Meta-Analysis

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Meta-analyses of ataluren randomized controlled trials in nonsense mutation Duchenne muscular dystrophy

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Aim: Assess the totality of efficacy evidence for ataluren in patients with nonsense mutation Duchenne muscular dystrophy (nmDMD). **Materials & methods:** Data from the two completed randomized controlled trials (ClinicalTrials.gov: NCT00592553; NCT01826487) of ataluren in nmDMD were combined to examine

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the intent-to-treat (ITT) populations and two patient subgroups (baseline 6-min walk distance [6MWD] ≥300–<400 or <400 m). Meta-analyses examined 6MWD change from baseline to week 48. **Results:** Statistically significant differences in 6MWD change with ataluren versus placebo were observed across all three meta-analyses. Least-squares mean difference (95% CI): ITT (n = 342), +17.2 (0.2–34.1) m, p = 0.0473; ≥300–<400 m (n = 143), +43.9 (18.2–69.6) m, p = 0.0008; <400 m (n = 216), +27.7 (6.4–49.0) m, p = 0.0109. **Conclusion:** These meta-analyses support previous evidence for ataluren in slowing disease progression versus placebo in patients with nmDMD over 48 weeks. Treatment benefit was most evident in patients with a baseline 6MWD ≥300–<400 m (the ambulatory transition phase), thereby informing future trial design.

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Duchenne muscular dystrophy (DMD) is a rare, X-linked, neuromuscular disease characterized by progressive muscle degeneration resulting in loss of ambulation and early death [1]. In 10–15% of boys, the disease is caused by a nonsense mutation in the *DMD* gene [2]. Ataluren (40 mg/kg/day) is an orally bioavailable molecule enabling functional dystrophin production [3] and is conditionally approved for the treatment of nonsense mutation DMD (nmDMD) in ambulatory patients aged 2 years or older in member states of the EU, Iceland, Israel, Kazakhstan, Liechtenstein, Norway and the Republic of Korea and approved for ambulatory patients aged 5 years or older in Brazil, Chile and the Ukraine (under special state registration in Ukraine) [4]. Two randomized, double-blind, placebo-controlled trials of ataluren in nmDMD have been conducted to date: one Phase IIb trial [5] and one Phase III trial (Ataluren Confirmatory Trial in DMD [ACT DMD]) [6]. Both trials assessed efficacy measures supported by regulatory authorities, including the 6-min walk test (6MWT) and timed function tests (TFTs) [7,8].

Over time, understanding of the sensitivity of the 6MWT across the spectrum of ambulatory phases in nmDMD has increased [6,9–11]. It is now known that patients with a baseline 6-min walk distance (6MWD) ≥400 m remain relatively stable in physical functioning over 48 weeks, whereas patients with a 6MWD <300 m are at highest risk of rapid decline and loss of ambulation over the same period of time [6,11]. The EMA [7] and US FDA [8] guidelines recommend stratifying patients by functional status.

Although neither trial met its primary end point, both trials reported a numerical benefit for ataluren-treated patients compared with those treated with placebo, as measured by the 6MWT [5,6,12]; in the Phase III trial, this difference was statistically significant in the prespecified subgroup with baseline 6MWD ≥300–<400 m (patients in the 'ambulatory transition' phase) but not in the overall intent-to-treat (ITT) population [6].

Rare disease clinical trials such as these are limited by the inclusion of relatively low numbers of participants. We therefore aimed to assess the efficacy of ataluren in nmDMD based on all the available evidence by conducting meta-analyses using the ITT populations of patients from both trials (receiving ataluren 40 mg/kg/day or placebo) and then using subgroups categorized by prespecified baseline 6MWD values.

Patients & methods

Study inclusion criteria

Inclusion criteria comprised all randomized placebo-controlled trials of patients with nmDMD who received ataluren 40 mg/kg/day or placebo. All completed randomized controlled trials of ataluren have been conducted by PTC Therapeutics Inc.; these two studies were included in the meta-analyses. These were the Phase IIb trial (ClinicalTrials.gov: NCT00592553; study start date, February 2008) [5] and ACT DMD (ClinicalTrials.gov: NCT01826487; study start date, March 2013) [6]. It was anticipated that these were the only randomized controlled trials of ataluren to date; however, the Ovid MEDLINE® (1946–present) and Embase® (1974–8 February 2019) databases were searched to identify any relevant randomized controlled trials performed by other study sponsors. The search strategy is presented in Supplementary Table 5.

Study design

The two trials were of similar design, both being randomized, double-blind, placebo-controlled, multicenter, international trials in patients with nmDMD. Both trials included ambulant boys with nmDMD confirmed by

gene sequencing and phenotypic evidence of dystrophinopathy, with elevated serum creatine kinase levels and ambulation difficulty. One key difference between the trials was the inclusion criterion regarding patients' baseline 6MWD. The Phase IIb trial specified boys were aged ≥5 years, with a screening 6MWD ≥75 m [5]. ACT DMD inclusion criteria were more strict, specifying boys aged ≥7 and ≤16 years, with a 6MWD of both ≥150 m and ≤80% of that predicted for their age and height [6]. ACT DMD also specified that patients should be receiving concomitant stable corticosteroid therapy. This was not specified in the Phase IIb trial; nonetheless, 71% of patients recruited into the Phase IIb study were receiving corticosteroids [5,6]. Full trial inclusion and exclusion criteria have been published previously [5,6]. Patients in both trials received ataluren 40 mg/kg/day (given orally in three doses: 10, 10 and 20 mg/kg for morning, midday and evening doses, respectively) or placebo for 48 weeks, except for some patients in the Phase IIb trial who received high-dose ataluren (80 mg/kg/day). However, as the approved dose of ataluren is 40 mg/kg/day [4], patients in the Phase IIb trial who received high-dose ataluren were not included in the meta-analyses presented in this manuscript.

