Denufosol Tetrasodium in Patients with Cystic Fibrosis and Normal to Mildly Impaired Lung Function

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Scientific knowledge on the subject: Abnormal ion transport and defective mucociliary clearance are fundamental pathophysiological defects that contribute to complications of cystic fibrosis lung disease. Whereas currently available therapies address these complications, the novel ion channel–regulating agent denufosol was designed to target the underlying pathophysiological defects and could potentially modify the course of the disease, particularly when administered early in the disease process.

What this study adds to the field: Results of this first large, phase 3 study of an ion channel regulator in cystic fibrosis patients with little or no baseline pulmonary function impairment suggest that denufosol has efficacy and safety profiles suitable for early intervention in cystic fibrosis lung disease.

This article has an online data supplement, which is accessible from this issue's table of content online at [www.atsjournals.org](http://www.atsjournals.org).

Author Contributions:

Conception, Design, Analysis and Interpretation: FA, RB, RW, RA, AS, TD, BR.
ABSTRACT

Rationale: Intervention for cystic fibrosis lung disease early in its course has the potential to delay or prevent progressive changes that lead to irreversible airflow obstruction.

Denufosol is a novel ion channel regulator designed to correct the ion transport defect and increase the overall mucociliary clearance in cystic fibrosis lung disease by increasing chloride secretion, inhibiting sodium absorption, and increasing ciliary beat frequency in the airway epithelium independently of cystic fibrosis transmembrane conductance regulator genotype.

Objective: To evaluate the efficacy and safety of denufosol in patients with cystic fibrosis who had normal to mildly impaired lung function characteristic of early cystic fibrosis

Methods: 352 patients ≥5 years old with cystic fibrosis who had forced expiratory volume at 1 second ≥75% of predicted normal were randomized to receive inhaled denufosol 60 mg or placebo three times daily in a Phase 3, randomized, double-blind, placebo-controlled, 24-week trial.

Measurements and Main Results: Main outcome measures included change in expiratory volume at 1 second from baseline to Week 24 endpoint and adverse events. Mean change from baseline to Week 24 endpoint in expiratory volume at 1 second (primary efficacy endpoint) was 0.048 L for denufosol (n=178) and 0.003 L for placebo (n=174; P=0.047). No significant differences between groups were observed for secondary endpoints including exacerbation rate and other measures of lung function. Denufosol was well tolerated with adverse event and growth profiles similar to placebo.

Conclusions: Denufosol improved lung function relative to placebo in cystic fibrosis patients with normal to mildly impaired lung function.

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INTRODUCTION

Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, which encodes a membrane glycoprotein that regulates chloride, sodium, and bicarbonate ion flux at the airway surface. ¹ Defective CFTR protein leads to abnormal ion transport and reduced airway surface liquid, which impair mucociliary clearance and increase susceptibility to infection and inflammation with resultant progressive airway damage. ²

Although the lungs of children with CF are thought to be normal at birth, many manifestations of pulmonary disease begin early in life. Recent studies demonstrate a significant reduction in lung function during the first 6 years of life in CF even in the absence of symptoms. ³,⁴ Likewise, structural damage to the lungs can occur in CF in the first few years of life. ⁵-⁷ Currently approved CF therapies are directed at complications such as thickened mucus and airway infection but do not address the basic pathophysiological defects that lead to loss of lung function in CF. Many patients continue to suffer progressive loss of lung function despite treatment of complications. ⁸ These findings have led to calls for intervention early in the course of CF lung disease with therapies having the potential to delay or prevent the progressive changes that lead to irreversible airflow obstruction. ⁹,¹⁰

Denufosol is a novel ion channel regulator with multimodal action designed to correct the ion transport defect independent of CFTR genotype. Denufosol increases chloride secretion through a calcium-activated chloride channel (CaCC), inhibits sodium absorption via the epithelial sodium channel (ENaC), and stimulates ciliary beat frequency (¹¹-¹⁸ and data on file, Inspire, Pharmaceuticals). These integrated actions enhance airway hydration and mucociliary clearance, ¹⁴,¹⁶ and are mediated by denufosol’s activation of P2Y₂ receptors on airway epithelia. Denufosol has the potential to be useful early in the disease process to maintain lung function or delay the progression of lung disease in CF.
Denufosol was generally well tolerated with little potential for adverse systemic effects given its very low systemic exposure following inhalation in early phase trials.\textsuperscript{16,19} In a phase 2, multiple-dose study in patients with CF, improvement in lung function over 28 days was demonstrated with denufosol relative to placebo.\textsuperscript{20} The phase 3 trial reported herein was designed to assess the safety and efficacy of denufosol versus placebo over 24 weeks in patients with CF and normal to mildly impaired baseline lung function characteristic of early CF lung disease. Some of the results of these studies have been previously reported in the form of abstracts.\textsuperscript{21-29}
METHODS

Patient Selection

Eligible patients were aged ≥5 years with a diagnosis of CF and a forced expiratory volume at 1 second (FEV\textsubscript{1}) ≥75% of predicted normal for age, height, and gender at screening. Patients were required to be clinically stable at baseline with no history of acute respiratory illnesses within the 4 weeks prior to screening and to demonstrate reproducible lung function (two FEV\textsubscript{1} values within 15% of each other on separate days) prior to randomization. Medications constituting the usual standard of care were permitted. Hypertonic saline, which was infrequently prescribed for CF lung disease at the time the trial was designed,\textsuperscript{30} was not permitted. Patients taking medications known to improve lung function were required to be taking stable doses prior to screening. 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. Informed consent was obtained from all study participants and/or guardians (including assent for minors as appropriate).

Study Design

This phase 3, randomized, double-blind, placebo-controlled trial, the Transport of Ions to Generate Epithelial Rehydration (TIGER-1) trial (Study 08-108), was conducted at 61 CF centers in the United States and one in Canada. The study consisted of a 24-week, double-blind, placebo-controlled treatment phase followed by a 24-week, denufosol-only, open-label extension (OLE) phase. Eligible patients were randomized in a ratio of 1:1 (stratified by study center) to receive denufosol tetrasodium inhalation solution 60 mg (Inspire Pharmaceuticals, Inc, Durham, NC) three times daily (TID) or placebo vehicle (0.9% wt/vol saline) TID. Study medication (4.2 mL) was delivered via the PARI LC\textsuperscript{®} Star Reusable Nebulizer (PARI Respiratory Equipment, Inc, Midlothian, VA) and the PARI PRONEB\textsuperscript{®} Ultra compressor (PARI Respiratory
Equipment, Inc). For patients taking multiple inhaled medications, the order of treatments was bronchodilator, dornase alfa, chest physiotherapy or vest, study drug, and inhaled antibiotic. Patients were assessed at clinic visits at approximately the same time of day.

