Results of the phase 3 ACT DMD trial of ataluren in patients with nonsense mutation Duchenne muscular dystrophy: a multicentre, randomised, double-blind, placebo-controlled trial

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PANEL: **RESEARCH IN CONTEXT**

Evidence before this study

Results from a phase 2a trial (NCT00264888) showed that ataluren improved dystrophin expression in the skeletal muscle of patients with nonsense mutation Duchenne muscular dystrophy (nmDMD) after 28 days of treatment. Results from the 6-minute walk test (6MWT) and other timed function tests (TFTs) from a 48-week, phase 2b trial (NCT00592553) showed a clinical benefit of ataluren (at a dose of 40 mg/kg/day) versus placebo in patients with nmDMD. In this phase 2b trial, a post-hoc, subgroup analysis showed that the treatment effect was more evident in patients predicted to be in the decline phase of disease (i.e. those aged 7−16 years with a baseline 6-minute walk distance [6MWD] ≥150 m and ≤80%-predicted for age and height). Furthermore, recent natural history studies have shown that patients with a baseline 6MWD >400 m show fewer declines across multiple measures of physical function. In contrast, patients with a baseline 6MWD <300 m are at higher risk of precipitous declines in 6MWD and loss of ambulation in the subsequent year.

Added value of this study

In the present phase 3 trial (NCT01826487), ataluren-treated boys (aged 7−16 years) in the intent-to-treat (ITT) population showed a 13·0-m least square (LS) mean difference, (standard error of the mean, SEM=10·4); p=0·213 (observed difference, 15·4 m), numerically favouring ataluren, in 6MWD after 48 weeks of treatment compared with placebo-treated boys. In addition, treatment with ataluren led to a statistically significant 42·9-m LS mean difference (15·9); p=0·007 (observed difference, 47·2 m) in 6MWD versus placebo in a pre-specified subgroup of patients with nmDMD in the mid-range (declining) stage of disease who had a baseline 6MWD ≥300 m to <400 m; a subgroup of patients in whom a treatment response, as measured by the 6MWT, is more likely to be observed over 48 weeks. This is owing to the limited sensitivity of the 6MWT (over a 48-week study) in patients with higher baseline function (defined as stable, baseline 6MWD ≥400 m), and because of the increased interpatient variability seen in patients with lower baseline ambulatory function (those at risk of loss of ambulation, baseline 6MWD <300 m). Ataluren-

treated patients in the ITT population showed less deterioration numerically, as measured by the TFTs, versus placebo; this treatment effect was more evident in patients with a baseline 6MWD ≥300 to <400 m. Ataluren-treated patients in the ≥300 to <400 m subgroup also experienced benefits in function versus placebo, as measured by the North Star Ambulatory Assessment (NSAA). Furthermore, a post-hoc analysis using data from the NSAA showed that patients in the ITT population and in the subgroup with baseline 6MWD ≥300 to <400 m experienced statistically significant reductions in the relative risk of loss of clinically meaningful milestones versus placebo (31% and 46% reduction, respectively; both p=0·010).

Implications of all the available evidence

These results demonstrate the clinical benefit of ataluren in a subgroup of patients with nmDMD with a baseline 6MWD ≥300 to <400 m, in whom the 6MWT is most likely to show a treatment benefit over a 48-week trial, owing to the increased sensitivity of this outcome measure in this subgroup.

SUMMARY

Background This trial examined the efficacy and safety of ataluren in ambulatory boys with nonsense mutation Duchenne muscular dystrophy (nmDMD).

Methods This 48-week, phase 3, multicentre, randomised, double-blind, placebo-controlled trial was conducted across 54 sites (18 countries). Key inclusion criteria: nmDMD; boys aged 7−16 years; and baseline 6-minute walk distance (6MWD) ≥150 m and ≤80%-predicted for age and height. Patients were randomised (1:1) to receive ataluren orally thrice daily (40 mg/kg/day) or placebo. The primary endpoint was change in 6MWD from baseline in the intent-to-treat (ITT) population. A pre-specified subgroup of patients with a baseline 6MWD ≥300 to <400 m was also assessed. Secondary endpoints included timed function tests (TFTs). ClinicalTrials.gov: NCT01826487 (completed).

Findings Patients were recruited (Mar 26, 2013–Aug 26, 2014), and randomised to receive ataluren (n=115) or placebo (n=115). The decrease in 6MWD after 48 weeks was less with ataluren (n=114) than with placebo (n=114): least square (LS) mean (standard error of mean, SEM) ∆ataluren *vs* placebo, ITT: 13·0 (10·4) m; p=0·213; ≥300 to <400 m subgroup: 42·9 (15·9) m; p=0·007. Ataluren-treated patients experienced less of a decline versus placebo for TFTs (LS mean ∆ataluren *vs* placebo, ITT: 10-m run/walk: −1·1 s, p=0·117; 4-stair climb: −1·4 s, p=0·058, 4-stair descend: −2·0 s, p=0·012); this was more evident in the ≥300 to <400 m subgroup. Ataluren was generally well tolerated (treatment-related adverse events: ataluren, 33·9% [39/115]; placebo, 20·9% [24/115]); most were mild to moderate in severity.

Interpretation Ataluren-treated ITT patients did not experience a statistically-significant change in 6MWD versus placebo over 48 weeks, although significant effects on other measures were observed. Change in 6MWD was statistically significant in the pre-specified ≥300 to <400 m subgroup; in whom a consistent treatment response is more likely to be observed over a 48-week period using this measure.

