Title	Chronic Ataluren (PTC124) Treatment of Nonsense Mutation Cystic Fibrosis
Authors	Michael Wilschanski[1]; Langdon L Miller[2]; David Shoseyov[1]; Hannah Blau[3]; Joseph Rivlin[4]; Micha Aviram[5]; Michael Cohen[1]; Shoshana Armoni[1]; Yasmin Yaakov[1]; Thea Pugatch[1]; Malena Cohen-Cymberknoh[1]; Nilsen L Miller[2]; Allen Reha[2]; Valerie J Northcutt[2]; Samit Hirawat[2]; Kerri Donnelly[2]; Gary L Elfring[2]; Temitayo Ajayi[2]; Eitan Kerem[1]
Author Affiliations	[1]Hadassah Hebrew University Hospital, Jerusalem, Israel; [2]PTC Therapeutics, South Plainfield, New Jersey, United States; [3]Schneider Children's Hospital, Petach Tikvah, Israel; [4]Carmel Hospital, Haifa, Israel; [5]Soroka Medical Center, Be'er Sheva, Israel.
Corresponding Author	Michael Wilschanski, MD Director, Pediatric Gastroenterology Hadassah University Hospital Mount Scopus POB 24035, Jerusalem, Israel 91240 Telephone: +011-972-2-584-5039 Fax: +011-972-2-584-4697 E-mail: michaelwil@hadassah.org.il
Address for Reprints	info@ptcbio.com
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Abstract

In a subset of patients with cystic fibrosis (CF), nonsense mutations (premature stop codons) disrupt production of full-length, functional cystic fibrosis transmembrane conductance regulator (CFTR). Ataluren (PTC124) allows ribosomal readthrough of premature stop codons in mRNA.

We evaluated drug activity and safety in patients with nonsense mutation CF who took ataluren 3 times daily (morning, midday, and evening) for 12 weeks at either a lower dose (4, 4, 8 mg/kg) or higher dose (10, 10, 20 mg/kg).

The study enrolled 19 patients (males/females 10/9; ages 19 to 57 years; dose: lower 12, higher 7) with a classic CF phenotype, ≥ 1 CFTR nonsense mutation allele, and an abnormal nasal total chloride transport. Both ataluren doses were similarly active, improving total chloride transport with a combined mean change of -5.4 mV ($p<0.001$), and on-treatment responses (at least -5 mV improvement) and hyperpolarizations (values more electrically negative than -5 mV) in 67% ($p<0.001$) and 59% ($p=0.002$) of patients. CFTR function was greater with time and was accompanied by trends toward improvements in pulmonary function and CF-related coughing. Adverse clinical and laboratory findings were uncommon and usually mild.

Chronic ataluren administration produced time-dependent improvements in CFTR activity and clinical parameters with generally good tolerability.

Introduction[1]

Cystic fibrosis (CF) is an autosomal recessive inherited disorder that results from mutations leading to dysfunction in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel located at the apical surface of epithelial cells [1]. In patients with CF, disruption of CFTR-mediated ion and water transport leads to abnormally viscous secretions that obstruct the lungs, pancreas, biliary tree, intestine, and other organs [2]. Pulmonary manifestations are prominent; patients commonly exhibit airway inflammation, infection, and obstruction leading to progressive lung damage [3,4]. The most prevalent symptom of the disease is coughing [5,6,7]. Ultimately, progressive lung dysfunction leads to respiratory failure and death in most patients [8,9]. Current supportive treatment options (eg, inhaled mucolytics, antibiotics, and osmotic agents) [10,11,12] provide benefit, but there is no approved therapy to correct CFTR production and function.

A nonsense mutation in DNA generates a UGA, UAG, or UAA premature stop codon within the protein-coding region of the corresponding mRNA. Premature stop codons result in disease when they disrupt ribosomal translation of the mRNA for a critical protein, such as the CFTR. Nonsense mutations in the CFTR gene cause CF in 5 to 10% of patients worldwide [13] and in >60% of CF patients in Israel [14]. Patients with nonsense mutation CF generally exhibit a severe CF phenotype [15,16,17].

Ataluren (PTC124) is an investigational, orally bioavailable, small-molecule drug designed to overcome the adverse effects of a nonsense mutation by inducing selective ribosomal readthrough of mRNA containing a premature stop codon and allowing translation of a full-length protein [18]. Preclinical evaluation indicated that ataluren generates functional CFTR in a nonsense mutation animal model of CF [19]. In a prior short-term proof-of-concept trial in

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nonsense mutation CF, we administered ataluren in two 14-day treatment courses separated by a 14-day washout period [20]. Drug administration regimens comprised morning, midday, and evening doses of ataluren; in the first course, patients received a lower dose level of 4, 4, and 8 mg/kg and in the second course they received a higher dose level of 10, 10, and 20 mg/kg of ataluren. Our results showed that short-term oral administration of ataluren as a nonsense mutation suppression therapy was generally well tolerated and improved the epithelial electrophysiological abnormalities caused by CFTR dysfunction.

We designed this study to extend those observations by evaluating the activity and safety of 12 weeks of continuous ataluren treatment in the same patients who had participated in the short-term study. The primary objective was to assess pharmacodynamic activity as determined by changes in nasal transepithelial potential difference (NPD). Secondary objectives included assessment of changes in pulmonary function and cough rate, evaluation of safety and study drug compliance, and characterization of ataluren plasma exposures over time. We have previously presented preliminary data from this study in abstract form [21,22].

Methods

Ethics

The institutional ethics committee and the Israeli Ministry of Health approved the study protocol. Each participant provided signed informed consent prior to conduct of any study procedures. We conducted the trial according to Good Clinical Practice and listed the study on www.clinicaltrials.gov (Identifier NCT00351078).

Eligibility Criteria

Patients were adults ≥18 years of age who had participated in the previous Phase 2 trial [20]. All patients had phenotypic evidence of CF and 2 disease-causing *CFTR* mutations, with at least 1 being a nonsense mutation as determined by gene sequencing (Ambry Genetics, Inc,

Aliso Viejo, CA). At the study baseline, all patients had a sweat concentration outside of the normal range (value >40 mEq/L in sweat collected by pilocarpine iontophoresis), abnormal total chloride transport as assessed by NPD (value more electrically positive than -5 mV during successive nasal perfusions with chloride-free amiloride followed by chloride-free amiloride with isoproterenol) [23,24], a forced expiratory volume in 1 second (FEV_1) ≥40% of predicted, and an oxygen saturation of ≥92% on room air. Exclusion criteria included pre-existing or ongoing clinically significant illnesses or complications of CF, serum bilirubin greater than normal limits, serum transaminase values ≥2 times normal limits, serum creatinine >1.5 times the upper limits of normal, or use of systemic or inhaled aminoglycosides within 14 days prior to starting ataluren. Patients could take stable regimens of other inhaled drugs and oral pancreatic enzymes. Female patients could not be pregnant or breast-feeding.