The protocol was approved by the Cystic Fibrosis Foundation (CFF) Therapeutics Development Network Protocol Review Committee before it was submitted for approval to each site’s institutional review board. In addition, an independent data safety monitoring committee founded under the auspices of the CFF was chartered to prospectively evaluate safety.

**Outcome Measures**

Safety was assessed via reports of adverse events (AEs), routine clinical laboratory assessments, physical examination (including vital signs, height, weight, and body mass index), chest x-rays, and, in a subset of patients, electrocardiograms.

Pharmacokinetic assessments were undertaken in a subset of patients to assess potential systemic effects of denufosol following inhalation. Details of this pharmacokinetic evaluation are provided in the online data supplement.

The primary efficacy endpoint for the placebo-controlled phase was the change in FEV₁ from baseline to Week 24 endpoint, defined as Week 24 or the last observation carried forward for patients withdrawing prior to Week 24. Three pulmonary function measurements, FEV₁, forced vital capacity (FVC), and forced expiratory flow at 25% to 75% of FVC (FEF₂₅₋₇₅%), were obtained using standardized spirometry equipment and interpreted by a centralized spirometry reading specialist. Spirometry was obtained after the study drug had been withheld for at least 6 hours and bronchodilators had been withheld for at least 2 hours. At least three acceptable forced expiratory maneuvers were obtained from each patient at each clinic visit in accordance with American Thoracic Society standards. The highest FEV₁ and FVC values from acceptable
curves were recorded even if the two values were from different curves. In addition, the 
FEF$_{25\%-75\%}$ was recorded from the curve with the highest sum of FEV$_1$ and FVC.

Because no universally accepted definition of a pulmonary exacerbation (PE) existed, 
two a priori definitions of PE, adapted from existing definitions, were evaluated.$^{31-33}$ According to 
the primary definition, selected because it was expected to occur more commonly in this mildly 
impaired patient population, a PE occurred if a patient experienced at least four of the following 
12 signs and symptoms, regardless of required treatment: change in sputum production 
(volume, color, consistency); new/increased hemoptysis; increased cough; increased dyspnea; 
malaise/fatigue/lethargy; decreased exercise tolerance; fever; weight loss/anorexia; sinus 
pain/tenderness or change in sinus discharge; FVC or FEV$_1$ decreased 10% from previous 
value; radiographic changes indicative of pulmonary infection; and changes in chest sounds.$^{23}$ 
The signs and symptoms used in this trial were adapted from those used in previous clinical 
trials$^{23,24}$ or documented as predictors of pulmonary exacerbation.$^{33}$ Further details of this PE 
definition are given in the online data supplement. The second PE definition, expected to occur 
rarely in this patient population, included episodes that, in the judgment of the treating 
physician, required intravenous antibiotics to treat at least one respiratory sign or symptom. 
Post hoc analyses and results characterizing the relationship between PEs and change in lung 
function are described in the online data supplement as are quality of life assessments and 
results.

**Statistical Analysis**

A statistical analysis plan was finalized prior to locking the database and unblinding of 
the treatment assignments. 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. Fisher’s exact test was used to compare the
incidence of AEs. 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\textsubscript{1} was defined as the average of the two FEV\textsubscript{1} measurements obtained on separate days prior to randomization. Similarly, the Week 24 time point was the average of two FEV\textsubscript{1} measurements collected on 2 separate days after 24 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\textsubscript{1}. Least squares (adjusted) means were estimated from the ANCOVA model. Lung function in the OLE was summarized using unadjusted means. One planned interim analysis was carried out to review safety data only. A nominal alpha penalty (0.0001) was invoked as a result of the interim analysis. The sample size, which was large given the aim of demonstrating denufosol’s utility as an agent for preserving, rather than rescuing, lung function,\textsuperscript{34} was chosen so that the study would have greater than 95% power to detect a treatment difference in change from baseline FEV\textsubscript{1} of 0.075 L.

A priori subgroup analyses for the primary endpoint included gender, age group, baseline status of \textit{Pseudomonas aeruginosa} infection, and baseline use of chronic inhaled antibiotics, dornase alfa, or macrolides. Overall interaction P values were based on the ANCOVA model with terms for pooled study site, treatment, subgroup, baseline FEV\textsubscript{1}, and treatment-by-subgroup interaction. The time to first PE was analyzed using a Kaplan-Meier estimate of the survival function. Treatment differences were evaluated via the log-rank test. For the \textit{post hoc} lung function analysis, reference equations from Wang et al.\textsuperscript{35} and Hankinson et al.\textsuperscript{36} were employed to calculate FEV\textsubscript{1} percent of predicted values. Three additional subgroups were defined \textit{post hoc}: occurrence of PE by the primary definition, genotype, and use of baseline pancreatic enzyme replacement. In an additional \textit{post hoc} analysis, FEV\textsubscript{1} values were transformed to Z-scores using the methods from Stanojevic et al.\textsuperscript{37} Finally, the potential for
improvements in FEV$_1$ and in this context the role of somatic growth in this young population were assessed in a post hoc exploratory analysis. The expected change in FEV$_1$ that would have occurred had patients experienced no loss of percent predicted lung function during the 24-week placebo-controlled phase and the subsequent 24-week open-label extension was projected using the predicted values as described by Wang et al.$^{35}$ and Hankinson et al.$^{36}$
RESULTS

Patients

A total of 352 patients were randomized (intent-to-treat population of 174 in the placebo group and 178 in the denufosol group) between August 2006 and October 2007. The majority of patients (n=315, 89%) completed the 24-week, placebo-controlled, double-blind phase of the trial; withdrawal rates were similar between groups (Figure 1). All but one patient who completed the 24-week, placebo-controlled phase (n=314) entered the 24-week, denufosol-only OLE phase. Completion rates for the extension phase were high (96%). The most common reason for withdrawal was patient decision, related to the time commitment associated with TID dosing. Withdrawals due to AEs were relatively rare (1%-3%) and occurred with comparable frequency between treatment groups. Withdrawals related to physician decisions were based on patient nonadherence. Compliance information is provided in the online data supplement.

