ATALUREN TREATMENT OF PATIENTS WITH NONSENSE MUTATION DYSTROPHINOPATHY

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Accepted 1 July 2014

ABSTRACT: *Introduction*: Dystrophinopathy is a rare, severe muscle disorder, and nonsense mutations are found in 13% of cases. Ataluren was developed to enable ribosomal readthrough of premature stop codons in nonsense mutation (nm) genetic disorders. *Methods*: Randomized, double-blind, placebo-controlled study; males ≥5 years with nm-dystrophinopathy received study drug orally 3 times daily, ataluren 10, 10, 20 mg/kg ($N = 57$); ataluren 20, 20, 40 mg/kg ($N = 60$); or placebo ($N = 57$) for 48 weeks. The primary endpoint was change in 6-Minute Walk Distance (6MWD) at Week 48. *Results*: Ataluren was generally well tolerated. The primary endpoint favored ataluren 10, 10, 20 mg/kg versus placebo; the week 48 6MWD $\Delta = 31.3$ meters, *post hoc* $P = 0.056$. Secondary endpoints (timed function tests) showed meaningful differences between ataluren 10, 10, 20 mg/kg, and placebo. *Conclusions*: As the first investigational new drug targeting the underlying cause of nm-dystrophinopathy, ataluren offers promise as a treatment for this orphan genetic disorder with high unmet medical need.

Muscle Nerve **50**: 477–487, 2014

Additional Supporting Information may be found in the online version of this article.

Abbreviations: 6MWD, six-minute walk distance; 6MWT, six-minute walk test; C_{0h}, plasma concentration before the morning dose; C_{2h}, plasma concentration 2 hours after the morning dose; CINRG, Cooperative International Neuromuscular Group; cITT, corrected intent-to-treat; CK, creatine kinase; DMD, Duchenne muscular dystrophy; ITT, intent-to-treat; MCID, minimal clinically important difference; MMRM, mixed-model repeated-measures; mRNA, messenger ribonucleic acid; nm, nonsense mutation; nmDMD, nonsense mutation Duchenne muscular dystrophy; PedsQL, pediatric quality of life inventory; PODCI, pediatric outcomes data collection instruction; TFTs, timed function tests; TSQM, treatment satisfaction questionnaire for medication.

Key words: Duchenne muscular dystrophy; genetic; pediatric; nonsense mutation; orphan

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Published online 5 July 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.24332

Approximately 13% of patients with dystrophinopathy have a nonsense mutation in the gene for dystrophin.[1] A nonsense mutation results in a

premature stop codon within the protein coding region of the corresponding messenger ribonucleic acid (mRNA) and causes premature termination of translation and generation of a truncated, unstable, nonfunctional protein. There are 3 different types of premature stop codons: opal, amber (uridine-adenosine-guanosine), and ochre (uridine-adenosine-adenosine). Nonsense-mediated mRNA decay (NMD) weakens mRNA, and readthrough of nonsense mutations allows for production of functional protein by altering the level of mRNA deterioration when readthrough activity is established.[2] Readthrough of a premature stop codon is a novel approach to treat genetic disorders due to a nonsense mutation. Aminoglycoside antibiotics such as gentamicin have been investigated for their potential ability to promote premature stop codon readthrough in patients with nonsense mutation dystrophinopathy.[2–5] The development of a safe, orally bioavailable drug that has readthrough activity would be beneficial to Duchenne muscular dystrophy (DMD) patients with nonsense mutations in their dystrophin gene.[4] To treat genetic disorders due to a nonsense mutation, ataluren (PTC124) has been developed as a first-in-class, investigational new drug designed to enable ribosomal readthrough of premature stop codons.

Ataluren's activity has been demonstrated independently in a large number of peer-reviewed articles of multiple disease models spanning many different organ systems, including models of dystrophinopathy.[2,3,6–21] A proof-of-concept Phase 2a trial demonstrated that ataluren produced dystrophin in patients with nonsense mutation dystrophinopathy.[22] A second Phase 2a study demonstrated that ataluren produced cystic fibrosis transmembrane conductance regulator (cftr) protein in nonsense mutation cystic fibrosis patients.[23,24]

Based on the results of the proof-of-concept clinical trials, we conducted a Phase 2b registration-directed study. Before this study, very few large-scale, randomized, controlled trials had been performed in dystrophinopathy, and none had been performed using a new chemical entity targeting the underlying cause of DMD.[25] This Phase 2b study is a randomized, double-blind, placebo-controlled international study that evaluated the efficacy and safety of 2 doses of ataluren in patients with nonsense mutation dystrophinopathy (referred to in this study as nonsense mutation DMD [nmDMD]). This trial in dystrophinopathy used the 6-Minute Walk Test (6MWT) as an outcome measure. As this was the first study for registration in DMD, there were no established primary or secondary endpoints from a regulatory perspective, and there was limited DMD natural history data available at the time the study was designed. Completion of this trial has provided a better understanding of the natural history of DMD using the 6MWT and has established the 6MWT as a validated primary endpoint in DMD clinical trials; in addition, the data from this trial have helped to identify the best secondary endpoints in DMD trials and lay the clinical trial groundwork for future therapies for this disease.[26]

MATERIALS AND METHODS

Participants. Patients were enrolled at 37 sites in 11 countries, which featured the following inclusion criteria: male, $\geq$5 years of age with a documented nonsense mutation in the dystrophin gene, onset of dystrophinopathy symptoms by age 9 years, elevated serum creatine kinase (CK), and difficulty ambulating but able to walk $\geq$75 meters unassisted during a 6MWT at screening. Stable use of concomitant glucocorticoids was allowed.

At each participating institution, institutional review boards/ethics committees and health authorities approved the study protocol. All parents/participants provided signed informed consent/assent before study initiation. The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice and was registered (Identifier NCT00592553) at www.clinicaltrials.gov.

Procedures. Patients were stratified prospectively by age ($<$9 or $\geq$9 years), use of glucocorticoids (yes or no), and baseline 6-Minute Walk Distance (6MWD) ($\geq$350 or $<$350 meters) and were randomized 1:1:1 to receive study drug orally 3 times daily for 48 weeks (ataluren 10, 10, 20 mg/kg, henceforth referred to as ataluren 40 mg/kg/day; or ataluren 20, 20, 40 mg/kg, referred to as ataluren 80 mg/kg/day; or placebo). Evaluations were performed at screening, baseline and every 6 weeks. The primary outcome measure was the 6MWD.[27,28] Secondary outcome measures of physical functioning included timed function tests ([TFTs] - stand from supine, 4-stair ascend, 4-stair descend, and 10 meter run/walk), functional test method grading, at-home activity, myometry (knee flexion and extension, elbow flexion and extension, and shoulder abduction), patient/caregiver-reported accidental falls, and the Pediatric Quality of Life Inventory (PedsQL) physical functioning and psychosocial domains,[29] Treatment Satisfaction Questionnaire for Medication (TSQM), verbal memory and attention, heart rate, and serum CK. Safety, study drug compliance, and ataluren plasma concentrations before (C_{0h}) and 2 h (C_{2h}) following the morning dose[30] were evaluated.

Biceps muscle dystrophin expression was also assessed (fully detailed in Supplementary Appendix – available online). Biopsy of the biceps brachii was performed at baseline (pretreatment sample)

and from the contralateral arm at Week 36 ± 14 days (posttreatment sample) to assess for production of dystrophin. Biopsies were performed at the 37 study sites in 11 countries (United States, United Kingdom, Italy, Australia, Germany, Canada, France, Sweden, Spain, Belgium, and Israel). Samples were shipped to Covance Central Lab and stored at $-80°C$.

Statistical Analysis. The study hypothesis was that mean change in 6MWD from baseline to 48 weeks would be 30 meters better in at least 1 ataluren arm versus placebo. Thirty meters was selected based on the 6MWD treatment effects seen in trials of drugs which have been approved for the treatment of other rare diseases with neuromuscular complications.[31,32] Based primarily on earlier observational 6MWD data in DMD patients,[27,28] as well data from other diseases,[31,32] the standard deviation of the change in 6MWD was hypothesized in the protocol to be 50 meters, and this standard deviation was assumed for sample size determination. The prespecified intent-to-treat (ITT) population included all randomized subjects with a valid 6MWT available at baseline and ≥ 1 postbaseline visit. Mixed-model repeated-measures (MMRM) analyses of changes from baseline to Week 48 were performed. Terms in the model included treatment, visit, treatment*visit, and the stratification factors. Original data were to be analyzed, if the data were normally distributed; otherwise, log-transformed data were to be analyzed, if the log-transformed data were normally distributed; otherwise, rank-transformed data were to be analyzed. Shapiro-Wilk testing was used to determine if the data were distributed normally. The MMRM model fit was improved *post hoc* by the addition of a baseline*visit interaction term.[33,34] The baseline values for 2 patients (1 placebo-dosed and 1 treated with ataluren 80 mg/kg) were replaced by their screening values, because their baseline 6MWDs were radically lower than their screening and Week 6 values due to lower-limb injuries before the baseline test. This is referred to as the corrected ITT (cITT) population. The *post hoc* analysis was performed on the untransformed data with deviations from assumptions addressed by means of a re-randomization test (10,000 iterations) using MMRM. Details of prespecified statistical methods and *post hoc* modifications are provided in the Supplementary Appendix.

The *P*-values of the primary and secondary outcome measures were adjusted for comparisons of 2 dose levels against placebo. All analyses were 2-sided at the 0.05 level of significance. Where *P*-values are described as nominal, they are not adjusted for multiplicity.

RESULTS

Patient Disposition and Characteristics. The ITT population included all 174 randomized patients, of whom 57 were assigned to placebo, 57 to ataluren 40 mg/kg/day, and 60 to ataluren 80 mg/kg/day (Supplementary Appendix, which is available online). One patient discontinued at Week 6 due to noncompliance. The remaining 173 patients completed 48 weeks. Patients ranged in age from 5 to 20 years (Table 1). All 3 premature stop codon types were represented. There was no significant difference among the 3 arms in any patient characteristic.

Median study drug compliance was >97%. Ataluren concentrations before and 2 h after the morning dose were dose-proportional and remained stable over time.

Seventy-one percent (124/174) of patients were receiving glucocorticoids, which was equal across treatment arms, and of these, 92 (74%) received glucocorticoids daily, 7 (6%) received glucocorticoids every other day, and 25 (20%) were on other regimens. Changes in glucocorticoid regimens were minimal during the study. No patients discontinued glucocorticoid use during the study.

