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Original Article

Long term effects of denufosol tetrasodium in patients with cystic fibrosis ☆

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Abstract

Rationale: Denufosol stimulates chloride secretion independent of the chloride channel which is dysfunctional in cystic fibrosis (CF) and therefore has the potential to benefit CF patients regardless of genotype.
Objectives: To assess the efficacy of denufosol in CF patients with mild lung function impairment age 5 years and older.
Methods: This multicenter, randomized, parallel group double-blind placebo-controlled trial was conducted at 102 CF care centers in Australia, Canada and the United States (NCT00625612) The active group (n=233) received 60 mg denufosol via inhalation three times daily The primary efficacy endpoint was change in FEV_1 in liters from Day 0 to week 48.
Measurements and main results: 685 patients were screened for the study and 466 patients (233 in each group) were randomized to study treatment. The adjusted mean change in $FEV_1$was 40 mL for denufosol and 32 mL for placebo with a resulting treatment effect of 8 mL (95% CI −0.040, 0.056). The average rate of change in FEV_1 percent of predicted over 0 to 48 weeks was −3.04% for placebo vs. −2.30 for denufosol (a difference of 24% relative to placebo) among all patients. The incidence of pulmonary exacerbation was 26% vs. 21% for the placebo and denufosol groups with no differences in the time to first event. The study treatments were well tolerated and there was no evidence of systemic effects in any safety parameter assessed.
Conclusions: In patients with CF treatment with denufosol for 48 weeks did not improve pulmonary function or reduce the incidence of pulmonary exacerbations.

Keywords: Cystic fibrosis; Ion transport; Denufosol; Lung function; Pulmonary exacerbation

☆ Contributions: 1) Conception and design (FR, TD, TN, AS, FA, and RM), acquisition of data (FR, TD, TN, AS, FA, MB, CW, and RM), analysis and interpretation of data (FR, TD, TN, AS, FA, CW, and RM); 2) drafting the article or revising it critically for important intellectual content (FR, TD, TN, AS, FA, CW, and RM); and 3) final approval of the version to be published (FR, TD, TN, AS, FA, MB, CW, and RM).

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1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR). CFTR functions as a chloride channel and is expressed in epithelial membranes throughout multiple organ systems [1]. Pathological changes due to the underlying genetic defect are evident in many organs, but lung disease accounts for most of the morbidity and mortality in patients [2]. Defective chloride secretion leads to reduced airway surface liquid, impaired mucociliary clearance, and resultant airway infection and inflammation [3]. This process starts early and significant structural and functional pulmonary changes have been documented in the first 6 years of life [4–9].

Currently available interventions such as mucolytics and antibiotics address the complications of CF lung disease and have been demonstrated to improve lung function [10–15]. Recent interest has focused on interventions which target the underlying ion channel abnormality as highlighted by the promising results for the CFTR potentiator ivacaftor in patients carrying at least one copy of the G551D mutation [16]. If introduced early, therapies directed against the underlying chloride channel defect could have the potential to delay the progression of lung disease [14–16].

Denufosol tetrasodium is an investigational compound delivered via nebulization. Denufosol acts on P2Y receptors expressed on the surface of airway epithelium to stimulate chloride secretion via calcium activated chloride channels (CACCs), inhibit sodium absorption via epithelial Na^+ channels (ENAC), and stimulate ciliary beat frequency; and thereby has the potential to benefit patients independent of CFTR genotype [17].

Previous studies with denufosol have demonstrated its potential to provide benefit to patients with CF. In a Phase 2 study [18] among patients with mild lung function impairment, patients receiving denufosol treatment had significantly better lung function after 28 days of treatment. Based on these effects in patients with mild lung function impairment the Phase III program for denufosol focused on this population of CF patients. The positive findings were supported by the first Phase 3 study (TIGER-1) which included a 24-week, placebo-controlled, double-blind Phase III trial in 352 CF patients where a moderate effect on forced expiratory volume in one second (FEV1) (45 mL or 2% versus baseline) was observed [19]. However, within this study these FEV_1 results were not corroborated with any statistically significant improvements in other a priori defined secondary efficacy endpoints, including pulmonary exacerbation rates, other measures of lung function, hospitalization or illness (missed work/school) rates, or quality of life.

The TIGER-1 study included a 24-week open-label extension phase immediately following the 24-week double blind phase. In this open-label extension, patients appeared to continue to improve in the group originally assigned to denufosol, with a change in inflection in the mean change in FEV_1 for those patients whose treatment was switched at Week 24 from placebo to denufosol. This suggested that a longer treatment period might be necessary to identify the time to maximum benefit of denufosol relative to placebo on lung function. Based on these findings, the current study (clinicaltrials.gov NCT00625612; TIGER-2) was designed to assess the efficacy of treatment over 48 rather than 24 weeks in a double blind trial. In addition, to increase power, the sample size was increased from 350 to 450 patients compared to the previous study, and patients with $FEV_1 > 110\%$ predicted were excluded as they were unlikely to improve lung function due to high baseline values.