Baseline demographic characteristics and use of concomitant medications were similar between the treatment groups (Table 1). Patients on average had mild lung function impairment (mean baseline percent predicted FEV, 92%). The majority of patients were taking multiple concomitant therapies (Table 1). The percentage of patients using ≥5 concomitant CF medications for lung disease during the 24-week double-blind phase was 71.3% in the denufosol group and 71.8% in the placebo group.

Safety

Study treatments were well tolerated. Nearly all patients reported at least 1 AE during the placebo-controlled phase of the study (Table 2). The most commonly reported events were similar between treatment groups except for sinusitis, rhinorrhea, and headache, which were reported at a significantly lower incidence in the denufosol group compared with the placebo group (P<0.05).
Changes in height, weight, and body mass index did not differ between the treatment groups during the placebo-controlled phase of the study. Moreover, no evidence of any systemic effects of denufosol on any safety parameter was found, an observation that is consistent with the minimal-to-no systemic exposure to denufosol observed in the subset of patients (n=70) who underwent pharmacokinetic evaluations after acute and chronic dosing of denufosol. Further details of the pharmacokinetic evaluations are provided in the online data supplement.

**Lung Function**

Mean change from baseline in FEV\textsubscript{1} at Week 24 endpoint (primary efficacy endpoint) was significantly greater for the denufosol group than for the placebo group (treatment effect, 0.045 L; P=0.047) (Figure 2). At Week 24 endpoint, the denufosol group had improved by 0.048 L from baseline (significantly different from 0) whereas the placebo group remained relatively unchanged from baseline (0.003 L improvement; not significantly different from 0). The groups did not significantly differ with respect to percent predicted FEV\textsubscript{1} or Z-scores, the results for which were similar to those for percent predicted FEV\textsubscript{1} (Figure 1 E1 in the online data supplement). No significant treatment-by-subgroup interactions in the analysis of change in FEV\textsubscript{1} were observed; the treatment effect was generally consistent across multiple subgroups (Figure 2). By the end of the OLE, lung function in the group receiving denufosol for the entire study had improved by 0.115 L (Figure 3A). After being switched to denufosol during the OLE, the placebo group for the double-blind phase of the study had a mean improvement of 0.078 L in FEV\textsubscript{1} at Week 48.

In the *post hoc* analysis on somatic growth in this population, the average projected improvement in FEV\textsubscript{1} at Week 24 was 82 mL in the denufosol group and 80 mL in the placebo group with a continued projected improvement of 154 mL in the denufosol group and 146 mL in
the placebo group by the end of the 48-week study. At Week 24, the observed changes were 47 mL (57% of projected change) in the denufosol group compared with 17 mL (21% of projected change) in the placebo group. Week 24 Endpoint values, which accounted for dropouts during the first 24 weeks, were 48 mL (64% of projected change) in the denufosol group and 3 mL (4% of projected change) in the placebo group. By the conclusion of the 48-week observation period with continued treatment, the denufosol group achieved a mean improvement of 115 mL (75% of projected change). After 24 weeks the placebo patients crossed over to denufosol and achieved an improvement of 78 mL (53% of projected change) at Week 48.

FVC and FEF\textsubscript{25\%-75\%} did not significantly differ between groups at Week 24 endpoint, but a trend toward improvement in FEF\textsubscript{25\%-75\%} with denufosol over placebo was observed (difference of 0.088 L/sec; \(P=0.072\)) (Figure 3B). At Week 24 endpoint, the mean change in FEF\textsubscript{25\%-75\%} for placebo was −0.047 L/sec compared with 0.041 L/sec for denufosol. Mean changes in FEF\textsubscript{25\%-75\%} at Week 24 endpoint were not significantly different from 0 for either group.

**Pulmonary Exacerbations**

During the double-blind phase, no significant differences between groups were observed in the time to first PE using either the primary definition or the secondary definition (19% placebo vs 26% denufosol, \(P=0.088\) for primary definition; 7.5% vs 9.0%, \(P=0.574\) for secondary definition) (Figure E2 in the online data supplement). Results of post hoc analyses to characterize the relationship between PEs and change in lung function are described in the online data supplement and shown in Figure E3 in the online data supplement.
DISCUSSION

Abnormal ion transport and defective mucociliary clearance are fundamental pathophysiological defects that contribute to complications of CF lung disease including mucus plugging, chronic bacterial infection, inflammation, and progressive airway damage. Whereas currently available therapies for CF lung disease target these complications, the novel ion channel–regulating agent denufosol was designed to target the underlying pathophysiological defects and could potentially modify the course of the disease, particularly when administered early in the disease process. This first large, phase 3 study of an ion channel regulator in patients with CF was designed to examine the safety and efficacy of denufosol in patients with little or no baseline pulmonary function impairment as is characteristic of early CF lung disease. Denufosol improved lung function relative to placebo by 45 mL (approximately 2% of the baseline) during 24 weeks of treatment. During continued treatment with denufosol during the OLE, the improvement observed during the placebo-controlled phase was maintained, a notable finding given that this improvement occurred in patients with little or no pulmonary function impairment who were treated with many concomitant medications.27 The majority of patients took ≥5 concomitant therapies for CF lung disease: 71.3% of the denufosol group and 71.8% of the placebo group during the double-blind phase. The difficulty in demonstrating improvement in FEV₁ in patients with mildly impaired baseline lung function is illustrated by the recent finding that azithromycin, which has been demonstrated to improve FEV₁ in CF patients with moderate-to-severe baseline lung function impairment,38 demonstrated some benefit but did not improve the primary endpoint of FEV₁ (or other pulmonary function endpoints) relative to placebo in a 24-week randomized, double-blind trial in 260 CF patients with mildly impaired baseline lung function.39