Data extraction

All data for individual patients from both trials receiving ataluren 40 mg/kg/day or placebo for 48 weeks were available and were analyzed by the sponsor of the trials. The following information was collated: patient baseline characteristics and demographics; change from baseline to week 48 in 6MWD (primary end point); time to persistent 10% worsening in 6MWD (secondary end point); and change from baseline to week 48 in three TFTs (time taken to walk/run 10 m, to climb four stairs and to descend four stairs; all secondary end points). A fourth TFT, time taken to stand from a supine position, was not included, because a high proportion of patients in both trials could not complete this task at baseline (Phase IIb, 23%; ACT DMD, 10%). Safety parameters were assessed throughout both trials and the safety outcomes are briefly summarized below.

Statistical analyses

The meta-analyses reported here were performed in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2009 guidelines, where applicable [13]. All completed randomized controlled trials of ataluren conducted by PTC Therapeutics Inc. to date have been included for analysis. Because these meta-analyses included only two randomized, placebo-controlled studies and no significant treatment–study interaction or treatment heterogeneity across the two studies were observed, the standard fixed-effects model approach was utilized – that is, the overall treatment effect was estimated via a weighted average of the two individual treatment effect estimates, where the weight was inversely proportional to the variance estimates. This approach is appropriate based on two conditions: the two studies are functionally identical; and the aim of these analyses is to compute the common effect size for the identified populations and not to generalize the outcomes to other populations [14]. Outcomes for the 6MWT and TFTs are reported as the least-squares (LS) mean difference ± 95% CIs. Time to persistent 10% 6WMD worsening is reported as the hazard ratio (HR) with 95% CI, indicating risk of persistent worsening.

Three meta-analyses were conducted. The first examined data from the ITT populations of the two trials (receiving ataluren 40 mg/kg/day or placebo). This meta-analysis was conducted to provide additional data with a more conservative approach, including a larger and more heterogeneous population than the meta-analysis specified in the ACT DMD statistical analysis plan – the latter examined patients in the Phase IIb study who met ACT DMD criteria and is reported elsewhere [6]. The second and third meta-analyses examined patients from the ITT populations with a prespecified baseline 6MWD of ≥300–<400 and <400 m, respectively. The subgroup of patients with a baseline 6WMD of ≥300–<400 m was prespecified in the Study 020 statistical analysis plan in order to determine the treatment effect in the 'ambulatory transition phase', in which 48-week changes in 6MWD are most likely to be observed [6]. The subgroup of patients with a baseline 6MWD <400 m was not prespecified in the Study 020 statistical analysis plan; the rationale for including this subgroup was that the upperbound inclusion criterion (specifying 6MWD <80% of that predicted) of ACT DMD failed to eliminate patients who were in the stable phase of the disease, with many patients (n = 84) still having baseline 6MWD values in excess of 400 m.

Results

Study characteristics

The search of the Ovid MEDLINE and Embase databases identified 143 articles relating to ataluren in nmDMD; however, as anticipated, only two randomized, placebo-controlled studies of ataluren have been conducted to date.

All randomized placebo-controlled trials of ataluren in patients with nmDMD were included [5,6]. In total, 114 patients received either ataluren 40 mg/kg/day (n = 57) or placebo (n = 57) in the Phase IIb trial and 228 patients received either ataluren (n = 114) or placebo (n = 114) in the Phase III trial [5,6].

Baseline patient demographics & characteristics

Baseline demographics and characteristics of boys included in both trials were similar, except for the aforementioned functional inclusion criterion and were similar between the ataluren- and placebo-treated groups. The mean (standard deviation [SD]) age of patients in both trials was comparable (ataluren vs placebo: Phase IIb, 8.8 [2.9] vs 8.3 [2.3] years; ACT DMD, 8.9 [1.8] vs 9.0 [1.7] years). The majority of patients in both trials were Caucasian (ataluren vs placebo: Phase IIb, 93.0% [53/57] vs 94.7% [54/57]; ACT DMD, 77.4% [89/115] vs 74.8% [86/115]). At baseline, mean (SD) 6MWD was slightly lower for patients in the Phase IIb trial (ataluren, 350.0 [97.6] m; placebo, 359.6 [87.7] m) than in ACT DMD (ataluren, 364.0 [73.3] m; placebo, 362.7 [81.4] m). Full details of baseline characteristics have been reported previously [5,6].

Meta-analysis of the entire ITT populations

This meta-analysis included data from all patients who received ataluren 40 mg/kg/day or placebo in the Phase IIb trial (ataluren, n = 57; placebo, n = 57) and ACT DMD (ataluren, n = 114; placebo, n = 114). The combined results from the two ITT populations demonstrated a difference in change in 6MWD from baseline to week 48 between ataluren- and placebo-treated patients, which was statistically significant in favor of ataluren (LS mean difference [95% CI], +17.2 [0.2–34.1] m; p = 0.0473; Figure 1A). Combined results also revealed statistically significant benefits in patients receiving ataluren versus placebo (LS mean [95% CI]) in time to climb four stairs (-1.7 [-2.9 to -0.4] s; p = 0.0078) and descend four stairs (-1.9 [-3.2 to -0.6] s; p = 0.0055), but the time to walk/run 10 m missed statistical significance (-1.1 [-2.2 to 0.1] s; p = 0.0677; Figure 2A). Patients who received ataluren versus placebo also had a statistically significantly reduced risk of persistent 10% 6MWD worsening (HR [95% CI], 0.68 [0.48–0.94]; p = 0.0215; Figure 3A). Treatment effects observed in the meta-analyses were improved compared with those observed in the individual trials for all outcomes. Between-trial differences were not statistically significant for any end point, indicating that it is appropriate to combine data in this meta-analysis (p = 0.4715, 0.9811, 0.4994, 0.8070 and 0.3258 for between-trial differences for change from baseline in 6MWD, time to walk/run 10 m, time to climb four stairs and time to descend four stairs and risk of persistent 10% 6MWD worsening, respectively).