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is a severe, progressive and rare neuromuscular, X-linked recessive disease.[1] Corticosteroids and better coordinated care have improved outcomes in DMD in the past several decades,[2,3] but these approaches do not specifically target dystrophin deficiency, which is the underlying cause of DMD.[4] Mutation-specific therapies, aimed at restoring dystrophin protein production, are therefore being explored. Ataluren promotes readthrough of nonsense mutations to produce full-length functional dystrophin protein.[4-7] Approximately 10–15% of patients with DMD have a nonsense mutation,[8] which introduces a premature stop codon into the dystrophin mRNA, leading to the translation of a truncated, non-functional protein. The readthrough mechanism of ataluren targets this mutation to treat the underlying cause of disease.[4]

Results from a phase 2a, open-label, dose-ranging, 28-day trial (NCT00264888) demonstrated an increase from baseline in the dystrophin/spectrin expression ratio in 61% (23/38) of patients with nonsense mutation DMD (nmDMD) after 28 days of treatment with ataluren (16, 40, or 80 mg/kg/day).[6] A phase 2b, randomised, double-blind, placebo-controlled trial (NCT00592553) showed a slowing of disease progression in patients receiving ataluren (40 mg/kg/day) versus placebo, as measured by a change in their 6-minute walk distance (6MWD) after 48 weeks (corrected intent-to-treat[†]: observed mean difference=31·3 m;[7] least square [LS] mean difference=31·7 m; nominal p=0·0197; adjusted p=0·0367);[9] but failed to achieve its primary endpoint. However, secondary outcome measures, including timed function tests (TFTs), supported these results and consistently favoured ataluren over placebo.[7,9] In a subgroup of patients who were in ambulatory decline (7−16 years old, with a baseline 6MWD ≥150 m and ≤80%-predicted for age and height), the observed mean difference in 6MWD between ataluren- and

[†]Baseline 6MWD values for two patients (placebo, n=1; ataluren 80 mg/kg/day, n=1) were lower than their screening values, owing to lower limb injuries that occurred before the baseline visit. These values were therefore replaced with the patients' screening values; this population is therefore referred to as the corrected intent-to-treat population.

placebo-treated patients was 49·9 m[7,9,10] (LS mean difference=45·6 m, nominal p=0·0096; adjusted p=0·0182; PTC Therapeutics, data on file).

The 6-minute walk test (6MWT) and TFTs are recommended in guidelines by the European Medicines Agency[13] and the US Food and Drug Administration for use in clinical trials of DMD.[14] These guidelines recommend stratifying patients according to disease status, functional status and/or developmental stage.[13,14] Natural history data have shown that patients with a baseline 6MWD >400 m show fewer declines in physical functioning than those with a 6MWD ≤400 m.[15,17,18] In addition, emerging magnetic resonance imaging data have shown that, as DMD progresses, fibrotic tissue and fat replace muscle fibres,[19] contributing to a patient's physical decline. Magnetic resonance spectroscopy data have shown that patients with >80% fat fraction in the vastus lateralis muscle are likely to have a 6MWD <300 m and are at increased risk of losing ambulation compared with those with a 6MWD ≥300 m.[20] Treatment effects using the 6MWT are therefore more likely to be observed in patients in the mid-range (declining) stage of disease (baseline 6MWD ≥300 to <400 m). This is owing to the limited sensitivity of the 6MWT (over 48 weeks) for patients with higher baseline function (defined as stable, 6MWD >400 m), and to the increased interpatient variability seen in patients with lower baseline ambulatory function (those at risk of loss of ambulation, 6MWD <300 m).

The aim of this phase 3 trial (ACT DMD, Ataluren Confirmatory Trial of Patients with nmDMD) was to evaluate the ability of ataluren to stabilise ambulation, as measured by the 6MWT, in patients with nmDMD in ambulatory decline compared with placebo over 48 weeks, and to determine the effect of ataluren on other measures of physical function. Based on an evolving understanding of the 6MWT,[15,16] a pre-specified analysis of patients with a baseline 6MWD ≥300 to <400 m was also performed.

METHODS

Study design

This was a phase 3, multicentre, randomised, double-blind, placebo-controlled trial evaluating the efficacy and safety of ataluren orally three times daily in ambulatory boys with nmDMD (NCT01826487). The trial comprised a 2-week screening period, followed by a 48-week blinded treatment period, in which patients received either ataluren or placebo. Subsequently, patients were eligible to enter an open-label extension (NCT02090959). Assessments were performed during screening, at baseline, and then every 8 weeks until the end of treatment. The study was conducted at 54 sites in 18 countries (Australia, Belgium, Brazil, Canada, Chile, Czech Republic, France, Germany, Israel, Italy, Poland, South Korea, Spain, Sweden, Switzerland, Turkey, UK, and the USA). The trial and any changes to the protocol were approved by the local regulatory authorities and the institutional review board of each site. The trial was conducted in accordance with the Declaration of Helsinki (2000) and the ethical principles of Good Clinical Practice, according to the International Conference on Harmonisation Harmonised Tripartite Guideline.

Patients

Patients who met the following inclusion criteria were eligible for enrolment: boys aged 7−16 years; phenotypic evidence of dystrophinopathy (onset of characteristic clinical symptoms or signs by 6 years of age, elevated serum creatine kinase levels and difficulty with ambulation); nmDMD, confirmed by gene sequencing; use of systemic corticosteroids for at least 6 months before the start of treatment, with no significant change in dosage/dosing regimen (not related to change in body weight) for at least 3 months before the start of treatment and an expectation that this would not change during the study; and a 6MWD ≥150 m and ≤80%-predicted for age and height during screening. Subsequently, patients were required to perform two valid 6MWTs on 2 separate days (with the second value ±20% of the first value). The mean of these two performances was taken as the baseline 6MWD, and was to be within ±20% of the screening 6MWD. Patients' laboratory results during

screening were required to be within normal ranges (with the exception of tests indicative of muscle breakdown). Key exclusion criteria included: treatment with systemic aminoglycoside antibiotics within 3 months of the start of treatment; initiation of systemic corticosteroids in the 6 months before the start of treatment; and change in systemic corticosteroid therapy within 3 months before the start of treatment (not related to change in body weight). A full list of exclusion criteria is provided in the **Supplementary Methods**. Written, informed consent was obtained from each patient's parents or guardians and assent was provided by the patient (where appropriate).

Randomisation and masking

Eligible patients were stratified based on age (<9 years and ≥9 years), duration of prior corticosteroid use (6 to <12 months and ≥12 months), and baseline 6MWD (<350 m and ≥350 m). Patients were randomised 1:1 to receive placebo or ataluren, using the permuted block randomisation technique, which allowed for the treatment arms to be balanced with respect to the stratification factors and patient numbers. A study site representative provided patient information to the interactive voice response/interactive web response system, which then assigned patients to their treatment arms. Patients, parents/caregivers, investigational site personnel, PTC Therapeutics employees, and all other study personnel remained blinded until every patient had completed the study and the database was locked. The identity of the study treatment was concealed using a placebo that was identical to the active drug in appearance, taste, odour, packaging and labelling.

