statistically significant difference between acebilustat (combined doses) and placebo was observed (p=0.4). Furthermore, change in $ppFEV_1$ in the PP population was not different. Analyses of the primary endpoint in predefined subgroups were consistent with the overall differences between acebilustat and placebo (data not shown). No clinically meaningful changes were observed in other spirometry measures (Supplementary Table S1).

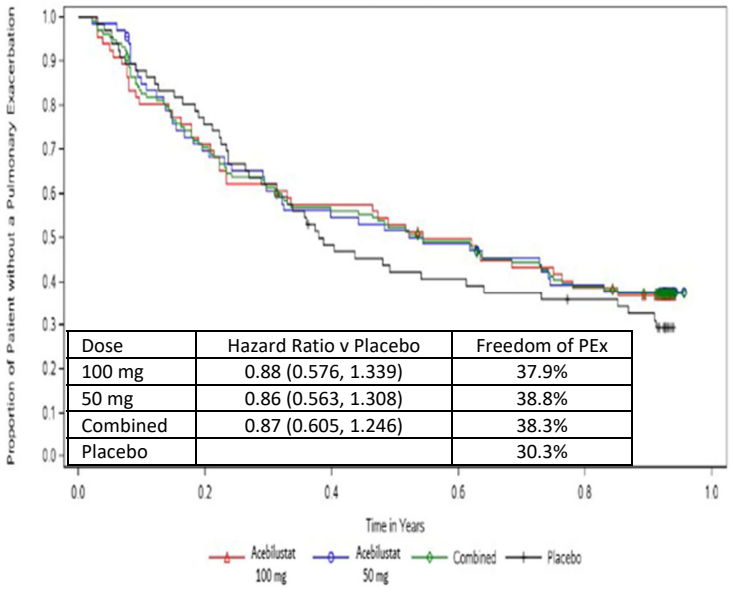
Given the mechanism of action of anti-inflammatory therapies, there is the potential for reduced frequency of pulmonary exacerbations, even without improvements in lung function. Therefore, measures of assessment of pulmonary exacerbations were an important secondary objective in this trial. Importantly, based on the sample size, this study was underpowered to demonstrate statistically significant differences in rates or hazard ratios for pulmonary exacerbations between acebilustat and placebo. Thus, these data are summarized using 95% 2-sided CIs for exploratory purposes and no changes reported with acebilustat therapy were statistically significant. In the FAP, the adjusted mean (95% CI) annualized pulmonary exacerbation rates based on the negative binomial regression model were 1.51 (1.26, 1.81) in the combined acebilustat groups,1.46 (1.13, 1.89) in the 50 mg dose group, 1.57 (1.22, 2.02) in the 100 mg dose group, and 1.56 (1.21, 2.01) in the placebo group (Fig. 2A). The time to first pulmonary exacerbation was no different in subjects receiving acebilustat (combined and individual dose groups) versus placebo (Fig. 2B). The hazard ratios versus placebo (95% CI) were 0.87 (0.605, 1.246) in the acebilustat combined group, 0.86 (0.563, 1.308) in the acebilustat 50 mg group, and 0.88 (0.576, 1.339) in the acebilustat 100 mg group. The

proportion of subjects who did not experience a pulmonary exacerbation during the study period was not different in the acebilustat groups (51 of 133 subjects [38%] in the combined acebilustat dose group, 26 of 67 subjects [39%] in the 50 mg group, and 25 of 66 subjects [38%] in the 100 mg dose group) than in the placebo group (20 of 66 subjects [30%]). While also not statistically significant, in the PP analysis, a number of favorable trends were observed (Fig. 2C). The time to first pulmonary exacerbation and the proportion of subjects who did not experience a pulmonary exacerbation during the study slightly favored the acebilustat group (Fig. 2D). The rates of pulmonary exacerbation leading to hospitalization and necessitating intravenous antibiotics are shown in Supplementary Table S2.