Meta-analysis of the ≥300–<400 m 6MWD subgroup

This meta-analysis of patient subgroup data from the ITT populations was based on 44 patients in the Phase IIb trial (ataluren, n = 22; placebo, n = 22) and 99 patients in ACT DMD (ataluren, n = 47; placebo, n = 52) who had a baseline 6MWD of ≥300–<400 m. In this subgroup, the meta-analysis demonstrated a statistically significant difference in change in 6MWD between ataluren-treated and placebo-treated patients, favoring ataluren (+43.9 [18.2–69.6] m; p = 0.0008; Figure 1B). Statistically significant benefits in patients receiving ataluren versus placebo in this subgroup were also seen in time taken to walk/run 10 m (-2.1 [-3.7 to -0.4] s; p = 0.0149), climb four stairs (-3.4 [-5.3 to -1.5] s; p = 0.0004) and descend four stairs (-4.3 [-6.2 to -2.3] s; p < 0.0001; Figure 2B). No significant difference in risk of persistent 10% 6MWD worsening in ataluren- versus placebo-treated patients was observed (0.66 [0.39–1.11]; p = 0.1162; Figure 3B). Treatment effects observed in the meta-analyses were improved compared with those observed in the individual trials for all outcomes except risk of persistent 10% 6MWD worsening, the p-value for which was lower in the Phase IIb trial than in the meta-analysis (p = 0.0415 vs p = 0.1162). Again, between-trial differences were not statistically significant for any end point and justified this analysis for this subgroup (p = 0.9141, 0.6506, 0.9243, 0.8596 and 0.1491 for between-trial differences for change from baseline in 6MWD, time to walk/run 10 m, time to climb four stairs and time to descend four stairs and risk of persistent 10% 6MWD worsening, respectively).

Meta-analysis of the <400 m 6MWD subgroup

This meta-analysis was based on data from a subgroup of patients from the ITT populations with a baseline 6MWD <400 m, including 72 patients in the Phase IIb trial (ataluren, n = 37; placebo, n = 35) and 144 patients in ACT DMD (ataluren, n = 71; placebo, n = 73). In this subgroup, the difference in change in 6MWD between ataluren- and placebo-treated patients was statistically significant, favoring ataluren (+27.7 [6.4–49.0] m; p = 0.0109; Figure 1C). Statistically significant benefits in patients receiving ataluren versus placebo in this subgroup were also