While the superiority of denufosol over placebo for the primary endpoint was expected, large denufosol-associated improvements versus baseline in FEV₁ and other pulmonary
function measures were not anticipated given that denufosol was designed to prevent or delay loss of lung function rather than as a rescue therapy. In the current study, the increase in FEV$_1$ over time that would have been expected in a healthy growing population was preserved to a greater degree by denufosol than by placebo. The decline in lung function in the placebo group is consistent with previous findings demonstrating that normal to mildly impaired baseline lung function (i.e., high baseline FEV$_1$) is an independent risk factor for lung function decline in young patients with CF.\textsuperscript{40} In a study of children ranging in age from 6 to 17 years, the better the baseline lung function, the steeper the decline in FEV$_1$ over a 3- to 6-year period.\textsuperscript{40}

Recent evidence supports the hypothesis that much of the decline in lung function in CF is associated with the occurrence of PEs.\textsuperscript{41-44} For example, in a case-control analysis of patients admitted for their first pulmonary exacerbation between 2001-2006 at a single pediatric center, approximately one in four patients with CF failed to recover to baseline lung function by 3 months after a pulmonary exacerbation despite treatment with intravenous antibiotics.\textsuperscript{42} Failure to recover to baseline was associated with pre-admission degree of decline in FEV$_1$, a finding that the authors suggested highlights opportunities for earlier intervention to improve lung function outcomes. In a similar analysis of 8479 pulmonary exacerbations treated with intravenous antibiotics in the CF Foundation Patient Registry from 2003 to 2006, 25% of exacerbations failed to recover to baseline FEV$_1$.\textsuperscript{43} Results of post hoc analyses summarized in the online data supplement and Figure E2 in the online data supplement show that patients with at least one PE in the current study had greater loss in lung function during the study than patients who did not experience an exacerbation, a finding confirming previous results.\textsuperscript{41} Although denufosol did not reduce the incidence of PEs, treatment with denufosol in patients with PEs was associated with a trend toward higher FEV$_1$ values at the Week 24 endpoint than treatment with placebo in patients with PEs (Figure E3 in the online data supplement). These results suggest that denufosol treatment either leads to PEs that are qualitatively different from
those experienced by patients receiving placebo or protects against the loss of lung function
associated with exacerbations. These possibilities are being explored prospectively in the
ongoing phase 3 TIGER-2 trial.

Denufosol was generally well tolerated in the current study as in previous studies. The
AE profile for denufosol was similar to that of placebo in keeping with the demonstrated low-to-nil systemic absorption of the drug. Low potential for systemic side effects is an important
consideration for early intervention therapy for CF, which could be administered chronically over
decades beginning during the first months of life. The incidence of adverse events related to
sinopulmonary disease was generally lower with denufosol than placebo. The potential clinical
significance of this finding is undetermined.

In conclusion, the results of this trial suggest that denufosol has efficacy and safety profiles
suitable for potential early intervention in CF lung disease. Designed to target basic defects in
CF lung disease across CFTR genotypes, denufosol increases chloride secretion, inhibits
sodium absorption, and increases ciliary beat frequency in the airways. Denufosol improved
lung function versus placebo in patients having minimally impaired to normal baseline lung
function characteristic of early CF lung disease. Because this study included spirometry as its
primary efficacy endpoint, children younger than 5 years were not enrolled. However,
considering the evidence that early inflammation and infection results in impaired lung function
and structural damage in early childhood, investigation in future studies of the effects of
denufosol during the first 5 years of life is warranted. A second phase 3 trial that enrolled
patients 5 years of age and older and that has a longer placebo-controlled treatment phase is
ongoing to further elucidate the utility of denufosol for patients with CF.
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Dr. Accurso serves as Director of the Cystic Fibrosis Therapeutics Development Biochemical Biomarker Laboratory and of the Sweat Analysis Laboratory. In addition, Dr. Accurso is site Principal Investigator of the Cystic Fibrosis Foundation Therapeutics Development University of Colorado Denver Site. In these capacities during the last five years, Dr. Accurso has consulted for or received grants and contracts from following companies: Corus Pharma, Inc., CSL Behring LLC, Digestive Care, Inc., Genentech, Inc., Gilead Sciences, Inc., Inspire Pharmaceuticals, Inc. KaloBios Pharmaceuticals, Inc., Mpex Pharmaceuticals, Inc., Novartis Pharmaceuticals, Corp., PTC Therapeutics, Inc., Solvay Pharmaceuticals, Inc., Sucampo Pharmaceuticals, Inc., Vertex Pharmaceuticals, Inc., and ViaSys Manufacturing, Inc. He has received consultancy fees from Inspire Pharmaceuticals, Inc. and PTC Therapeutics, Inc. He has received travel reimbursement from Inspire Pharmaceuticals Inc. He and his family have no ownership or stock/ stock options in any of these companies. He has also received grant support from the Cystic Fibrosis Foundation and the National Institute of Health. Dr. Moss discloses receipt of consultancy fees from Novartis, Genentech, Mpex, Vertex, PTC, Aridis,
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APPENDIX