2. Methods

2.1. Patient selection

Patients were eligible if they were at least 5 years of age, had a confirmed diagnosis of CF and a FEV_1 between 75% and 110% of predicted normal for age, height and sex at screening [20,21]. Patients were required to be clinically stable, as evidenced by no acute respiratory illnesses within 4 weeks of screening, and able to demonstrate reproducible FEV_1 measurements (±15%) on two separate days [22]. All concomitant medications except hypertonic saline were allowed during the study. Clinic visits for patients taking inhaled antibiotics on a cyclical basis were scheduled so that baseline and endpoint evaluations of lung function occurred at the same relative time in each patient's cycle. The study protocol was approved by the Cystic Fibrosis Foundation Therapeutics Development Network Protocol Review Committee. An independent data monitoring committee was chartered to prospectively evaluate safety. The study was approved by the institutional review boards of all participating centers and informed consent was obtained from all study participants and/or guardians (including assent for minors as appropriate).

2.2. Study design

Subjects were enrolled from a total of 102 centers in the United States (82), Canada (9), Australia/New Zealand (11) over the period from Feb. 2008 to Oct. 2009. The study had a 48-week, placebo-controlled treatment period with scheduled clinic visits at screening, Weeks 0, 4, 12, 24, 36 and 48. A web based computer generated randomization sequence was used stratified by study center assuring an allocation ratio of denufosol to placebo was 1:1. The targeted study size was 450 subjects (225 per group). Study treatment was denufosol tetrasodium inhalation solution, 60 mg (Inspire Pharmaceuticals, Durham, NC) three times daily (TID) or placebo vehicle (0.9% wt/vol saline) TID. The treatment was delivered via the PARI LC Star Reusable Nebulizer (PARI Respiratory Equipment Inc., Midlothian, VA) and the PARI PRONEB Ultra Compressor (PARI Respiratory Equipment Inc.). The order of concomitant treatments administration was prescribed in the study protocol as follows: bronchodilator, dornase alfa, chest physiotherapy or vest, study drug, and inhaled antibiotic. Use of hypertonic saline was excluded. At selected sites, spontaneously expectorated sputum samples were collected for measuring the concentration of denufosol pre-dose and at 5, 15, and 60 minutes post-dose during a Week 4 clinical visit.

2.3. Outcome measures

The primary efficacy endpoint was the change from baseline to Week 48, defined as the Week 48 value or the last observation carried forward, in FEV_1 (liters). There were three key secondary efficacy endpoints: (1) the rate of change in FEV_1 percent of predicted over the 48-week treatment period; (2) the time to first pulmonary exacerbation requiring intravenous antibiotics to treat at least one respiratory sign or symptom; and (3) change from baseline to Week 48 endpoint in maximal mid-expiratory flow ($FEF_{25\%–75\%}$ L/s). Three definitions of pulmonary exacerbation were used: (1) the treatment with intravenous antibiotics for at least one respiratory symptom; (2) the presence of 4 or more out of 12 respiratory and systemic signs or symptoms; and (3) the diagnosis by the investigators. Pulmonary function measurements were obtained using standardized spirometry equipment according to ATS standards (ATS/ERS 2005) and interpreted by a centralized spirometry reading specialist (nSpire Health™, Longmont, CO). Clinic spirometry was conducted after the study drug had been withheld for at least 6 h and bronchodilator for at least 2 h. Multiple exploratory efficacy endpoints were also evaluated (clinicaltrials.gov NCT00625612). Safety was assessed by reports of adverse events, clinical laboratory results, physical examination results, and incidences of new pulmonary infections, hospitalizations and changes from screening lung function.

2.4. Pulmonary pharmacokinetics

At selected sites, spontaneously expectorated sputum samples were collected for measuring the concentration of denufosol pre-dose and at 5, 15, and 60 min post-dose on Week 4. Samples were collected in 50% EtOH solution containing EDTA, stored at −20 °C and shipped into the central lab for analysis. Concentrations of denufosol in sputum were determined using a liquid chromatography tandem mass spectrometry (LC/MS/MS) by Enthalpy Analytical, Durham, NC with a detection limit of quantification of 1 ng/ml.

2.5. Statistical analysis

All analyses were pre-specified in the statistical analysis plan, which was finalized prior to unblinding the study for analysis. The primary analysis of efficacy was based on data from all randomized patients (intent-to-treat population). To reduce the variance of treatment effect estimates for the primary efficacy analysis, baseline FEV_1 was defined as the average of the two FEV_1 measurements obtained on separate days within a 4 week period prior to randomization. Similarly, the Week 48 time point was the average of two FEV_1 measurements collected on 2 separate days after 48 weeks of treatment. The treatment effect was assessed via an analysis of covariance (ANCOVA) model with effects for treatment, pooled study site, and baseline FEV_1. Least squares (adjusted) means were estimated from the ANCOVA model. The sample size was chosen so that the study would have 90% power to detect a treatment difference in change from baseline FEV_1 of 0.075 L at 48 weeks. The analysis of change from baseline $FEF_{25–75\%}$ at Week 48 endpoint was based on an ANCOVA model similar to the analysis for the primary endpoint. A proportional hazards model with effects for treatment, pooled study site, and baseline FEV_1 was used for the analysis of the time to first pulmonary exacerbation. The rate of change in FEV_1 percent of predicted was analyzed using a normality-based mixed effects model, including treatment, pooled site, study week, baseline measurement as fixed effects, and patient as a random effect, and using a spatial power covariance structure.