Study Treatment

PTC Therapeutics provided ataluren as vanilla-flavored granules in aluminum foil sachets. We instructed patients to mix the granules with plain water, apple juice, or milk to make a suspension for oral administration. Patients were to take ataluren 3 times per day (morning, midday, and evening) for 84 days (12 weeks) of continuous daily treatment followed by 28 days (4 weeks) of follow-up without study drug. While ataluren can be given either fasting or fed, we recommended ingestion within 30 minutes after a meal based on Phase 1 pharmacokinetic data indicating that plasma concentrations were better sustained when the drug is taken with food [25]. We based allocation to ataluren dose level on the magnitude of the change in total chloride transport we had observed in the prior short-term study. If a patient's best total chloride transport improvement had occurred at the lower dose level (4 mg/kg in the morning, 4 mg/kg at midday, and 8 mg/kg in the evening) in the prior study, then the patient was to be assigned to the 4-, 4-, 8-mg/kg dose level in this study. If a patient's best total chloride transport improvement had occurred at the higher dose level (10 mg/kg in the morning, 10 mg/kg at midday, and

20 mg/kg in the evening) in the prior study, then the patient was to be assigned to the 10-, 10-, 20-mg/kg dose level in this study.

Study Assessments

We assessed NPD in each nostril using standardized methods [26]. Warmed solutions were perfused through a nasal catheter while a voltage tracing was recorded. Solutions comprised Ringer's lactate to obtain a basal potential difference, amiloride to inhibit the epithelial sodium channel, a chloride-free of sodium and calcium gluconate with amiloride to induce an electrogenic chloride gradient, and isoproterenol in chloride-free gluconate with amiloride to stimulate CFTR secretion of chloride ions from the epithelium. The chloride transport during the perfusions of gluconate (intrinsic chloride transport) and isoproterenol (stimulated chloride transport) constituted the total CFTR effect (total chloride transport), the primary measure of the study. For each parameter, we analyzed the average of the available values from the left and right nostrils. We had NPD tracings independently reviewed by an external expert (Dr. M Sinaasappel, Rotterdam, the Netherlands), blinded as to study timepoint; the reported values are those of the independent reviewer.

FEV_1 and forced vital capacity (FVC) were assessed according to American Thoracic Society/European Respiratory Society guidelines [27,28]. We quantified cough using the VivoMetrics LifeShirt™ as previously described [7]. This device incorporates chest-wall, motion-sensing transducers; electrocardiographic electrodes, a unidirectional throat microphone, and a 3-axis accelerometer into a lightweight vest; integrated input from the motion sensors and microphone provides an analysis of cough rate [29]. Following each clinic visit, patients were to wear the LifeShirt vest for 24 hours at home during normal activities of daily living. Adverse events and laboratory abnormalities were assessed using conventional clinical techniques. We quantified ataluren compliance based on data from patient-completed compliance logs. Ataluren plasma concentrations were assayed using a validated bioanalytical method [25].

We obtained activity and safety assessments at the beginning of treatment, every 28 days during treatment, and at 28 days after the end of treatment. Before and at 3 hours after the morning ataluren dose on Days 1, 28, 56, and 84, we collected blood samples for determination of ataluren plasma concentrations. In addition, we performed post-study safety assessments at ~6-month intervals.

Statistics

The sample size was bounded by the number of patients (n=23) that had been enrolled to the prior Phase 1 study. We predefined total chloride transport response ($\geq$-5 mV improvement) and hyperpolarization (equal to or more electrically negative than -5 mV) based on past experience [23,24,30,31,32]. We intended to enroll $\geq$18 evaluable patients to achieve a power $\geq$0.90 (exact binomial test, 1-sided α=0.05) to reject a <10% spontaneous total chloride transport response or hyperpolarization proportion (null hypothesis) in favor of a $\geq$40% pharmacologically induced response or hyperpolarization proportion (alternative hypothesis). With a sample size of 18 patients we also had power >0.90 (paired t-test, 2-sided α=0.05) to detect a change in total chloride transport of similar magnitude and variability as was observed in our prior ataluren study [20].

We used the standard equation to determine body mass index (BMI). Similarly, pulmonary function values were calculated as %-predicted values for age, height, and sex [33]. On-study relative changes were expressed using %-predicted values at baseline for reference. LifeShirt data were analyzed using device-specific software with predefined algorithms that counted coughs and differentiated between wake and sleep status [7]. Consistent with past methods [7,29], a cough was defined as $\geq$1 tussive blasts, each separated by <1 second and occurring within the same expiratory breath. We determined waking and sleeping cough rates by dividing the number of coughs within a wake or sleep period by the time spent wearing the

LifeShirt during that period. We characterized individual cough duration in milliseconds and cough intensity as the amplitude of the cough signal measured in mV.

We evaluated activity variables in all patients with sufficient baseline and on-study measurements for the test of interest. Safety and compliance were assessed in all patients who received ≥ 1 dose of ataluren. We assessed the proportion of patients with a total chloride transport response relative to the null-hypothesis response rate of 10% using a 1-sided exact binomial test. We analyzed changes in NPD parameters, relative pulmonary function, and cough rates using paired t-tests. Changes in the proportions of patients with hyperpolarized total chloride transport values were assessed using McNemar's test. Effects by dose were assessed using an unpaired t-test for best on-treatment changes and Fisher's exact test for response and hyperpolarization proportions. As was specified in the protocol, we considered p-values ≤ 0.05 to be statistically significant. Adverse events and laboratory abnormalities were coded using the Medical Dictionary for Regulatory Activities (http://www.meddramsso.com) and worst severity was graded using the Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0 (http://ctep.cancer.gov/forms). We calculated compliance for each patient as the proportion of actual relative to planned ataluren doses. Standard regression techniques were employed.

Results

Patients

Of the 23 patients who participated in the preceding Phase 2a study [20], 4 could not enroll in this extension study; 2 had time constraints precluding participation and 2 were not eligible (1 developed a CF exacerbation during screening, and 1 had an FEV_1 <40% and required chronic oxygen supplementation). Of the 19 patients who agreed to participate in this trial, 12 were allocated to the lower 4-, 4-, 8-mg/kg dose level and 7 patients to the higher 10-, 10-, 20-mg/kg dose level. One patient receiving 4, 4, 8 mg/kg had a Grade 1 elevation in alanine aminotransferase (ALT) on Day 52; he elected to withdraw from the study despite investigator

encouragement to continue with study participation. One patient receiving 10, 10, 20 mg/kg developed distal ileal obstruction syndrome (DIOS) on Day 80; she did not resume ataluren after the event because the end of the planned treatment period was Day 84. Thus, 17 patients completed the planned 84 days (12 weeks) of study drug treatment.

Baseline patient characteristics defined a population with typical characteristics for adults with nonsense mutation CF (*Table 1*). The majority of patients were young adults. Consistent with the autosomal genetics of the disease, similar proportions of males and females participated, although there were more males receiving the lower ataluren dose level. For all patients, sweat chloride values and nasal total chloride transport values were abnormal. Spirometry revealed that most patients had substantial obstructive pulmonary dysfunction, with 14 (78%) of patients having abnormal FEV_1 values (ie, <90% of predicted). Chronic pathological airway infection was nearly universal. Most patients required pancreatic enzyme replacement therapy for exocrine pancreatic insufficiency and several needed insulin for control of CF-related diabetes. Mild to moderate (Grade 1 to 2) liver enzyme abnormalities suggesting CF-related hepatobiliary disease were also evident in a few subjects. Three nonsense mutation genotypes were represented, with W1282X being the most common. For 5 patients, nonsense mutations were present on both alleles. The UGA premature stop codon type predominated. The minimum period from the last ataluren dose in the prior Phase 2a study to the first dose in this study was >8 months in all patients.