Procedures

Patients received either placebo or ataluren (PTC Therapeutics International Limited, Ireland) dosed orally three times daily (10, 10 and 20 mg/kg of body weight for morning, midday, and evening doses) for 48 weeks. Doses were to be given 6 h apart on the same day, with a 12-h interval between evening and morning doses on the next day. Patients' clinical and medical histories were recorded during screening. Vital signs, height, and weight

178 measurements, and concomitant medications were recorded, and laboratory assessments
179 were performed during screening, at baseline, and every 8 weeks until the end of treatment.
180 A physical examination was performed during screening, at baseline, at 24 weeks, and at
181 the end of treatment. Additionally, patients' physical function was assessed using the
182 6MWT,[16,23] TFTs,[23] and the North Star Ambulatory Assessment (NSAA)[24] during screening,
183 at baseline, and every 8 weeks until the end of treatment. A second 6MWT was performed at
184 baseline and at week 48, and the average of the two was used from these visits.
185
186 **Outcomes**
187 The primary efficacy endpoint was to determine the ability of ataluren to slow disease
188 progression in patients in ambulatory decline, as assessed by the 6MWT. The secondary
189 efficacy endpoint was to determine the effect of ataluren on proximal muscle function, as
190 assessed by TFTs (10-m run/walk, 4-stair climb, 4-stair descend). The following exploratory
191 efficacy endpoints were also examined: change in physical function, as assessed by the
192 percentage of patients who lost ambulation, and by the NSAA (total score);[24] parent-reported
193 health-related quality of life (HRQoL), as assessed by the Pediatric Outcomes Data
194 Collection Instrument (PODCI); and the activities of daily living (ADL)/disease status survey.
195 Endpoints were also evaluated in a pre-specified subgroup of patients who had a baseline
196 6MWD ≥300 to <400 m. Post-hoc analyses included the following: a sensitivity analysis for
197 the 6MWT, including intervals of baseline distance, a composite TFT endpoint (linear
198 combination of 10-m run/walk, 4-stair climb and 4-stair descend), the time to loss of ability to
199 perform the 4-stair climb and 4-stair descend, and the percentage of patients who lost
200 function across each of the individual 17 items in the NSAA. Lastly, a pre-specified meta-
201 analysis was performed using data from the intent-to-treat (ITT) population of this trial and a
202 subgroup of patients from the ITT population of the phase 2b trial[7] (who met the ACT DMD
203 entry criteria). Full details for PODCI, ADL and post-hoc analyses are presented in the
204 **Supplementary Methods**.
205

Adverse events (AEs) were captured throughout the 48-week treatment period.

Statistical analyses

Patient populations

The as-treated population comprised all randomised patients who received any study treatment, with treatment assignments designated according to actual study treatment received. This population was used to analyse safety and treatment administration. The ITT population comprised all patients who were randomised, with study drug assignment designated according to initial randomisation. Patients in this population were required to have a valid baseline 6MWD value and at least one valid post-baseline 6MWD value. This population was used to analyse all efficacy parameters. Both the ITT population and the ≥300 to <400 m subgroup were pre-specified in the statistical analysis plan.

Hypothesis and statistical power

The study hypothesis was that there would be a difference of at least 30 m in change from baseline to week 48 between ataluren- and placebo-treated patients in the decline phase of disease. In the phase 2b study, the standard deviation (SD) of the change in observed 6MWD from baseline to week 48 was 72 m in patients receiving ataluren 40 mg/kg/day.[7] With 1:1 randomisation, 210 patients would be required (ataluren, n=105; placebo, n=105) to detect a difference of 30 m in 6MWD with at least 85% power (α=0·05). Assuming that ~5% of patients discontinue prematurely, a total of 220 patients (ataluren, n=110; placebo, n=110) would need to be enrolled.

Statistical analysis of primary and secondary endpoints

The primary analysis of this study evaluated change in 6MWD from baseline to week 48 in the ITT population using an analysis of covariance (ANCOVA) model. This model included treatment group and the stratification factors for age, duration of corticosteroid use at baseline, and baseline 6MWD category, as well as baseline 6MWD as a covariate. If

patients were unable to perform the 6MWT due to disease progression, a value of zero was used. Within-treatment group multiple imputations on the actual scale were applied to handle missing values via the Markov chain Monte Carlo method; 100 imputations were conducted, which was expected to be adequate given the anticipated amount of missing data. The MIANALYZE procedure (SAS® software, Version 9.3, [2011], SAS Institute Inc., Cary, NC, USA) combined the results from the respective invocations of multiple imputations, producing a final estimate of treatment effect and corresponding standard error.

The secondary efficacy endpoints were evaluated in a similar manner to the primary endpoint; however, if the time taken to perform a TFT exceeded 30 s or if a patient could not perform the test owing to disease progression, a value of 30 s was used. A post-hoc composite TFT endpoint and the time to loss of ability to perform the 4-stair climb and 4-stair descend were also analysed (**Supplementary Methods**). The change in a range of functions was also measured using the NSAA; a validated, internationally-used tool for examining treatment effect in patients with DMD.[25] For the NSAA,[26] patients were rated on a scale of 0−2 for each of the 17 items by the study investigator. A score of 0 indicated that the patient was unable to perform the function, a score of 1 indicated that the patient performed the function with difficulty (independent of physical assistance from another person using a modified method), and a score of 2 indicated that the patient performed the function (without modification/assistance). The sum of the 17 activity scores was used to form an ordinal total score (max score=34). If 13−16 functions were performed, the total score was calculated as follows: ([sum of the scores] × [17/number of activities completed]). If fewer than 13 activities were performed, the total score was considered missing. Ordinal scores were transformed to a linear total score (0−100) for further analysis.[27] A post-hoc analysis of loss of individual functions on the NSAA was also carried out by examining the percentage of patients who shifted from a score of 1−2 at baseline to a score of 0 after 48 weeks of treatment. A p value was obtained using a permutation test with 1000 permutations of treatment assignments within the original eight strata combinations to account for the correlation between the 17

items on the NSAA. Details for the PODCI and ADL/disease status survey are included in the **Supplementary Methods**. No adjustment for multiple comparisons with respect to subgroups was made;[28] all p values for this study can be considered nominal.