The primary efficacy endpoint served as a gatekeeper for the analyses of the key secondary efficacy endpoints. If there was a statistically significant treatment effect for the primary efficacy endpoint, the Hochberg procedure [23] was to be applied to the three key secondary efficacy endpoints for the ITT population. Therefore, the family-wise error rate was to be controlled at an alpha level of 0.05 for the primary endpoint and three key secondary efficacy endpoints. All other p-values for secondary analyses were considered descriptive and exploratory in nature.

A priori subgroup analyses for the primary endpoint included gender, age group, country, region, genotype, baseline status of *Pseudomonas aeruginosa* infection, and baseline use of chronic inhaled antibiotics, dornase alfa, or macrolides, and occurrence of pulmonary exacerbation during the study. Overall interaction P-values were based on the ANCOVA model with terms for pooled study site, treatment, subgroup, baseline FEV_1, and treatment-by-subgroup interaction. Treatment effects (and 95% CI) for each subgroup category were estimated using an ANCOVA model with effects for pooled study site, treatment, and baseline FEV_1.

Safety was evaluated by comparing the incidence of AEs and changes in clinical laboratory tests, physical exam findings, vital signs, weight, height, body mass index, electrocardiograms, and X-rays.

All analyses were performed using SAS Version 9.0 or higher.

3. Results

3.1. Patients

A total of 685 patients were screened for the study and 466 patients (233 in each group) were randomized to study treatment in the United States [384 patients (82%)], Canada [29 patients (6%)], and Australia/New Zealand [53 patients (11%)] (Fig. 1). The treatment groups were balanced with respect to demographic and background characteristics (Table 1). On average, the patients had mild lung function impairment, with a baseline mean percent of predicted FEV_1 of 90%. 40% of patients overall (36% of placebo and 44% of denufosol subjects) were positive for *P. aeruginosa*. 24% of patients had been hospitalized for a pulmonary exacerbation in the previous year. The baseline concomitant medication use was similar in both treatment groups.

Study completion rates were high and similar between treatment groups (82% for denufosol versus 83% for placebo). The most common reason for study withdrawal was based on patients' decision, associated with the time commitment required for TID dosing. The rate of withdrawal due to adverse events was 6% in both groups. The average dosing compliance, estimated

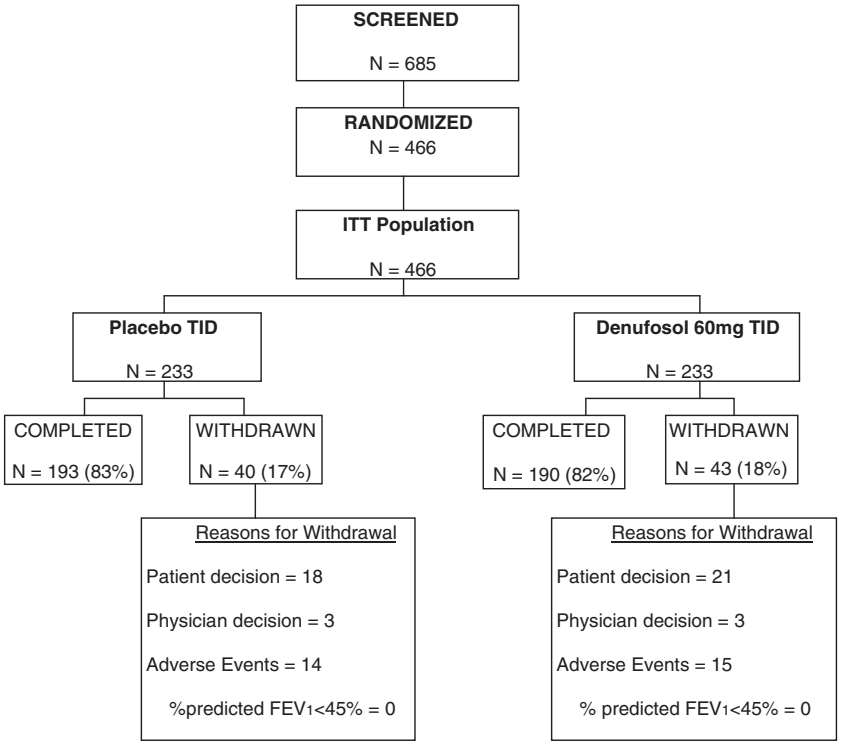

Fig. 1. Patient disposition. ITT = intent-to-treat; TID = three times daily.

using returned versus dispensed vials, was 89% for placebo and 88% for denufosol.