A Rate of Pulmonary Exacerbation (95% CI) – FAP

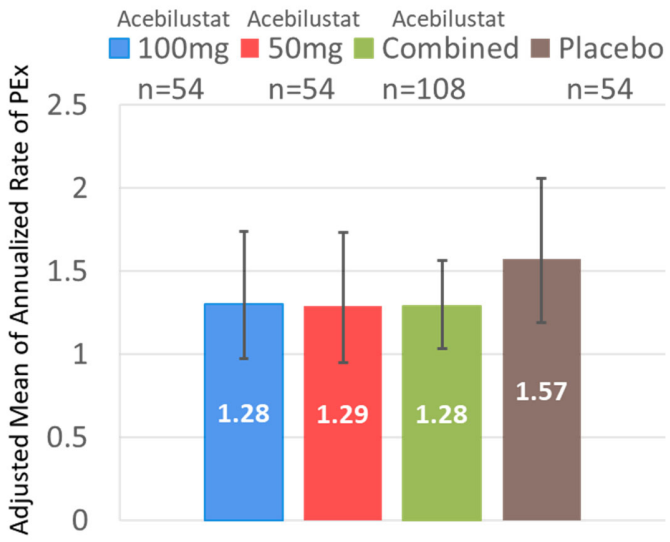


B Time to First Pulmonary Exacerbation – FAP

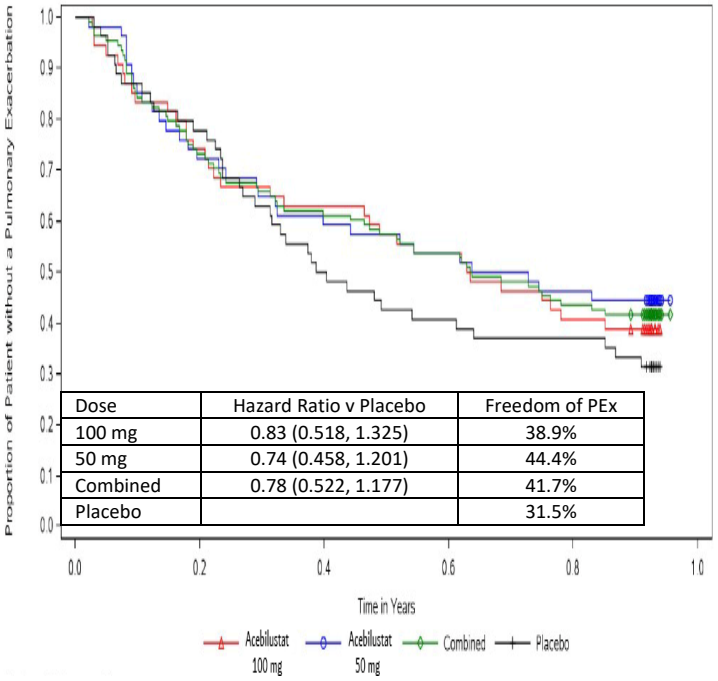


Dose	Hazard Ratio v Placebo	Freedom of PEx
100 mg	0.88 (0.576, 1.339)	37.9%
50 mg	0.86 (0.563, 1.308)	38.8%
Combined	0.87 (0.605, 1.246)	38.3%
Placebo		30.3%

C Rate of Pulmonary Exacerbation (95% CI) – PP



D Time to First Pulmonary Exacerbation – PP



Dose	Hazard Ratio v Placebo	Freedom of PEx
100 mg	0.83 (0.518, 1.325)	38.9%
50 mg	0.74 (0.458, 1.201)	44.4%
Combined	0.78 (0.522, 1.177)	41.7%
Placebo		31.5%

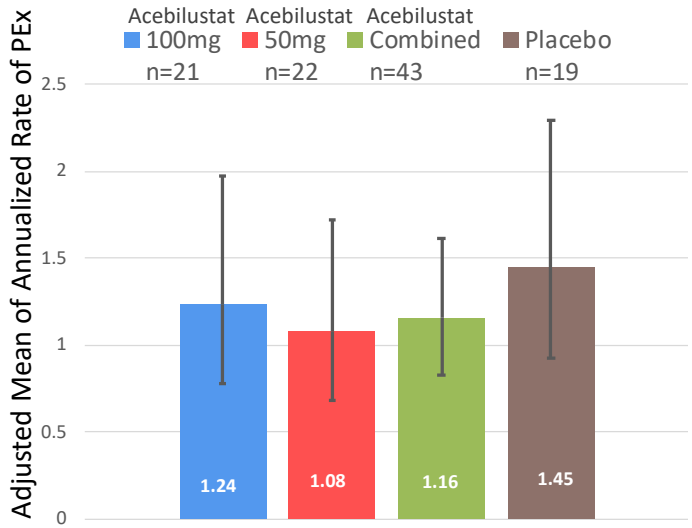
Figure 2. Analyses of Rate of Pulmonary Exacerbations by Dose Group for Subjects in the FAP (A) and PP (C) populations and Kaplan-Meier Plots for Time to First Pulmonary Exacerbation for Subjects in the FAP (B) and PP (D) Populations.

CI, confidence interval; FAP, full analysis population; HR, hazard ratio; PEx, pulmonary exacerbation; PP, per protocol population

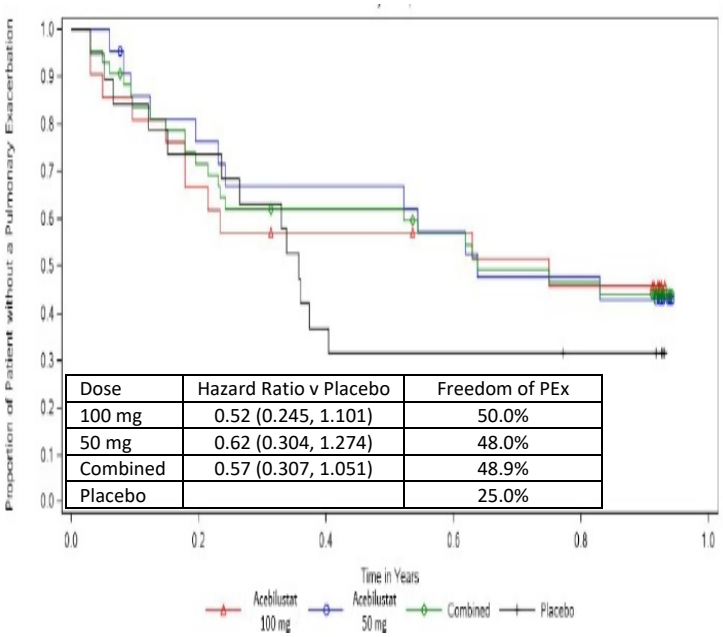
3.3 Exploratory subgroup analyses

The mean annualized rate of pulmonary exacerbation in subjects with baseline ppFEV$_1$ >75 (mild lung disease) was 35% lower in the combined acebilustat dose groups versus placebo. The adjusted mean (95% CI) annualized rate of pulmonary exacerbations in this pre-specified population was 1.04 (0.74, 1.46) in the combined acebilustat groups; 1.28 (0.84, 1.96) in the 50 mg dose group and 0.84 (0.49, 1.44) in the 100 mg dose group versus 1.61 (1.07, 2.42) in the placebo group (Fig. 3A). The effect of acebilustat versus placebo on time to first pulmonary exacerbation mirrored this trend (Fig. 3B). Hazard ratios (95% CI) versus placebo were 0.57 (0.307, 1.051) in the acebilustat combined group, 0.62 (0.304, 1.274) in the acebilustat 50 mg group, and 0.52 (0.245, 1.101) in the acebilustat 100 mg group. Additionally, almost half of the subjects in the higher ppFEV$_1$ group receiving acebilustat did not have a pulmonary exacerbation during the study (23 of 47 subjects [49%] in the combined acebilustat group; 12 of 25 subjects [48%] in the 50 mg acebilustat group; 11 of 22 subjects [50%] in the 100 mg acebilustat group), while only 6 of 24 subjects (25%) in the placebo group did not have a pulmonary exacerbation. These numerical reductions in freedom from exacerbations during the trial were not statistically significant. Results of individuals with one or two pulmonary exacerbations in the year prior to enrollment did not substantially differ from those with one.