Safety. Ataluren was generally well tolerated at both dose levels (Supplementary Appendix). There were no study discontinuations due to adverse events. No ataluren-related serious adverse events were reported. Most treatment-emergent adverse events were mild or moderate. Investigator attributions of drug-related adverse effects showed similar frequencies across the placebo- and ataluren-treated arms. Changes in laboratory and physical parameters generally were not significant clinically.

Efficacy. ***Primary Endpoint.*** In the ITT population, mean declines in 6MWD at Week 48 of 42.6 and 12.9 meters were observed for placebo and ataluren 40 mg/kg/day, respectively ($\Delta = 29.7$ meters, nominal $P = 0.149$, MMRM on ranks). This difference was consistent with the targeted treatment effect size of 30 meters. In the corrected ITT population, mean declines in 6MWD at Week 48 of 44.1 and 12.8 meters were observed for placebo and ataluren 40 mg/kg/day, respectively (see Fig. 1; $\Delta = 31.3$ meters, $P = 0.056$, re-randomization MMRM adjusted for multiplicity). The difference in the mean change in 6MWD from baseline to Week 48 between placebo and ataluren 80 mg/kg/day was negligible.

Progression of 6MWD was defined *a priori* based on time to persistent 10% 6MWD worsening relative to baseline. In the ITT population, 26% of patients in the ataluren 40 mg/kg/day arm, as compared with 44% of patients in the placebo arm, had experienced persistent 10% 6MWD worsening by Week 48. This corresponds to a 48%

Table 1. Patient characteristics.

Characteristic	Treatment arm		
	Placebo *N*=57	40 mg/kg/day *N*=57	80 mg/kg/day *N*=60
Demographics			
Age, years			
Mean (SD)	8.3 (2.33)	8.8 (2.91)	8.4 (2.53)
Median	8.0	8.0	8.0
Range	5–15	5–20	5–16
Race, *n* (%)			
Caucasian	54 (94.7)	53 (93.0)	50 (83.3)
Black	0 (0.0)	1 (1.8)	1 (1.7)
Asian	1 (1.8)	1 (1.8)	4 (6.7)
Hispanic	1 (1.8)	1 (1.8)	2 (3.3)
Other	1 (1.8)	1 (1.8)	3 (5.0)
Body height, cm			
Mean (SD)	123 (11.8)	125 (15.3)	126 (13.8)
Median	122	121	126
Range	104–163	99–173	99–173
Body weight, kg			
Mean (SD)	29 (9.1)	31 (12.1)	32 (12.8)
Median	26	27	28
Range	16–55	16–76	17–84
Stop codon type, *n* (%)			
UGA	31 (54.4)	29 (50.9)	23 (38.3)
UAG	12 (21.1)	17 (29.8)	19 (31.7)
UAA	14 (24.6)	11 (19.3)	18 (30.0)
Stratification factors			
Age group, *n* (%)			
<9 years	32 (56)	32 (56)	34 (57)
≥9 years	25 (44)	25 (44)	26 (43)
Glucocorticoid use, *n* (%)			
Yes	40 (70)	41 (72)	43 (72)
No	17 (30)	16 (28)	17 (28)
Baseline 6MWD, *n* (%)			
≥350 m	35 (61)	32 (56)	33 (55)
<350 m	22 (39)	25 (44)	27 (45)
Functional characteristics			
6MWD, m, mean (SD)	361 (87.5)	350 (97.6)	361 (99.7)
%-predicted 6MWD, mean (SD)	61.9 (16.26)	59.6 (18.06)	61.6 (17.78)
Climb 4 stairs, s, mean (SD)	6.0 (5.67)	6.9 (6.47)	7.5 (7.46)
Descend 4 stairs, s, mean (SD)	5.5 (5.75)	6.1 (5.98)	6.7 (7.21)
10-m run/walk, s, mean (SD)	6.7 (2.67)	7.4 (4.37)	7.4 (4.36)
Supine to stand, s, mean (SD)	11.5 (11.44)	10.8 (9.92)	12.3 (11.19)
Falls/day*, mean (SD)	0.5 (0.94)	0.3 (0.48)	0.4 (0.60)

**Baseline falls/day data were available for 48, 48, and 54 patients in the placebo, ataluren 40 mg/kg/day, and ataluren 80 mg/kg/day treatment arms, respectively.*

SD, standard deviation; UAA, uridine-adenosine-adenosine; UAG, uridine-adenosine-guanosine; UGA, uridine-guanosine-adenosine

reduction in the risk of 10% 6MWD worsening (Figure 2; hazard ratio of ataluren 40 mg/kg/day vs. placebo of 0.52, nominal $P = 0.039$). Similar results were observed for the corrected ITT population (hazard ratio = 0.51, nominal $P = 0.033$). The proportion of patients with persistent 10% 6MWD worsening at Week 48 in the ataluren 80 mg/kg/day arm was similar to placebo.

Recently, an age- and height-based equation[35] has been used to convert 6MWD to %-predicted 6MWD in patients with DMD,[36] which accounts for maturational differences in 6MWD. This equation was applied to 6MWD data in the corrected ITT population. At baseline, this DMD cohort performed the 6MWT at approximately 60% predicted. Mean declines in %-predicted 6MWD at Week 48 of 7.6% and 2.7% were observed for placebo and ataluren 40 mg/kg/day, respectively ($\Delta = 4.9\%$, $P = 0.055$, re-randomization MMRM adjusted for multiplicity). The mean decline for ataluren 80 mg/kg/day was 7.7%. The significance level of this %-predicted analysis is consistent with the results of the analysis of the 6MWD in meters, in which a *P*-value of 0.056 was observed.

Age, glucocorticoid use, and baseline 6MWD were prespecified as stratification factors, because

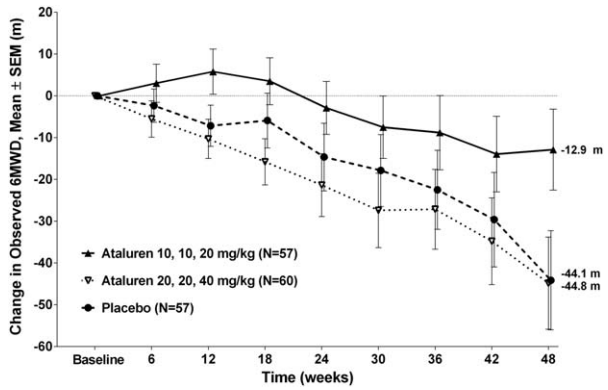

FIGURE 1. Change in 6MWD. Mean changes in the ataluren 10, 10, 20 mg/kg and placebo arms were −12.86 and −44.14 meters, respectively, resulting in a difference of 31.28 meters.

these variables were likely to have prognostic significance. Analyses of mean changes in 6MWD within the 6 subgroups defined by the 3 stratification factors showed that all ataluren 40 mg/kg/day subgroups performed better relative to the corresponding placebo subgroup. The largest mean differences between ataluren 40 mg/kg/day and placebo were observed in patients <9 years old, patients receiving glucocorticoids, and patients with baseline 6MWD <350 meters.

The natural history of changes in ambulation as measured by the 6MWT,[26] indicates that patients greater than 350 meters at baseline generally do not demonstrate substantial changes in their 6MWDs in 48 weeks while those less than 350 meters tend to show large declines. Based on this, and the fact that a baseline value of 350 meters was a prespecified stratification factor, an analysis was performed on the prespecified group with baseline 6MWD < 350 meters. In this prespecified subgroup with baseline 6MWD < 350 meters in patients treated with ataluren 40 mg/kg/day the mean change of 6MWD from baseline to Week 48 was 68.2 meters better than placebo-dosed patients (nominal $P = 0.0053$, see Figure 3).

Further analysis was performed on patients likely to be in the ambulatory decline phase of the disease

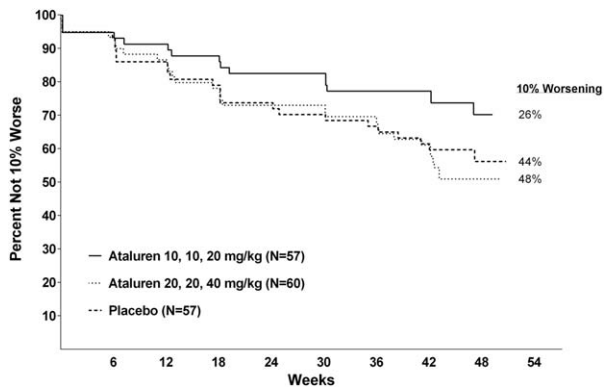

FIGURE 2. Time to persistent ≥10% worsening in 6MWD.

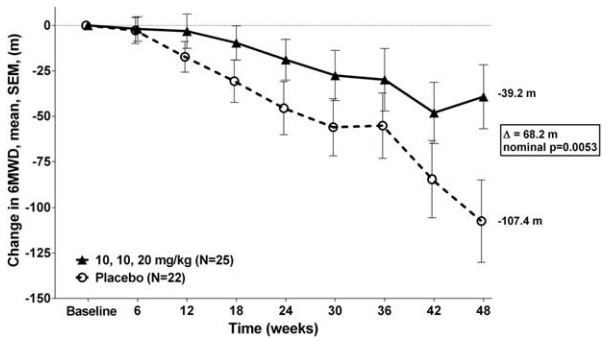

FIGURE 3. Mean change in 6MWD from baseline to week 48 in the < 350 meters 6MWD subgroup.

subsequent to age 7. This subgroup consisted of nmDMD patients aged 7 to 16 with a baseline %-predicted 6MWD ≤80% and, to minimize heterogeneity, who were taking corticosteroids and had a baseline 6MWD ≥150 meters. In this decline-phase subgroup, in patients treated with ataluren 40 mg/kg/day, the mean change of 6MWD from baseline to Week 48 was 49.9 meters better than placebo-dosed patients (nominal $P = 0.0096$; see Fig. 4). Separation between ataluren 40 mg/kg/day and placebo in the decline-phase subgroup occurred early (6 weeks), and by 24 weeks there was a 30 meter treatment difference between ataluren 40 mg/kg/day in comparison to the placebo group (nominal $P = 0.047$).

The baseline characteristics for the decline-phase subgroup and the < 350 meters subgroup were balanced across the 3 treatment arms (see Table 2).

Although the effect of ataluren was most evident in patients in the ambulatory decline-phase, the activity of ataluren was seen across the disease spectrum. As shown in Figure 5, the Phase 2b patients can be categorized based on %-predicted 6MWD at baseline. All patients, including milder patients (>70% predicted), showed a favorable effect for ataluren, and the overall results were not driven by milder patients.