3.2. Lung function

There was no benefit attributable to denufosol with respect to the primary efficacy endpoint, change from baseline in FEV_1 at Week 48 endpoint. The adjusted mean change was 40 mL for denufosol and 32 mL for placebo with a resulting treatment effect of 8 mL (95% CI −0.040, 0.056; p-value=0.742; Table 2). The mean response was similar for the two groups at all visits during the placebo-controlled portion of the study (Fig. 2). In both treatment groups, a decline in FEV_1 in liters and percent of predicted was apparent at Week 4 (Fig. 2). Furthermore, no benefit in FEV_1 change from baseline was apparent for any of the pre-specified subgroups (Fig. 3), nor was any treatment-by-subgroup interaction significant. Treatment differences were not statistically significant for any other measure of lung function, including two key secondary efficacy endpoints of change from baseline $FEF_{25\%–75\%}$ at Week 48 endpoint and the rate of change in FEV_1 percent of predicted (Table 2).

The average rate of change in FEV_1 percent of predicted over 0 to 48 weeks was −3.04% for placebo vs. −2.30 for denufosol with a treatment difference (95% CI) of 0.73% (−1.01, 2.50). The estimated treatment difference was 24% relative to placebo among all patients. Given the significant decline in lung function in both groups from baseline to the Week 4 visit, we also analyzed the rate of change including only the post-baseline values. Excluding the baseline measure (Week 0), the rate of decline was not significantly different, although a non-significant trend favoring denufosol was observed (−2.32 for placebo vs. −1.40 for denufosol; a relative difference of 40%). Although the magnitude of the slope was affected by inclusion or exclusion of the baseline value, the standard deviation of the slope was not.

3.3. Pulmonary exacerbations

The time to the first pulmonary exacerbation for any of the three definitions did not differ between treatment groups (Fig. 4). As evidenced by hazard ratios (95% CI) ranging from 0.7 (0.5, 1.1) for the primary definition (IV antibiotics to treat at least one respiratory sign or symptom) to 0.9 (0.7, 1.2) for the other two definitions of event, the risk of event was lower for denufosol-treated subjects as for placebo. The overall incidence of pulmonary exacerbation by the primary definition (IV antibiotics to treat at least one respiratory sign or symptom) in the 48-week study period was 26% vs. 21% for the placebo and denufosol groups, respectively (Table 3). The frequency of exacerbations by the two other definitions was higher in both groups (39% vs. 37% and 52% vs. 47%, respectively). There was no difference between the groups with respect to the incidence or incidence density of pulmonary exacerbation according to any definition.

3.4. Pulmonary pharmacokinetic profile of denufosol

Twenty patients participated in the sputum PK sub-study. Eleven patients were randomized to receive denufosol, and nine were randomized to receive placebo. The demographics for these patients were similar to those of the Intent-to-Treat population (data not shown). Levels of denufosol in sputum after termination of inhalation were high (C_{max}=393,480 ng/g±441,535 ng/g in terms of mass of denufosol per mass of collected sputum,

Table 1
Demographic and baseline characteristics (ITT population).

	Placebo (*N*=233)	Denufosol, 60 mg (*N*=233)	Total (*N*=466)
Age, mean (SD), years	**14.7 (8.91)**	15.5 (8.89)	15.1 (8.90)
Age category, n (%)			
5–11 years	99 (42%)	92 (39%)	191 (41%)
12–18 years	81 (35%)	73 (31%)	154 (33%)
≥19 years	53 (23%)	68 (29%)	121 (26%)
Male, n (%)	126 (54%)	128 (55%)	254 (55%)
White, n (%)	221 (95%)	224 (96%)	445 (95%)
CFTR genotype, n (%)			
Δ F508 homozygous	120 (52%)	122 (52%)	242 (52%)
Δ F508 heterozygous	80 (34%)	80 (34%)	160 (34%)
Other/unknown	33 (14%)	31 (13%)	64 (14%)
BMI, mean (SD), kg/m^2	19.42 (3.705)	19.64 (3.771)	19.53 (3.736)
Baseline FEV_1 (L), mean (SD)	2.33 (0.961)	2.48 (1.019)	2.40 (0.992)
Baseline % of predicted FEV_1, mean (SD)	88.7 (10.12)	90.7 (10.74)	89.7 (10.47)
Pseudomonas aeruginosa positive respiratory culture at baseline, n (%)	84 (36%)	102 (44%)	186 (40%)
History of hospitalization for exacerbation in prior year, n (%)	58 (25%)	54 (23%)	112 (24%)
Missed days of school/work in prior 28 days, n (%)	38 (16%)	29 (12%)	67 (14%)
Baseline use of concomitant medications:			
Pancreatic enzymes, n (%)	209 (90%)	206 (88%)	415 (89%)
Bronchodilators, n (%)	196 (84%)	199 (85%)	395 (85%)
Dornase alfa, n (%)	176 (76%)	184 (79%)	360 (77%)
Chronic inhaled antibiotics, n (%)	**86 (37%)**	**95 (41%)**	181 (39%)
Chronic macrolides use, n (%)	80 (34%)	88 (38%)	168 (36%)
Chronic inhaled tobramycin use, n (%)	75 (32%)	83 (36%)	158 (34%)

Definition of abbreviations: ITT = intent-to-treat.

T_{max}=5 min±0 min), but the observed half-life of denufosol in sputum was short ($T_{1/2}$=17.2 min±8.99 min).