Professors C M McDonald and E Mercuri had full access to all study data and were responsible for submission of the manuscript.

RESULTS

Patients were recruited between Mar 26, 2013 and Aug 26, 2014. Of 291 screened patients, 230 were enrolled and randomised to receive either ataluren (n=115) or placebo (n=115). A total of 228 patients (ataluren, n=114; placebo, n=114) met the eligibility criteria for inclusion in the ITT population (**Figure 1**). Overall, 4% of patients (n=9) discontinued the study (ataluren, n=5; placebo, n=4). Two patients from the as-treated population (one from each treatment arm) were prematurely discontinued from the study when dystrophin gene sequencing did not confirm the presence of a nonsense mutation in the dystrophin gene. In addition, two patients discontinued (one in each treatment arm) owing to AEs; these were constipation, possibly related to the study drug (ataluren, n=1) and disease progression (placebo, n=1). Patient demographics and type of concomitant corticosteroid usage are shown in **Table 1**, and were similar at baseline for both treatment arms.

For the primary efficacy endpoint, the LS mean change (standard error of the mean, SEM) in 6MWD from baseline to 48 weeks in the ITT population was −47·7 (9·3) m for ataluren- and −60·7 (9·3) m for placebo-treated patients. This resulted in a 13·0 (10·4) m difference (p=0·213) favouring ataluren (**Figure 2A**). The observed difference was 15·4 m. This effect was more evident in the pre-specified subgroup of patients with a baseline 6MWD ≥300 to <400 m; the LS mean change (SEM) in 6MWD from baseline to 48 weeks was −27·0 (12·6) m in ataluren-treated patients and −69·9 (12·1) m in placebo-treated patients. This resulted in a 42·9 (15·9) m difference (p=0·007), favouring ataluren (**Figure 2B**). The observed difference was 47·2 m. A post-hoc sensitivity analysis was also performed to assess the change in 6MWD from baseline in other patient subgroups. The largest change from baseline in difference between ataluren- and placebo-treated patients was for the pre-specified ≥300 to <400 m subgroup (**Supplementary Table 1**). In addition, loss of ambulation was reduced in ataluren- versus placebo-treated patients; overall, 8% (9/114) of ataluren-treated patients lost ambulation (unable to perform the 6MWT) compared with 12%

(14/114) of placebo-treated patients. The majority of these patients had severely impaired ambulation at baseline (6MWD <300 m). For those with a baseline 6MWD ≥300 to <400 m, no ataluren-treated patient (0/47) lost ambulation versus 8% (4/52) of placebo-treated patients after 48 weeks of treatment (**Supplementary Table 2**).

The TFTs were key secondary efficacy endpoints. In the ITT population, ataluren-treated patients experienced less of a decline than placebo-treated patients, as measured by the TFTs, after 48 weeks of treatment (LS mean difference, ataluren *vs* placebo [SEM], 10-m run/walk: −1·1 [0·7] s, p=0·117; 4-stair climb: −1·4 [0·8] s, p=0·058; 4-stair descend: −2·0 [0·8] s, p=0·012; **Table 2**). However, only the 4-stair descend was statistically significant. This treatment effect favouring ataluren was more evident in the subgroup of patients with a baseline 6MWD ≥300 to <400 m (10-m run/walk: −1·8 [1·0] s, p=0·066; 4-stair climb: −3·5 [1·2] s, p=0·003; 4-stair descend: −4·4 [1·2] s, p<0·001; **Table 2**). A post-hoc composite TFT analysis was performed (10-m run/walk, 4-stair climb and 4-stair descend), and showed that patients receiving ataluren exhibited less deterioration than those receiving placebo. This endpoint showed a statistically significant difference (SEM) of −1·6 (0·7) s between ataluren- and placebo-treated patients, favouring ataluren (p=0·023; **Supplementary Figure 1A**). This effect was more evident in patients with a baseline 6MWD ≥300 to <400 m (−3·5 [1·0] s, p<0·001; **Supplementary Figure 1B**). The time to loss of ability to perform the 4-stair climb and 4-stair descend also favoured ataluren- versus placebo-treated patients (**Supplementary Figures 2A and 2B**).

In the ITT population, a positive LS mean treatment difference (SEM) of 0·8 (0·5) points (p=0·128) (ordinal scale) was observed in the pre-specified total NSAA score numerically favouring ataluren-treated patients. In the linear transformed score, there was a 1.5-point advantage (1·4) for ataluren-treated patients versus placebo (p=0·268). This treatment effect

was more evident in individuals with a baseline 6MWD ≥300 to <400 m, based on observed total score (LS mean difference [SEM], 1·7 points [0·8]; p=0·037) and linear transformed score (4.3-point advantage [2·1] favouring ataluren, p=0·041). A post-hoc analysis to assess the loss of ability to perform each of the 17 individual items of the NSAA was also performed. The proportion of ataluren- and placebo-treated patients able to perform each function at baseline was balanced (**Supplementary Table 3**). Every patient (ataluren, n=114; placebo, n=114) performed each of the 17 functions (totalling 1938 total functions per treatment arm). Ataluren- and placebo-treated patients had 273/1938 and 282/1938 functions assessed as "0" (inability to perform the activity) at baseline, respectively. In the ITT population, after 48 weeks of treatment, ataluren-treated patients lost 12% (203/1665) of functions compared with 18% (294/1656) of functions lost by placebo-treated patients from baseline. This equates to a 31% reduced risk of loss of function for ataluren-treated patients versus those receiving placebo (p=0·010; **Figure 3**). This observation was more evident in patients with a baseline 6MWD ≥300 to <400 m (reduced risk=46%, p=0·010). Results from the PODCI and ADL/disease status survey also favoured ataluren over placebo (**Supplementary Figure 3** and **Supplementary Figure 4**, respectively).