Secondary Endpoints. In TFTs, ataluren-treated patients demonstrated smaller increases in the

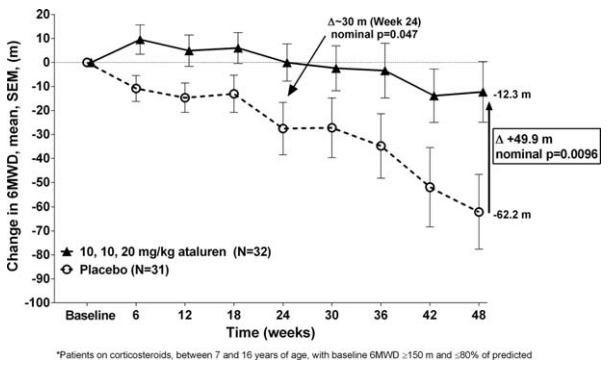

FIGURE 4. Mean change in 6MWD from baseline to week 48 in the decline-phase subgroup.

Table 2. Frequency of patients within levels of stratification factors.

Parameter	Placebo	Ataluren 40 mg/kg/day	Ataluren 80 mg/kg/day
	Decline phase subgroup		
	N=31	*N*=32	*N*=33
< 9 years	39%	41%	36%
≥ 9 years	61%	59%	64%
< 350 meters baseline 6MWD	42%	44%	42%
≥ 350 meters baseline 6MWD	58%	56%	58%
Corticosteroid use	100%	100%	100%
	< 350 meters 6MWD subgroup		
	N=22	*N*=25	*N*=27
< 9 years	36%	44%	48%
≥ 9 years	64%	56%	52%
< 350 meters baseline 6MWD	100%	100%	100%
Corticosteroid use	64%	68%	59%
No corticosteroid use	36%	32%	41%

time it takes to climb 4 steps, descend 4 steps, and run/walk 10 meters relative to placebo (Table 3.). The log of the threshold for the clinically meaningful difference in TFTs was estimated to be 0.4 s,[37] which corresponds to ~1.5 s on the untransformed scale. These trends were more prominent at the 40 mg/kg/day dose, which meets the threshold (~1.5 s) and suggests clinically meaningful differences in TFTs.[37] Positive trends for ataluren 40 mg/kg/day were also seen in functional method grading (Table 3.); see detailed explanation in Supplementary Appendix. Differences between ataluren versus placebo for mean changes in supine to stand were small for both dose levels, and at baseline 23% of patients were unable to stand from supine within the predefined maximum of 30 seconds (vs. ≤3% patients in other TFTs), limiting the ability to demonstrate a treatment effect with this test.

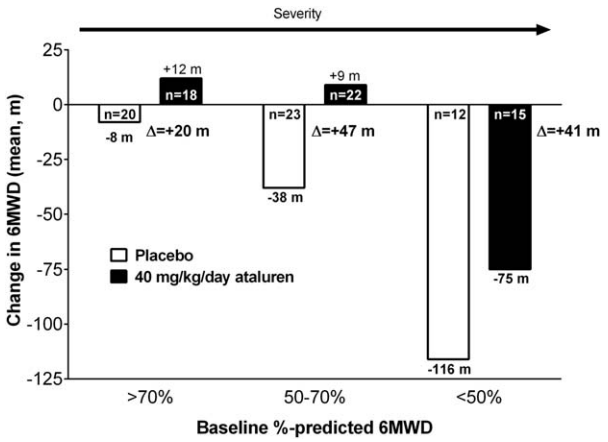

FIGURE 5. Mean change in 6MWD by disease severity.

Compared with the results of the overall population, in the prespecified baseline 6MWD < 350 meters subgroup, the results favoring ataluren were even greater in values of 10 meter run/walk (3.5 s), the time to climb 4 steps (6.4 s), and the time to descend 4 steps (5.0 s). Also similar to these results, the ambulatory decline-phase subgroup exhibited greater differences in the TFTs of 4-stair climb, 4-stair descend, and 10-meter run/walk of ataluren over placebo compared with the overall study population. In this subgroup, the results favoring ataluren were nearly twice the 1.5 s clinically meaningful threshold in the 10-meter run/walk (2.8 s), the time to climb 4 steps (2.9 s), and the time to descend 4 steps (2.9 s) (see Fig. 6).

Positive trends favoring ataluren 40 mg/kg/day versus placebo were seen in patient-reported physical functioning, as measured by the PedsQL. The difference in the mean change in physical functioning score was 3.4 at Week 48. This was more pronounced in the ambulatory decline-phase subgroup with a difference of 6.1 in the mean change in physical functioning score, favoring ataluren 40 mg/kg/day over placebo at Week 48.

Accidental falling is the most common cause of limb fractures in boys with DMD,[38] and ~35 to 40% of lower-limb fractures result in permanent loss of ambulation.[39] This patient-reported outcome was monitored by patient/caregiver diaries. The results show reductions in accidental falling for ataluren versus placebo; the relative ratios of the estimated fall rates at Week 48 were 0.38 (95% CI = 0.16, 0.94) for ataluren 40 mg/kg/day versus placebo.

Additional positive trends favoring ataluren 40 mg/kg/day versus placebo were seen across the other secondary outcome measures of physical functioning, including activity and wheelchair use in the community setting, as well as myometric evaluation of muscle strength, (see Supplementary Appendix). In 5- to 6-year-old patients treated with ataluren 40 mg/kg/day, a 30 meter treatment benefit in 6MWD versus placebo was seen. In addition, 5- to 6-year-old patients treated with ataluren 40 mg/kg/day showed stable or improved TFTs, whereas patients treated with ataluren 80 mg/kg/day or dosed with placebo showed worsening over 48 weeks. These younger patients treated with ataluren 40 mg/kg/day also showed an improvement across all myometry measures compared with patients dosed with placebo (For further 5 to 6 year old data, see Supplementary Appendix). Secondary outcome measures unrelated to physical functioning did not show a difference between ataluren and placebo.

Table 3.. Timed function test scores (corrected ITT population).

	Mean time and change from baseline to week 48 (Δ) in seconds					
	Placebo		40 mg/kg/day ataluren		80 mg/kg/day ataluren	
Endpoint*	Baseline	Week 48	Baseline	Week 48	Baseline	Week 48
Climb 4 stairs, s	6.0	10.8	6.9	9.3	7.7	11.2
	Δ=4.8		Δ=2.4		Δ=3.5	
Descend 4 stairs	5.5	9.6	6.1	8.5	6.8	9.7
	Δ=4.0		Δ=2.4		Δ=3.0	
Run/walk 10 m	6.8	9.8	7.4	9.1	7.8	10.2
	Δ=3.0		Δ=1.7		Δ=2.4	
Supine to stand	11.4	14.6	10.8	14.0	12.4	15.4
	Δ=3.2		Δ=3.2		Δ=3.0	

Comparison of change from baseline to week 48 between ataluren and placebo, mean (95% CI)		
	40 mg/kg/day ataluren vs Placebo	80 mg/kg/day ataluren vs Placebo
Climb 4 stairs	−2.4 (−4.8, 0.0)	−1.3 (−4.0, 1.4)
Descend 4 stairs	−1.6 (−4.2, 1.0)	−1.1 (−3.9, 1.7)
Run/walk 10 m	−1.4 (−3.7, 0.9)	−0.7 (−3.0, 1.7)
Supine to stand	−0.0 (−2.5, 2.4)	−0.2 (−2.6, 2.2)

**For timed function tests, negative differences between ataluren and placebo represent better outcomes in ataluren-treated patients.*

CI, confidence interval; Corrected ITT, corrected intent-to-treat population.

Based on an analysis of patients with pre- and posttreatment muscle biopsy samples, a mean change from pretreatment to posttreatment of 2.8% in dystrophin/spectrin ratio was observed in the ataluren 40 mg/kg/day dose group: 1.3% in the ataluren 80 mg/kg/day dose group and 0.09% in the placebo group. The method used and results are further detailed in the Supplementary Appendix.

Exposure-Response. The inverse dose-response observed with ataluren was evaluated further by means of assessment of exposure-response using ataluren C_{2h} (plasma concentration 2 h postmorning dose), which correlates with ataluren area under the concentration-time curve (Supplementary Appendix). In patients who received ataluren 40 mg/kg/day, mean C_{2h} across all visits ranged between 3.4 and 19.2 μg/ml. In patients who received ataluren 80 mg/kg/day, mean C_{2h} ranged between 6.5 and 42.1 μg/ml. Approximately 40% of 80 mg/kg/day patients had a mean C_{2h} that overlapped with the range observed in 40 mg/kg/day patients (<19.3 μg/ml). This cutoff point was used to analyze changes in 6MWD and timed function tests by dividing the higher-dose arm into low-concentration (<19.3 μg/ml) and high-concentration (≥19.3 μg/ml) groups. This allowed assessment of whether patients who received 80 mg/kg/day, but with exposure in the range of 40 mg/kg/day, performed better than patients with higher exposure. Consistent with the inverse dose-response relationship, less mean decline in 6MWD and better performance of timed function tests was seen in the group of high-dose patients with mean C_{2h} <19.3 μg/ml (Figure 7).

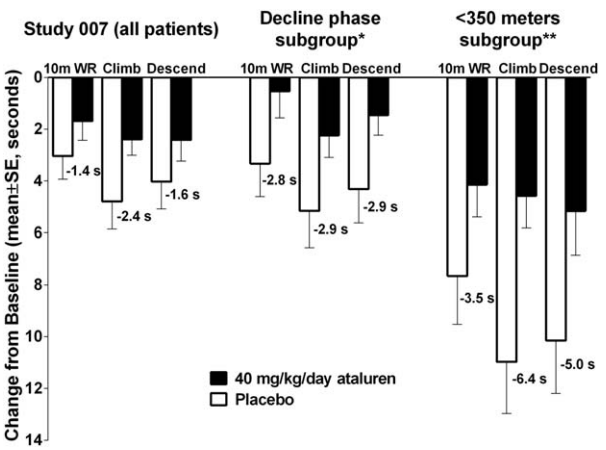

FIGURE 6. Timed function tests change from baseline to week 48 in Study 007 overall population versus decline-phase subgroup.

We also performed an exposure-response analysis by performing a step-wise scan across the entire ataluren plasma concentration range (see Fig. 8). *P*-values for the concentration cuts within the range of exposure for the 40 mg/kg/day dose are significant statistically, whereas the *P*-values for exposures outside that range (i.e., the high exposure range of the 80 mg/kg/day dose) are not.