3.5. Safety

The study treatments were well tolerated and there was no evidence of systemic effects in any safety parameter assessed, including clinical laboratories, adverse events, and anthropomorphic measures. Nearly all patients reported at least one adverse event (see Table 4). The most commonly reported adverse events occurred with similar frequency in the two groups and were consistent with CF lung disease. The proportion of patients experiencing at least a 20% decline in FEV_1 percent of predicted at any time during the study was similar for the two treatments (15% for placebo vs. 9% for denufosol).

4. Discussion

This Phase 3 study was designed to assess the efficacy of denufosol over an extended time period and in a larger sample

Table 2
Lung function results (ITT population).

	Statistic	Placebo *N*=233	Denufosol, 60 mg *N*=233	Treatment effect [a] (95% CI)	P-value
Primary endpoint: FEV_1 (liters) change from baseline to Week 48 endpoint	LS mean (SE) mean (SE)	0.032 (0.017) 0.032 (0.018)	0.040 (0.017) 0.039 (0.019)	0.008 (0.024) (−0.040, 0.056)	0.742
Key secondary endpoint: FEV_1 percent of predicted rate of change over 0–48 weeks (%/year)	Slope (SE) SD	−3.04 (0.627) 6.74	−2.30 (0.630) 5.58	0.73 (0.889) (−1.01,2.50)	0.410
Key secondary endpoint: $FEF_{25–75\%}$ (liters/s) change from baseline to Week 48 endpoint	LS mean (SE) mean (SE)	−0.018 (0.034) −0.005 (0.035)	−0.034 (0.034) −0.047 (0.034)	−0.017 (0.048) (−0.111, 0.077)	0.728
FEV_1 percent of predicted (%) change from baseline to Week 48 endpoint	LS mean (SE) mean (SE)	−3.03 (0.553) −2.95 (0.581)	−2.76 (0.553) −2.84 (0.549)	0.27 (0.785) (−1.27, 1.81)	0.731
FEV_1 percent of predicted rate of change over 4–48 weeks (%/year)	Slope (SE) SD	−2.32 (0.698) 6.77	−1.40 (0.703) 5.17	0.92 (0.991) (−1.02,2.86)	0.354

Values displayed for the change scores are the simple means (and their standard errors), the adjusted means (and their standard errors) obtained from an ANCOVA model with effects for pooled site and baseline value, and the treatment effect (differences in adjusted means) and its 95% confidence interval. Values displayed for rates of change are the slope estimate (and its standard error), the standard deviation, and the treatment effect (difference in slopes) and its 95% confidence interval. The rate of change was analyzed using a normality-based mixed effects model, including treatment, pooled site, study week, baseline measurement as fixed effects, and patient as a random effect, and using a spatial power covariance structure. P-values for all endpoints were obtained from the same models used to estimate the treatment effect.

Definition of abbreviations: ITT = intent-to-treat; Week 48 endpoint = Week 48 value, if present, and last observation carried forward otherwise.

[a] The treatment effect is the absolute difference (denufosol minus placebo) in summary statistics. Positive values favor denufosol.