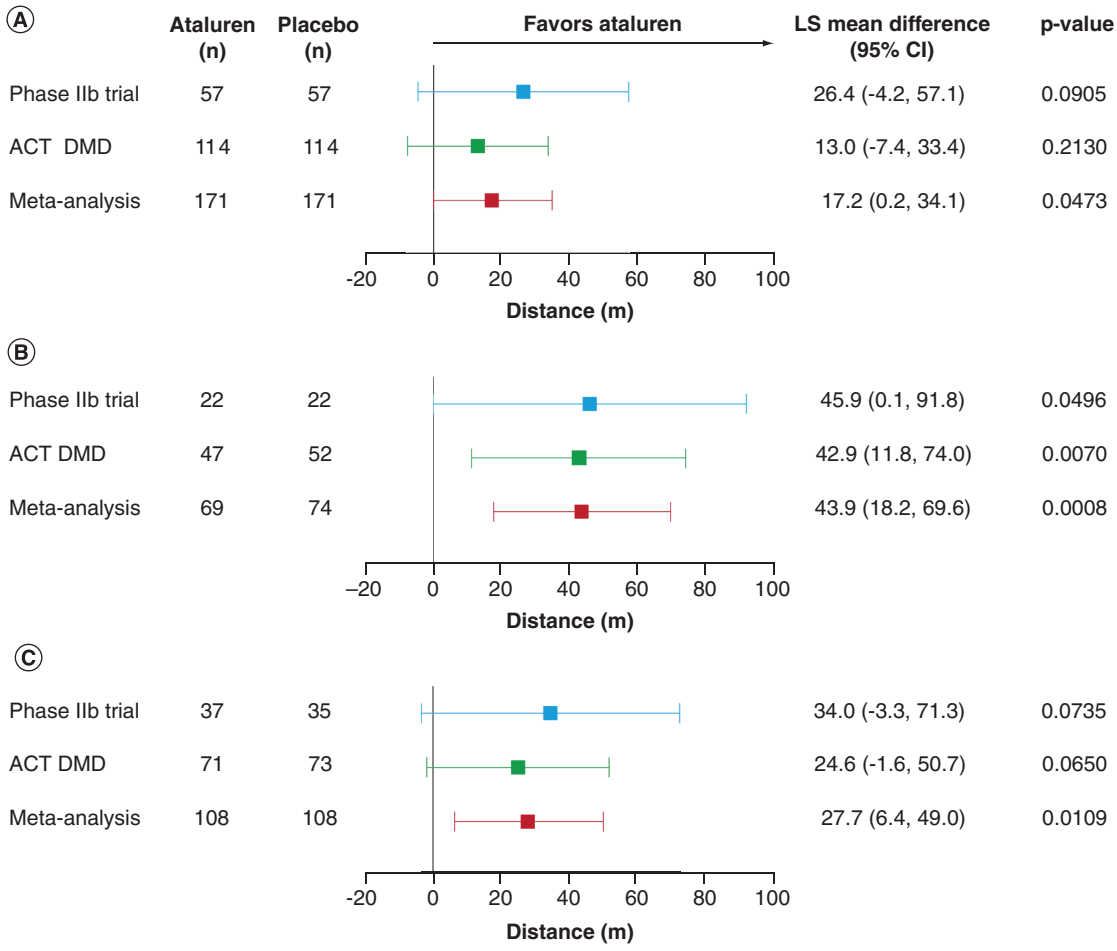
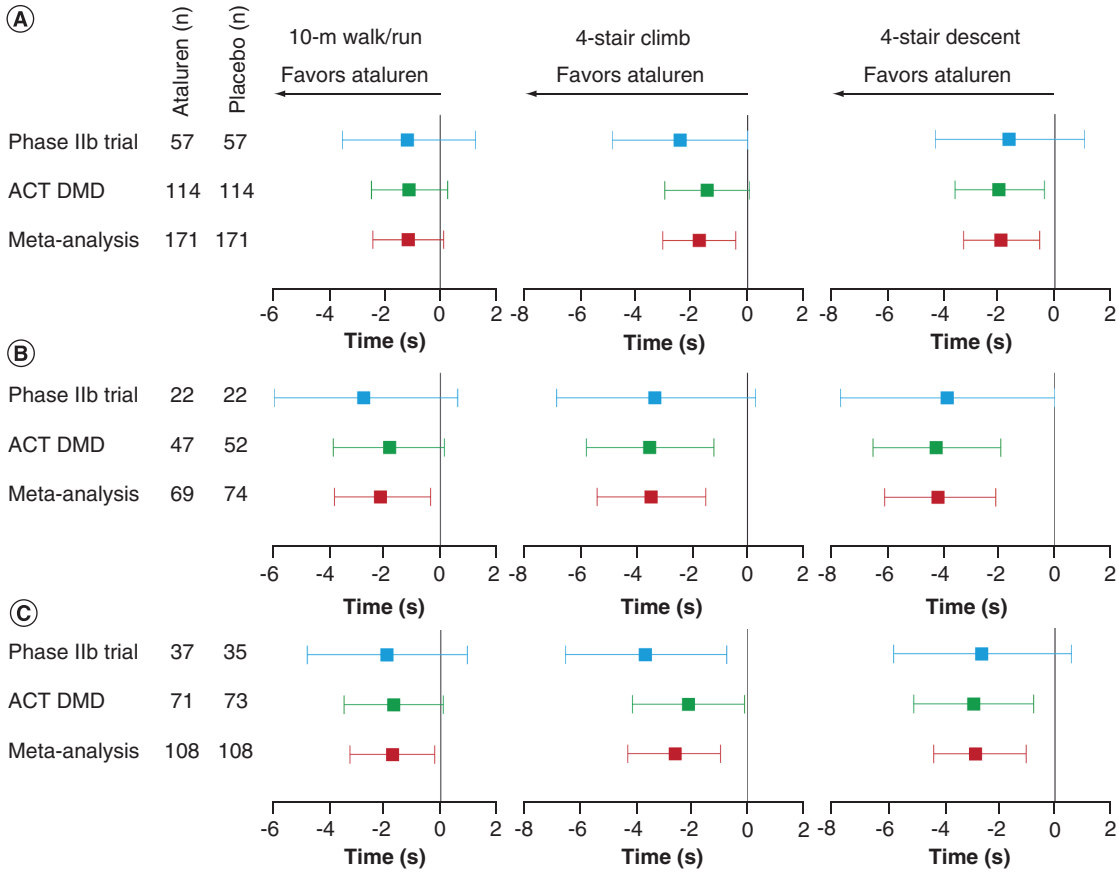


Figure 1. Least-squares mean differences in change in 6-min walk distance from baseline to week 48. LS mean differences between ataluren and placebo groups were assessed by **(A)** meta-analysis of the intent-to-treat population, **(B)** meta-analysis of patients with a baseline 6MWD ≥300–<400 m and **(C)** meta-analysis of patients with a baseline 6MWD <400 m, using a fixed-effects model. Data show LS mean difference ± 95% CIs.
6MWD: 6-min walk distance; ACT DMD: Ataluren Confirmatory Trial in Duchenne Muscular Dystrophy; LS: Least-squares.

seen in time to walk/run 10 m (-1.7 [-3.2 to -0.3] s; p = 0.0197), climb four stairs (-2.6 [-4.3 to -1.0] s; p = 0.0013) and descend four stairs (-2.8 [-4.3 to -1.1] s; p = 0.0015; Figure 2C). Furthermore, patients who received ataluren experienced a statistically significantly reduced risk of persistent 10% 6MWD worsening relative to those who received placebo (0.62 [0.45–0.85]; p = 0.0028; Figure 3C). Treatment effects observed in the meta-analyses were improved compared with those observed in the individual trials for all outcomes, except for time to walk/run 10 m, the p-value for which was lower in the Phase III trial than in the meta-analysis (p = 0.0050 vs p = 0.0197). Between-trial differences were not statistically significant for any end point and thus justified this analysis for this subgroup (p = 0.6826, 0.8992, 0.3876, 0.8702 and 0.8269 for between-trial differences for change from baseline in 6MWD, time to walk/run 10 m, time to climb four stairs and time to descend four stairs and risk of persistent 10% 6MWD worsening, respectively).