DISCUSSION

Before this study, there was no accepted primary endpoint identified as suitable for evaluating efficacy in clinical trials of patients with

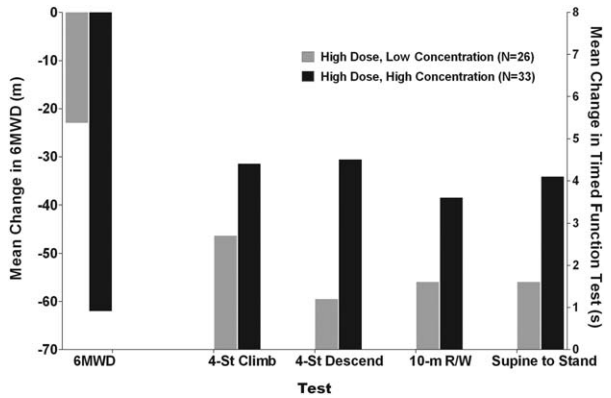

FIGURE 7. Mean change in 6MWD and timed function tests by concentration.

dystrophinopathy. The major goal of intervention during the ambulatory phase of dystrophinopathy is to maintain walking ability for as long as possible.[40] The 6MWT, a standardized assessment of ambulation,[41] had been used to evaluate efficacy in various diseases,[31,32] including myotonic dystrophy type 1.[42] An observational study showed that the 6MWT is feasible and reliable in dystrophinopathy.[27] Subsequent studies have further established the 6MWT as a clinically meaningful outcome measure in dystrophinopathy, and natural history studies show that patients increase in walking ability in the early years, stabilize, then enter a decline phase, which leads, potentially rapidly, to complete loss of ambulation.[28,43,44]

The results from this Phase 2b study showed that ataluren at a dose of 40 mg/kg/day demonstrated clinical benefit and a favorable clinical benefit/risk profile in ambulatory patients $\geq$5 years old with dystrophinopathy due to a nonsense mutation. This study was planned in collaboration with regulatory authorities and academic investigators, and the design and results of this trial lay the groundwork for future clinical trials in dystrophinopathy.

The results showed that ataluren 40 mg/kg/day slowed the rate of decline of walking ability and achieved the targeted mean 30-meter difference between ataluren and placebo in 6MWD over 48 weeks. No effect was observed in the 80 mg/kg/day dose. The 30-meter target was based upon 6MWD data from registration-directed studies for other drugs in other diseases with neuromuscular manifestations.[31,32,42] Differences in mean change in 6MWD between active and placebo ranged from 28 to 44 meters in these previous studies.

Several lines of evidence support the clinical meaningfulness of a 30-meter difference in 6MWT. These include: (1) 2 distribution-based methods show that a 28.5 to 31.7 meters difference in 6MWD should be considered the minimal clinically important difference (MCID), and it is clinically relevant for nmDMD patients.[26] Although not statistically significant, patients treated with ataluren 40 mg/kg/day demonstrated a 31.3-meter difference in change in 6MWD relative to placebo. (2) A 30-meter improvement over placebo in the 6MWT is in the range in which other drugs have been approved in multiple inherited conditions, including mucopolysaccharidosis and Pompe disease. (3) Evidence of the clinical relevance of these results comes from a recent report which showed that a 30-meter change in 6MWD over 48 weeks was considered a clinically meaningful change based on the patient/parent-reported Pediatric Outcomes Data Collection Instruction (PODCI), a quality of life measure, in DMD patients with disease status[45] similar to this study. (4) Recent results of longitudinal 6MWT natural history data in DMD demonstrate that each 30-meter decrease in baseline 6MWD predicts increasing risk of loss of ambulation over the following 2 years (Eugenio Mercuri, MD, unpublished data adapted from a longitudinal multicentric cohort study[46]).

Consistent with ataluren's activity in DMD patients, a time-to-event analysis of the 6MWT showed that ataluren-treated patients were less likely to lose walking ability, defined as a 10% reduction in 6MWD from baseline. This analysis showed that ataluren 40 mg/kg/day substantially slowed disease progression in nmDMD patients. The separation between ataluren and placebo occurred early in the study and continued to the end of the study; by Week 48, 74% of patients who received ataluren 40 mg/kg/day did not experience disease progression versus 56% of patients who received placebo ($P = 0.0386$; see Fig. 2).

Delaying ambulatory decline provides the direct clinical benefit of affording boys with nmDMD a longer period of self-sufficiency. Importantly, slowing the loss of walking ability may also have beneficial effects that could not be measured within a 48-

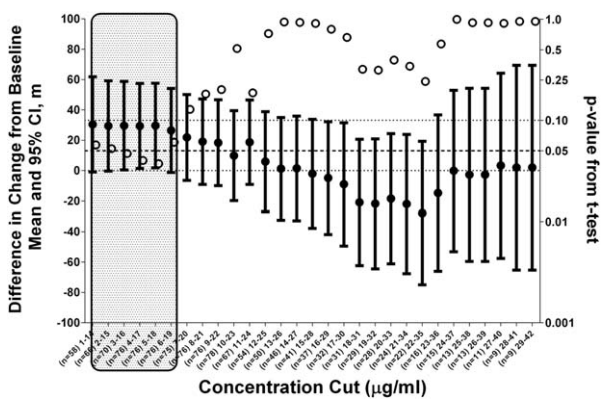

FIGURE 8. Difference in change from baseline and statistical significance by concentration. Note: Shaded area represents exposure range for 40 mg/kg/day ataluren.

week timeframe. For example, maintenance of ambulatory capacity has been associated with prevention or delay of onset and reduced severity of scoliosis and the need for major surgery.[47,48]

The variability of the 6MWT over 48 weeks in this disease was unknown at the time the study was designed. By Week 48, however, it was evident that there was considerable heterogeneity in the rate of disease progression in nmDMD. This contributed to the higher-than-anticipated standard deviation ranging from 72–90 meters (see Supplementary Table 1).

Within all subgroups created by the stratification factors, ataluren 40 mg/kg/day treated patients performed better than placebo patients. This was true for subgroups based on age, baseline 6MWD, and glucocorticoid use. Clinically meaningful differences in disease progression due to ataluren treatment were also found in both the decline-phase subgroup, which included patients aged >7, on steroids, and baseline values of 6MWD from 150 meters to 80% predicted (Δ 6MWD = 49.9 meters), and in the prespecified subgroup with baseline 6MWD < 350 meters (Δ 6MWD = 68.2 meters). These treatment effects over a 48-week study duration in a placebo-controlled trial of DMD are substantial, clinically meaningful, and unprecedented in a corticosteroid-treated population of boys with DMD. The minimal decline in 6MWD among placebo-treated patients with baseline 6MWD $\geq$350 meters (Δ 6MWD = −9 meters) indicates that these patients are in a more stable phase of the disease.

Timed function tests (TFTs) are well-established and sensitive to changes in disease status.[43,44,49,50] Over 48 weeks, ataluren-treated patients showed less decline in TFTs than placebo. More recent data have shown that TFTs are important endpoints, and, like the 6MWT, are predictive of the time for a patient to become nonambulatory.[26] Natural history data from the Cooperative International Neuromuscular Group (CINRG) and from Study 007 show that a >6-s time to climb 4 stairs is predictive of a greater likelihood of 10% progression in the 6MWD, whereas a >8-s stair climb predicts greater likelihood of loss of ambulation over 1 year.[26] The methods used by patients to perform these tests were evaluated by means of functional method grading, which was a novel assessment introduced in this study. It consisted of a 6-point scale that assessed functional ability independently for each timed function test (the complete methodology is detailed in the Supplementary Appendix).

Patients treated with ataluren 40 mg/kg/day trended toward less decline in muscle function compared with placebo as measured by TFTs. The results were not statistically significant, but they met the threshold for clinically meaningful differences, supporting the primary endpoint results. Among the TFTs, the largest effect for ataluren 40 mg/kg/day was seen in stair-climbing, which is one of the most difficult activities of daily living for patients with DMD.[51] These data are particularly important, given that Mazzone et al. have reported that the 6MWT and TFTs provide complementary information and should be used in combination in dystrophinopathy clinical trials.[43] Positive trends favoring ataluren 40 mg/kg/day versus placebo were also seen for accidental fall frequency, functional method grading, activity and wheelchair use in the community setting, myometry, and patient-reported physical functioning. The consistency of these findings supports an ataluren 40 mg/kg/day treatment effect on physical functioning in ambulatory patients with nmDMD.

The lack of effect on 6MWD at 80 mg/kg/day is consistent with nonclinical data and the exposure-response analysis. A bell-shaped concentration-response curve for production of dystrophin has been observed in cultured myotubes isolated from *mdx* mice[2] and from patients with nmDMD when they were exposed to ataluren.[22] Consistent with these findings, recently published results showed that ataluren promoted readthrough of a nonsense mutation in the dystrophin gene of a zebrafish DMD model.[52] A bell-shaped dose-response relationship of ataluren activity in promoting dystrophin expression was also observed, consistent with the bell-shaped dose response curve observed in human and mouse myotubes.

In the current study, an analysis of 6MWD and timed function tests by ataluren C_{2h} showed that ataluren 80 mg/kg/day patients with lower concentrations (i.e., those in the range observed with the 40 mg/kg/day dose) experienced better outcomes than those patients with higher concentrations (Fig. 7; see Supplementary Appendix for further details). A bell-shaped exposure-response relationship has also been seen with aminoglycoside antibiotics (e.g., gentamicin) and other compounds evaluated for their ability to promote readthrough of premature stop codons.[53–55]

The dystrophin expression results were difficult to interpret due to generally poor sample quality as determined by the central laboratory pathologist, including freezing artifact, orientation, and fibrotic replacement (See Supplementary Appendix). Furthermore, a sensitive and reliable method for quantifying dystrophin is not currently available.[56] This issue has been recognized in the DMD research community, where an initiative to develop and validate a reliable dystrophin quantification protocol is ongoing.[57]

Even though the samples were of poor quality, all tissue obtained was immunostained for dystrophin and spectrin (used as the control) in a dual label protocol. A quantitative method for assessing the ratio of dystrophin/spectrin intensity values (similar to that used in the Phase 2a proof of concept study[22]) was used whereby 4 readings per sample were generated and used to produce mean pre- and posttreatment dystrophin and spectrin intensity values. A small positive trend was observed for ataluren compared with placebo.

Ataluren was generally well tolerated at both dose levels over 48 weeks. Adverse event profiles were similar in ataluren- and placebo-treated patients. No patients discontinued from the study due to an adverse event, and no ataluren-related serious adverse events were reported.