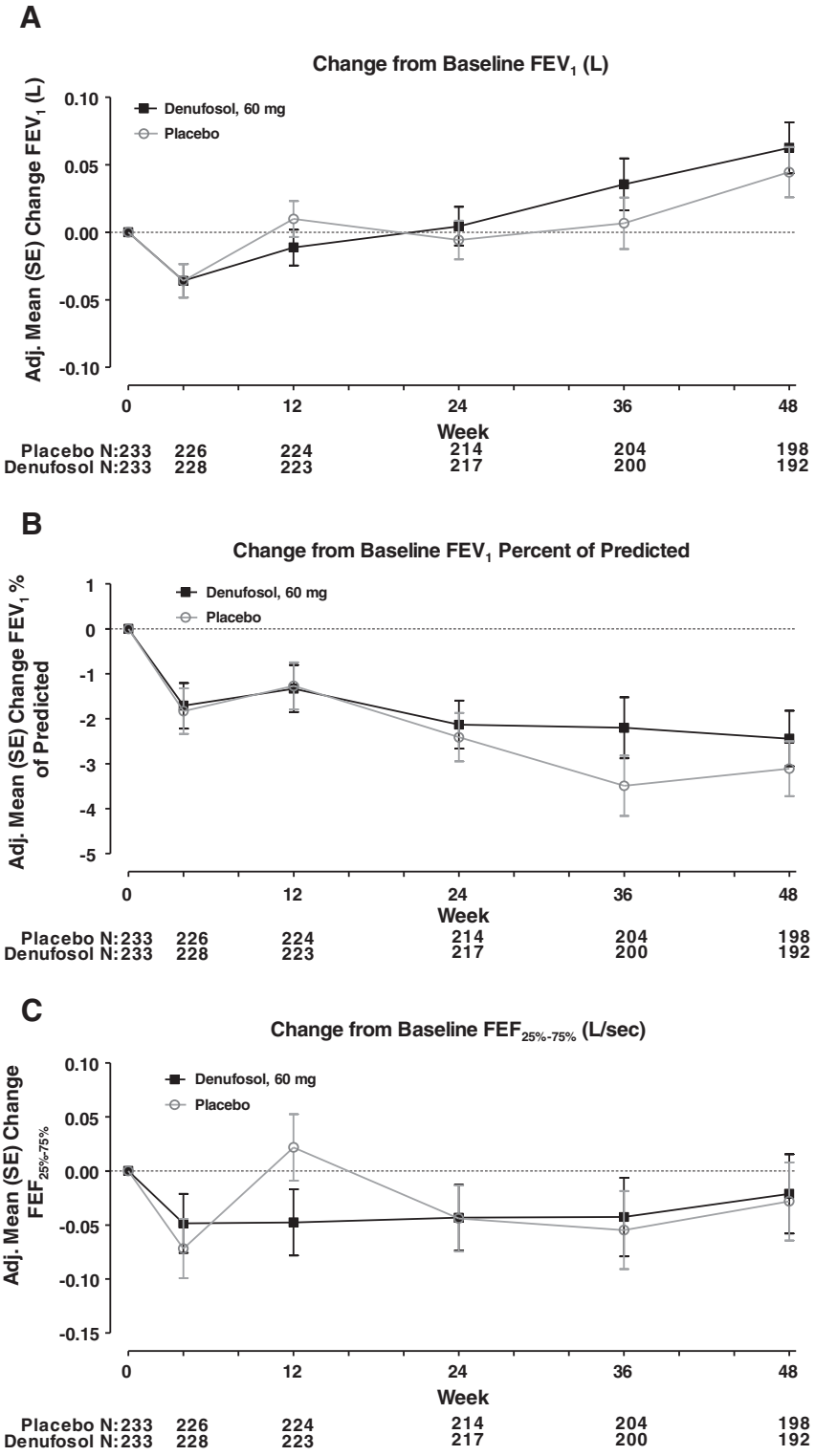

Fig. 2. Change from baseline in lung function by visit during the 48-week placebo-controlled period. Results are displayed for subjects with data at each time point. Values displayed are the adjusted means (and their standard errors) from an ANCOVA model with effects for pooled site and baseline value. CFB = change from baseline. (A) FEV_1 (L); (B) FEV_1 percent of predicted (%); (C) $FEF_{25\%–75\%}$ (L/s).

size compared to previous studies. No significant effects were observed on either lung function or pulmonary exacerbations over the 48 week double blind placebo controlled treatment period.

Results for lung function differed from those obtained among subjects treated with denufosol for 48 weeks (double-blind and open-label periods) in the previous Phase III study [19]. The mean change in FEV_1 at 48 weeks for denufosol subjects was

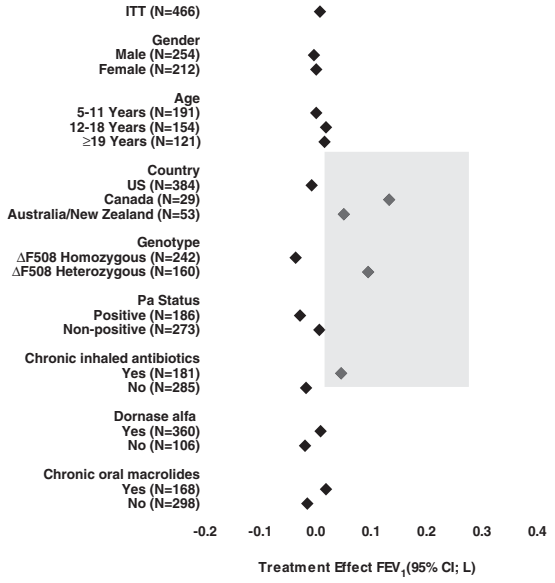

Fig. 3. Forest plot of the treatment effect (and 95% CI) for the change from baseline FEV_1 at Week 48 endpoint for prespecified subgroups. Values displayed are the treatment effects (differences in adjusted mean change from baseline) and their 95% confidence intervals, estimated from an ANCOVA model with effects for pooled site and baseline value. ITT = intent-to-treat.

0.115 L, approximately three times the magnitude of change observed in the present study among denufosol-treated subjects. Although the mean change in FEV_1 in the first 24 weeks for placebo was similar in the TIGER-2 versus the TIGER1 1 trial (−0.003 L vs. 0.003 L), the mean response among denufosol subjects was quite different (0.001 L vs. 0.048 L). Interestingly, a decline in FEV1 was observed in the first 4 weeks in both studies which could represent regression to the mean in patients with good baseline lung function who are enrolled into the study at a point of maximal clinical stability and are much more likely to worsen than improve their lung function.

There were no obvious differences in study design and/or patient populations studied to explain these differences. Most patients in the two studies had little or no impairment in lung function (mean baseline FEV_1 percent of predicted 90% vs. 92%). In the present study a greater percentage of patients was hospitalized for pulmonary exacerbation in the previous year (24% vs. 19%), which may reflect the slightly lower baseline pulmonary function values.

Denufosol has been shown to be pharmacologically active in a number of in vitro models where a robust increase in chloride secretion, inhibition of sodium absorption and stimulation of the ciliary beat frequency could be demonstrated in airway epithelial cell cultures from CF patients [24]. The non-clinical evidence would suggest that denufosol has the desirable pharmacological activity targeting the defective ion transport and impaired ciliary beat frequency underlying CF.