A Rate of Pulmonary Exacerbation (95% CI) in Subjects with Baseline ppFEV$_1$ >75 – FAP

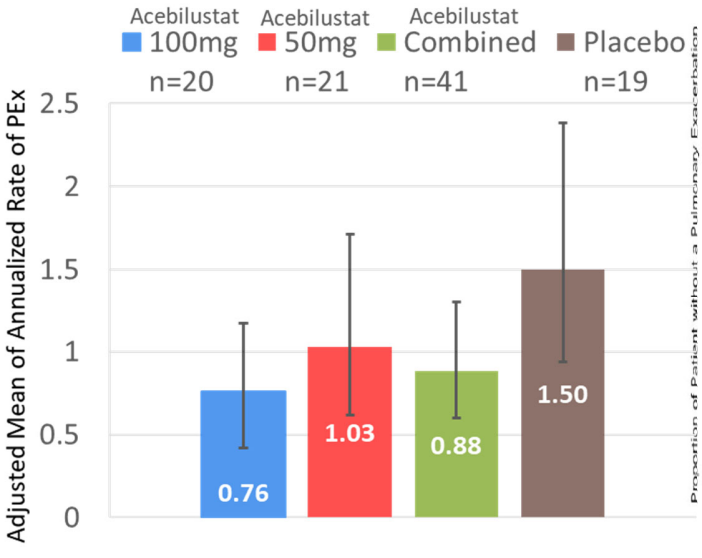


B Time to First Pulmonary Exacerbation in Subjects with Baseline ppFEV$_1$ >75 – FAP

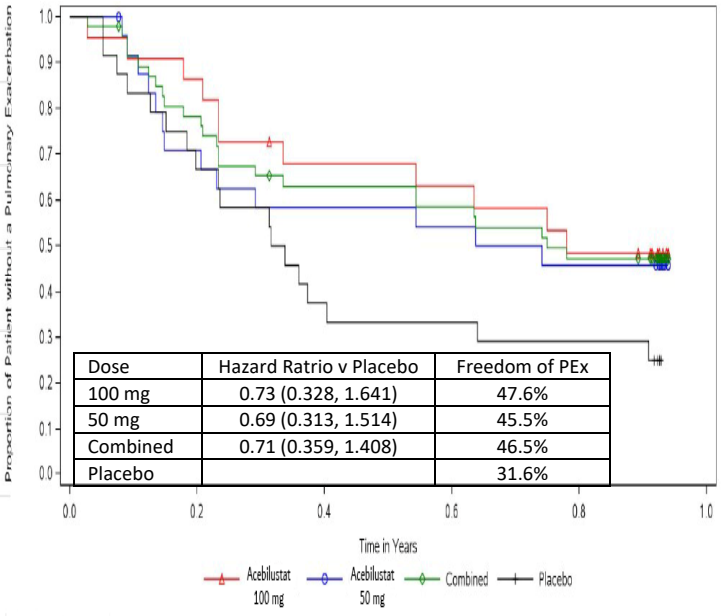


Dose	Hazard Ratio v Placebo	Freedom of PEx
100 mg	0.52 (0.245, 1.101)	50.0%
50 mg	0.62 (0.304, 1.274)	48.0%
Combined	0.57 (0.307, 1.051)	48.9%
Placebo		25.0%

C Rate of Pulmonary Exacerbation (95% CI) in Subjects taking CFTR Modulators – FAP



D Time to First Pulmonary Exacerbation in Subjects taking CFTR Modulators – FAP



Dose	Hazard Ratrio v Placebo	Freedom of PEx
100 mg	0.73 (0.328, 1.641)	47.6%
50 mg	0.69 (0.313, 1.514)	45.5%
Combined	0.71 (0.359, 1.408)	46.5%
Placebo		31.6%

Figure 3. Pre-specified Analyses in the FAP of Rate of Pulmonary Exacerbations by Dose Group for Subjects with Baseline $ppFEV_1$ >75 (A) and Subjects taking CFTR Modulator Therapy (C) and Kaplan-Meier Plots for Time to First Pulmonary Exacerbation for Subjects with Baseline $ppFEV_1$ >75 (B) Subjects taking CFTR Modulator Therapy (D).

CFTR, cystic fibrosis transmembrane conductance regulator CI, confidence interval; FAP, full analysis population; HR, hazard ratio; PEx, pulmonary exacerbation; $ppFEV_1$, predicted forced expiratory volume in 1 second

Similar to the findings in those with higher lung function, the mean annualized rate of pulmonary exacerbations in subjects receiving concomitant CFTR modulator therapy was 20% lower in the combined acebilustat dose groups versus placebo, although this was not statistically significant. The adjusted mean (95% CI) annualized rates of pulmonary exacerbations were 1.16 (0.83, 1.61) in the combined acebilustat group, 1.08 (0.67, 1.74) in the 50 mg dose group, 1.24 (0.78, 1.96) in the 100 mg dose group and 1.45 (0.91, 2.31) in the placebo group (Fig. 3C). The effect of acebilustat versus placebo on time to first pulmonary exacerbation slightly favored acebilustat (Fig. 3D). Hazard ratios (95% CI) versus placebo were 0.71 (0.359, 1.408) in the acebilustat combined group, 0.69 (0.313, 1.514) in the acebilustat 50 mg group and 0.73 (0.328, 1.641) in the acebilustat 100 mg group. Among these subjects, 20 of 43 (47%) in the combined acebilustat group did not have a pulmonary exacerbation (10 of 22 [46%] in the acebilustat 50 mg dose group and 10 of 21 [48%] in the acebilustat 100 mg dose group), compared with 6 of 19 subjects (32%) in the placebo group. None of the differences in exacerbations were statistically significant.