Nasal Transepithelial Potential Difference

Considering the enrollment of 19 patients and the plan to assess NPD at 5 study visits (1 at baseline, 3 on study, and 1 at follow-up), we intended to perform a total of 95 NPD evaluations. Of these, 3 tests were missed because 1 patient withdrew from the study and 4 tests were not completed for other reasons (2 due to rhinitis and 2 due to upper respiratory tract infection). Of the remaining 88 NPD assessments, recordings in both nostrils were available

from 58 (66%) timepoints and recordings from one nostril were available from 30 (34%)

timepoints. Reasons for not obtaining readings in both nostrils related to chronic conditions (eg,

deviated septum) or acute factors (eg, rhinitis, nasal bleeding) that prevented adequate testing in

one of the nostrils.

We predefined a response as an on-treatment improvement of least -5.0 mV from

baseline in total chloride transport. As summarized based on the magnitude of the best on-study

total chloride transport improvements for each patient (*Figure 1*), responses were observed in

7/11 (64%; 90% CI 35-86%, <0.001) evaluable patients receiving the lower ataluren dose and in

4/7 (57%; 90% CI 23-87%, <0.001) evaluable patients receiving the higher ataluren dose. When

considering all evaluable patients across both dose levels, the response rate was 11/18 (61%;

90% CI 39-80%; p<0.001). Based on these data, the primary hypothesis of the study was

satisfied, ie, a low response rate of <10% that might be due to random fluctuations in total

chloride conductance was excluded with substantial confidence. There was no statistically

significant difference in response rate between the 2 dose levels (p = 1.0, Fisher's exact test).

Ataluren treatment was associated with an on-treatment total chloride response in both patients

with the G542X and W1282X mutations. Surprisingly, the 1 evaluable patient with the

3849+10kB C→T mutation, who had responded in the prior short-term study [20], did not meet

criteria for response in this study. An examination of the data in the 5 patients who had nonsense

mutations on both alleles did not suggest that these patients were more likely to have an

improvement in total chloride transport than patients who had a nonsense mutation on only

1 allele.

When considering mean total chloride transport values at each dose level and for both

dose groups together, ataluren induced statistically significant improvements (*Figure 2*).

Changes were time-dependent, with progressively better mean values as treatment continued.

Mean improvements at the end of treatment on Day 84 were -6.8 mV (p=0.004) for those

receiving the lower ataluren dose and were -3.4 mV (p=0.025) for those receiving the higher

ataluren dose, resulting in a mean change for both dose levels combined of -5.4 mV (p<0.001).

Changes in total chloride transport were due to changes in both intrinsic and stimulated chloride transport values; respective mean improvements in intrinsic and stimulated chloride transport on Day 84 were -5.1 mV (p=0.015) and -1.7 mV (p=0.029) for those receiving the lower ataluren dose and were -3.0 mV (p=0.037) and -0.4 mV (p=0.736) for those receiving the higher ataluren dose. Respective mean intrinsic and stimulated chloride transport changes for both dose levels together were -4.2 mV (p=0.002) and -1.2 mV (p=0.080). Dose-dependency was not obvious in these analyses; unpaired t-tests indicated no statistically significant differences between the dose groups for changes in total (p=0.17), intrinsic (p=0.35), or stimulated (p=0.34) chloride transport by Day 84. We did not observe significant ~~changes~~improvements in basal potential difference or ~~sodium transport~~amiloride response values (*Figure 3*) or meaningful correlations between changes in total chloride transport and changes in basal potential difference or amiloride response values.

An examination of individual patient values (*Figure 4*) confirmed that all patients had abnormal total chloride transport at baseline and that ataluren treatment induced statistically significant improvements in the proportions of patients with hyperpolarized total chloride transport values (where hyperpolarization was predefined as an on-treatment value equal to or more electrically negative than -5.0 mV). Altogether, 10 of 18 (56%) evaluable patients had a total chloride transport hyperpolarization at one timepoint during ataluren treatment across both dose levels. Confirming the evaluation of mean changes (*Figure 2*), changes in individual values were generally time-dependent, with progressive improvements with increasing duration of ataluren treatment. No difference in total chloride transport hyperpolarization by dose was apparent (p=1.0, Fisher's exact test).

Rather than using the mean NPD values obtained from both nostrils (as in the primary analyses), we also analyzed outcome considering the single best (most hyperpolarized) total chloride transport value available at each visit; the findings of this sensitivity analysis confirmed

the pattern and statistical significance of the primary NPD findings (data not shown). Analyses of total chloride response, change, and hyperpolarization by age, sex, and baseline total chloride transport value revealed no significant influence of these patient factors on NPD outcomes (data not shown).

Pulmonary Function Assessment

Pulmonary function tests were performed at baseline and on-treatment in 18 patients during the course of the study (Figure 4*Figure 4*). Changes were not statistically significant; however, the aggregate mean value moved higher through Day 84 for the combined dose groups (with changes of 4.5% and 3.5% for relative %-predicted FEV_1 and FVC, respectively). Changes in lung function moved in general correspondence with changes in total chloride transport values over the same period (*Figure 2*). With cessation of ataluren, mean changes reverted toward baseline by Day 112.

Cough Quantitation

Altogether, 18 patients were evaluable for cough quantitation. For these patients, cough data were collected at every visit (1 at baseline, 3 on-treatment, and 1 at follow-up); the median [range] duration of recording was 24 [6-26] hours; of these, 15 [5-20] hours were recorded as waking and 9 [0-15] hours were recorded as sleeping. Of the 90 evaluable assessments, 86 of 90 (96%) included at least 20 hours worth of data; of the remaining 4 assessments, 2 included at least 12 hours of data. At baseline, patients had a mean [range] of 734 [324-1569] coughs/day. As expected, baseline mean [range] waking cough rates of 43 [20-82] coughs/hour markedly exceeded sleeping cough rates 2 [0.1-7] coughs/hour. By Day 84, ataluren was associated with reductions in waking cough frequency of 21% and 25% in the lower and higher ataluren dose groups, respectively (Figure 5*Figure 5*); the overall reduction of 23% for the combined dose groups was statistically significant (p=0.006, paired t-test). For the combined groups, there was

also a time-dependent decrease in median cough duration that achieved greatest significance (p=0.007, paired t-test) at Day 84; cough intensity was not significantly changed (data not shown). Sleeping cough rates remained low and generally unaltered throughout the study.

Drug Compliance and Safety

There were no physician-prescribed dose interruptions or reductions. Median patient compliance was 96% (range: 91-102%) for the 4-, 4-, 8-mg/kg dose regimen and 99% (range: 78-99%) for the 10-, 10-, 20-mg/kg dose regimen. With both regimens, planned doses were only infrequently missed (~4%) or reduced (<0.2%) by the patients.

We recorded all treatment-emergent adverse events and laboratory abnormalities regardless of their potential relationship to ataluren therapy (***Table 2***). Most adverse events were mild (Grade 1) or moderate (Grade 2) in severity and showed no obvious dose-dependent increase in frequency or severity. Three patients had Grade 1 dysuria without associated urinary abnormalities; ataluren was continued and symptoms abated with hydration after periods of 2, 2, and 20 days. One patient had a transient ischemic attack while coughing. This event was not considered ataluren-related given a past history of such events in this patient and the fact that cough-induced hemiplegic migraine or paradoxical embolization are known complications of CF [34,35,36]. Serious adverse events requiring hospitalization included fungal infection of a central venous catheter, DIOS, and a CF exacerbation.