There are a number of possible explanations for the failure of the study to support the in vitro data and earlier promising findings. Although pharmacologically active in vitro, it is possible that denufosol's short half-life in the pulmonary tissue observed in this study contributed to the lack of clinical efficacy. Even though the

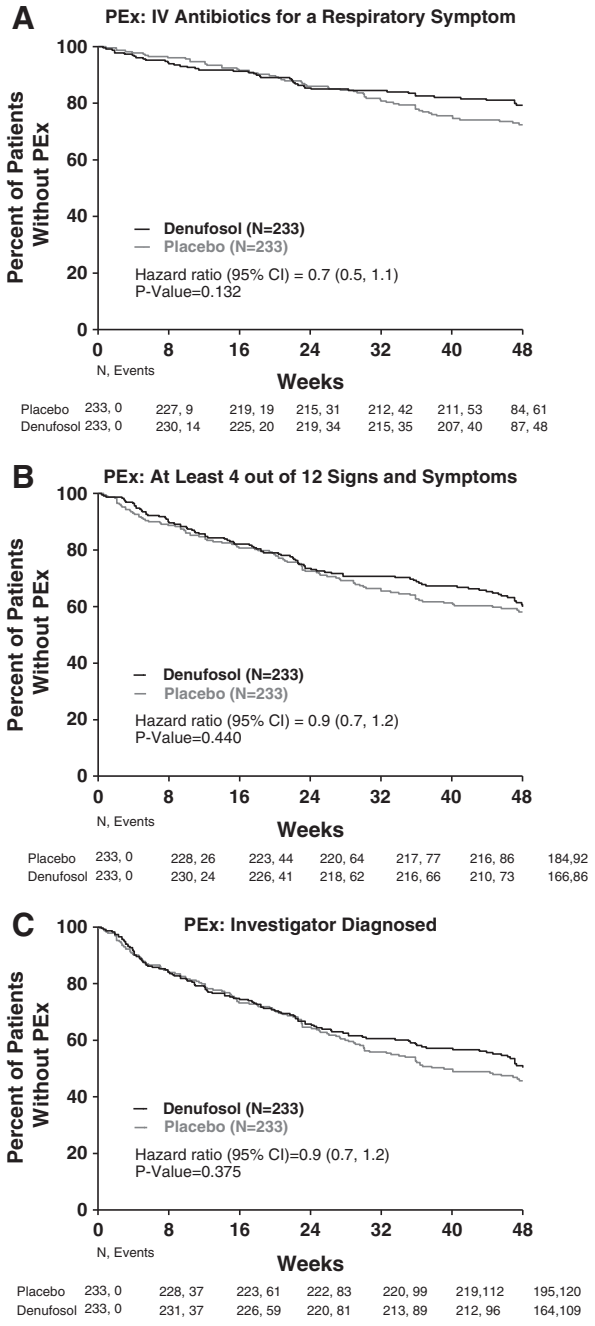

Fig. 4. Kaplan–Meier estimate of the time to first pulmonary exacerbation for three definitions of exacerbation. (A) Treatment with IV antibiotics for at least one respiratory sign or symptom. (B) Presence of at least 4 out of 12 signs and symptoms. (C) Investigator-defined event. Hazard ratios, their 95% confidence intervals, and P-values were obtained from a proportional hazards model with effects for treatment, pooled study site, and baseline FEV_1.

treatment was administered three times per day, the 17 minute half-life determined in the pulmonary PK sub-study may have been insufficient to result in a clinical benefit. The observed half life differs from what was predicted from in vitro and ex vivo studies [24]. Denufosol half-life was 25 h in ex vivo human CF sputum, but reduced to 3 h in cultured, well differentiated human nasal epithelial cells. These observations were consistent with a postulated degradation mechanism via hydrolysis by extracellular

Table 3
Incidence and incidence density of pulmonary exacerbations (ITT population).