The following principal investigators and primary research coordinators participated in the TIGER-1 trial: R. Ahrens, (University of Iowa Hospitals and Clinics, Iowa City, IA); R. Anbar, D. Lindner (SUNY Upstate Medical University Hospital, Syracuse, NY); P. Anderson (University of Arkansas for Medical Sciences, Little Rock, AR); A. Atlas (The Respiratory Center for Children, Morristown Memorial Hospital, Morristown, NJ); Y. Berthiaume, N. Beaudoin (Centre de recherche du CHUM, Montréal, QC, Canada); D. Bisberg, P. Pock (Saint Barnabas Medical Center, Livingston, NJ); S. Boas (Cystic Fibrosis Institute, Glenview, IL); D. Borowitz, J. Smith (State University of New York at Buffalo, Buffalo, NY); A.S. Chidekel (Alfred I. duPont Hospital for Children, Wilmington, DE); B. Chipps (Capital Allergy & Respiratory Disease Center, Sacramento, CA); J.F. Chmiel, B. Ksenich (University Hospitals of Cleveland, Cleveland, OH); J.P. Clancy (University of Alabama at Birmingham, Birmingham, AL); J. Colombo, D. Heimes (University of Nebraska Medical Center, Omaha, NE); C. Daines (Cincinnati Children’s Hospital Medical Center, Cincinnati, OH); J. DeCelie-Germana, S. Galvin (Cystic Fibrosis Center of Schneider Children’s Hospital, North Shore-Long Island Jewish Health System, Great Neck, NY); R. Deterding (The Children’s Hospital, Aurora, CO); E. DiMango (Columbia University Medical Center, New York, NY); H. Dorkin (Children’s Hospital, Boston, MA); A. Dozor, I. Gherson (New York Medical College, Hawthorne, NY); J. Dunitz (University of Minnesota Medical Center, Fairview, Minneapolis, MN); M. Egan, J. Young (Yale School of Medicine, New Haven, CT); G. Elliott, J. Gadd (Pediatric Pulmonary Clinic, Richmond, VA); P. Fornos (Alamo
Clinical Research Associates, San Antonio, TX); M. Franco (Miami Children's Hospital, Miami, FL); D. Froh, R. Kelly (University of Virginia, Charlottesville, VA); D. Geller, D. Cook (Nemours Children's Clinic, Orlando, FL); R. Gibson, A. Genatossio (University of Washington/Seattle Children's Hospital, Seattle, WA); G. Gong (Phoenix Children's Hospital, Phoenix, AZ); G. Graff (Penn State Milton S. Hershey Medical Center, Hershey, PA); K. Hardy, A. Robles (Bay Area Pediatric Pulmonary Medical Corporation, Oakland, CA); D. Hicks (Children's Hospital of Orange County, Orange, CA); M. Howenstine, L. Bendy (James Whitcomb Riley Hospital for Children, Indianapolis, IN); K. Jones, A. Gardner (Louisiana State University Health Sciences Center, Shreveport, LA); J. Kanga, B. Owsley (University of Kentucky, Lexington, KY); J. Kreindler (Children's Hospital of Pittsburgh, Pittsburgh, PA); C. Lapin, G. Drapeau (Connecticut Children's Medical Center, Hartford, CT); R. Lee, M. Dillard (Naval Medical Center Portsmouth, Portsmouth, VA); T. Liou (University of Utah, Salt Lake City, UT); K.S. McCoy, P. Olson (Nationwide Children's Hospital, Columbus, OH); S. Millard (Spectrum Health Hospitals, Grand Rapids, MI); R. Moss, C. Dunn/Z. Davies (Stanford University, Palo Alto, CA); C. Nakamura, T. Brascia (Children's Lung Specialists, Ltd, Las Vegas, NV); S. Nasr (University of Michigan Health System, Ann Arbor, MI); C. Oermann, L. Traplena (Baylor College of Medicine, Houston, TX); C. Prestidge (Dallas Cystic Fibrosis Center/Children's Medical Center of Dallas, Dallas, TX); A. Prestridge, D. Delute (Children's Memorial Hospital, Chicago, IL); C. Ren (University of Rochester, Rochester, NY); G. Retsch-Bogart, D. Towle/C. Barlow (University of North Carolina at Chapel Hill, Chapel Hill, NC); S. Reyes (Oklahoma Children's Memorial Hospital, Oklahoma City, OK); D. Roberts, V. Roberts (Pediatric Breathing Disorders, Anchorage, AK); M. Rock, L. Makholm (University of Wisconsin Hospital and Clinics, Madison, WI); R. Rubenstein, C. Kubrak/C. Murray (The Children's Hospital of Philadelphia, Philadelphia, PA); D. Schaeffer, E. DeLuca (Nemours Children's Clinic, Jacksonville, FL); M. Schechter, J. Peabody (Emory University, Atlanta, GA); D. Schellhase (Arkansas Children's Hospital Research Institute, Little 21
Rock, AR); P. Walker, C. Grece (Saint Vincent’s Catholic Medical Center, New York, NY); M. Wall, A. Guzik (Oregon Health Sciences University, Portland, OR); D. Weiner, A. Horn (Children’s Hospital of Pittsburgh, Pittsburgh, PA); D.B. Willey-Courand (University of Texas Health Science Center at San Antonio, San Antonio, TX); R.W. Wilmott, V. Kociela (Saint Louis University, St. Louis, MO); M. Woo (Children’s Hospital Los Angeles, Los Angeles, CA); J. Wooldridge, L. Duan (Cincinnati Children’s Hospital Medical Center, Cincinnati, OH); P. Zeitlin (Johns Hopkins University School of Medicine, Baltimore, MD); J. Zuckerman, R. Kennedy-DuDevoir (Maine Medical Center, Portland, ME).
REFERENCES


31. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, Rosenstein BJ, Smith AL, Wohl ME, for the Pulmozyme Study Group. Effect of


with Pseudomonas aeruginosa: a randomized controlled trial. *JAMA* 2010;303:1707-1715.