Treatment-emergent laboratory abnormalities were infrequent (***Table 2***). We observed Grade 2 or 3 elevation in serum glucose in several patients; this finding appeared to be a CF-related phenomenon in patients with a past history of hyperglycemia. In nonclinical studies and clinical trials in healthy volunteers, substantially higher ataluren doses than we used in this study have not caused perturbations in glucose metabolism [18,25]. Three patients had Grade 1 increases in creatinine; in 2 patients these abnormalities were each ascribed to DIOS and gentamicin administration while in 1 patient a Grade 1 elevation abated with planned cessation

of ataluren on Day 84. Occasional sporadic fluctuations in serum aminotransferase values were seen but these generally did not persist despite continued ataluren administration. We monitored plasma ACTH and cortisol because of preclinical data in dogs suggesting that ataluren administration can be associated with adrenalitis [18]; however, no patient showed a pattern suggestive of clinical adrenocortical insufficiency. Collectively, the observed laboratory abnormalities were not clinically significant.

Long-term ($\geq$25 months), post-study safety data were available for all study participants. No notable new health conditions were reported.

Pharmacokinetics

Ataluren pharmacokinetics showed rapid drug absorption, dose-proportional differences in mean plasma exposure between dose levels, and generally steady maintenance of plasma concentrations above target trough concentrations (data not shown). The observed mean concentration values were similar to those observed in these same patients in the preceding Phase 2a ataluren study [20].

Discussion

Despite impressive gains in prognosis through early diagnosis, aggressive supportive care, and specialty care, CF remains a disabling and life-threatening disorder [2]. Treating disease manifestations persists as an important goal of drug development, but improved understanding of the molecular basis of CF has shifted the investigational focus toward therapies that address the underlying pathophysiologic defect [37]. Simultaneously, progress in gene sequencing has permitted development of pharmacogenetic profiling [38]. Nonsense mutation suppression therapy with ataluren builds on these evolving paradigms. Application of ataluren constitutes a targeted therapeutic approach to genetic disease that couples genetic patient selection with a treatment designed to overcome a specific mutational abnormality.

In a preceding Phase 2a dose-ranging study in patients with nonsense mutation CF [20], we had administered ataluren in two 14-day treatment periods separated by a 14-day washout period. While pharmacological proof of concept for ataluren readthrough of premature stop codons in CFTR mRNA was observed [20], total treatment duration was limited. This extension study evaluated 12 weeks of continuous ataluren administration, the longest course of treatment yet reported in patients with CF. This trial confirms the pharmacodynamic activity of ataluren in mediating statistically significant improvements in CFTR-mediated total chloride conductance and documents the persistence of pharmacological effect over time. Trends toward improvements in pulmonary function and significant reductions in coughing coincident with changes in ataluren-mediated CFTR activity support the potential for clinical benefit. The longer-term exposure data derived from this study support the safety profile of ataluren administration as a potential chronic therapy for nonsense mutation CF.

Considering the short half-lives of ataluren [25] and of CFTR protein [39] and continuous epithelial cell turnover [40] during the 8- to 12-month period between the trials, no carryover effects from the end of the preceding study to the start of this extension study were expected or observed. Indeed, the mean (±SEM) change in baseline total chloride transport between the studies was only -0.04 (±1.00) mV (n=19). Over this inter-treatment interval, only 2 patients (10.5%) had spontaneous changes of at least -5 mV (8.0 to -0.6 mV and 4.0 to -1.5 mV) and all patients had persistently abnormal total chloride transport (values less than -5.0 mV). By contrast, during ataluren administration, 61% of patients experienced an on-treatment total chloride transport response (at least a -5.0 mV change) and 56% showed hyperpolarization (values of at least -5.0 mV), with marked improvements from uniformly abnormal values at baseline to consistently hyperpolarized values by Day 84. However, by Day 112, in the absence of ataluren pharmacological effect for 28 days, all values were again abnormal, corroborating the presence of pharmacological activity during treatment. Total chloride transport improvements were noted in patients with both G542X and W1282X premature stop codon mutations,

confirming data from our prior study [20] and from other ataluren trials [41] indicating that multiple nonsense mutation genotypes can be responsive to ataluren therapy. The data also demonstrate that in patients with these genotypes, amino acid substitution at the site of the nonsense mutation does not create structural disruptions of the CFTR that prevent chloride channel activity.

The time-dependent pattern of the ataluren-induced changes in total chloride transport in this study is notable. Given that responses have been observed more rapidly in other studies [20,41], the reason for the progressive improvements over an extended period is not clear. Changes in pharmacokinetics were not a likely explanation for this observation when considering that mean ataluren plasma exposures were generally consistent throughout the trial. The data may indicate that ongoing cell turnover during continuous ataluren-mediated CFTR generation results in recruitment of an increasingly greater proportion of CFTR-positive epithelium. Such conjecture may be consistent with the results from in vitro studies evaluating the functional consequences associated with CFTR gene transfer [40] or the mixing of CF and non-CF cells [42]; these experiments suggest that the magnitude of chloride transport correction is dependent not only on the amount of CFTR expressed per cell but also on the proportion of epithelial cells expressing CFTR.

Our study was not powered to document statistically significant changes in pulmonary function. However, given the available sample size, the trends toward pulmonary function improvement we did observe in the study are encouraging. It is further notable that the combined mean improvements in FEV_1 and FVC occurred coincident with the greatest mean improvement in total chloride transport and that there was reversion of effect to the baseline mean upon cessation of ataluren therapy. While the open-label design mandates interpreting these data with caution, the pattern of changes is consistent with a pharmacological effect. Presumably, a time-dependent, multistep process leading from pharmacological action to clinical benefit is required; putative steps may include CFTR production, recruitment of additional

CFTR-expressing epithelium during mucosal turnover, increasing periciliary layer hydration, cilia mobilization and regrowth, and decreased inflammation and infection – all ultimately resulting in reduced airway obstruction.

In patients with CF, coughing is the most common patient complaint [5,6]. Chronic cough is the source of frustration, irritability, and anger for patients and often interferes with daily activities and social events [43]. Therapies designed to restore CFTR expression in epithelial cells offer the potential to increase epithelial mucous hydration and cause secondary reductions in airway irritation; a decrease in cough frequency could serve as a symptomatic integration of disease-modifying pharmacological effects. The inadequacies of relying solely upon subjective cough assessment have been long recognized [44] and guidelines for cough assessment now recommend that cough be evaluated objectively with mobile, compact, continuous recording systems that employ sensitive and specific automated cough recognition and digital data processing [45,46,47]. The LifeShirt device incorporates all of these attributes [29] and – as we documented in a cross-sectional evaluation of outpatient cough quantitation in patients with CF [7] – substantiates very high rates of awake coughing in patients with CF. The results of longitudinal cough assessment in this study confirm that repeated 24-hour cough recording is well tolerated by patients and is practical for clinic staff, resulting in excellent compliance throughout the trial. By Day 84 of the study, we observed consistent reductions in mean awake cough rate (by ~20%) in both ataluren dose groups. Because the reductions occurred contemporaneously with improvements in CFTR function and pulmonary function, the cough data further supported a time-dependent ataluren-mediated effect. The disabling nature of chronic cough, the high rates of cough in patients with CF, and these data suggesting responsiveness to therapeutic intervention all support the potential of objective cough quantitation as an important new measure of clinical benefit in studies of therapies aimed at correcting the underlying pathophysiology of CF.