A lesson learned from this trial is that the study was underpowered, given the unexpectedly large standard deviation of the 6MWD scores over 48 weeks. However, the encouraging results of this double blind, placebo-controlled, long-term study suggest that ataluren may have a clinically meaningful effect in patients with nonsense mutation dystrophinopathy. Because dystrophin stabilizes muscle function but does not build strength, a dystrophin restoration therapy for DMD patients would be anticipated to preserve muscle function and delay disease progression. For this reason, in a 48-week trial, the efficacy of ataluren should be expected to be more notable in patients who have marked disease progression. The study results confirm this *post hoc*, as it was demonstrated that ataluren's effect is most evident in DMD patients with advanced disease, i.e., patients who have begun a phase of decline in their ambulatory ability.

Collectively, these data indicate that ataluren has clinical activity and a favorable safety profile. There is currently a lack of other disease-modifying treatment options for patients with nonsense mutations. As the first investigational new drug to address the underlying cause of dystrophinopathy, ataluren represents an important advance in personalized, genetic-based treatment of nonsense mutation disease.

This study was sponsored by PTC Therapeutics, funded in part by a grant from the FDA Office of Orphan Products. We thank the patients and their families for their participation in this study; individuals who were instrumental in the conduct of this study and the collection of data, particularly Principal Investigators (see Supplementary Appendix), supporting investigators, clinical coordinators, the clinical evaluator trainers (Ted Abresch, Kim Coleman, Michelle Eagle, Julaine Florence, Ed Gappmaier, Allan Glanzman, and Erik Henricson), clinical evaluators, and study coordinators; Laura Taylor of Nationwide Children's Hospital for assistance with dystrophin quantification; Zejiang Yang of INC Research for statistical programming support. We also thank the patient advocacy organizations (including Valerie Cwik, MD and the Muscular Dystrophy Association and Giovanna Spinella, MD and the Parent Project Muscular Dystrophy) for the collaboration and support which made this trial possible.

Steven Moore was partially supported by the Iowa Wellstone MDCRC NS053672.

We gratefully acknowledge the support of the Clinical Translational Research Center at the Children's Hospital of Philadelphia (UL1-RR-024134). The study in Newcastle was supported by the staff of the MRC Translational Centre for neuromuscular diseases, especially Mr. Geoffrey Bell, the Muscular Dystrophy Campaign, and the TREAT-NMD network. The Newcastle team acknowledges the support of the National Institute for Health Research through the Newcastle Clinical Research Facility and across the UK by means of the support for the Medicines for Children Research Network. We gratefully acknowledge the support of Stefania Corti, Francesca Magri, Valeria Lucchini at the Milan, Italy study site.

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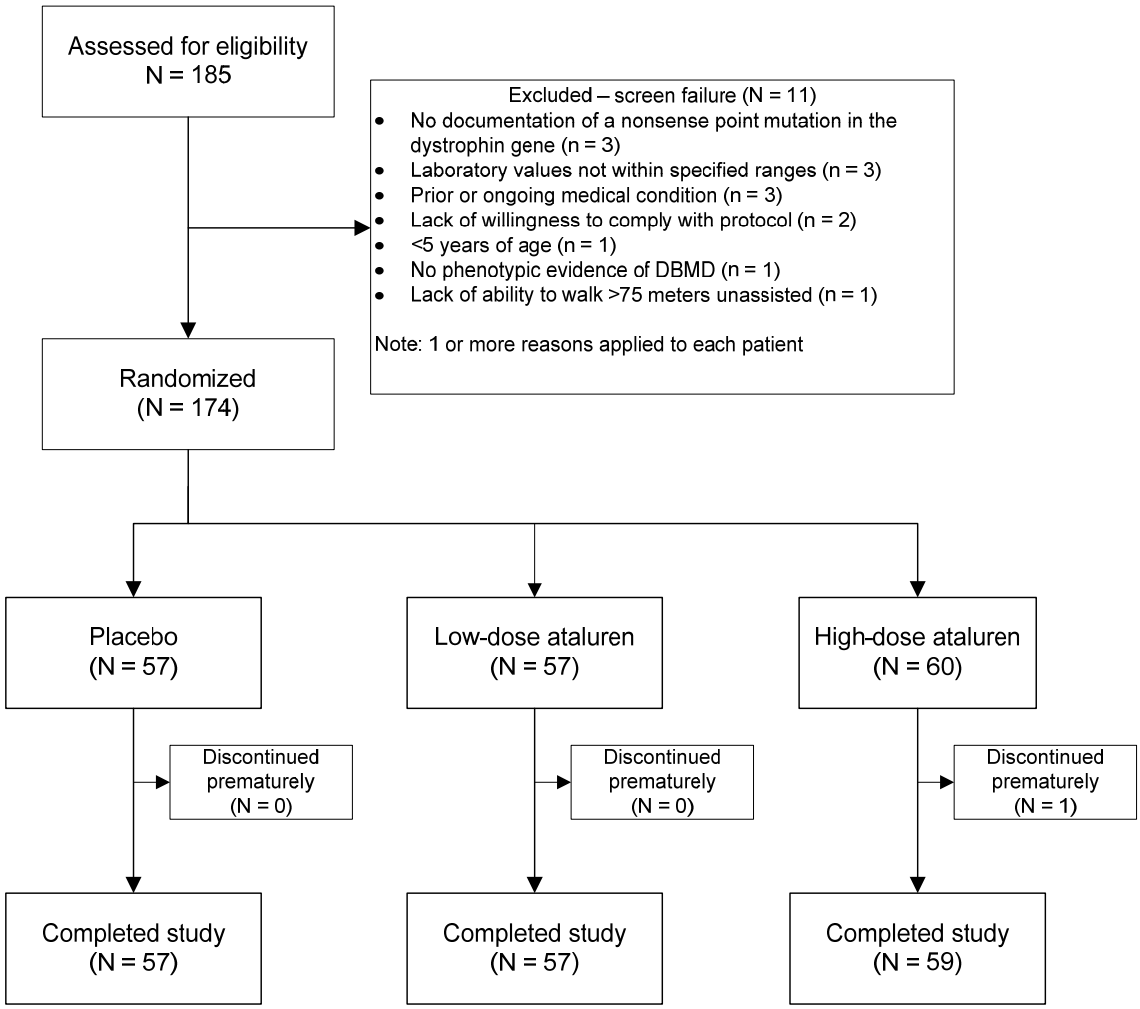
SUPPLEMENTARY APPENDIX

Patient Disposition

Patient disposition is shown in Supplementary Figure 1.

Supplementary Figure 1: Patient Disposition



Statistical Analysis Issues

As this study represented the first use of the 6MWT in a dystrophinopathy (referred to as nmDMD in this paper) therapeutic trial, the nature of an optimal statistical approach for analysis of these data was difficult to predict a priori. Several issues were identified after un-blinding of the results, including:

- An important term was missing from the pre-specified statistical analysis model
- Rank-transformation was not the optimal method for addressing the non-normal distribution of the 6MWD data
- Two patients had lower-limb injuries at baseline that substantially affected their walking ability and led to aberrant baseline 6MWD values

Pre-Specified Analysis of Change in 6MWD

As specified in the statistical analysis plan, an MMRM analysis of the change in 6MWD from baseline to Week 48 was performed. Included in the model were treatment, baseline 6MWD, age (<9 or ≥9 years), glucocorticoid use (yes or no), visit, and treatment-by-visit interaction as fixed factors. Because baseline 6MWD was included in the model as a covariate, the stratification factor of baseline 6MWD (≥350 or <350 meters) was excluded from this model.

The intent in the statistical analysis plan was to analyze 6MWD on its original scale (meters), unless the original data were non-normally distributed. Normality of changes in 6MWD from baseline was tested using the Shapiro-Wilk W-test at the 0.05 significance level. If there was a significant degree of non-normality, then log-transformed or, if necessary, rank-transformed data were to be used in the analysis.

Because the untransformed data and log-transformed data both exhibited significant non-normality, rank-transformed data were analyzed. The resulting nominal p-values for comparisons of mean changes in rank-transformed 6MWD from baseline to Week 48 were 0.1490 for ataluren 40 mg/kg/day vs placebo and 0.4756 for ataluren 80 mg/kg/day vs placebo.

In reviewing the results of the MMRM analysis as well as a pre-specified supportive ANCOVA, a marked discrepancy was observed. Only 5/174 (2.9%) patients had missing 6MWD data at Week 48. Consequently, the ANCOVA on the original data (in which the 5 missing values at Week 48 were replaced with the last observation carried forward [LOCF]) and the MMRM analysis on original data at Week 48 would be expected to yield similar results. Instead, the p-values for the difference between ataluren 40 mg/kg/day and placebo that were obtained with the MMRM on original data (0.0905) and with the ANCOVA of original LOCF Week 48 data (0.0445) were at variance.

Post-Hoc Corrections to Analysis of Change in 6MWD

It was determined that the pre-specified MMRM should have included a baseline-by-visit (baseline*visit) interaction term in the model. This term is critical in allowing the model to take into account the effect of baseline at each visit. The reason for this is that the relationship of the baseline 6MWD to post-baseline 6MWD can potentially vary over time, and the interaction allows for unrestricted modeling of the change in this relationship. Inclusion of the baseline*visit interaction term has become standard practice for repeated-measures analysis.[33,34] This interaction term proved to be highly statistically significant ($p<0.001$). Adding this term to

the MMRM model resulted in a p-value of 0.0446 for ataluren 40 mg/kg/day vs placebo, and thus resolved the discrepancy between the MMRM (p=0.0446) and ANCOVA (p=0.0445) results.