44. Sanders DB, Bittner RC, Rosenfeld M, Redding GJ, Goss CM. Pulmonary exacerbations are associated with subsequent FEV₁ decline in both adults and children with cystic fibrosis. *Pediatr Pulmonol* 2010 Oct 21 [Epub ahead of print]
Table 1. Demographic and Baseline Characteristics (ITT Population).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (n=174)</th>
<th>60 mg (n=178)</th>
<th>Total (N=352)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>14.9 (9.33)</td>
<td>14.3 (8.77)</td>
<td>14.6 (9.04)</td>
<td>0.498</td>
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<tr>
<td>Age range, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5-11 y</td>
<td>77 (44.3)</td>
<td>81 (45.5)</td>
<td>158 (44.9)</td>
<td>0.737</td>
</tr>
<tr>
<td>12-18 y</td>
<td>64 (36.8)</td>
<td>59 (33.1)</td>
<td>123 (34.9)</td>
<td></td>
</tr>
<tr>
<td>≥19 y</td>
<td>33 (19.0)</td>
<td>38 (21.3)</td>
<td>71 (20.2)</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>85 (48.9)</td>
<td>96 (53.9)</td>
<td>181 (51.4)</td>
<td>0.370</td>
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<tr>
<td>Caucasian, n (%)</td>
<td>163 (93.7)</td>
<td>170 (95.5)</td>
<td>333 (94.6)</td>
<td>0.426</td>
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<tr>
<td>CF genotype, n (%)</td>
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<td>0.238</td>
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<tr>
<td>Δ F508 homozygous</td>
<td>94 (54.0)</td>
<td>88 (49.4)</td>
<td>182 (51.7)</td>
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<tr>
<td>Δ F508 heterozygous</td>
<td>51 (29.3)</td>
<td>65 (36.5)</td>
<td>116 (33.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (4.0)</td>
<td>15 (8.4)</td>
<td>22 (6.3)</td>
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<tr>
<td>Unknown/Not done</td>
<td>22 (12.6)</td>
<td>10 (5.6)</td>
<td>32 (9.1)</td>
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<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>19.29 (3.43)</td>
<td>19.72 (3.85)</td>
<td>19.50 (3.65)</td>
<td>0.226</td>
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<tr>
<td>Baseline FEV₁, mean (SD), L</td>
<td>2.31 (0.91)</td>
<td>2.33 (0.96)</td>
<td>2.32 (0.93)</td>
<td>0.835</td>
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<tr>
<td>Range</td>
<td>0.8-4.7</td>
<td>0.9-5.0</td>
<td>0.8-5.0</td>
<td></td>
</tr>
<tr>
<td>Baseline FEF₂₅₋₇₅%, mean (SD), L/sec</td>
<td>2.39 (1.00)</td>
<td>2.32 (1.03)</td>
<td>2.35 (1.02)</td>
<td>0.594</td>
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<tr>
<td>Range</td>
<td>0.5-5.3</td>
<td>0.5-6.1</td>
<td>0.5-6.1</td>
<td></td>
</tr>
<tr>
<td>Baseline % of predicted FEV₁, mean (SD)</td>
<td>91.9 (11.3)</td>
<td>92.7 (11.4)</td>
<td>92.3 (11.3)</td>
<td>0.422</td>
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<tr>
<td>Range</td>
<td>63-128</td>
<td>74-126</td>
<td>63-128</td>
<td></td>
</tr>
<tr>
<td>Measure</td>
<td>Group A</td>
<td>Group B</td>
<td>Group C</td>
<td>p-value</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Baseline % of predicted FEF&lt;sub&gt;25%-75%&lt;/sub&gt;, mean (SD)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>83.9 (24.8)</td>
<td>81.3 (24.5)</td>
<td>82.6 (24.6)</td>
<td>0.351</td>
</tr>
<tr>
<td>Range</td>
<td>31-184</td>
<td>34-143</td>
<td>31-184</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa positive, n (%)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>84 (48.3)</td>
<td>74 (41.6)</td>
<td>158 (44.9)</td>
<td>0.173</td>
</tr>
<tr>
<td>Hospitalized for exacerbation, n (%)</td>
<td>36 (20.7)</td>
<td>30 (16.9)</td>
<td>66 (18.8)</td>
<td>0.353</td>
</tr>
<tr>
<td>Days missed (school/work), mean (SD), n‡</td>
<td>0.41 (1.54)</td>
<td>0.29 (0.96)</td>
<td>0.35 (1.28)</td>
<td>0.765</td>
</tr>
<tr>
<td>Range</td>
<td>0-10.0</td>
<td>0-7.0</td>
<td>0-10.0</td>
<td></td>
</tr>
<tr>
<td>Baseline Use:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic enzyme use, n (%)</td>
<td>165 (94.8)</td>
<td>159 (89.3)</td>
<td>324 (92.0)</td>
<td>0.054</td>
</tr>
<tr>
<td>Bronchodilator use, n (%)</td>
<td>137 (78.7)</td>
<td>146 (82.0)</td>
<td>283 (80.4)</td>
<td>0.433</td>
</tr>
<tr>
<td>Dornase alfa use, n (%)</td>
<td>134 (77.0)</td>
<td>137 (77.0)</td>
<td>271 (77.0)</td>
<td>0.868</td>
</tr>
<tr>
<td>Macrolide use, n (%)</td>
<td>70 (40.2)</td>
<td>69 (38.8)</td>
<td>139 (39.5)</td>
<td>0.652</td>
</tr>
<tr>
<td>Chronic inhaled antibiotic use, n (%)</td>
<td>66 (37.9)</td>
<td>65 (36.5)</td>
<td>131 (37.2)</td>
<td>0.794</td>
</tr>
<tr>
<td>Chronic inhaled tobramycin use, n (%)</td>
<td>65 (37.4)</td>
<td>61 (34.3)</td>
<td>126 (35.8)</td>
<td>0.563</td>
</tr>
</tbody>
</table>

BMI denotes body mass index, CF cystic fibrosis, FEF<sub>25%-75%</sub> forced expiratory flow at 25% to 75% of forced vital capacity, FEV<sub>1</sub> forced expiratory volume at 1 second, ITT intent-to-treat, and SD standard deviation.

*Could not be calculated for children aged 5 to 7 years.

†For the previous year.

‡During the 28 days prior to visit 1.
Table 2. Most Common* Adverse Events (Safety Population†) in the Double-Blind Phase.

<table>
<thead>
<tr>
<th>Adverse event, n (%)</th>
<th>Placebo (n=175)</th>
<th>Denufosol 60 mg (n=177)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients who reported any adverse event</td>
<td>169 (97)</td>
<td>165 (93)</td>
</tr>
<tr>
<td>Cough</td>
<td>104 (59)</td>
<td>97 (55)</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>34 (19)</td>
<td>26 (15)</td>
</tr>
<tr>
<td>Pharyngolaryngeal pain</td>
<td>32 (18)</td>
<td>34 (19)</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>31 (18)</td>
<td>18 (10)‡</td>
</tr>
<tr>
<td>Productive cough</td>
<td>30 (17)</td>
<td>30 (17)</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>31 (18)</td>
<td>17 (10)‡</td>
</tr>
<tr>
<td>Pseudomonal infection</td>
<td>23 (13)</td>
<td>15 (8)</td>
</tr>
<tr>
<td>Pulmonary exacerbation</td>
<td>51 (29)</td>
<td>61 (34)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>27 (15)</td>
<td>36 (20)</td>
</tr>
<tr>
<td>Headache</td>
<td>44 (25)</td>
<td>20 (11)§</td>
</tr>
</tbody>
</table>

*Adverse events ≥10% in any group.