Safety outcomes

The spectrum and severity of adverse events (AEs) were consistent across the two trials. In brief, the majority of patients experienced AEs that were mild to moderate in severity (Phase IIb trial: ataluren, 82.5%; placebo, 82.5%; ACT DMD: ataluren, 83.5%; placebo, 79.1%). AEs were considered possibly or probably related to the study drug



		LS mean difference (95% CI)	p-value	LS mean difference (95% CI)	p-value	LS mean difference (95% CI)	p-value
A	Phase IIb trial	-1.1 (-3.4, 1.2)	0.3509	-2.4 (-4.8, 0.0)	0.0488	-1.6 (-4.2, 1.0)	0.2268
	ACT DMD	-1.1 (-2.4, 0.3)	0.1170	-1.4 (-2.9, 0.1)	0.0580	-2.0 (-3.5, -0.4)	0.0120
	Meta-analysis	-1.1 (-2.2, 0.1)	0.0677	-1.7 (-2.9, -0.4)	0.0078	-1.9 (-3.2, -0.6)	0.0055
B	Phase IIb trial	-2.7 (-5.9, 0.5)	0.1000	-3.3 (-6.8, 0.3)	0.0715	-4.0 (-7.8, -0.1)	0.0419
	ACT DMD	-1.8 (-3.8, 0.1)	0.0660	-3.5 (-5.7, -1.2)	0.0030	-4.4 (-6.6, -2.1)	<0.001
	Meta-analysis	-2.1 (-3.7, -0.4)	0.0149	-3.4 (-5.3, -1.5)	0.0004	-4.3 (-6.2, -2.3)	<0.0001
C	Phase IIb trial	-1.9 (-4.7, 0.9)	0.1823	-3.7 (-6.5, -0.8)	0.0116	-2.6 (-5.7, 0.5)	0.1018
	ACT DMD	-1.7 (-3.4, 0.0)	0.0050	-2.1 (-4.1, -0.2)	0.0340	-2.9 (-5.0, -0.8)	0.0070
	Meta-analysis	-1.7 (-3.2, -0.3)	0.0197	-2.6 (-4.3, -1.0)	0.0013	-2.8 (-4.3, -1.1)	0.0015

Figure 2. Least-squares mean differences in change in time to complete timed function tests from baseline to week 48. LS mean differences between ataluren and placebo groups were assessed by **(A)** meta-analysis of the ITT population, **(B)** meta-analysis of patients with a baseline 6MWD ≥300–<400 m and **(C)** meta-analysis of patients with a baseline 6MWD <400 m, using a fixed-effects model. Data show LS mean difference ± 95% CIs.
6MWD: 6-min walk distance; ACT DMD: Ataluren Confirmatory Trial in Duchenne Muscular Dystrophy; LS: Least-squares.

in a similar proportion of patients across both trials (Phase IIb: ataluren, 45.6%; placebo, 52.6%; ACT DMD: ataluren, 33.9%; placebo, 20.9%). No individuals discontinued owing to AEs in the Phase IIb trial; two patients discontinued owing to AEs in ACT DMD (ataluren, n = 1 [constipation]; placebo, n = 1 [disease progression]). No deaths were reported in either trial [5,6].