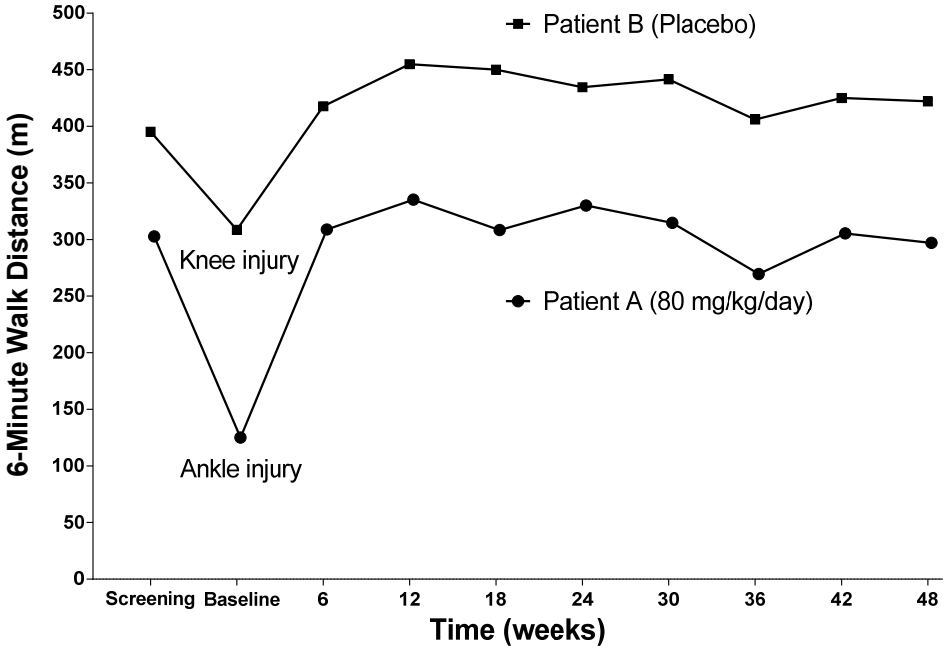
A permutation test (10,000 re-randomizations) was pre-specified as a sensitivity analysis to address the possible effect of biased coin (dynamic) randomization. However, the permutation test can also address other departures from assumptions (eg, non-normality of the data, unknown variance/covariance structure). Thus, the pre-specified analysis on rank-transformed data in the case of non-normality was not necessary. Also, the pre-specified rank-transformation analysis is not as sensitive to treatment differences in many situations as the analysis on the original scale (meters) of distance walked, mainly because it only uses the relative ordering of the distances walked without accounting for the information in the corresponding magnitudes of the distances walked. In patients with nmDMD, 6MWD can decline rapidly and substantially due to disease progression and thus the magnitudes of the distances walked are clinically important. For these reasons, the permutation test provides a more accurate assessment than the pre-specified rank test of the treatment differences in this study.

Two Patients Had Invalid Baseline 6MWD Values

Following un-blinding of the study results, it was noted that 2 patients had baseline 6MWD values that were markedly different than their previous values at screening (up to 6 weeks earlier) and their subsequent values at Week 6. Both patients also were noted to have lower-limb injuries at baseline. Lower-limb injuries are a known source of 6MWD variability.[41]

Through a comprehensive analysis, it was verified that Patients A and B were the only two patients in whom the baseline 6MWT results were affected by lower-limb injuries. Patient A, randomized to the ataluren 80 mg/kg/day treatment arm, had a screening 6MWD value of 303 meters. After spraining his ankle "a couple of days" before his baseline visit (as stated in the case report form), he had a 6MWD value of only 125 meters at baseline. At Week 6, his 6MWD value returned to 309 meters (Supplementary Figure 2). A similar pattern was evident for Patient B, randomized to the placebo treatment arm, who had injured his right knee the "day before [the] test." His 6MWD value was 395 meters at screening. His baseline 6MWD value, obtained a day after his injury, was 309 meters. At Week 6, his 6MWD value was 418 meters. Considering the marked differences between these patients' baseline 6MWD values and their respective screening and Week 6 values, it is clear that these patients' lower-limb injuries, documented as adverse events, led to baseline 6MWD values that were aberrantly low and that did not accurately reflect their pre-treatment ambulatory ability.

Supplementary Figure 2: 6MWD Values for 2 Patients with Lower-Limb Injuries Prior to Baseline in Study 007



As these baseline 6MWTs were not representative of these patients' true baseline ambulatory ability, these 6MWTs should have been classified as invalid (in accordance with the study manual), and the patients should not have been randomized until their injuries had resolved and a valid baseline 6MWD was obtained.

As defined in the statistical analysis plan, a valid 6MWT available at baseline and from at least one post-baseline visit was required for inclusion in the ITT population. Because the baseline 6MWTs performed by Patients A and B were incorrectly classified as valid, these patients were wrongly included in the original ITT population.

To address this issue and to include only truly valid 6MWTs in a cITT analysis, the invalid baseline values were replaced with the valid screening value for Patients A and B. These are the results presented in this article. Similar results are obtained by excluding Patients A and B from the analysis.

Results of Post-Hoc Analysis of Change in 6MWD

Upon discovery of the trial issues discussed above, a post-hoc corrected analysis of mean change in 6MWD over 48 weeks was performed. The baseline 6MWD values for the two patients with lower-limb injuries at baseline were replaced with their screening values. An MMRM analysis on original data, with baseline-by-visit interaction included in the model, was performed. The permutation test was applied to address departures from assumptions.

The difference between ataluren 40 mg/kg/day and placebo in mean change in observed 6MWD from baseline to Week 48 was 31.3 meters (post-hoc nominal p=0.028, adjusted for multiplicity

p=0.056). These results represent the most scientifically appropriate and valid analysis of the 6MWD data in this study.

6MWD Variability

As the study progressed, the variability of 6MWD increased. The primary reason for this is the substantial heterogeneity in the rate of disease progression in nmDMD (Supplementary Table 1).

Supplementary Table 1: Standard Deviation of Change in Observed 6MWD Across All Visits (ITT)

Visit	Treatment Arm		
	Placebo	**Ataluren 40 mg/kg/day**	**Ataluren 80 mg/kg/day**
	N=57	**N=57**	**N=60**
Week 6	33.4	34.0	41.6
Week 12	41.2	40.1	45.7
Week 18	52.7	42.4	49.5
Week 24	63.6	47.5	64.6
Week 30	66.9	55.5	73.4
Week 36	70.7	65.4	75.6
Week 42	87.3	66.5	84.3
Week 48	90.0	72.0	89.2

Abbreviations: 6MWD = 6-minute walk distance, ITT = intent-to-treat population, SD = standard deviation

Secondary Endpoints

Timed function tests and functional method grading

Timed function tests included time taken to stand from a supine position, time taken to run/walk 10 meters, time taken to climb 4 standard-sized stairs, and time taken to descend 4 standard-sized stairs.[50,58-60]

During the test for standing from a supine position, the method used by the patient was categorized and reported as follows:

1. Unable to stand from supine, even with use of a chair
2. Assisted Gowers – requires furniture for assist in arising from supine to full upright posture

3. Full Gowers – rolls over, stands up with both hands “climbing up” the legs to above the knees to achieve full upright posture.
4. Half Gowers – rolls over, stands up with one hand support on lower legs
5. Rolls to the side and/or stands up with one or both hands on the floor to start to rise
6. Stands up without rolling over or using hands

During the test for running or walking 10 meters, the method used by the patient was categorized and reported as follows:

1. Unable to walk independently
2. Unable to walk independently but can walk with support from a person or with assistive device (full leg calipers [knee-ankle-foot orthoses - KAFOs] or walker)
3. Highly adapted gait, wide-based lordotic gait, cannot increase walking speed.
4. Moderately adapted gait, can pick up speed but cannot run.
5. Able to pick up speed but runs with a double stance phase (ie, cannot achieve both feet off the ground).
6. Runs and gets both feet off the ground (with no double stance phase).

During the test for stair-climbing, the method used by the patient was categorized and reported as follows:

Ascending the stairs

1. Unable to up climb 4 standard stairs
2. Climbs 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), using both arms on one or both handrails.
3. Climbs 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), using one arm on one handrail.
4. Climbs 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), not needing handrail.
5. Climbs 4 standard stairs alternating feet, needs handrail for support.
6. Climbs 4 standard stairs alternating feet, not needing handrail support.

Descending the stairs

1. Unable to descend 4 standard stairs
2. Descends 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), using both arms on one or both handrails.
3. Descends 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), using one arm on one handrail.
4. Descends 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), not needing handrail
5. Descends 4 standard stairs alternating feet, needs handrail for support

6. Descends 4 standard stairs alternating feet, not needing handrail support.

For patients who became unable to perform a timed function test due to disease progression, a method of "1" was assigned for each visit at which the patient was no longer able to perform the test.

These parameters were monitored during screening, at baseline, and every six weeks during treatment. Before the study began, clinical evaluators from each of the 37 participating clinical sites participated in a timed function test training and standardization session to harmonize the testing protocol and logistics across the sites. A centralized re-training session was also held ~1 year after study start.

Results for timed function tests and functional method grading are described in the article.

Frequency of Accidental Falls

The number of accidental falls per day was collected via patient/caregiver diaries, completed on a daily basis throughout the trial.

Results for frequency of accidental falls are described in the article.

Myometry

Upper and lower extremity myometry was performed using a hand-held myometer (MicroFET 2, Hoggan Health Industries, West Jordan, Utah, USA) following standardized procedures.[61,62] Muscle groups evaluated included knee flexors, knee extensors, elbow flexors, elbow extensors, and shoulder abductors. Bilateral assessments were done and three measurements were recorded from each muscle group on each side if possible. These parameters were monitored during screening, at baseline, and every six weeks during treatment. Before the study began, clinical evaluators from each of the 37 participating clinical sites participated in a myometry training and standardization session to harmonize the testing protocol and logistics across the sites. A centralized re-training session was also held ~1 year after study start.

Myometric evaluation of limb strength is less sensitive to changes in disease status compared to timed function tests, and muscle strength, although severely affected in ambulatory patients with DMD, deteriorates at a much slower rate than muscle function.[50] In the current study, mean changes from baseline to Week 48 in the placebo arm were small, demonstrating that little decline in myometry occurs over this timeframe, thus making it difficult to show a slowing of progression in muscle strength. Nevertheless, most of the myometry parameters showed less mean decline over 48 weeks for ataluren-treated patients versus placebo (Supplementary Table 1). However, differences were below the 2-pound threshold considered to be clinically meaningful.[37]

Supplementary Table 2: Changes in Myometry from Baseline to Week 48 (ITT Population)

Endpoint, lbs	Placebo		Ataluren 40 mg/kg/day			Ataluren 80 mg/kg/day		
	Base-line, mean	Δ at Wk 48, mean	Base-line, mean	Δ at Wk 48, mean	Difference,[a] mean (95% CI)	Base-line, mean	Δ at Wk 48, mean	Difference,[a] mean (95% CI)
Knee flexion	11.06	0.38	12.08	-0.07	-0.46 (-1.66, 0.75)	12.45	0.39	0.01 (-1.19, 1.20)
Knee extension	12.96	-1.85	12.81	-0.63	1.22 (-0.15, 2.59)	12.71	-0.59	1.26 (-0.10, 2.62)
Elbow flexion	8.14	-0.35	7.66	-0.10	0.25 (-0.41, 0.91)	8.72	-0.50	-0.15 (-0.80, 0.51)
Elbow extension	6.77	-0.51	6.19	0.10	0.60 (-0.05, 1.26)	6.81	-0.28	0.22 (-0.43, 0.87)
Shoulder abduction	5.76	-0.28	5.81	-0.08	0.21 (-0.50, 0.90)	6.37	-0.96	-0.68 (-1.39, 0.02)