There was no difference at 48 weeks in BMI between the acebilustat and placebo groups: adjusted mean (95% CI) change from baseline based on the ANOVA model was 0.07 kg/m^2 (−0.12, 0.26) in the combined acebilustat groups (0.11 kg/m^2 [−0.16, 0.37] in the 50 mg dose group and 0.03 kg/m^2 [−0.24, 0.31] in the 100 mg dose group) and −0.02 kg/m^2 (−0.29, 0.26) in the placebo group.

Cystic Fibrosis Questionnaire–Revised respiratory domain scores, inflammatory biomarkers, and sputum bacterial densities showed no differences in acebilustat treated subjects compared to placebo; the results are outlined in the online supplement.

3.4. Safety

Acebilustat was generally well tolerated. AEs reported in a greater percentage of subjects in either acebilustat group than in the placebo group (difference ≥5 percentage points) were cough, hemoptysis, headache, oropharyngeal pain, rhinorrhea, sinus hypersecretion, rash, arthralgia, and diarrhea. AEs most commonly reported in either acebilustat dose group were infective pulmonary exacerbation of CF which consists of both protocol-defined and non-protocol defined exacerbations. Consistent with reporting of the secondary endpoint, a lower percentage of subjects taking acebilustat versus placebo had infective pulmonary exacerbations (Table 2). AEs were considered by the investigators to be not related or unlikely related in 53 subjects (79.1%) in the 50 mg dose group, 44 subjects (66.7%) in the 100 mg dose group, and 47 subjects (71.2%) in the placebo group. Most AEs were of mild or moderate severity in all treatment groups. AEs leading to treatment discontinuation were reported in four subjects in the acebilustat 100 mg dose group (one subject each with *Burkholderia cepacia* complex and *Mycobacterium abscessus* identified by respiratory tract culture as part of clinical standard care at the site, as well as one patient each with aspartate aminotransferase increased, and one with dizziness and loss of consciousness), four subjects in the acebilustat 50 mg dose group (one subject each with infective pulmonary exacerbation of CF, γ-glutamyl transferase increased, transaminases increased, and headache), and two subjects in the placebo group (one with infective pulmonary exacerbation of CF, and one with infective pulmonary exacerbation of CF and headache). Serious AEs were reported in 32 to 41% of subjects across treatment groups. There were no deaths during the study.

Event, n (%)	Placebo (n = 66)	Acebilustat 50 mg (n = 67)	Acebilustat 100 mg (n = 66)
Any AE	65 (98.5)	67 (100)	66 (100)
Treatment-related AE[a]	18 (27.3)	14 (20.9)	22 (33.3)
AEs by severity			
Mild	18 (27.3)	14 (20.9)	19 (28.8)
Moderate	36 (54.5)	34 (50.7)	31 (47.0)
Severe	11 (16.7)	19 (28.4)	16 (24.2)
Serious AE	21 (31.8)	26 (38.8)	27 (40.9)
AE leading to discontinuation	2 (3.0)	4 (6.0)	4 (6.1)
AE leading to death	0	0	0
Most common AEs (≥10% incidence in any group)			
Infective pulmonary exacerbation of CF	53 (80.3)	47 (70.1)	45 (68.2)
Cough	22 (33.3)	27 (40.3)	29 (43.9)
Hemoptysis	11 (16.7)	17 (25.4)	12 (18.2)
Nasopharyngitis	13 (19.7)	11 (16.4)	14 (21.2)
Headache	7 (10.6)	16 (23.9)	9 (13.6)
Sputum increased	11 (16.7)	13 (19.4)	7 (10.6)
Fatigue	7 (10.6)	9 (13.4)	9 (13.6)
Oropharyngeal pain	4 (6.1)	7 (10.4)	9 (13.6)
Nasal congestion	9 (13.6)	7 (10.4)	8 (12.1)
Pyrexia	7 (10.6)	6 (9.0)	8 (12.1)
Diarrhea	4 (6.1)	9 (13.4)	5 (7.6)
Nausea	5 (7.6)	5 (7.5)	8 (12.1)
Chest discomfort	7 (10.6)	3 (4.5)	8 (12.1)
Abdominal pain upper	5 (7.6)	7 (10.4)	3 (4.5)
Sinusitis	5 (7.6)	3 (4.5)	7 (10.6)

Table 2. Adverse events.

[a]AE possibly, probably, or definitely related to treatment

AE, adverse event; CF, cystic fibrosis

No clinically meaningful differences in hematology (including absolute neutrophil counts) or clinical chemistry (including liver function tests) parameters were observed between treatment groups in changes from baseline or shifts from normal values to high or low values during the study.

4. Discussion

No difference between acebilustat and placebo was observed for the primary endpoint, absolute change in $ppFEV_1$ from baseline at Week 48. Given the mechanism of action of acebilustat as an anti-inflammatory agent, and prior experience with ibuprofen [6], an acute increase from baseline in $ppFEV_1$ from a bronchodilatory or mucociliary clearance effect was unlikely. The use of change from baseline in $ppFEV_1$ as the primary endpoint relied on a hypothesized cumulative benefit on $ppFEV_1$ that was not observed, perhaps because the number of exacerbations in this study was low overall and below what was anticipated despite enrolling a population with a prior history of exacerbation. To demonstrate a reduction in decline of lung function versus placebo would have required many more subjects or a longer trial [24], as was the case with the 4-year high dose ibuprofen study [6], and beyond the scope of this Phase 2 effort.