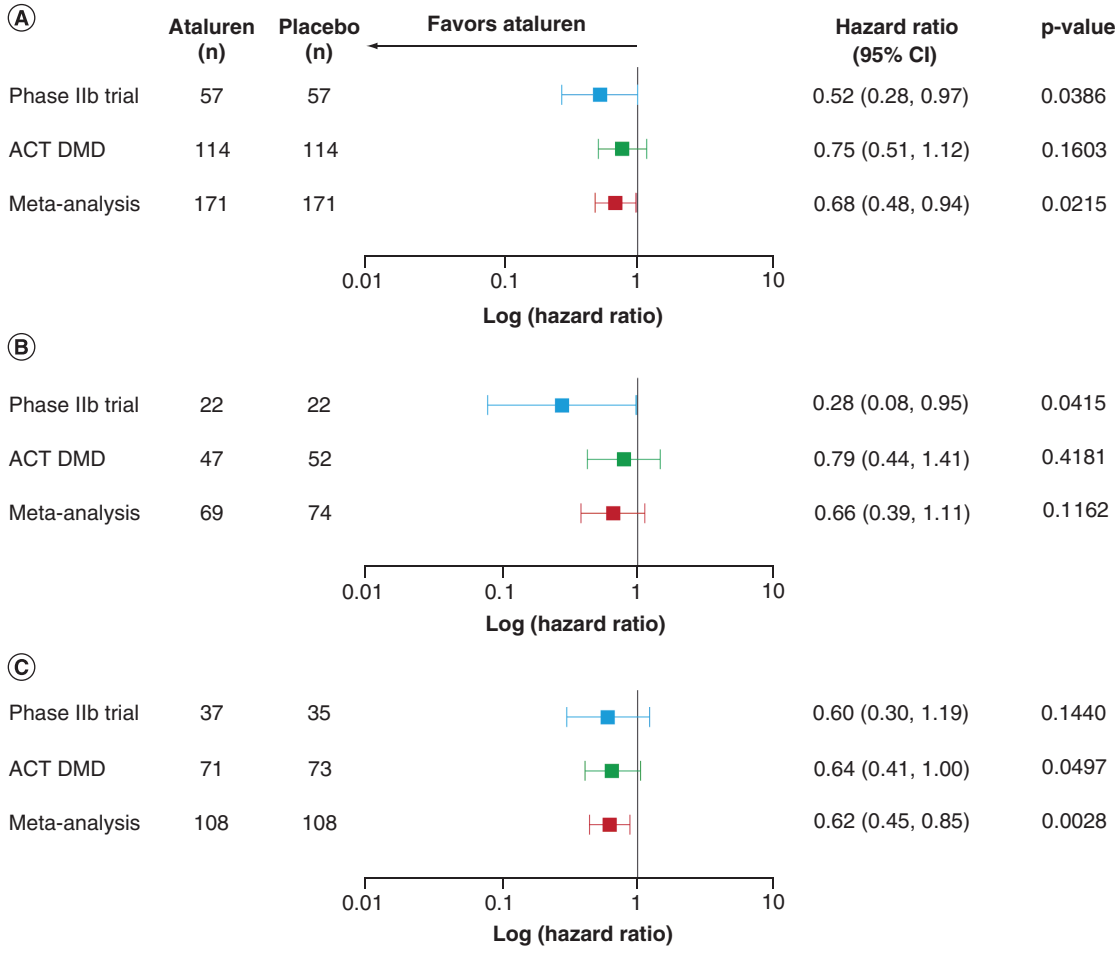


Figure 3. Least-squares mean differences in risk of persistent 10% 6-min walk distance worsening between ataluren and placebo groups. Assessments were by **(A)** meta-analysis of the intent-to-treat population, **(B)** meta-analysis of patients with a baseline 6MWD ≥300–<400 m and **(C)** meta-analysis of patients with a baseline 6MWD <400 m, using a fixed-effects model. Data show hazard ratio ± 95% CIs.
6MWD: 6-min walk distance; ACT DMD: Ataluren Confirmatory Trial in Duchenne Muscular Dystrophy.

Discussion

Ataluren (40 mg/kg/day) is the first approved therapy that specifically targets the underlying cause of DMD in patients with nonsense mutations. Using meta-analyses of data from the two randomized, placebo-controlled clinical trials [5,6], ataluren treatment results in a statistically significant slowing of disease progression, as measured by the 6MWT, compared with placebo over 48 weeks of treatment. Furthermore, using this approach, a statistically significant slowing of disease progression was also observed in several of the TFTs, which could be performed by virtually all patients at baseline. Treatment effects observed in the meta-analyses were improved compared with those observed in the individual trials for almost all outcomes.

The meta-analysis of the combined ITT populations demonstrated a 17.2 m treatment benefit with ataluren compared with placebo as measured by the 6MWT. Evidence suggests that a change in 6MWD of less than 30 m can be clinically meaningful in terms of patients' self-reported health-related quality of life [15]. Understanding of the 6MWT and its sensitivity in the different ambulatory phases of DMD has evolved over recent years [9–11]. Natural history data show that change in 6MWD over 48 weeks appears to be most demonstrable in patients who are in the 'ambulatory transition' phase (i.e., those with baseline 6MWD ≥300–<400 m) [6]. Our meta-analyses examining patients with a baseline 6MWD of ≥300–<400 m and of <400 m indeed showed substantial treatment benefits for ataluren (+43.9 and +27.7 m, respectively) over 48 weeks versus placebo. These analyses were performed

to investigate the efficacy of ataluren in patients in the 'ambulatory transition' and 'ambulatory decline' phases of the disease, respectively – that is, the groups likely to experience deterioration in their ability to perform the 6MWT within 48 weeks. When a patient's baseline 6MWD is less than 325–350 m, or lower than 55% of that predicted for their age and height, the patient is at a significantly increased risk of loss of ambulation in the following year [9,15,16]. Maintaining effective ambulation is of paramount importance in DMD, because there is a clear link between ambulatory capacity and other functional outcomes, such as, fall frequency and independence [9] and in the longer term, with respiratory and orthopedic comorbidities, such as scoliosis, that significantly affect quality and duration of life [17,18]. At the same time, an important consideration is the extensive and progressive loss of leg muscle mass in children with advanced pathology and limited walking ability. In these patients, the progressive loss of target tissue for dystrophin restoration (which has been extensively documented by magnetic resonance spectroscopy and imaging [11,19]) reduces the possibility of detecting a large effect within the relatively short duration of a clinical trial.

As well as challenges in assessing patients in precipitous ambulatory decline, the 6MWT exhibits limited sensitivity in patients who are at the milder end of the spectrum with stable ambulatory ability (those with a baseline 6MWD of ≥400 m). Patients in this subgroup generally show fewer changes in physical ability over a typical 48-week study than those with a 6MWD <400 m [6,10,11,20] and corticosteroid treatment can help to maintain relatively stable ambulation in these patients for a few years. Given these confines, subgroup analyses are of great importance in DMD. Although clinical trials should be as inclusive as possible, subgroup analyses should be preplanned, given the inherent variability in the progression of this disease; a recent study showed that only 28% of the variation in changes in 6MWD can be explained by age, baseline 6MWD and corticosteroid use [21]. Guidelines from the EMA (December 2015) and draft guidance from the FDA (June 2015), published after the designs of the Phase IIb trial and ACT DMD were finalized, suggest stratification of patients based on baseline functional status [7,8]. Data from these subgroup analyses based on baseline ambulatory ability (using the 6MWT alone or in combination with other prognostic factors) support these recommendations, which will hopefully help to improve assessment of patients during future clinical trials.

In addition to the 6MWD data, these meta-analyses demonstrated a benefit of ataluren in all TFTs. In the ITT population, ataluren was associated with a benefit of 1.1–1.9 s versus placebo across the three tests; this was a 1.7- and 1.9-s benefit versus placebo in the four-stair climb and four-stair descent, respectively, which meets the cutoff of 1.5 s estimated to be clinically meaningful for TFTs [5]. Moreover, an even greater benefit was observed in the ≥300–<400 m (2.1–4.3 s) and <400 m (1.7–2.8 s) subgroups across all three TFTs, indicating the value of stratification for these secondary outcome measures too. Similarly, a benefit of ataluren in reducing the risk of persistent 10% 6MWD worsening was observed in the ITT population and <400 m subgroup. However, this difference missed statistical significance in the ≥300–<400 m subgroup. This could be because this specific end point is most responsive when including patients with baseline 6WMD <300 m (i.e., those who are most likely to lose 10% of baseline 6MWD over 48 weeks). For this subgroup, use of a slope analysis could be recommended, because the time to a 6MWD of zero (loss of ambulatory ability) can be affected by therapeutic intervention and its timing; the latter is also considered when assessing the treatment effect [22].