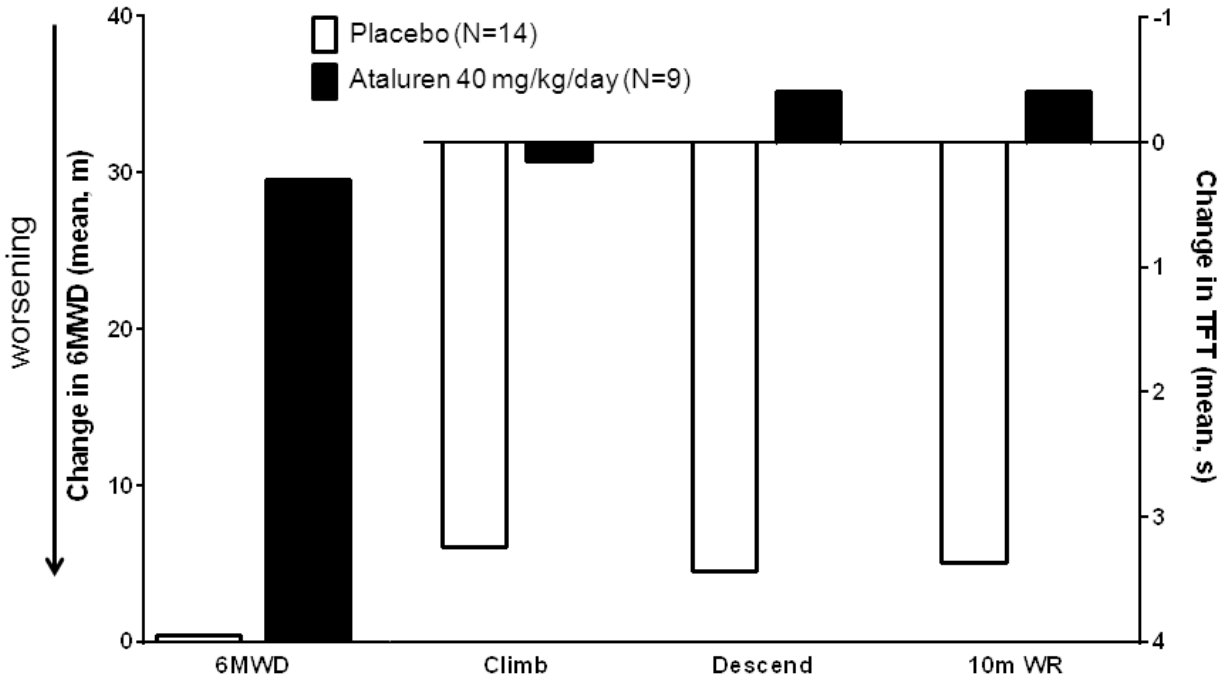
[a] Positive differences between ataluren and placebo represent better outcomes in ataluren-treated patients.

Abbreviation: ITT = intent-to-treat population

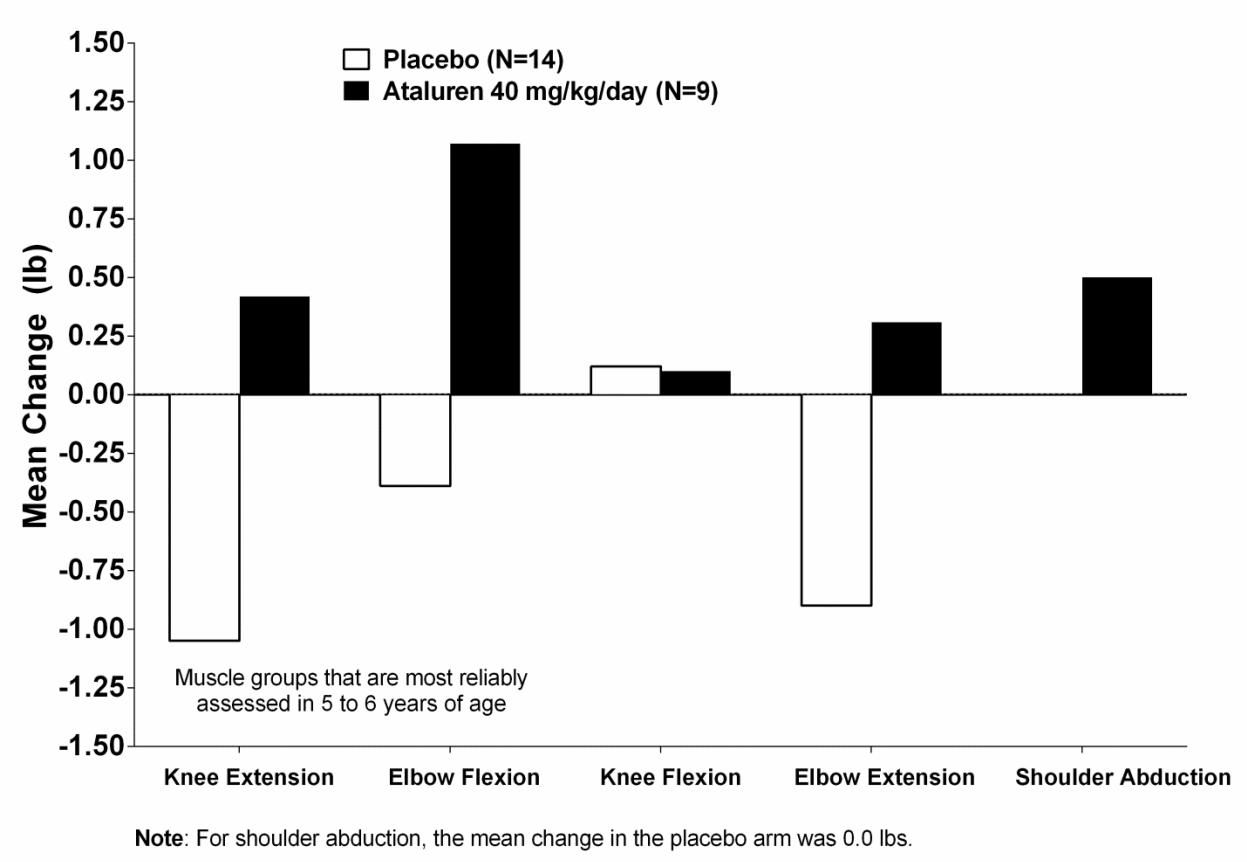
6MWD, TFTs, and Myometry in Younger Patients

In natural history studies, a large percentage of strength decrement in DMD occurs prior to age 7. As a dystrophin restoration therapy, ataluren is expected to stabilize disease by preserving muscle function, but is not expected to increase strength. In the younger population, 5 to 6 years of age, positive trends that favored ataluren 40 mg/kg/day vs placebo were seen in 6MWD, TFTs (see Supplementary Figure 3), and specific to this age group, the most reliably measured forms of myometry, knee extension and elbow flexion (See Supplementary Figure 4).

Supplementary Figure 3: Change from Baseline to Week 48 in 6MWD and TFTs in Study 007 Patients Aged 5 to 6 years


Placebo (N=14)
Ataluren 40 mg/kg/day (N=9)
worsening
Change in 6MWD (mean, m)
Change in TFT (mean, s)
40
30
20
10
0
-1
0
1
2
3
4
6MWD
Climb
Descend
10m WR

Supplementary Figure 4: Change from Baseline to Week 48 in Myometry, Measured by Force Exerted, in the Study 007 Patients Aged 5 to 6 Years



Note: For shoulder abduction, the mean change in the placebo arm was 0.0 lbs.

Activity in the Community Setting as Assessed by Step Activity Monitoring:

Step activity for each patient was captured using an ankle pedometer (StepWatch™ Activity Monitor, Orthocare Innovations LLC., Mountlake Terrace, Washington, USA). Before the study began, clinical evaluators from each of the 37 participating clinical sites participated in a SAM training and standardization session to harmonize the testing protocol and logistics across the sites. A centralized re-training session was also held ~1 year after study start. Within the trial, step activity monitoring was performed in the community setting for periods of ~9 days following each clinic visit.

The proportions of time during which the patient is moving at 0 (no activity), 1 to 15 (low activity), 16 to 30 (medium activity), or >30 (high activity) steps per minute were calculated. In the assessment of time spent at different activity levels, the largest differences between ataluren and placebo in mean changes at Week 48 were observed at the 40 mg/kg/day dose level, which showed trends toward less time spent at no activity (0 steps/minute) and more time spent at medium activity (16 to 30 steps/minute) (Supplementary Table 3). Differences were smaller and inconsistent at the 80 mg/kg/day dose level.

Supplementary Table 3: Proportions of Time Spent at No, Low, Medium, and High Activity (ITT Population)

Endpoint, %[a]	Placebo		Ataluren 40 mg/kg/day			Ataluren 80 mg/kg/day		
	Base-line, mean	Δ at Wk 48, mean	Base-line, mean	Δ at Wk 48, mean	Difference,[b] mean (95% CI)	Base-line, mean	Δ at Wk 48, mean	Difference,[b] mean (95% CI)
No activity	48.17	4.08	51.88	2.78	-1.30 (-5.51, 2.90)	49.41	4.07	-0.01 (-4.16, 4.13)
Low activity	32.86	-1.11	32.38	-1.12	-0.01 (-2.90, 2.88)	32.91	-2.06	-0.95 (-3.80, 1.90)
Medium activity	11.84	-1.92	10.00	-0.69	1.23 (-0.25, 2.71)	11.11	-1.35	0.57 (-0.89, 2.03)
High activity	7.17	-1.03	5.78	-0.96	0.07 (-1.18, 1.31)	6.59	-0.66	0.37 (-0.87, 1.60)

[a] No activity = 0 steps/minute; low activity = ≤15 steps/minute; medium activity = 16-30 steps/minute; high activity = >30 steps/minute

[b] For no activity, negative differences between ataluren and placebo represent better outcomes in ataluren-treated patients. For medium and high activity, positive differences between ataluren and placebo represent better outcomes in ataluren-treated patients.

Abbreviation: ITT = intent-to-treat population

Even during the ambulatory phase of the disease, as the condition advances, patients sometimes require the use of a wheelchair. Thus, in conjunction with this assessment, the patient/caregiver reported the number of days that they used a wheelchair during the step activity monitoring data collection period.