To assess the totality of data collected from this phase 3 trial and the earlier phase 2b trial,[7] a pre-specified meta-analysis was performed. This analysis showed that when 6MWD data for the ITT populations from both trials were combined, a 20·0-m (SEM, 8·2) treatment benefit was observed for ataluren- versus placebo-treated patients over 48 weeks (**Supplementary Figure 5A**). Similarly, when TFT data for the ITT population from both trials were combined, ataluren-treated patients experienced less of a decline than placebo-treated patients (∆ataluren *vs* placebo [SEM], −1·3 to −1·9 [0·6–0·7] s) (**Supplementary Figure 5B)**. Further detail provided in the **Supplementary Material**.

The mean (SD) duration of drug exposure for ataluren- and placebo-treated patients was 332·3 (39·6) and 333·3 (39·7) days, respectively. Ataluren was generally well tolerated, with a high compliance with the dosing regimen. At least one treatment-emergent AE (TEAE) was reported for most patients (ataluren *vs* placebo: 89·6% [103/115] *vs* 87·8% [101/115]) and the majority of reported TEAEs were mild to moderate in severity. Treatment-related (possible or probable) AEs were slightly higher in ataluren- versus placebo-treated patients (33·9% [39/115] vs 20·9% [24/115]) (**Table 3**). Severe TEAEs are summarised in **Supplementary Table 4**. Serious AEs (SAEs) were reported in eight patients (ataluren, n=4; placebo, n=4); four of these patients reported more than one serious AE. All reported SAEs, except one in the placebo group, were considered to be unrelated to treatment. The SAE that occurred in a placebo-treated patient was abnormal hepatic function possibly related to treatment. No new safety signals were identified during the course of this 48-week trial. Additional safety information is reported in the **Supplementary Material**.

DISCUSSION

Dystrophin is a structural protein necessary for preserving the integrity of muscle fibres.[4] Treatments focusing on dystrophin restoration, such as ataluren, are expected to preserve existing muscle function, thereby stabilising or slowing disease progression in patients with DMD. The slowing of disease progression and motor decline is viewed by the DMD physician community as a realistic expectation for the effect of dystrophin restoration therapies,[29] and patients and their caregivers consider this to be a highly valuable benefit of therapy.[30] Data from this phase 3 trial show a positive safety profile for ataluren and demonstrate the clinical efficacy of ataluren (40 mg/kg/day) versus placebo in ambulatory patients with nmDMD in stabilising/slowing disease progression over 48 weeks. Together, these findings show a favourable risk−benefit profile for ataluren, despite this trial not meeting its primary endpoint. For the ITT population, the change in 6MWD between ataluren- and placebo-treated patients was 13·0 m (15·4 m, observed), favouring ataluren. While this primary endpoint did not reach statistical significance (p=0·213), the treatment effect in patients with a baseline 6MWD ≥300 to <400 m was more evident (42·9 m, p=0·007; 47·2 m, observed). Stratifying patients by baseline function is advisable, because of the decreased sensitivity of the 6MWT in patients with higher baseline function and the increased interpatient variability in patients with a baseline 6MWD <300 m (PTC Therapeutics, data on file). It is important to note that a change in 6MWD of <30 m, may be clinically meaningful from the view point of patients' self-reported abilities and HRQoL.[31]

Additionally, fewer ataluren-treated patients lost ambulation compared with those who received placebo over 48 weeks, in both the ITT population and in patients with a baseline 6MWD ≥300 to <400 m. Furthermore, a pre-specified meta-analysis of 6MWD and TFT data from the phase 2b trial[7] and this phase 3 trial showed a statistically significant treatment benefit of ataluren versus placebo.

391 The TFTs are key secondary endpoints that are predictive of loss of function, including
392 ambulation.[16] Across the TFTs, a 1·1−2·0 s-benefit in ataluren-treated patients versus
393 placebo in the ITT population was observed after 48 weeks. This treatment effect was more
394 evident in patients with a baseline 6MWD ≥300 to <400 m (1·8−4·4 s). These findings are
395 similar to those from a recent one-year DMD trial of prednisone, in which a 1·7-s benefit and
396 a 1·6-s benefit were observed for prednisone- versus placebo-treated patients when
397 performing the 10-m run/walk and the 4-stair climb, respectively.[32] When examining the
398 composite TFT endpoint, a statistically significant treatment effect of ataluren versus placebo
399 was observed.

400

401 The clinical benefit of ataluren was also supported using the NSAA, a DMD-specific
402 exploratory efficacy endpoint that provides information on a wide spectrum of functions that
403 are important in everyday life.[17] For the total observed and linear transformed NSAA scores,
404 ataluren-treated ITT patients with a baseline 6MWD ≥300 to <400 m experienced a
405 statistically significant benefit versus placebo over 48 weeks. Furthermore, a post-hoc
406 analysis of data showed that ataluren-treated patients in both populations (ITT and the pre-
407 specified ≥300 to <400 m subgroup) experienced a statistically significant reduction in loss of
408 clinically meaningful milestones across the 17 NSAA functions versus placebo (both
409 p=0·010). This finding suggests a broader context of benefit in motor function experienced
410 by patients receiving ataluren versus those receiving placebo.

411

412 Ataluren was generally well tolerated and no new safety signals were identified. Overall,
413 efficacy and safety data from this trial demonstrate a favourable risk−benefit profile for
414 ataluren in patients with nmDMD, particularly when considering the serious, ultimately fatal
415 nature of this disorder and the high unmet medical need for disease-modifying therapies.