Acebilustat did not result in change in the frequency of pulmonary exacerbations. Formally powering a trial to determine statistically significant differences in pulmonary exacerbations would have required a much larger sample size [25], a design not feasible in Phase 2 testing. Although the trial was underpowered to detect changes in pulmonary exacerbations, acebilustat treatment was associated with favorable trends in a number of outcomes related to pulmonary exacerbations, consistent with the proposed anti-inflammatory effect of acebilustat. These included the rate of pulmonary exacerbations, an increase in the time to first pulmonary exacerbation, and a higher proportion of subjects with no pulmonary exacerbations versus placebo. The favorable trends on exacerbations were also evident in pre-specified subgroups. In subjects who had higher lung function ($ppFEV_1$ >75 at baseline), consistent with an earlier stage of CF lung disease and most likely to benefit from anti-inflammatory drugs, overall there were fewer exacerbations in the acebilustat group, although this difference was not statistically significant. Supporting this finding, the magnitude of effect of

acebilustat on pulmonary exacerbations was also more prominent in those using concomitant CFTR modulators, a group that may more closely resemble those with mild CF lung disease due to partial restoration of CFTR activity.

Reducing pulmonary exacerbations is a critical goal of CF therapy, as exacerbations are associated with significant morbidity, decline in lung function, and early death [26-28]. Trials of longer duration are likely to be needed to detect the effect of combination treatment on the trajectory of lung function decline. Although CFTR modulators have been shown to reduce the rate of lung function decline, subjects receiving CFTR modulator treatment still experience exacerbations and a gradual loss of lung function [17-20], perhaps because the CFTR modulators used during the course of this study (predominantly lumacaftor/ivacaftor, but also ivacaftor) have not induced a substantial effect on airway inflammation [20]. These findings suggest that targeting neutrophil-mediated inflammation in CF has the potential to confer benefit by reducing pulmonary exacerbations in subjects with an earlier stage of CF lung disease. Furthermore, our results suggest that individuals with CF treated with CFTR modulator therapy represent a reasonable population in which single agent anti-inflammatory therapy has the potential to be beneficial, perhaps due to fewer overlapping pro-inflammatory pathways expected in patients with an earlier stage of lung disease.

Direct targeting of LTB_4 inflammatory signaling was investigated in a clinical trial of amelubant, an antagonist of the BLT1 receptor, but this trial in children and adults with mild to moderate CF lung disease was terminated early due to an increase in serious pulmonary AEs in adults [29]. Amelubant, at the doses used in the study, may have had an overly potent effect on the BLT1 receptor, impairing antibacterial defenses, and permitting increased infection [30, 31]. Its mechanism as a receptor antagonist may also have paradoxically increased LTB_4 presence in the airways as a deleterious consequence. As acebilustat acts to reduce LTB_4 synthesis by inhibiting the LTA_4H enzyme, it is likely to downregulate signaling through the BLT1 receptor

rather than block signaling. This mechanistic difference is reflected in the acceptable safety profile observed for acebilustat in this study in which acebilustat was safe and well tolerated in adult subjects with CF. While targeting a single inflammatory pathway may have benefit in regards to safety, the persistent effects of other chemoattractants not affected by acebiulstat may explain the negative results in this study.

Neutrophil elastase is a key marker of inflammation associated with lung function decline in subjects with CF [32]. In a phase 1 trial of acebilustat, sputum levels of neutrophil elastase, as well as sputum neutrophil DNA and serum high-sensitivity C-reactive protein, were reduced with acebilustat treatment compared with placebo [21]. We did not observe similar changes in this study. This may be due to limitations related to sputum collection, processing, transportation, and analyses from multiple clinical sites including the need to freeze samples to conduct centralized analysis, which has the propensity to release intracellular neutrophil elastase, a challenge that was not encountered during the phase 1 study. Alternatively, differences in a more generalized study population may have played a factor.

The results of this study highlight the challenges of designing a clinical trial for an anti-inflammatory agent at the phase 2 stage of drug development. Although reduction in rate of pulmonary exacerbations is a more clinically meaningful measure of anti-inflammatory drug activity, this study was powered based on change in $ppFEV_1$, which allowed a more feasible sample size and reasonable study duration. A trial based on FEV1 rate of decline would have required even more subjects and/or longer duration than a trial using either FEV1 change or pulmonary exacerbation rate as the endpoint. This trial design issue was an attempt to address the obvious challenge of assessing impact of anti-inflammatory agents early in clinical development, since biomarkers of sputum are not necessarily predictive of intermediate term benefit [33], whereas exacerbation trials are not feasible. By intention, we enrolled a population at risk for future pulmonary exacerbations which we hypothesized would enable the detection of

cumulative effects on FEV_1 through drug-inhibited (vs. unchecked) inflammation, in addition to positive trends in pulmonary exacerbations [22]. Despite this study population enrichment strategy, the rate of exacerbations was relatively low, which may have abrogated the potential to detect efficacy of an anti-inflammatory agent. With the advent of highly effective CFTR modulator therapy for the majority of people with CF [34, 35], trials with larger sample sizes and/or longer duration will be required to detect improvements in the frequency of pulmonary exacerbations since these events are less frequent, emphasizing the critical need for proximal biomarkers of airway inflammation to assess efficacy in early phases of drug development. This poses a major challenge for the development of anti-inflammatory therapies in CF, even though continued inflammation may be occurring.