The focus of this study was on observing temporal changes in study parameters; however, within the constraints of the limited sample size, we did evaluate for any substantive differences in outcome by dose. The ataluren dosing regimens (respective morning, midday, and evening doses of either 4, 4, 8 mg/kg or 10, 10, 20 mg/kg) evaluated in this longer-term trial were the same as those administered in our prior study [20] and in a separate short-term ataluren study in children [41]. In this trial, there was a numerically, but not a statistically greater total chloride transport at the lower dose level than at the higher dose level. These data contrast with data from a separate study in children with CF suggesting that numerically but not statistically greater activity and durability of ataluren-mediated chloride transport at the higher dose level than at the lower dose level. In this trial as in the other CF studies, the general similarity of the activity of dose levels was not likely due to similarities of exposure given that ataluren plasma concentrations increased proportional to dose. It is notable that dose selection for this trial attempted to allocate patients to the lowest dose that had been associated with the best response in the prior short-term study; because this scheme was not random, it may have resulted in assignment of the most responsive patients to the lowest dose level, thus hindering an unbiased evaluation of dose-response. Given that ataluren was generally well tolerated at both dose levels, the data do not suggest a safety constraint on use of either dose level in future studies.

The data from this Phase 2 study have supported the initiation of a Phase 3, international, randomized, double-blind, placebo-controlled study of ataluren in >200 children and adults with nonsense mutation CF (http://clinicaltrials.gov/ct2/show/NCT00803205). Based on the collective Phase 2 findings from this and other studies [20,41], the dose level of 10, 10, 20 mg/kg has been selected for that trial. Given the time-dependent findings observed in this study, the Phase 3 trial will assess ataluren effects over 48 weeks. The Phase 3 trial is designed to evaluate change in FEV_1 as the primary endpoint. Major secondary clinical endpoints include exacerbation frequency and quantitative change in cough rate. Nasal NPD evaluation of change in total chloride transport is also included as an important secondary pharmacodynamic endpoint

in order to provide a critical link between the pharmacological action of ataluren on disease pathophysiology and the projected clinical effects of the drug on pulmonic and extrapulmonic CF manifestations.

Contributors

MW, LLM, NLM, AR, VJN, SH, KD, GLE, TA, and EK were involved in the design of the study; in the collection, analysis, and interpretation of NPD and clinical data; in the writing of the report; and in the decision to submit the paper for publication. DS, HB, JR, MA, MC, SA, YY, TP, MCC participated in various aspects of trial conduct and patient referral. LLM, NLM, VJN, SH, KD, GLE, and TA were involved in regulatory interactions with the Israeli Ministry of Health, and in representing study results to the US Food and Drug Administration and the European Medicines Agency. All authors had full access to all of the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest Statement

MW, DS, MC, SA, YY, TP, MCC, and EK have received funding support for the conduct of this study and have also received compensation for time, effort, and travel expenses related to data discussions and presentations from PTC Therapeutics, Inc. LLM, NLM, AR, VJN, SH, GLE, and TA have been employees of PTC Therapeutics, Inc. and hold financial interests in the company.

Acknowledgements

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Table 1. Baseline Patient Characteristics

Characteristic	Ataluren Dose Level		Combined (N=19)
	4, 4, 8 mg/kg (N=12)	10, 10, 20 mg/kg (N=7)	
Age, median, years	21	27	26
[range]	[19-57]	[19-45]	[19-57]
Gender, number of subjects			
Male	8	2	10
Female	4	5	9
BMI, median	22.3	21.8	21.8
[range]	[15.4-27.8]	[19.0-26.6]	[15.4-27.8]
Sweat chloride, median,* mEq/L	88	89	88
[range]	[47-109]	[49-106]	[47-109]
Nasal total chloride transport, median,† mV	1.6	-0.3	0.7
[range]	[-1.6-9.3]	[-2.3-1.9]	[-2.3-9.3]
Pulmonary function, median % predicted‡			
FEV_1	63	65	65
[range]	[44-106]	[46-92]	[44-106]
FVC	82	77	78
[range]	[63-109]	[57-101]	[57-109]
Pathological lung infection, n	12	6	18
Pseudomonas aeruginosa	11	6	17
Mycobacterium abscessus	2	0	2
Methicillin-resistant *Staphylococcus aureus*	1	0	1
None	0	1	1
Pancreatic insufficiency, n			
Exocrine	11	6	17
Endocrine	2	5	7
Abnormalities of liver-related serum parameters, n	3	2	5
ALT	0	2	2
AST	1	0	1
Alkaline phosphatase	2	1	3
GGT	0	0	0
Bilirubin	1	0	1
LDH	1	0	1
Genotype, n			
Allele 1 *Allele 2*			
G542X (UGA) ΔF508	2	0	2
G542X (UGA) W1282X (UGA)	0	1	1
G542X (UGA) N1303K	0	1	1
W1282X (UGA) ΔF508	8	2	10
W1282X (UGA) W1282X (UGA)	1	2	3
W1282X (UGA) 3849+10kB C→T§ (UAA)	0	1	1
3849+10kB C→T§ (UAA) ΔF508	1	0	1
Time from last ataluren dose in prior study, median, months	10.5	10.5	10.5
[range]	[9.3-12.2]	[8.5-11.5]	[8.5-12.2]

*   Upper limit of normal = 40 mEq/L

†   Upper limit of normal = -5 mV [23,24]

‡   Based on normative data for age, sex, and height [33]

§   Mutation results in an elongated mRNA containing an in-frame premature UAA stop codon [48].

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma glutamyl transferase, LDH = lactate dehydrogenase; BMI = body mass index; FEV_1 = forced expiratory volume in 1 second, FVC = forced vital capacity