416

LIMITATIONS AND STRENGTHS

The entry criteria employed in this trial were selected to enrich for patients likely to be in ambulatory decline (patients aged 7−16 years; with a baseline 6MWD ≥150 m and ≤80%-predicted for age and height, and with use of systemic corticosteroids for ≥6 months before the start of treatment). However, these criteria allowed for inclusion of a broad subset of study patients with a baseline 6MWD (142·5–526·0 m) and ultimately failed to enrich for patients in ambulatory decline. Patients with a higher range of ambulatory ability (baseline 6MWD ≥400 m) accounted for 37% of patients in this study. These patients tend to remain stable in natural history and placebo studies over a 48-week period; and the inclusion of these patients in the ITT population may have attenuated the treatment effect of ataluren. More stringent entry criteria with regard to baseline 6MWD subgroups would likely have increased the overall effect observed, as seen for patients with a baseline 6MWD ≥300 to <400 m. Owing to the limited sensitivity of the 6MWT (over a 48-week study) in patients with higher baseline function (6MWD ≥400 m), and because of the increased interpatient variability seen in patients with lower baseline ambulatory function (6MWD <300 m), an effect was more likely to be observed in the mid-range subgroup (6MWD ≥300 m to <400 m) over 48 weeks. In addition, because of the slowly progressive nature of the disorder, a longer treatment duration is recommended in current regulatory guidelines for DMD,[13,14] which were not available when this study was designed. Lastly, the clinical endpoints in this trial were effort-dependent and/or susceptible to rater bias; efforts to develop objective, non-invasive measures for DMD studies should continue.

Although the change in 6MWD in the ITT population was not statistically significant, the benefit observed in patients with a baseline ≥300 to <400 m supports the clinical benefit of ataluren versus placebo in patients with nmDMD, especially when considering the totality of supporting evidence. The 48-week data presented here confirm the clinical benefit of ataluren in terms of preserving muscle function, and its favourable risk−benefit profile.

FUTURE RESEARCH DIRECTIONS

Future and ongoing trials should assess the long-term benefits of ataluren in patients with nmDMD. The delay in loss of ambulation seen with ataluren will hopefully extrapolate to longer-term benefits in both upper limb and pulmonary function in non-ambulatory patients with DMD. Future research should therefore determine whether these and other outcome measures not assessed here, but relevant to non-ambulatory patients, also respond to treatment with ataluren. The treatment of younger boys (<5 years old) with ataluren would also be of interest, as treatment initiated earlier is likely to result in the greatest long-term benefit.[14] An additional trial to examine the long-term efficacy and safety of ataluren in patients with nmDMD is currently planned.

Contributors

CMM, CC, RET, RSF, KF, NG, PH, AK, JK, FM, ANO, US, TS, PBS, HLS, HT, MT, JJV, TV, BW, GE, HK, XL, JM, TO, PR, MS, RJS, SWP and EM and the **Clinical Evaluator Training Group authors**[†] contributed to the conception or design of the work; the acquisition, analysis, or interpretation of data for the work; drafted the report and revised it critically for important intellectual content; and gave final approval of the version that was submitted. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declaration of interests

CMM has acted as a consultant on DMD clinical trials to BioMarin, Catabasis, Eli Lilly, Italfarmaco, Mitobridge, Pfizer, PTC Therapeutics, Santhera Pharmaceuticals, and Sarepta Therapeutics; and has received research support for clinical trials from BioMarin, Eli Lilly, PTC Therapeutics, and Sarepta Therapeutics.

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CC has collaborated on clinical trials with Acceleron, Biogen, BioMarin, Eli Lilly, Ionis Pharmaceuticals, Pfizer, and PTC Therapeutics.

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RET has nothing to disclose.

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NG is a site Principal Investigator for the PTC Therapeutics extension study of ataluren in DMD and has acted as a consultant and/or advisory board member for BioMarin, Biogen, Bristol-Myers Squibb, Eli Lilly, Italfarmaco, PTC Therapeutics, Roche, and Summit Therapeutics.

PH has acted as a consultant for Marathon Pharmaceuticals, PTC Therapeutics, and Sarepta Therapeutics.

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TS has received speaking/expert consultancy fees from Biogen, BioMarin, and PTC Therapeutics.

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REFERENCES

1. Bushby K, Finkel R, Birnkrant DJ et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol* 2010; **9**: 77–93.

2. Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for Duchenne muscular dystrophy. *Cochrane Database Syst Rev* 2008; CD003725.

3. Eagle M, Baudouin SV, Chandler C, Giddings DR, Bullock R, Bushby K. Survival in Duchenne muscular dystrophy: improvements in life expectancy since 1967 and the impact of home nocturnal ventilation. *Neuromuscul Disord* 2002; **12**: 926–9.

4. Peltz SW, Morsy M, Welch EM, Jacobson A. Ataluren as an agent for therapeutic nonsense suppression. *Annu Rev Med* 2013; **64**: 407–25.

5. Welch EM, Barton ER, Zhuo J et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 2007; **447**: 87–91.

6. Finkel RS, Flanigan KM, Wong B et al. Phase 2a study of ataluren-mediated dystrophin production in patients with nonsense mutation duchenne muscular dystrophy. *PLoS One* 2013; **8**: e81302.

7. Bushby K, Finkel R, Wong B et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve* 2014; **50**: 477–87.

8. Pichavant C, Aartsma-Rus A, Clemens PR et al. Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. *Mol Ther* 2011; **19**: 830–40.

9. Haas M, Vlcek V, Balabanov P et al. European Medicines Agency review of ataluren for the treatment of ambulant patients aged 5 years and older with Duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene. *Neuromuscul Disord* 2015; **25**: 5–13.

10. McDonald C, Reha A, Elfring G, Peltz SW, Spiegel R. Timed function tests and other physical function outcomes in ataluren-treated patients with nonsense mutation Duchenne muscular dystrophy (nmDMD). *Neuromuscul Disord* 2014; **24**: 861.

11. European Medicines Agency. Translarna™ summary of product characteristics. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002720/WC500171813.pdf (accessed 19 October 2016).

12. PTC Therapeutics. 2014. PTC Therapeutics receives conditional approval in the European Union for Translarna™ for the treatment of nonsense mutation Duchenne muscular dystrophy. Available from: http://ir.ptcbio.com/releasedetail.cfm?releaseid=863914. (accessed 27 October 2016).

13. European Medicines Agency. Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy. Report No. EMA/CHMP/236981/2011, Corr. 1. 2015. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/12/WC500199239.pdf (accessed 19 October 2016).

14. US Food and Drug Administration. Duchenne muscular dystrophy and related dystrophinopathies: developing drugs for treatment. Guidance for industry. 2015. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/UCM450229.pdf (accessed 19 October 2016).

15. Pane M, Mazzone ES, Sivo S et al. Long term natural history data in ambulant boys with Duchenne muscular dystrophy: 36-month changes. *PLoS One* 2014; **9**: e108205.