This study has several strengths. Most importantly, all relevant trial data were included, allowing complete analysis of the available evidence. Furthermore, the two trials were of relatively similar design and statistical heterogeneity between the two trials was not observed for any end point, supporting the validity of combining results for these analyses. Summarized safety results confirm that ataluren has a good safety profile in the population studied.

The limitations of this study include the relatively short 48-week duration of each trial; because of the slowly progressive nature of DMD, especially in children with 6MWD abilities of >400 m, DMD regulatory guidelines now recommend a longer treatment duration for studying dystrophin restoration therapies [7,8]. It is also important to emphasize that different outcome measures are needed for patients at different stages of DMD. A lack of a treatment effect on the 6MWT or other gross motor function tests in patients becoming nonambulatory does not exclude a treatment benefit in patients at this stage and at more advanced disease stages – rather, the benefit may be evident only when other end points (e.g., upper limb or respiratory function tests) are used. In addition, we attempted to minimize publication bias by including all completed randomized controlled trials of ataluren to date. However, only two randomized controlled trials have been conducted, both by PTC Therapeutics Inc. and no other studies (by any other sponsor) were identified during the literature searches.

Since the present study was conducted, results from an additional study have been published that support the effectiveness of ataluren: the Strategic Targeting of Registries and International Database of Excellence (STRIDE;

ClinicalTrials.gov: NCT02369731) Registry [23,24]. STRIDE is an ongoing registry providing real-world evidence on long-term outcomes for patients with nmDMD receiving ataluren during routine clinical practice. In this study, the mean (SD) ataluren exposure was 639.0 (362.9) days, or 372.6 patient-years (n = 213), which is longer than the 336 days of ataluren use in each of the Phase IIb and ACT DMD Phase III clinical trials [23]. In the STRIDE registry, 89.2% of patients received corticosteroids [23]. Similar to in ataluren clinical trials, adverse events in most patients in the registry were mild or moderate and not related to ataluren [24]. In order to assess the long-term effectiveness of ataluren in the registry, results were compared between propensity-score matched patient populations from STRIDE, which comprises patients receiving ataluren plus the standard of care and the Cooperative International Neuromuscular Research Group Duchenne Natural History Study, which comprises patients receiving the standard of care only [24]. Kaplan–Meier analyses showed that patients in the STRIDE registry had a statistically significantly delayed age at loss of ambulation by approximately 3.5 years and delayed age at worsening of performance in timed function tests versus patients in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study [24]. Compared with clinical trials, the STRIDE registry comprises a more heterogeneous population of patients, with a wider range of ages and ambulatory abilities and is therefore more representative of the real-world experiences of patients [23]. These results therefore add to the evidence that ataluren has demonstrated effectiveness in patients with nmDMD in clinical practice over a longer period of time than can be afforded by clinical trials [24].

Conclusion

In conclusion, these meta-analyses show that ataluren provides a slowing of disease progression compared with placebo in patients with nmDMD. The most compelling result is that these significant differences were observed for patients in the entire ITT population. Moreover, the treatment benefit of ataluren was most evident in patients in the ambulatory transition phase of the disease. This study therefore highlights the importance of stratifying patients according to baseline ambulatory ability to overcome limitations in sensitivity of the 6MWT. This study has demonstrated the value of meta-analyses for rare disease clinical trials where enrollment of relatively low numbers of participants and greater than expected variability on outcome measures can limit the ability to observe statistically significant findings in a single trial. Future trials should assess the long-term benefits of ataluren and include prespecified subgroup analyses of data from the population of patients in whom the 6MWT is highly sensitive.

Summary points

- Ataluren is an orally bioavailable treatment for patients with nonsense mutation Duchenne muscular dystrophy (nmDMD) designed to enable functional dystrophin production.
- We aimed to assess the available evidence for the efficacy of ataluren in clinical trials of patients with nmDMD.
- Two randomized, double-blind, placebo-controlled trials of ataluren in nmDMD have been conducted to date: one Phase IIb trial (NCT00592553) and one Phase III trial (Ataluren Confirmatory Trial in DMD; NCT01826487).
- We conducted meta-analyses using the intent-to-treat (ITT) populations and two patient subgroups (baseline 6-min walk distance [6MWD] $\geq$300–<400 or <400 m) from both trials (receiving ataluren 40 mg/kg/day or placebo).
- Baseline demographics and characteristics were similar, the spectrum and severity of adverse events were consistent and ataluren was well tolerated across both trials.
- Statistically significant differences in change in 6MWD with ataluren versus placebo were observed for the ITT population ($p = 0.0473$), the $\geq$300–<400 m subgroup ($p = 0.0008$) and the <400 m subgroup ($p = 0.0109$).
- The combined results from the two ITT populations also demonstrated statistically significant benefits in two out of the three timed function tests, namely the time to climb four stairs ($p = 0.0078$) and descend four stairs ($p = 0.0055$) and a statistically significantly reduced risk of persistent 10% 6MWD worsening ($p = 0.0215$) in patients receiving ataluren versus placebo.
- The treatment benefit of ataluren was most evident in patients in the ambulatory transition phase of the disease; this study therefore highlights the importance of including in future trials prespecified subgroup analyses of data from the population of patients in whom the 6MWT is highly sensitive within the limited time constrains of a clinical trial.
- In conclusion, ataluren provided greater slowing of disease progression than placebo in patients with nmDMD over 48 weeks.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/cer-2020-0095

Author contributions

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Ethical conduct of research

Both trials were performed in accordance with the Declaration of Helsinki (2000) and the principles of Good Clinical Practice. The trials and any changes to the protocols were approved by the local regulatory authorities and the institutional review board/ethics committee of each site. Written informed consent/assent was obtained from all patients/guardians in both trials. The two trials were registered (ClinicalTrials.gov: NCT00592553 and NCT01826487).