Table 2. Treatment-Emergent Adverse Events and Laboratory Abnormalities

| Event*	Ataluren Dose Level							
	4, 4, 8 mg/kg				10, 10, 20 mg/kg			
	N=12				N=7			
	Adverse Event CTCAE Grade, Number of Subjects (%)†							
	1	2	3	4	1	2	3	4
Adverse Events								
CF exacerbation	3 (25.0)	2 (16.7)						
Diarrhea	1 (8.3)							
DIOS							1 (14.3)	
Fungal infection				1 (8.3)				
Nasopharyngitis	1 (8.3)				1 (14.3)			
Pseudomonas infection	1 (8.3)							
Respiratory tract infection	1 (8.3)							
Rhinitis	2 (16.7)				2 (28.6)			
Urinary tract infection					1 (14.3)			
Viral infection	1 (8.3)							
Arthralgia					1 (14.3)			
Transient ischemic attack	1 (8.3)							
Dysuria	2 (16.7)				1 (14.3)			
Cough	7 (58.3)				4 (57.1)			
Dyspnea	1 (8.3)							
Rash					1 (14.3)			
Laboratory Abnormalities‡								
Hemoglobin ↓	1 (8.3)				1 (14.3)			
Platelets ↓	1 (8.3)							
Sodium ↓	1 (8.3)				2 (28.6)			
Potassium ↑	2 (16.7)				1 (14.3)			
Bicarbonate ↓					1 (14.3)			
BUN ↑	4 (33.3)							
Creatinine ↑	2 (16.7)				1 (14.3)			
Calcium ↓	5 (41.7)				3 (42.9)			
Uric acid ↑	4 (33.3)							
Glucose ↑	6 (50.0)	2 (16.7)			1 (14.3)	1 (14.3)	4 (57.1)	
Albumin ↓	1 (8.3)							
Bilirubin ↑	1 (8.3)							
AST ↑	1 (8.3)				4 (57.1)			
ALT ↑	1 (8.3)	1 (8.3)			2 (28.6)			
GGT ↑					1 (14.3)			
Creatine kinase ↑	1 (8.3)					1 (14.3)		
LDH ↑	2 (16.7)	1 (8.3)			1 (14.3)			
Alkaline phosphatase ↑	3 (25.0)							
Total cholesterol ↑	1 (8.3)				1 (14.3)			
Triglycerides ↑	2 (16.7)	1 (8.3)			1 (14.3)			

*   Blank spaces in table are intentionally blank.
†   A patient with >1 occurrence of the same type of event is only listed once, by worst severity grade.
‡   Only patients with shifts to worse grades are listed
Abbreviations: ↑ = increase, ↓ = decrease; ACTH = adrenocorticotropic hormone, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, GGT = gamma glutamyl transferase, LDH = lactate dehydrogenase; CF = cystic fibrosis; CTCAE = Common Terminology Criteria for Adverse Events; GGT = gamma-glutamyltransferase
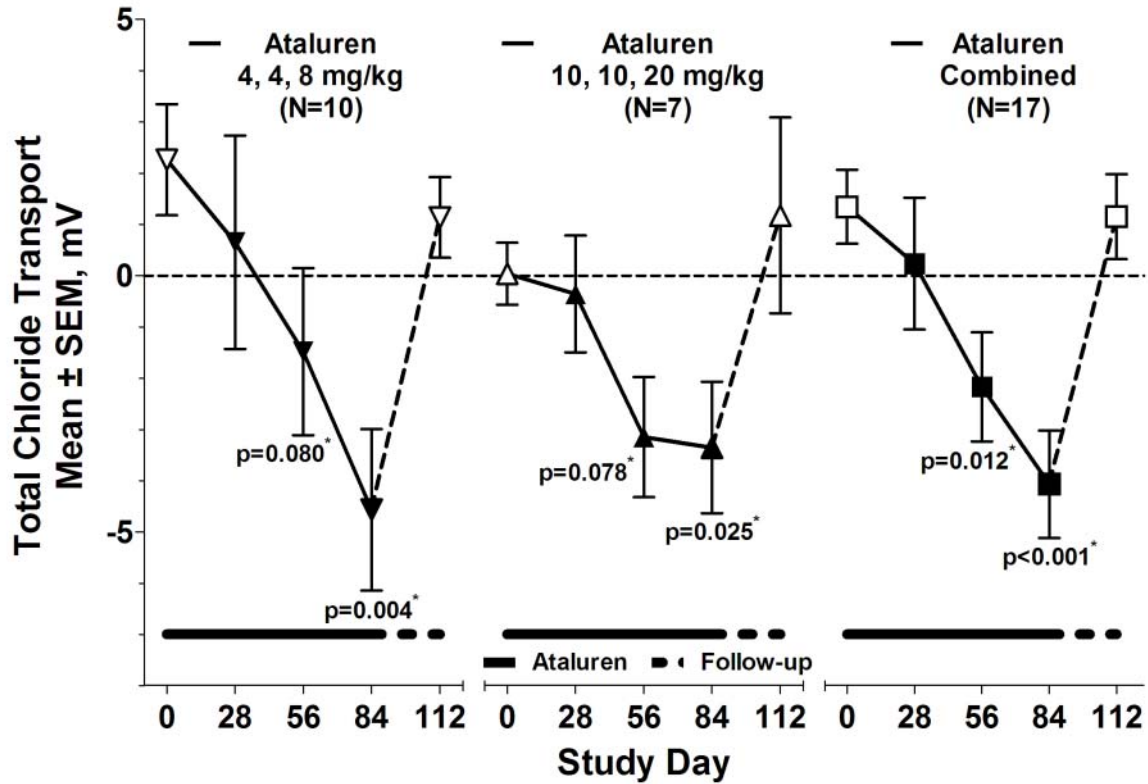
Figure 1. Individual Best Total Chloride Transport Changes
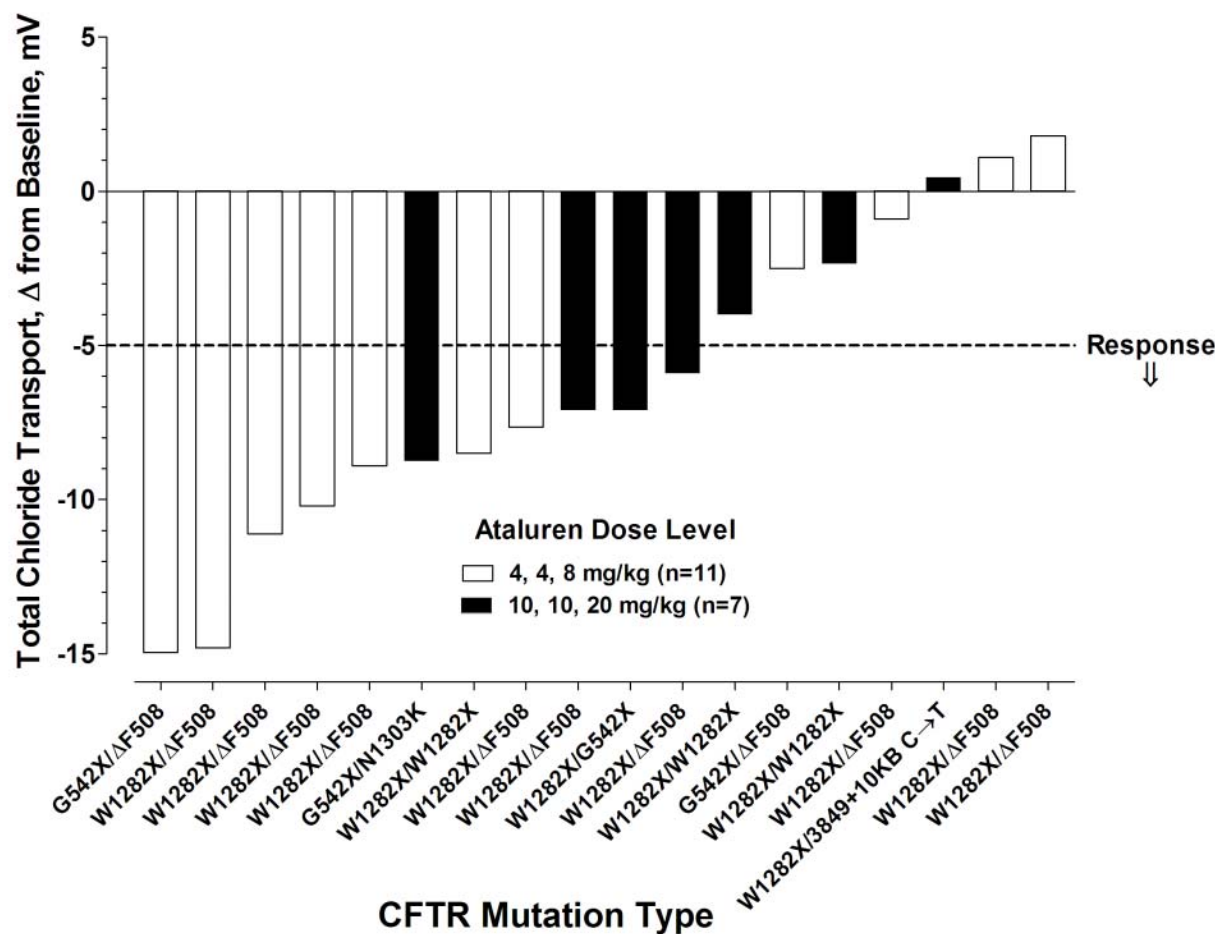
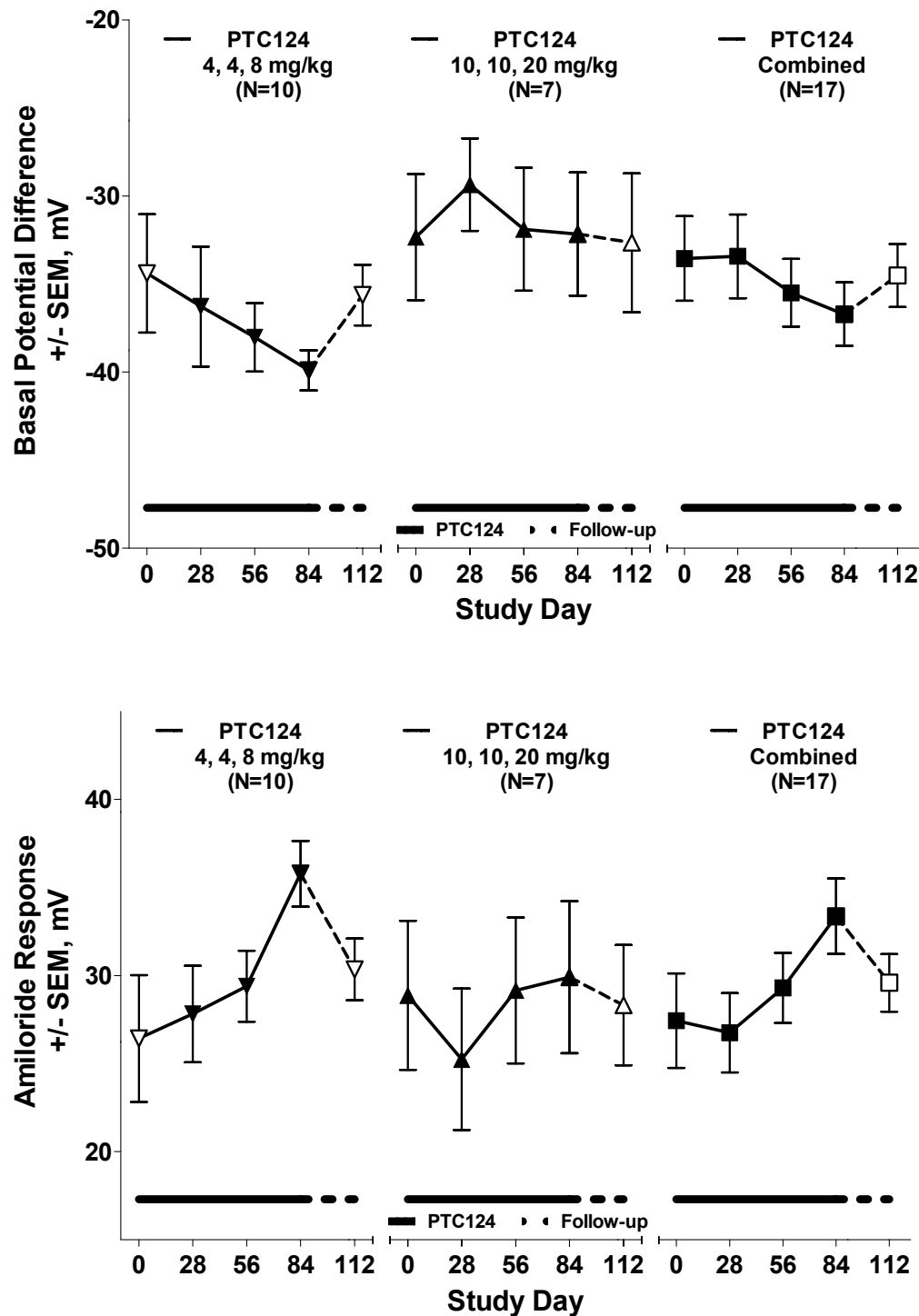