In summary, acebilustat had no effect on change in $ppFEV_1$, although positive trends of the effect of acebilustat on rate of and time to first pulmonary exacerbation were observed, particularly in subjects with higher lung function and in those receiving concomitant CFTR modulator therapy. Given the importance of reducing pulmonary exacerbations, this study provides important information on the challenges of developing anti-inflammatory therapy for the treatment of people with cystic fibrosis.

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Supplementary Material

Inclusion criteria

1. 18–30 years of age inclusive at the time of screening
2. Documented, confirmed diagnosis of pulmonary cystic fibrosis (CF), defined as:
    a. CF signs and symptoms, *and*
    b. Either two CF transmembrane conductance regulator mutations on genetic testing *or* sweat chloride ≥60 mEq/L
3. Medically stable, in the opinion of the investigator
4. ≥1 pulmonary exacerbation in the 12 months before screening, based on the investigator's judgement
5. Resolution of any pulmonary exacerbation of CF ≥14 days before screening, in the opinion of the investigator
6. On a stable regimen of CF treatments with no change for ≥14 days before screening and between screening and baseline
7. If on ivacaftor or ivacaftor-lumacaftor combination, on a stable regimen for ≥8 weeks before baseline
8. No clinical or radiologic evidence of clinically significant lung abnormalities (eg, major atelectasis, pneumothorax), per the investigator's site procedures
9. Percentage of predicted forced expiratory volume in 1 second ($ppFEV_1$) ≥50 at screening
10. Resting oxygen saturation >92% on room air
11. Body mass index (BMI) ≥17.0 kg/m^2
12. No smoking (including electronic cigarettes) for ≥6 months before screening and agreement not to use such products for the duration of the study
13. If female and of childbearing potential, must have a negative pregnancy test at screening (unless surgically sterile) and must agree to use effective contraception from screening throughout duration of study
14. Able to perform spirometry as per European Respiratory Society/American Thoracic Society guidance [36]
15. Able to swallow investigational product whole
16. Able to comply with study procedures, in the opinion of the investigator
17. Provided informed consent to participate in the study

Exclusion criteria

1. In the opinion of the investigator, any significant clinical, laboratory, radiologic, or spirometric sign of unstable or unexpectedly deteriorating respiratory disease within 14 days before screening or between screening and baseline (including but not limited to features suggestive of a pulmonary exacerbation as suggested by the modified Fuchs' criteria)
2. A medical condition that is unstable, could be adversely impacted by participation in the study, or could impact assessment of the study results, in the opinion of the investigator

3. History of organ transplantation
4. History of alcoholism or drug abuse in the opinion of the investigator
5. Clinically significant hemoptysis (eg, >approximately 30 cc per episode, or clinically significant in the opinion of the investigator) within 180 days before screening
6. Colonization with organisms associated with a more rapid decline in respiratory function in CF patients (eg, all *Burkholderia* species, *Mycobacterium abscessus*) Subjects with a history of a positive culture could be considered free of colonization if she/he has had six subsequent respiratory tract cultures negative for these bacteria within the past 24 months prior to screening, with one of these cultures obtained within 6 months prior to screening
7. Active allergic bronchopulmonary aspergillosis at screening or at baseline
8. Any clinically significant electrocardiographic abnormality, in the opinion of the investigator
9. Positive serology for human immunodeficiency virus (HIV)-1 or -2 antibody, hepatitis C virus antibody, or hepatitis B surface antigen at screening
10. Aspartate aminotransferase or alanine aminotransferase $\geq 2 \times$ the upper limit of normal (ULN) at screening
11. Bilirubin >1.25 × ULN at screening. Subjects with known Gilbert's syndrome can be included with bilirubin >1.25 × ULN
12. Any patient with cirrhosis of the liver
13. Any patient with portal hypertension
14. Neutrophil count $<1.5 \times 10^9$/L at screening
15. Platelet count <150,000/μL at screening
16. Clinically unstable pancreatic function, in the opinion of the investigator. Evidence of unstable pancreatic function could include:
    a. Clinically significant weight loss (≥5% after a previously stable period)
    b. Evidence of uncontrolled hyperglycemia or recent hypoglycemia
    c. Change in pancreatic enzyme requirements in the 60 days before screening
17. Use of systemic corticosteroids or systemic antimicrobial therapy (other than chronic antimicrobial use, e.g., azithromycin, flucloxacillin, itraconazole) within 14 days before screening or between screening and baseline
18. Regular use (>3 times per week) of a high-dose non-steroidal anti-inflammatory drug (eg, >1.6 g ibuprofen/day) within 60 days before screening or between screening and baseline
19. Participation in a clinical trial for any medical/device product within 30 days before screening (participation in a noninterventional or observational study is permitted)
20. Pregnant or nursing women

Statistical analysis

Target enrollment was 65 subjects in each treatment arm (N = 195). The sample size calculation was performed with the following assumptions: 1:1:1 ratio of 50 mg acebilustat, 100 mg acebilustat, and placebo (2:1 ratio of acebilustat to placebo), difference between average

treatment effect (change from baseline) of 50 mg and 100 mg acebilustat combined versus placebo of 3.5 percentage points in $ppFEV_1$ at 48 weeks with a common standard deviation (SD) of 7 percentage points, power of at least 90%, and one-sided alpha = 0.05. Using a two-sample t-test for the mean difference with the above assumptions, a sample size of 156 subjects (52:52:52) was required for the primary overall comparison of acebilustat versus placebo. Because it was also of interest to be able to have a sufficient number of subjects to support analyses in the PP, the number of randomized subjects was increased to 195 (65:65:65) with the estimation that approximately 80% of randomized subjects would be included in the PP. The primary efficacy analysis of change from baseline to Week 48 in $ppFEV_1$ included all randomized subjects who received ≥1 dose of study drug according to their randomized treatment group (full analysis population [FAP]) and was based on an analysis of variance (ANOVA) in which the average of the Week 48 change from baseline in $ppFEV_1$ for the two dose groups was compared with that for the placebo group. The last data point (including early termination visit) recorded between Week 32 and 48 was used in calculating the change from baseline. $ppFEV_1$ was determined using the equations of Hankinson et al [2]. The ANOVA model contained a separate term for each dose group with the average over the two acebilustat doses created by averaging the parameter estimates from the ANOVA model. The model included stratification for the factors used for randomization. Subjects with no recorded data between Week 32 and 48 were treated as missing the primary endpoint. Missing primary endpoint values were imputed using the average placebo (according to the randomized treatment assignment) change from baseline to Week 48.