†The safety population differed from the intent-to-treat (ITT) population because 1 patient was randomized to denufosol but was treated with placebo. This patient is counted in the denufosol group for the ITT population and in the placebo group for the safety population.

‡P<0.05 vs placebo.

§P<0.01 vs placebo.
FIGURE LEGENDS

Figure 1. Patient Disposition. FEV$_1$ denotes forced expiratory volume at 1 second, ITT intent-to-treat, phys physician, pt patient, TID three times daily, and W/D withdrawal.

Figure 2. Effect of Denufosol Treatment on Lung Function. Treatment effect and 95% confidence interval (CI) for the change in FEV$_1$ from baseline to Week 24 endpoint for the intent-to-treat (ITT) population and by subgroup. FEV$_1$ denotes forced expiratory volume at 1 second.

Figure 3. Change in Lung Function During the 48-Week Study. (A) Mean change from baseline FEV$_1$ by visit during the 48-week study. (B) Mean change from baseline FEF$_{25\%-75\%}$ by visit during the 48-week study. Results are displayed for subjects with data at each time point. Error bars represent standard error; shaded area represents the open-label extension phase of the study during which all patients received denufosol. CFB denotes change from baseline, FEF$_{25\%-75\%}$ forced expiratory flow at 25% to 75% of forced vital capacity, and FEV$_1$ forced expiratory volume at 1 second.
Figure 1

Screened (N=461)

Randomized (ITT Population) (N=352)

Received double-blind study drug (N=352)

Placebo TID (n=174)

Completed (n=156; 90%)
Withdrawn (n=18; 10%)

Open-label extension Denufosol 60 mg TID (n=155)

Completed (n=146; 94%)
Withdrawn (n=9; 6%)

Reason for W/D
Pt decision (n=10)
Phys decision (n=1)
Adverse event (n=2)
Other (n=5)

Denurosol 60 mg TID (n=178)

Completed (n=159; 89%)
Withdrawn (n=19; 11%)

Reason for W/D
Pt decision (n=12)
Phys decision (n=1)
Adverse event (n=5)
Other (n=1)

Open-label extension Denufosol 60 mg TID (n=159)

Completed (n=156; 98%)
Withdrawn (n=3; 2%)

Reason for W/D
Pt decision (n=2)
Other (n=1)

Screening failure (n=109)
Reason for screening failure (multiple may apply)
FEV1 <75% predicted or not reproducible (n=57)
Exclusionary medication use and/or not clinically stable (n=36)
Withdraw informed consent (n=8)
Lab or chest x-ray results (n=8)
Other (n=5)
A priori–specified subgroups

**Gender (P=0.539)**
- Male (n=181)
- Female (n=171)

**Age (P=0.840)**
- 5-11 years (n=158)
- 12-18 years (n=123)
- >18 years (n=71)

**Pseudomonas aeruginosa (P=0.971)**
- Positive (n=158)
- Nonpositive (n=193)

**Chronic inhaled antibiotics (P=0.374)**
- Yes (n=131)
- No (n=221)

**Dornase alfa (P=0.146)**
- Yes (n=271)
- No (n=81)

**Chronic oral macrolides (P=0.420)**
- Yes (n=139)
- No (n=213)
Figure 3

A

Mean CFB FEV, L

-0.05
0.00
0.05
0.10
0.15

-0.05
0.00
0.05
0.10

Week

Placebo

Denufosol

B

Mean CFB FEF_{25\%-75\%}, L/sec

-0.2
-0.1
0.0
0.1
0.2

-0.2
-0.1
0.0
0.1

Week
Online Data Supplement

Denufosol Tetrasodium in Patients with Cystic Fibrosis and Normal to Mildly Impaired Lung Function

Frank J Accurso, MD; Richard B Moss, MD; Robert W Wilmott, MD; Ran D Anbar, MD; Amy E Schaberg, BSN, RN; Todd A Durham, MS; Bonnie W Ramsey, MD; and the TIGER-1 Investigator Study Group
METHODS

**Compliance**

Compliance with study medication was assessed throughout the study by reconciling the number of vials dispensed, the number of vials returned unused, and the duration of time between visits. It was deemed an unacceptable infection risk to the patient to require them to keep and return used study vials.

**Pharmacokinetics**

Blood was drawn for pharmacokinetic evaluation in a subset of patients to assess the potential systemic exposure to denufosol following inhalation. By prospective design, approximately twice as many patients were assessed for exposure following the first dose compared with chronic dosing. Plasma concentrations of the drug were assessed by high-performance liquid chromatography with tandem mass spectrometric detection (HPLC/MS/MS) pre-dose and 5, 15, and 60 minutes postdose on the first day of dosing (n=62; acute dosing) and at the same time points after 12 and 24 weeks of treatment (n=30 and n=28, respectively; chronic dosing). The assay for detection of denufosol was validated to have a lower limit of quantitation of 0.5 ng/mL. One site’s values were excluded from the analysis due to sample contamination (denufosol detected in plasma samples collected predose on first day of dosing).

**Pulmonary Exacerbation**

Because there is no universally accepted definition of a pulmonary exacerbation, two definitions were generated for use in this trial based on a constellation of 12 potential signs and symptoms evaluated in previous studies.\(^1,2\) Decreased exercise tolerance and fever\(^1\) (also noted in Fuchs et al.\(^2\) as temperature above 38 °C) were included as were eight additional signs and symptoms\(^2\): change in sputum; new or
increased hemoptysis; increased cough; increased dyspnea; malaise, fatigue, or lethargy; weight loss or anorexia; decrease in pulmonary function by 10% or more from a previously recorded value; and radiograph changes indicative of pulmonary infection. The remaining two signs and symptoms were adapted from those used by Fuchs et al.\textsuperscript{2}: sinus pain/tenderness or change in sinus discharge (listed as separate signs and symptoms in Fuchs et al.\textsuperscript{2}); and changes in chest sounds, regardless of treatment (described as a change in physical examination of the chest in Fuchs et al.\textsuperscript{2}). A sign-and-symptom–based definition of pulmonary exacerbation, based on the presence of any four out of 12 signs and symptoms from Fuchs et al.,\textsuperscript{2} was also used in the previous trial.\textsuperscript{3}

A post hoc analysis was undertaken to characterize the relationship between PEs and change in lung function by describing the outcome in terms of change in FEV\textsubscript{1} from baseline to Week 24 endpoint separately for those patients who did and those who did not experience a PE according to the primary definition.