Abbreviation: CFTR = cystic fibrosis transmembrane

Figure 2. Mean Total Chloride Transport

Abbreviation: SEM = standard error of the mean
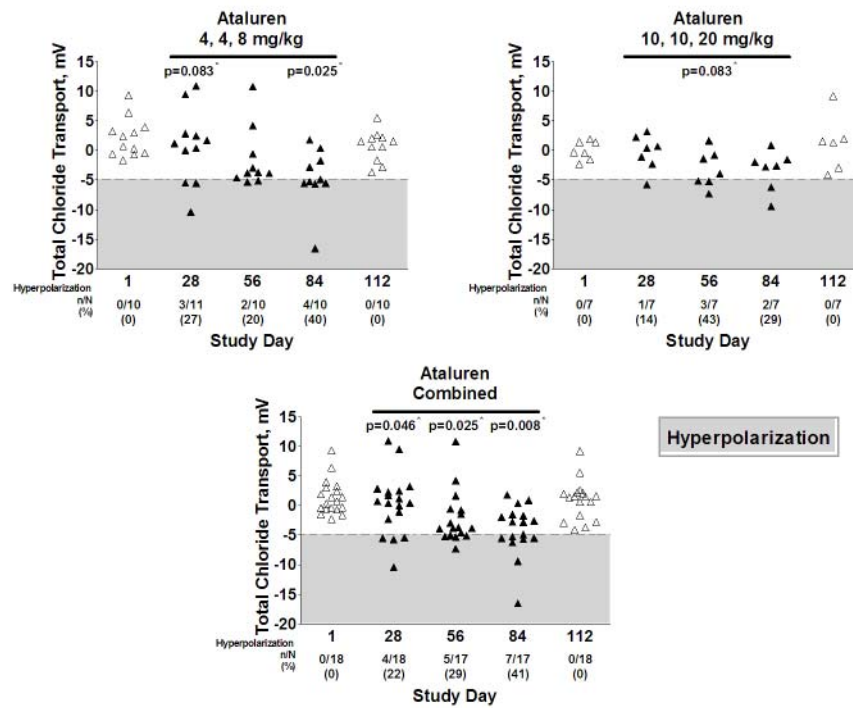* Paired t-test comparing on-study timepoint versus corresponding baseline

Figure 3. Mean Basal Potential Difference and ~~Sodium Transport~~<u>Amiloride Response</u>