	Randomization to Week 24			Randomization to Week 48		
	Placebo *N*=233	Denufosol, 60 mg *N*=233	P-value*	Placebo *N*=233	Denufosol, 60 mg *N*=233	P-value*
PEx: IV antibiotics for at least one respiratory sign or symptom						
Patients with ≥ 1PEx, n(%)	31 (13%)	35 (15%)	0.624	61 (26%)	49 (21%)	0.169
Number of PEx per patient, n(%):	202 (87%)	199 (85%)		172 (74%)	185 (79%)	
0	26 (11%)	31 (13%)		49 (21%)	36 (15%)	
1	5 (2%)	2 (1%)		9 (4%)	7 (3%)	
2	0	1 (<1%)		3 (1%)	4 (2%)	
3	0	0		0	1 (<1%)	
>3						
Number of PEx/patient-year [a]	0.35	0.36	0.891	0.39	0.35	0.215
PEx: ≥4 out of 12 signs and symptoms						
Patients with ≥ 1PEx, n(%)	64 (27%)	63 (27%)	0.879	92 (39%)	87 (37%)	0.525
Number of PEx per patient, n(%):	169 (73%)	171 (73%)		141 (61%)	147 (63%)	
0	46 (20%)	52 (22%)		56 (24%)	57 (24%)	
1	14 (6%)	7 (3%)		23 (10%)	19 (8%)	
2	4 (25)	3 (1%)		9 (4%)	7 (3%)	
3	0	0		4 (2%)	3 (1%)	
>3						
Number of PEx/patient-year [a]	0.85	0.73	0.173	0.77	0.67	0.093
PEx: investigator-diagnosed						
Patients with ≥ 1PEx, n(%)	83 (36%)	82 (35%)	0.885	120 (52%)	110 (47%)	0.273
Number of PEx per patient, n(%):	150 (64%)	152 (65%)		113 (48%)	124 (53%)	
0	60 (26%)	55 (24%)		64 (27%)	57 (24%)	
1	17 (7%)	20 (9%)		35 (15%)	28 (12%)	
2	4 (2%)	5 (2%)		12 (5%)	15 (6%)	
3	2 (1%)	1 (<1%)		9 (4%)	9 (4%)	
>3						
Number of PEx/patient-year [a]	1.15	1.14	0.801	1.12	1.08	0.505

*P-values for the percent of patients with ≥ 1 PEx were obtained from the Cochran–Mantel–Haenszel chi squared test stratified by pooled study site. P-values for the comparison of PEx/patient-year were obtained from a Poisson regression model with effects for pooled study site and baseline FEV_1 value.

[a] Patient-years at risk does not include days during a pulmonary exacerbation.

ectonucleotidase enzymes expressed by the pulmonary and nasal epithelia. Ectonucleotidase enzymes are up-regulated in CF airway epithelia, thus potentially accounting for the increased hydrolysis and shorter half-life of denufosol in CF airways [25].

Given the short half-life and the implied short duration of action therapy adherence may have been a key factor in the study outcome. While the data based on returned vials suggest high rates of adherence, the reliability of using vial counts as a

Table 4
Most common [a] adverse events (safety population [b]).

	Placebo *N*=232	Denufosol, 60 mg *N*=232	P-value
Patients with any adverse events, n (%)	225 (97%)	222 (96%)	0.623
Cough	140 (60%)	137 (59%)	0.850
CF lung disorder (CF specific pulmonary exacerbation)	78 (34%)	75 (32%)	0.843
Headache	47 (20%)	58 (25%)	0.267
Nasal congestion	45 (19%)	54 (23%)	0.365
Pyrexia	57 (25%)	50 (22%)	0.509
Oropharyngeal pain	46 (20%)	47 (20%)	>0.999
Lung disorder (non-CF specific pulmonary exacerbation)	45 (19%)	38 (16%)	0.468
Sputum increased	40 (17%)	37 (16%)	0.803
Rhinorrhea	43 (19%)	31 (13%)	0.163
Pseudomonas test positive	21 (9%)	26 (11%)	0.539
Pulmonary function test decreased	15 (6%)	26 (11%)	0.101
Fatigue	22 (9%)	23 (10%)	0.999

[a] Adverse events ≥ 10% in either group.

[b] The safety population differed from the intent-to-treat population because two patients (one in each group) were randomized but were not treated with study drug.

measure of adherence has been challenged. As this study required a 15 minute nebulization period per dose thrice daily over a 48-week study duration, actual adherence rates may be significantly lower based on the current knowledge of adherence to inhaled drug therapies in CF patients [26,27].

A third potential explanation for the failure of this study is that FEV_1 over 1 year may not be as sensitive to change in a CF study population with mild lung function impairment. This notion is supported by other recent studies in which previously approved therapies have failed to demonstrate a treatment benefit when studied in patients with less advanced lung disease [28,29]. Most drug development programs in CF have conducted their Phase III program in patients with moderate disease as it is more likely to improve lung function in this population. This poses a challenge for treatments aiming to prevent lung function decline which will be of highest benefit to patients prior to manifestation of significant pulmonary damage.

Finally, it is possible that another measure of disease progression could be better suited to capture treatment effects in the studied population [8]. Identifying a more appropriate endpoint is not without challenge. $FEF_{25\%–75\%}$, a measure which has been proposed as a more sensitive measure of lung function in patients with early disease, would require even more subjects than FEV_1 due to its higher intra- and inter-subject variability and did not demonstrate any treatment benefits in this study. We did not include imaging techniques or measures of airway inflammation that could potentially be influenced by preventative treatment, but these outcome measures have not yet been validated for Phase III trials in CF patients.

In summary, the data from this trial do not suggest that patients with mild lung function impairment will benefit from treatment with denufosol 60 mg administered three times daily. Importantly, the differing outcomes from two large studies of denufosol reinforce the importance of demonstrating efficacy in independent studies. While the CFTR potentiator ivacaftor has recently been shown to have remarkable benefits in CF patients carrying at least one copy of the G551D mutations, these advances in the treatment of CF lung disease have not fundamentally changed the long-term prognosis for most patients. Despite the negative results of this study, ion transport restoration independent of CFTR therefore remains a viable therapeutic target for the treatment of CF lung disease.

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Appendix A. Supplementary data

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