16. McDonald CM, Henricson EK, Abresch RT et al. The 6-minute walk test and other endpoints in Duchenne muscular dystrophy: longitudinal natural history observations over 48 weeks from a multicenter study. *Muscle Nerve* 2013; **48**: 343–56.

17. Mazzone E, Martinelli D, Berardinelli A et al. North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. *Neuromuscul Disord* 2010; **20**: 712–16.

18. Pane M, Mazzone ES, Fanelli L et al. Reliability of the Performance of Upper Limb assessment in Duchenne muscular dystrophy. *Neuromuscul Disord* 2014; **24**: 201-6.

612 19. Willcocks RJ, Rooney WD, Triplett WT et al. Multicenter prospective longitudinal
613 study of magnetic resonance biomarkers in a large Duchenne muscular dystrophy cohort.
614 *Ann Neurol* 2016; **79**: 535–47.
615 20. Sweeney L, Vandenborne K. Developing skeletal muscle MRI/MRS as a biomarker
616 for DMD therapeutic development.
617 http://www.parentprojectmd.org/site/DocServer/Session_8_-_Sweeney_b.pdf?docID=15384
618 (accessed 22 September 2016).
619 21. ClinicalTrials.gov registered trial (NCT02255552). Confirmatory study of eteplirsen in
620 DMD patients (PROMOVI). 2016. Available from:
621 https://clinicaltrials.gov/ct2/show/NCT02255552 (accessed 27 October 2016).
622 22. ClinicalTrials.gov registered trial (NCT02500381). Study of SRP-4045 and SRP-4053
623 in DMD patients (ESSENCE). Available from:
624 https://clinicaltrials.gov/ct2/show/NCT02500381 (accessed 27 October 2016)

625

626 23. McDonald CM, Henricson EK, Abresch RT et al. The 6-minute walk test and other
627 clinical endpoints in Duchenne muscular dystrophy: reliability, concurrent validity, and
628 minimal clinically important differences from a multicenter study. *Muscle Nerve* 2013; **48**:
629 357–68.
630 24. Scott E, Eagle M, Mayhew A et al. Development of a functional assessment scale for
631 ambulatory boys with Duchenne muscular dystrophy. *Physiother Res Int* 2012; **17**: 101–9.
632 25. Ricotti V, Ridout DA, Pane M et al. The NorthStar Ambulatory Assessment in
633 Duchenne muscular dystrophy: considerations for the design of clinical trials. *Journal of*
634 *Neurology, Neurosurgery & Psychiatry* 2016; **87**: 149–55.
635 26. Mayhew A, Cano S, Scott E, Eagle M, Bushby K, Muntoni F. Moving towards
636 meaningful measurement: Rasch analysis of the North Star Ambulatory Assessment in
637 Duchenne muscular dystrophy. *Dev Med Child Neurol* 2011; **53**: 535-42.

638 27. Mayhew AG, Cano SJ, Scott E et al. Detecting meaningful change using the North
639 Star Ambulatory Assessment in Duchenne muscular dystrophy. *Dev Med Child Neurol* 2013;
640 **55**: 1046−52.
641 28. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*
642 1990; **1**: 43–6.
643 29. Merlini L, Sabatelli P. Improving clinical trial design for Duchenne muscular
644 dystrophy. *BMC Neurol* 2015; **15**: 153.
645 30. Peay HL, Hollin I, Fischer R, Bridges JF. A community-engaged approach to
646 quantifying caregiver preferences for the benefits and risks of emerging therapies for
647 Duchenne muscular dystrophy. *Clin Ther* 2014; **36**: 624–37.
648 31. Henricson E, Abresch R, Han JJ et al. The 6-minute walk test and person-reported
649 outcomes in boys with Duchenne muscular dystrophy and typically developing controls:
650 longitudinal comparisons and clinically-meaningful changes over one year. *PLoS Curr* 2013;
651 **5**: ecurrents.md.9e17658b007eb79fcd6f723089f79e06.
652 32. Hu J, Ye Y, Kong M et al. Daily prednisone treatment in Duchenne muscular
653 dystrophy in southwest China. *Muscle Nerve* 2015; **52**: 1001−7.

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TABLES

Table 1: **Patient demographics at baseline (as-treated population)**

Characteristic	Ataluren (n=115)	Placebo (n=115)
Age, years	9·0 (7–10)	9·0 (8–10)
Male, n (%)	115 (100%)	115 (100%)
Race, n (%)		
Caucasian	89 (77%)	86 (75%)
Black	1 (1%)	1 (1%)
Asian	7 (6%)	6 (5%)
Hispanic	4 (4%)	8 (7%)
Other	7 (6%)	4 (4%)
Not reported	7 (6%)	10 (9%)
Height, cm	125·6 (118–132)	126·0 (118–133)
Weight, kg	29·3 (23–37)	27·0 (24–34)
Body mass index, kg/m^2	18·4 (16–22)	17·9 (16–20)
Age at diagnosis, years	4·0 (3·3–6·8)	4·0 (2·3–6·9)
Time from diagnosis to randomisation, years	4·8 (2·2–5·5)	4·7 (2·1–5·9)
Phenotype diagnosis, n (%)		
Waddling gait	83 (72%)	76 (66%)
Gowers' manoeuvre	83 (72%)	91 (79%)
Calf hypertrophy	91 (79%)	92 (80%)
6MWD, m	375·2 (314–421)	370·5 (314–422)
6MWD <300 m, n (%)	25 (22%)	22 (19%)
6MWD ≥300 to <400 m, n (%)	47 (41%)	52 (45%)
6MWD ≥400 m, n (%)	43 (37%)	41 (36%)
Concomitant corticosteroid use		
Deflazacort	50 (44%)	54 (47%)
Prednisone	38 (33%)	37 (32%)
Prednisolone	29 (25%)	28 (24%)

Data are median (25th and 75th percentiles) unless otherwise indicated.6MWD=6-minute walk distance

Table 2: **LS mean change from baseline to week 48 (SEM) in time to perform each TFT for ataluren- and placebo-treated patients (ITT population and a subgroup of patients with a baseline 6MWD ≥300 to <400 m)**