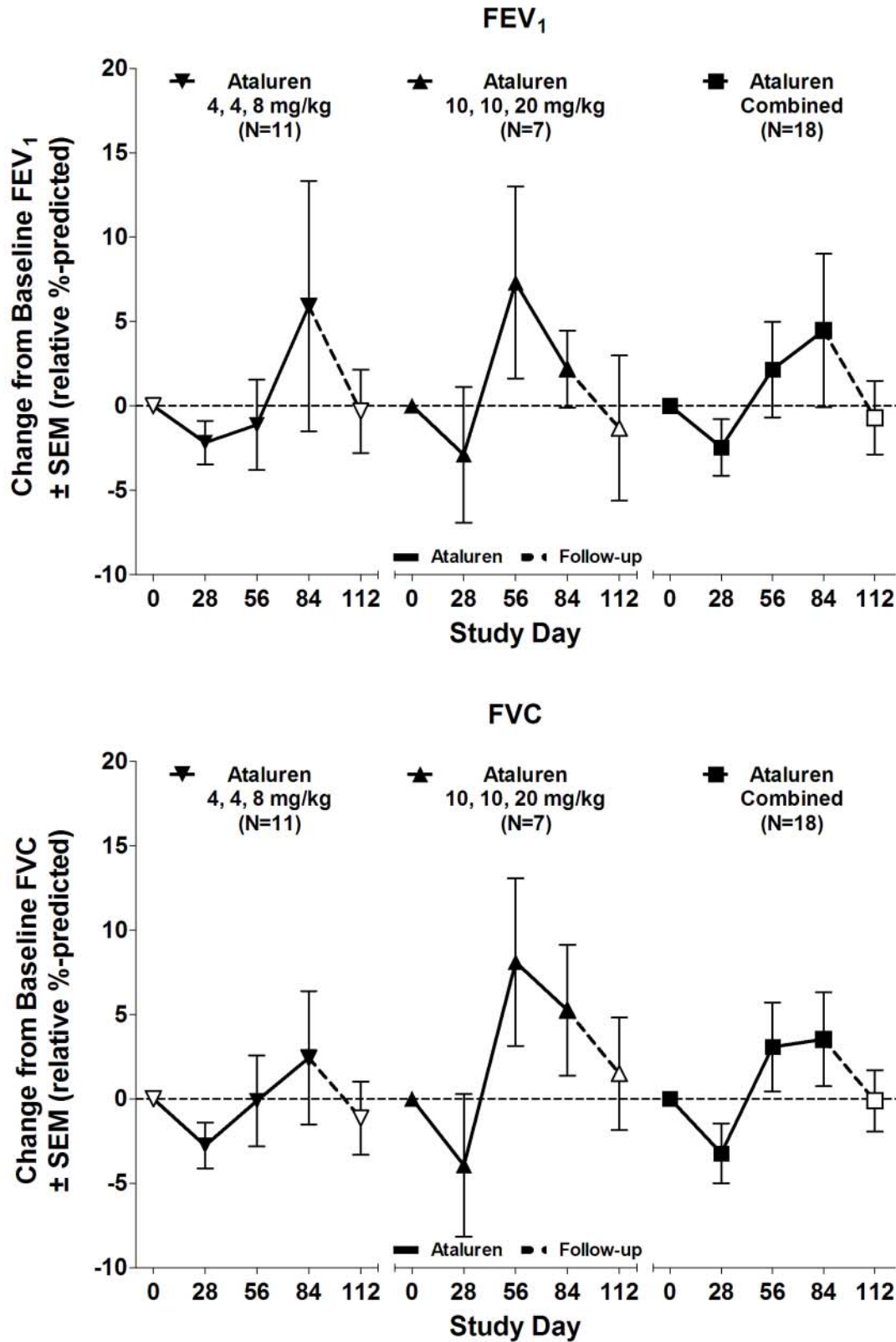**Abbreviation:** SEM = standard error of the mean

* Paired t-test comparing on-study timepoint versus corresponding baseline

Figure 34. Individual Total Chloride Transport Values
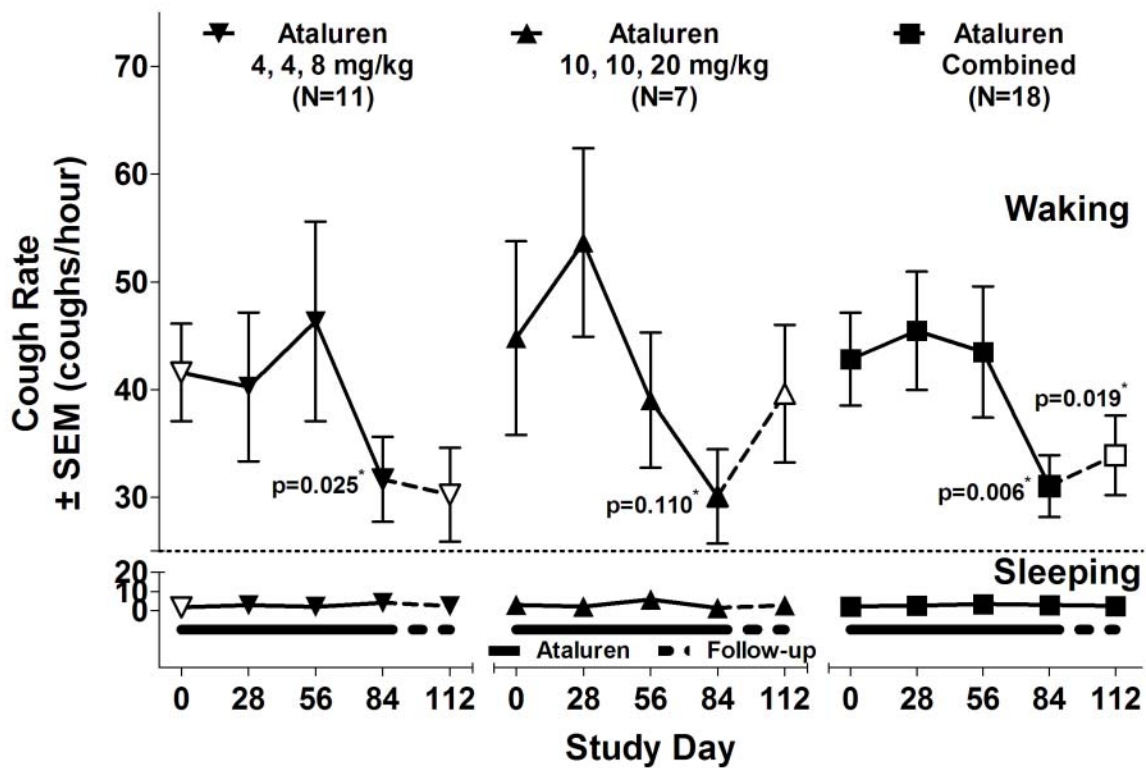

*   McNemar's test comparing on-study timepoint versus corresponding baseline

Figure 45. Change in Mean Pulmonary Function Values

Abbreviations: FEV$_1$ = forced expiratory volume in 1 second, FVC = forced vital capacity, SEM = standard error of the mean

Figure ~~56~~. Mean Waking and Sleeping Cough Rates

Abbreviations: SEM = standard error of the mean
*   Paired t-test comparing on-treatment timepoint versus corresponding baseline