Data sharing statement

Individual de-identified participant data or any patient-level data behind the results reported in this article will not be made available; only the results of this study will be shared, since this article reports on meta-analyses of data from previously published trials. The protocols and statistical analysis plans of the two trials discussed in this article will be made available following article publication with no defined end date. Access to the protocols and statistical analysis plans will be provided upon request.

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References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Birnkrant DJ, Bushby K, Bann CM *et al.* Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis and neuromuscular, rehabilitation, endocrine and gastrointestinal and nutritional management. *Lancet Neurol.* 17(3), 251–267 (2018).
2. Pichavant C, Aartsma-Rus A, Clemens PR *et al.* Current status of pharmaceutical and genetic therapeutic approaches to treat DMD. *Mol. Ther.* 19(5), 830–840 (2011).
3. Peltz SW, Morsy M, Welch EM, Jacobson A. Ataluren as an agent for therapeutic nonsense suppression. *Annu. Rev. Med.* 64, 407–425 (2013).

• **Reviews the process that led to the identification, characterization and clinical testing of ataluren.**

4. European Medicines Agency. Translarna™ summary of product characteristics. www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002720/WC500171813.pdf
5. Bushby K, Finkel R, Wong B *et al.* Ataluren treatment of patients with nonsense mutation dystrophinopathy. *Muscle Nerve* 50(4), 477–487 (2014).

•• **Describes the safety and efficacy results of ataluren in a Phase IIb randomized, double-blind, placebo-controlled trial.**

6. McDonald CM, Campbell C, Torricelli RE *et al.* Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, Phase III trial. *Lancet* 390(10101), 1489–1498 (2017).

•• **Describes the safety and efficacy results of ataluren in a Phase III randomized, double-blind, placebo-controlled trial.**

7. 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). www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/12/WC500199239.pdf

8. US Food and Drug Administration. Duchenne muscular dystrophy and related dystrophinopathies: developing drugs for treatment. *Guidance for Industry.* (2015). www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/UCM450229.pdf
9. 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* 48(3), 343–356 (2013).
10. 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* 9(10), e108205 (2014).
11. Sweeney L, Vandenborne K. Developing skeletal muscle MRI/MRS as a biomarker for DMD therapeutic development. http://join.parentprojectmd.org/site/DocServer/Session_8_-_Sweeney_b.pdf?docID=15384
12. 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.* 25(1), 5–13 (2015).

•• **Summarizes the scientific review of the application leading to the conditional approval of ataluren in the EU, based on the results of the Phase IIb trial.**

13. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 339, b2535 (2009).
14. Comprehensive Meta-Analysis. Chapter 13. Fixed-effect versus random-effects models. www.meta-analysis.com/downloads/Meta-analysis%20Fixed-effect%20vs%20Random-effects%20models.pdf
15. Henricson E, Abresch R, Han JJ *et al.* The 6-minute walk test and person-reported outcomes in boys with Duchenne muscular dystrophy and typically developing controls: longitudinal comparisons and clinically-meaningful changes over one year. *PLoS Curr.* 5, (doi:10.1371/ecurrents.md.9e17658b17007eb17679fcd17656f723089f723079e723006 2013).
16. McDonald CM, Henricson EK, Abresch RT *et al.* The 6-minute walk test and other clinical endpoints in Duchenne muscular dystrophy: reliability, concurrent validity and minimal clinically important differences from a multicenter study. *Muscle Nerve* 48(3), 357–368 (2013).
17. McDonald CM, Mercuri E. Evidence-based care in Duchenne muscular dystrophy. *Lancet Neurol.* 17(5), 389–391 (2018).
18. McDonald CM, Henricson EK, Abresch RT *et al.* Long-term effects of glucocorticoids on function, quality of life and survival in patients with Duchenne muscular dystrophy: a prospective cohort study. *Lancet* 391(10119), 451–461 (2018).
19. Willcocks RJ, Rooney WD, Triplett WT *et al.* Multicenter prospective longitudinal study of magnetic resonance biomarkers in a large Duchenne muscular dystrophy cohort. *Ann. Neurol.* 79(4), 535–547 (2016).
20. Pane M, Mazzone ES, Sivo S *et al.* The 6minute walk test and performance of upper limb in ambulant Duchenne muscular dystrophy boys. *PLoS Curr.* 6, e108205 (2014).
21. Goemans N, Vanden Hauwe M, Signorovitch J, Swallow E, Song J, Collaborative Trajectory Analysis P. Individualized prediction of changes in 6-minute walk distance for patients with Duchenne muscular dystrophy. *PLoS One* 11(10), e0164684 (2016).
22. Riebling P, Souza M, Elfring GL *et al.* Slope analysis of 6-minute walk distance as an alternative method to determine treatment effect in trials in Duchenne muscular dystrophy. *Eur. J. Paediatr. Neurol.* 21, e94 (abstr) (2017).
23. Muntoni F, Desguerre I, Guglieri M *et al.* Ataluren use in patients with nonsense mutation Duchenne muscular dystrophy: patient demographics and characteristics from the STRIDE registry. *J. Comp. Eff. Res.* 8(14), 1187–1200 (2019).

•• **Describes the demographics and characteristics of patients receiving ataluren in the STRIDE registry.**

24. Mercuri E, Muntoni F, Osorio AN *et al.* Safety and effectiveness of ataluren: comparison of results from the STRIDE Registry and CINRG DMD Natural History Study. *J. Comp. Eff. Res.* 9(5), 341–360 (2020).

•• **Describes interim data showing the safety of ataluren in the STRIDE registry and effectiveness results from the STRIDE registry compared with those from the Cooperative International Neuromuscular Research Group Duchenne Natural History Study.**