The primary analysis was based on the average of the Week 48 change from baseline in pp FEV_1 for the two acebilustat doses compared with placebo. If the primary analysis (aggregate acebilustat effect versus placebo) reached the 0.05 level of significance (1-sided), the individual acebilustat doses were to be compared to the placebo arm using Dunnett's procedure at the 0.05 (2-sided) alpha level. No other adjustments for multiplicity were used and other statistical analyses were viewed as supportive. The primary analysis was repeated for those subjects in the FAP who met all inclusion/exclusion criteria and had ≥80% of assigned treatment doses by Week 48 and completed the Week 48 visit according to the actual treatment received (per-protocol population [PP]).

The number of protocol-defined pulmonary exacerbations reported through Week 48/Early Termination was annualized where a year was defined by 52 weeks and analyzed using a negative binomial regression. The two active doses were compared to placebo individually as well as pooled. Point estimates, standard errors, and 95% confidence intervals (CIs) for the rate

of number of pulmonary exacerbations are presented. These analyses were limited to the observed data on the FAP and PP.

Time to first protocol-defined pulmonary exacerbation was analyzed using a Cox proportional hazards model. The two active doses were compared to placebo individually as well as pooled. Plots of the distribution of time to first exacerbation were produced using Kaplan-Meier (KM) methodology. In these analyses, subjects were censored as of their last assessment for pulmonary exacerbations. The percent of subjects free of pulmonary exacerbations was part of this determination of time to first exacerbation. These analyses were limited to the observed data on the FAP and PP.

Change from baseline at Week 48 in BMI was analyzed using ANOVA as described above for the primary efficacy variable.

The primary endpoint (both by individual dose and combined active doses) as well as time to first pulmonary exacerbation and number of pulmonary exacerbations were examined for subgroups based on the stratification factors for randomization: baseline ppFEV$_1$ (≤75 and >75), number of pulmonary exacerbations in the 12 months before screening (1 or >1), use of CFTR-modulating therapy such as ivacaftor or ivacaftor plus lumacaftor (yes/no).

The change from baseline at Week 48 in serum hs-CRP was summarized using ANOVA to estimate the means and 95% CIs for each individual treatment group and the pooled acebilustat treatment groups and the mean differences between the acebilustat groups (individual and pooled) and placebo. Sputum DNA and elastase data were transformed using the $\log_{10}$ transformation and summarized using the same methods as serum hs-CRP.

Colony forming units (CFUs) for sputum bacterial density and for individual species (*Pseudomonas aeruginosa*, *Burkholderia cepacia* complex, *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus* [including methicillin-resistant and small colony variants of *S aureus*]) and change from baseline in CFUs were analyzed descriptively by treatment group at 8, 24, and 48 weeks. CFUs were $\log_{10}$ transformed. In addition to basic descriptive statistics, 95% CIs for the mean differences between the acebilustat groups and placebo for the absolute change from baseline for both total bacterial load and organism were determined.

Cystic Fibrosis Questionnaire–Revised (CFQ-R) respiratory domain scores were analyzed descriptively by treatment group. In addition to the basic descriptive statistics, 95% CIs for the

mean differences between the acebilustat groups and placebo for the absolute change from baseline were determined.

Safety analyses included all subjects randomized and receiving ≥1 dose of study drug, based on actual treatment received.

Supplementary Tables and Figures

Table S1

Analysis of lung function secondary endpoints[a] (Observed Data).

Parameter	Placebo (n = 57)	Acebilustat 50 mg (n = 59)	Acebilustat 100 mg (n = 59)	Acebilustat combined doses (n = 118)
Relative change in $ppFEV_1$				
Change from baseline at 48 weeks (adjusted mean [95% CI]), %	−3.40 (−6.59, −0.22)	−4.81 (−7.84, −1.79)	−2.38 (−5.37, 0.60)	−3.60 (−5.72, −1.47)
Absolute change in ppFVC				
Change from baseline at 48 weeks (adjusted mean [95% CI]	−1.41 (−3.32, 0.51)	−2.06 (−3.88, −0.25)	−1.57 (−3.37, 0.23)	−1.82 (−3.10, −0.54)
Absolute change in $ppFEF_{25\%–75\%}$				
Change from baseline at 48 weeks (adjusted mean [95% CI],	−4.38 (−7.20, -1.57)	−4.79 (−7.46, −2.11)	−1.61 (−4.26, 1.03)	−3.20 (−5.08, −1.32)

[a]Based on ANOVA models

CI, confidence interval; $ppFEF_{25\%–75\%}$, percentage of predicted forced expiratory flow at middle half of forced vital capacity; $ppFEV_1$, predicted forced expiratory volume in 1 second; ppFVC, percentage of predicted forced vital capacity.

Table S2

Rate of pulmonary exacerbations leading to hospitalizations and necessitating treatment with intravenous antibiotics[a].