Group	Endpoint	LS mean change at week 48 (SEM), s					
		Ataluren	Placebo	LS mean difference (SEM), s	p value	Combined mean at baseline, s*	Difference, %†
ITT	10-m run/walk	2·36 (0·60)	3·43 (0·60)	−1·1 (0·7)	0·117	6·71	15·9%
	4-stair climb	3·88 (0·66)	5·31 (0·66)	−1·4 (0·8)	0·058	6·14	23·3%
	4-stair descend	2·78 (0·69)	4·75 (0·69)	−2·0 (0·8)	0·012	4·90	40·2%
≥300 to <400 m subgroup	10-m run/walk	0·92 (0·79)	2·76 (0·76)	−1·8 (1·0)	0·066	6·52	28·2%
	4-stair climb	2·27 (0·91)	5·73 (0·88)	−3·5 (1·2)	0·003	5·65	61·2%
	4-stair descend	0·54 (0·92)	4·90 (0·89)	−4·4 (1·2)	<0·001	4·34	100·5%

P values obtained via ANCOVA with multiple imputation.

*Mean baseline values for time taken to perform each TFT for the total population (ataluren- and placebo-treated patients).

†Difference, % = (LS mean difference/combined mean at baseline) x 100.

6MWD=6-minute walk distance, ANCOVA=analysis of covariance, ITT=intent-to-treat, LS=least square, SEM=standard error of the mean; TFT=timed function test.

Table 3: **Reported TEAEs (as-treated population)**

Parameter, n (%)	**Ataluren (n=115)**	**Placebo (n=115)**
Patients with ≥1 TEAE	103 (89·6%)	101 (87·8%)
TEAE by severity[†]		
Mild[‡]	61 (53·0%)	54 (47·0%)
Moderate[‡]	35 (30·4%)	37 (32·2%)
Severe[‡]	7 (6·1%)	9 (7·8%)
TEAEs by relatedness		
Unrelated	44 (38·3%)	47 (40·9%)
Unlikely	20 (17·4%)	30 (26·1%)
Possible	27 (23·5%)	18 (15·7%)
Probable	12 (10·4%)	6 (5·2%)
MedDRA system organ class/preferred term, **TEAEs reported for ≥5% of patients**		
Gastrointestinal disorders	52 (45·2%)	48 (41·7%)
Vomiting	26 (22·6%)	21 (18·3%)
Diarrhoea	20 (17·4%)	10 (8·7%)
Abdominal pain upper	9 (7·8%)	13 (11·3%)
Nausea	7 (6·1%)	7 (6·1%)
Constipation	3 (2·6%)	10 (8·7%)
Abdominal pain	7 (6·1%)	5 (4·3%)
General disorders and administration site conditions	29 (25·2%)	32 (27·8%)
Pyrexia	16 (13·9%)	12 (10·4%)
Disease progression	9 (7·8%)	14 (12·2%)
Infections and infestations	63 (54·8%)	50 (43·5%)
Nasopharyngitis	24 (20·9%)	22 (19·1%)
Upper respiratory tract infection	11 (9·6%)	6 (5·2%)
Rhinitis	8 (7·0%)	4 (3·5%)
Injury, poisoning, and procedural complications	35 (30·4%)	34 (29·6%)
Falls	21 (18·3%)	20 (17·4%)
Musculoskeletal and connective tissue disorders	32 (27·8%)	32 (27·8%)
Pain in extremity	10 (8·7%)	14 (12·2%)

Back pain	11 (9·6%)	8 (7·0%)
Nervous system disorders	28 (24·3%)	23 (20·0%)
Headache	21 (18·3%)	21 (18·3%)
Respiratory, thoracic, and mediastinal disorders	34 (29·6%)	30 (26·1%)
Cough	19 (16·5%)	13 (11·3%)
Oropharyngeal pain	7 (6·1%)	6 (5·2%)

Patients who had the same adverse event more than once were counted only once for that adverse event.

[†]No life-threatening or fatal TEAEs were reported.

[‡]Mild: sign or symptom not easily tolerated, but not expected to have a clinically significant effect on the patient's overall health and well-being, does not interfere with the patient's usual functions and is not likely to require medical attention; moderate: sign or symptom causes interference with usual activity or affects clinical status and may require medical intervention; severe: sign or symptom is incapacitating or significantly affects clinical status and likely requires medical intervention and/or close follow-up.

MedDRA=medical dictionary for regulatory activities, TEAE=treatment-emergent adverse event.

FIGURES LEGENDS

Figure 1: **Trial profile**

Note: Two patients from the as-treated population (one from each treatment arm) were prematurely discontinued from the study when dystrophin gene sequencing did not confirm the presence of a nonsense mutation in the dystrophin gene. This meant that they did not have at least one valid post-baseline 6MWD value, a requirement for the ITT population.
6MWD=6-minute walk distance, ITT=intent-to-treat.

Figure 2: **LS mean change (SEM) from baseline to week 48 in 6MWD for ataluren- and placebo-treated patients in (A) the ITT population and in (B) the subgroup of patients with a baseline 6MWD ≥300 to <400 m (pre-specified, mid-range)**

ANCOVA model based on change from baseline as the dependent variable, and independent variables included stratification for age (<9 or ≥9 years old), duration of previous corticosteroid use (6 to <12 months or ≥12 months) and baseline 6MWD (<350 m or ≥350 m), treatment, and baseline 6MWD as a covariate. *P* values obtained via ANCOVA via multiple imputations.
6MWD=6-minute walk distance, ANCOVA=analysis of covariance, ITT=intent-to-treat, LS=least square, SEM=standard error of the mean.

Figure 3: **Percentage of patients who lost the ability to perform each individual item in the NSAA over 48 weeks (ITT population)**

A score of 0 indicated that the patient was unable to perform the function, a score of 1 indicated that the patient performed the function with difficulty (ie, the patient completed the activity independent of physical assistance from another person using a modified method), and a score of 2 indicated that the patient performed the function (without modification or assistance). P value obtained via resampling analysis methods. This was a post-hoc analysis.

710 ITT=intent-to-treat, L=Left, NSAA=North Star Ambulatory Assessment, R=Right.

711