**Quality of Life**

Quality of life was assessed at baseline and at 12-week intervals with a cystic fibrosis–specific questionnaire, the Cystic Fibrosis Questionnaire–Revised (CFQ-R).\textsuperscript{4} The patient or the patient’s proxy (for young children) completed age-appropriate versions of the questionnaire. For each CFQ-R domain, the possible score ranged from 0 to 100, with higher scores indicating better health-related quality of life.
RESULTS

Compliance

Compliance rates were high and were similar between treatment groups. During the double-blind phase, 91% of placebo patients and 88% of denufosol patients took at least 70% of their medication. The average number of daily doses administered was similar between treatment groups (placebo, 2.68; denufosol, 2.65).

Pharmacokinetics

The majority of patients had no measurable level of denufosol following the first dose (71%) and the last dose (71%), whereas 45% had no measurable concentrations at any time point during 24 weeks of TID dosing. Overall, the systemic exposure to denufosol following 24 weeks of treatment was generally low (mean $C_{\text{max}}$, 0.35 ng/mL $\pm$ 0.15 ng/mL) and transient ($T_{\text{max}}$, 5 minutes). The safety profile during the OLE phase was generally similar with respect to the nature and extent of adverse events observed during the placebo-controlled phase.

Additional Post Hoc Subgroup Analyses of FEV$_1$

Additional subgroup analyses of forced expiratory volume at 1 second (FEV$_1$) at Week 24 endpoint (the primary efficacy time point) included the following: cystic fibrosis transmembrane conductance regulator genotype ($\Delta F508$ homozygous, $\Delta F508$ heterozygous, and other/unknown) and pancreatic enzyme use at baseline (yes, no). The treatment difference in adjusted mean change in FEV$_1$ at Week 24 endpoint was similar according to genotype: 0.033 L (95% confidence interval [CI]: $-0.031, 0.097$) for $\Delta F508$ homozygous; 0.078 L (95% CI: $-0.001, 0.157$) for $\Delta F508$ heterozygous; and
0.031 L (95% CI: −0.147, 0.208) for other/unknown. The magnitude of the treatment effect for pancreatic enzyme users (0.048 L; 95% CI: 0.001, 0.095) was similar to that for nonusers (0.066 L; 95% CI: −0.065, 0.196). The P value for the test of the treatment-by-subgroup interaction was 0.646 for genotype and 0.960 for pancreatic enzyme use.

**Pulmonary Exacerbations**

In the post hoc analysis to characterize the relationship between PEs and change in lung function, the mean change in FEV$_1$ from baseline to Week 24 endpoint in patients who had a PE (n=80) was significantly worse than in patients who did not experience a PE (n=272; P=0.006) (Figure E3A). The mean change in FEV$_1$ was −0.034 L (95% CI: −0.082, 0.014) for those experiencing a PE compared with 0.043 L (95% CI: 0.018, 0.068) for those who did not experience a PE. In patients experiencing a PE, the denufosol group had a mean change in FEV$_1$ of 0.002 L (95% CI: −0.065, 0.068) compared with −0.099 (95% CI: −0.189, −0.010) for placebo. Patients who did not experience PEs had similar results; the denufosol group had a mean change in FEV$_1$ of 0.064 L (95% CI: 0.028, 0.099) compared with 0.022 L (95% CI: 0.012, 0.057) for the placebo group. Additionally, similar results were obtained when the data were expressed as percent predicted (Figure E3B).

**Quality of Life**

Mean baseline CFQ-R scores were generally high (>65) for all domains; mean baseline scores for the respiratory domain were approximately 78 in both treatment groups. Quality of life was maintained while patients were enrolled in the study. No significant differences between treatment groups were observed at Week 24 endpoint.
with the exception of the emotion domain (feeling worried, useless, and sad, and not living a normal life) for which there was a small treatment effect in favor of placebo (−3.25; P=0.01).

REFERENCES


FIGURE LEGENDS

Figure E1. (A) FEV₁ percent predicted by visit during the 48-week study. (B) Mean Z-scores score FEV₁ by visit during the 48-week study. Error bars represent standard error; shaded areas represent the open-label extension phase of the study during which all patients received denufosol. FEV₁ denotes forced expiratory volume at 1 second.
Figure E2. Time to first pulmonary exacerbation. (A) Pulmonary exacerbation (PE) based on the primary definition. (B) PE based on the secondary definition.

Figure E3. Change in FEV$_1$ by Occurrence of Pulmonary Exacerbation According to Primary Definition. (A) Mean change in FEV$_1$ from baseline to Week 24 endpoint by occurrence of pulmonary exacerbation. (B) Mean change in FEV$_1$ percent predicted from baseline to Week 24 endpoint by occurrence of pulmonary exacerbation. Error bars represent 95% confidence intervals (CIs). CFB denotes change from baseline, FEV$_1$ forced expiratory volume at 1 second, and PE pulmonary exacerbation.
Figure E1

A

![Graph A: Mean FEV₁, predicted, % over weeks for Placebo and Denufosol]

B

![Graph B: Mean (SE) Z-Score for FEV₁ over weeks for Placebo and Denufosol]
Figure E2

(A) Patients without PE, %

- Placebo
- Denufosol

(B) Patients without PE, %

- Placebo
- Denufosol

Week
Figure E3

A

Adjusted mean CFB FEV₁ (95% CI), L

PEs (n=80)
No PEs (n=272)

PEs
Denufosol (n=47)
Placebo (n=33)

No PEs
Denufosol (n=131)
Placebo (n=141)

B

Adjusted mean CFB FEV₁ percent predicted (95% CI), %

PEs (n=80)
No PEs (n=272)

PEs
Denufosol (n=47)
Placebo (n=33)

No PEs
Denufosol (n=131)
Placebo (n=141)