Pulmonary exacerbations	Placebo	Acebilustat 50 mg	Acebilustat 100 mg	Acebilustat combined doses
Leading to hospitalization FAP				
N	66	67	66	133
Adjusted mean (95% CI) annualized rate	0.45 (0.27, 0.76)	0.44 (0.26, 0.74)	0.67 (0.42, 1.06)	0.54 (0.38, 0.77)
Leading to hospitalization PP				
N	54	54	54	108
Adjusted mean (95% CI) annualized rate	0.47 (0.27, 0.81)	0.30 (0.16, 0.56)	0.44 (0.25, 0.77)	0.36 (0.24, 0.55)
Necessitating administration of intravenous antibiotics FAP				
N	66	67	66	133
Adjusted mean (95% CI) annualized rate	0.63 (0.41, 0.95)	0.64 (0.42, 0.97)	0.85 (0.58, 1.24)	0.74 (0.56, 0.98)
Necessitating administration of intravenous antibiotics PP				
N	54	54	54	108
Adjusted mean (95% CI) annualized rate	0.65 (0.41, 1.02)	0.48 (0.29, 0.79)	0.68 (0.44, 1.06)	0.57 (0.41, 0.80)

[a]Based on negative binomial regression models

CI, confidence interval; FAP, full analysis population; PP, protocol population.

Table S3

Change from baseline in sputum and serum biomarkers[a].

Parameter	Placebo	Acebilustat 50 mg	Acebilustat 100 mg	Acebilustat combined doses
Sputum DNA				
N	42	42	33	75
Adjusted mean (95% CI) change from baseline at 48 weeks	−0.05 (−0.22, 0.12)	0.17 (0.01, 0.34)	0.06 (−0.13, 0.25)	0.12 (-0.01, 0.24)
Mean (95% CI) difference vs placebo	NA	0.23 (−0.01, 0.46)	0.11 (−0.15, 0.37)	0.17 (-0.04, 0.38)
Sputum elastase				
N	41	43	33	76
Adjusted mean (95% CI) change from baseline at 48 weeks	−0.08 (−0.25, 0.08)	0.17 (0.02, 0.33)	−0.02 (−0.20, 0.16)	0.08 (-0.04, 0.20)
Mean (95% CI) difference vs placebo	NA	0.26 (0.03, 0.48)	0.06 (−0.18, 0.31)	0.16 (-0.04, 0.36)
Serum hs-CRP				
N	51	55	51	106
Adjusted mean (95% CI) change from baseline at 48 weeks	−1.87 (−5.91, 2.17)	−0.33 (−4.21, 3.56)	2.16 (−1.84, 6.16)	0.92 (-1.87, 3.70)
Adjusted mean (95% CI) Difference vs placebo	NA	1.54 (−4.06, 7.14)	4.03 (−1.65, 9.71)	2.79 (-2.12, 7.69)

[a]Based on ANOVA model. CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein.

Table S4

Change from baseline in bacterial density (CFU/g) at Week 48*.

Bacterium	Placebo (n = 36)	Acebilustat 50 mg (n = 38)	Acebilustat 100 mg (n = 32)	Acebilustat combined doses (n = 70)
Total bacterial load				
Mean (SD) change in density	−0.74 (2.7)	0.32 (2.5)	−0.49 (3.4)	−0.05 (3.0)
Mean (95% CI) difference vs placebo	NA	1.06 (-0.16, 2.28)	0.25 (-1.23, 1.73)	0.69 (-0.48, 1.86)
Pseudomonas aeruginosa				
Mean (SD) change in density	0.36 (2.4)	0.41 (1.9)	−0.66 (2.4)	−0.08 (2.2)
Mean (95% CI) difference vs placebo	NA	0.05 (-0.95, 1.05)	−1.02 (-2.19, 0.16)	−0.44 (-1.36, 0.48)
Achromobacter xylosoxidans				
Mean (SD) change in density	0.14 (1.3)	−0.02 (0.1)	0.18 (0.7)	0.07 (0.5)
Mean (95% CI) difference vs placebo	NA	−0.16 (-0.58, 0.26)	0.03 (-0.49, 0.55)	−0.07 (-0.42, 0.28)
Stenotrophomonas maltophilia				
Mean (SD) change in density	−0.21 (1.0)	0.26 (1.4)	−0.37 (1.4)	−0.03 (1.5)
Mean (95% CI) difference vs placebo	NA	0.47 (-0.11, 1.06)	−0.16 (-0.75, 0.44)	0.18 (-0.35, 0.72)
Staphylococcus aureus				
Mean (SD) change in density	−1.23 (2.8)	0.14 (2.5)	−0.50 (2.9)	−0.15 (2.7)
Mean (95% CI) difference vs placebo	NA	1.37 (0.15, 2.60)	0.73 (-0.64, 2.09)	1.08 (-0.03, 2.18)

CI, confidence interval; CFU, colony forming unit; NA, not applicable; SD, standard deviation.

- No growth of Burkholderia cepacia complex

Table S5

Change from baseline in CFQ-R respiratory symptom domain scores.

Parameter	Placebo (n = 54)	Acebilustat 50 mg (n = 57)	Acebilustat 100 mg (n = 54)	Acebilustat combined doses (n = 111)
Mean (SD) change from baseline in CFQ-R respiratory symptoms domain score at 48 weeks	−3.09 (18.6)	−3.90 (14.6)	−2.88 (11.7)	−3.40 (13.2)
Mean (SEM) difference vs placebo (95% CI)	NA	−0.81 (3.2) (-7.09, 5.46)	0.21 (3.0) (-5.72, 6.13)	−0.32 (2.5) (-5.29, 4.66)

CFQ-R, Cystic Fibrosis Questionnaire–Revised; CI, confidence interval; NA, not applicable; SD, standard deviation; SEM, Standard Error of the Mean.

Supplementary Appendix 1

EMPIRE-CF Investigators

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