Patient-reported wheelchair use also showed a positive trend favoring ataluren 40 mg/kg/day vs placebo. At baseline, mean percentage of days of wheelchair use was 13.2% for placebo and 13.2% for ataluren 40 mg/kg/day. Mean percentage of days of wheelchair use increased from baseline to Week 48 by 11.5 % for placebo compared to 4.0% for ataluren 40 mg/kg/day. Thus, the difference was 7.5% favoring ataluren 40 mg/kg/day vs placebo. Differences were smaller at the 80 mg/kg/day dose level.

Health-Related Quality of Life:

Patient-reported HRQL was assessed by the PedsQL, which comprises physical functioning and psychosocial functioning (ie, emotional functioning, social functioning, and school functioning) scales. The physical functioning scale is most directly applicable to the clinical manifestations of nmDMD. Mean change in physical functioning score at Week 48 was -1.0 for placebo and 2.4 for ataluren 40 mg/kg/day. Thus, a difference in mean change in physical functioning score at Week 48 of 3.4 favoring ataluren 40 mg/kg/day vs placebo was observed, below the MCID[63]

but trending in the same direction as other measurements of physical functioning. No difference between ataluren 80 mg/kg/day and placebo was observed for the physical functioning scale.

Other Secondary Outcome Measures:

Changes in other secondary efficacy endpoints, not related to physical activity, (PedsQL domains for emotional, social, and school functioning; TSQM; digit span task; heart rate monitoring; serum CK) were generally small and no clear differentiation between ataluren and placebo was observed.

Muscle biopsy dystrophin expression

As noted in the article, mean change from pre-treatment to post-treatment dystrophin/spectrin ratio for the 40 mg/kg/day ataluren dose was 2.8%, 1.3% for the 80 mg/kg/day ataluren dose, and 0.09% for placebo, but interpretation of these results is compromised due to poor sample quality of the muscle biopsies.

The muscle biopsy assessments in this study posed greater challenges than in the Phase 2a, open-label study, in which increases in dystrophin expression were observed in response to ataluren treatment (PTC124-GD-004-DMD).[22] In the Phase 2a study the entire extensor digitorum brevis muscle was studied (excised from one foot during screening and the other foot at the end of treatment). In contrast, in this Phase 2b study of ataluren biopsies were obtained from the biceps branchii (partial muscle excised from one arm during baseline and the other arm at Week 36), rather than from the foot, so as not to interfere with the 6MWT, the primary endpoint in this study.

The Phase 2a study was small in scope (conducted at only three sites in one country) and brief in duration (28 days). In contrast, in this Phase 2b study, muscle biopsy of the biceps brachii was performed pre-treatment and at week 36 of the study, at the 37 study sites in 11 countries (US, UK, Italy, Australia, Germany, Canada, France, Sweden, Spain, Belgium, Israel). Samples were frozen and shipped to a regional laboratory for long-term storage. Periodically, pre-treatment and post-treatment sample pairs for each patient were shipped from the regional laboratory to a central pathology laboratory. Upon receipt at the central pathology laboratory, samples were accessioned and 2-3 cryosections (10 μm in thickness) were cut from each sample and stored at -80°C for subsequent hematoxylin and eosin (H&E) staining and dual-label immunostaining. H&E-stained sections were reviewed by the central pathology laboratory for muscle fiber orientation, presence of freezing artifacts, and severity of dystrophic pathology.

Although favorable dystrophin/spectrin ratios were observed in ataluren-treated patients, the majority of muscle biopsy samples were compromised (Supplementary Table 4), which confounded interpretation of the dystrophin expression results. Freezing artifacts were very common and may have been introduced at the time the biopsy was initial frozen or during shipping and storage. Many biopsies were also poorly oriented, resulting in a mixture of cross, oblique, and longitudinal sections of muscle fibers. The oblique and longitudinal orientations are more likely to have tangential sections through the sarcolemma, which may compromise quantitative assessment of dystrophin expression. In addition, the severity of endomysial fibrosis with or without fatty replacement ranged from mild to severe. Cryosection quality was sometimes poor in samples with severe fibrofatty replacement. Overall, the poor quality of the histological specimens precluded meaningful interpretation of the muscle biopsy dystrophin expression data.

Supplementary Table 4: Morphologic Features of Muscle Biopsy Samples

Morphologic Feature	N[a] (%)
Freezing artifacts	
None	74/342 (21.6)
Mild to moderate	123/342 (36.0)
Severe	145/342 (42.4)
Orientation	
Cross	206/342 (60.2)
Mixed	135/342 (39.5)
Longitudinal	1/342 (0.3)
Endomysial fibrosis with or without fatty replacement	
Mild	172/342 (50.3)
Moderate	122/342 (35.7)
Severe	48/342 (14.0)

[a] 174 patients were enrolled in the study, 348 muscle biopsies were planned, and 342 muscle biopsies were assessed for morphologic features by the central laboratory pathologist.

Furthermore, a sensitive and reliable method for quantifying dystrophin is not currently available.[56] This issue is well recognized in the Duchenne muscular dystrophy (DMD) research community, where an initiative to develop and validate a dystrophin quantification protocol has been organized.[57]

Recent observations of the potential for nonsense mutation suppression to result in the emergence of novel immunogenic epitopes[5] were not specifically examined in this study.

Pharmacokinetics

In this Phase 2b trial, ataluren plasma concentrations were measured before (C_{0h}) and 2 hours (C_{2h}) after the morning dose every 6 weeks for 48 weeks. The mean plasma concentrations were dose-proportional and stable over time.

In a prior Phase 2a trial, 24-hour pharmacokinetic profiling was performed after 4 weeks of treatment. The Pearson correlation coefficients for the relationships between C_{0h} and C_{2h} with area under the concentration-time curve from 0 to 24 hours (AUC_{0-24}) were 0.64 and 0.85, respectively. These data indicate that C_{2h} is highly correlated with AUC_{0-24} and support the use of C_{2h} data to examine the ataluren exposure-response relationship in the Phase 2b trial.

Safety

Treatment–emergent adverse events are summarized in Supplementary Table 5.

Supplementary Table 5: Overview of Treatment-Emergent Adverse Events (As-Treated Population)

Parameter, n (%)	Treatment Arm			All Patients
	Placebo	10, 10, 20	20, 20, 40	
	N=57	N=57	N=60	N=174
Patients with ≥1 adverse event	56 (98.2)	55 (96.5)	57 (95.0)	168 (96.6)
Adverse events by severity[a]				
Grade 1 (mild)	21 (36.8)	16 (28.1)	20 (33.3)	57 (32.8)
Grade 2 (moderate)	26 (45.6)	31 (54.4)	27 (45.0)	84 (48.3)
Grade 3 (severe)	9 (15.8)	8 (14.0)	10 (16.7)	27 (15.5)
Grade 4 (life-threatening)	0	0	0	0
Adverse events by relatedness[a]				
Unrelated	14 (24.6)	8 (14.0)	11 (18.3)	33 (19.0)
Unlikely	16 (28.1)	17 (29.8)	13 (21.7)	46 (26.4)
Possible	20 (35.1)	25 (43.9)	29 (48.3)	74 (42.5)
Probable	6 (10.5)	5 (8.8)	4 (6.7)	15 (8.6)
Discontinuations due to adverse events	0	0	0	0
Serious adverse events	3 (5.3)	2 (3.5)	2 (3.3)	7 (4.0)
Deaths	0	0	0	0

[a] The number (%) is based upon the worst category that was reported as assessed by the investigator.

Adverse events occurring in over 10% of patients across all 3 treatment arms during the 48-week treatment period are shown in Supplementary Table 6.

Supplementary Table 6: Treatment-Emergent Adverse Events with a By-Patient Frequency of >10% (As-Treated Population)

MedDRA Preferred Term,[a] n (%)	Treatment Arm			All Patients
	Placebo	40 mg/kg/day	80 mg/kg/day	
	N=57	N=57	N=60	N=174
Vomiting	22 (38.6)	32 (56.1)	27 (45.0)	81 (46.6)
Headache	14 (24.6)	22 (38.6)	15 (25.0)	51 (29.3)
Diarrhoea	14 (24.6)	11 (19.3)	17 (28.3)	42 (24.1)
Nasopharyngitis	13 (22.8)	13 (22.8)	10 (16.7)	36 (20.7)
Pyrexia	12 (21.1)	14 (24.6)	7 (11.7)	33 (19.0)
Cough	11 (19.3)	9 (15.8)	13 (21.7)	33 (19.0)
Abdominal pain upper	9 (15.8)	9 (15.8)	13 (21.7)	31 (17.8)
Upper respiratory tract infection	10 (17.5)	9 (15.8)	11 (18.3)	30 (17.2)
Nausea	7 (12.3)	8 (14.0)	10 (16.7)	25 (14.4)
Fall	7 (12.3)	11 (19.3)	6 (10.0)	24 (13.8)
Abdominal pain	4 (7.0)	7 (12.3)	10 (16.7)	21 (12.1)
Influenza	8 (14.0)	6 (10.5)	7 (11.7)	21 (12.1)
Procedural pain	7 (12.3)	6 (10.5)	8 (13.3)	21 (12.1)
Pain in extremity	6 (10.5)	7 (12.3)	8 (13.3)	21 (12.1)
Back pain	5 (8.8)	9 (15.8)	6 (10.0)	20 (11.5)

[a] Adverse events with a frequency of >10% across all 3 treatment arms are displayed from highest to lowest incidence across all 3 treatment arms using MedDRA Preferred Terms. Patients who had the same adverse event more than once are counted only once for that adverse event. Adverse events with a frequency of ≤10% across all 3 treatment arms are not shown.

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities

All reported serious adverse events are summarized in Supplementary Table 7.

Supplementary Table 7: Serious Adverse Events (As-Treated Population)

MedDRA Preferred Term	Age,[a] years	Treatment Duration,[a] weeks	Relationship per Investigator	Treatment Arm
Abdominal pain	9	7.6	Possibly	Placebo
Appendicitis	12	27.6	Unrelated	40 mg/kg/day
Dehydration	9	0	Unrelated	Placebo
Dehydration	12	25.9	Unrelated	40 mg/kg/day
Femur fracture	16	47.9	Unlikely	Placebo
Grand mal convulsion	13	44.6	Unlikely	Placebo
Influenza	10	47.9	Unlikely	Placebo
Lower limb fracture	9	32.7	Unrelated	80 mg/kg/day
Supraventricular tachycardia	14	46.1	Unlikely	80 mg/kg/day
Varicella	9	23.9	Unrelated	Placebo

[a] Age and treatment duration at start date of event

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, NA= not applicable

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