Eteplirsen for the Treatment of Duchenne Muscular Dystrophy

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Objective: In prior open-label studies, eteplirsen, a phosphorodiamidate morpholino oligomer, enabled dystrophin production in Duchenne muscular dystrophy (DMD) with genetic mutations amenable to skipping exon 51. The present study used a double-blind placebo-controlled protocol to test eteplirsen's ability to induce dystrophin production and improve distance walked on the 6-minute walk test (6MWT).
Methods: DMD boys aged 7 to 13 years, with confirmed deletions correctable by skipping exon 51 and ability to walk 200 to 400 m on 6 MWT, were randomized to weekly intravenous infusions of 30 or 50 mg/kg/wk eteplirsen or placebo for 24 weeks (n = 4/group). Placebo patients switched to 30 or 50 mg/kg eteplirsen (n = 2/group) at week 25; treatment was open label thereafter. All patients had muscle biopsies at baseline and week 48. Efficacy included dystrophin-positive fibers and distance walked on the 6MWT.
Results: At week 24, the 30 mg/kg eteplirsen patients were biopsied, and percentage of dystrophin-positive fibers was increased to 23% of normal; no increases were detected in placebo-treated patients ($p \leq 0.002$). Even greater increases occurred at week 48 (52% and 43% in the 30 and 50 mg/kg cohorts, respectively), suggesting that dystrophin increases with longer treatment. Restoration of functional dystrophin was confirmed by detection of sarcoglycans and neuronal nitric oxide synthase at the sarcolemma. Ambulation-evaluable eteplirsen-treated patients experienced a 67.3 m benefit compared to placebo/delayed patients ($p \leq 0.001$).
Interpretation: Eteplirsen restored dystrophin in the 30 and 50 mg/kg/wk cohorts, and in subsequently treated, placebo-controlled subjects. Duration, more than dose, accounted for dystrophin production, also resulting in ambulation stability. No severe adverse events were encountered.

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Duchenne muscular dystrophy (DMD) is a progressive, disabling genetic neuromuscular disorder caused by an absence of dystrophin, with an incidence of 1 in 5,000 newborn boys.[1] Affected boys develop muscle weakness in the first years of life, lose the ability to walk during childhood, and succumb to respiratory and

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Members of the Eteplirsen Study Group are listed in the Appendix on page 10.

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

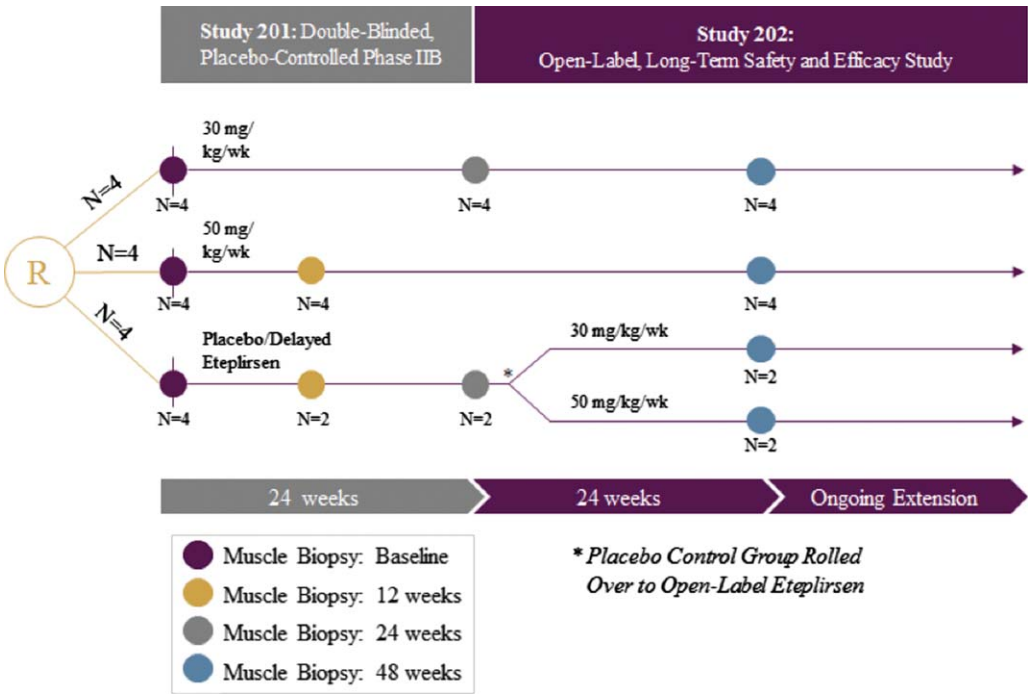

FIGURE 1: Study schema. Twelve Duchenne muscular dystrophy patients were randomized (R) to 1 of 3 cohorts in double-blind, placebo-controlled Study 201: Cohort 1, eteplirsen 30 mg/kg/wk; Cohort 2, eteplirsen 50 mg/kg/wk; and Cohort 3, placebo-treated, referred to as "placebo/delayed." At week 25, placebo/delayed patients in Cohort 3 switched to open-label treatment with either 30 or 50 mg/kg/wk eteplirsen. Patients were maintained on the same dose of eteplirsen under open-label extension Study 202. All patients underwent biceps biopsies at baseline and deltoid biopsies at week 48 for analysis of dystrophin. Additional biceps biopsies were obtained at week 12 (from patients in Cohort 2 and 2 patients in Cohort 3) or week 24 (from patients in Cohort 1 and 2 patients in Cohort 3). The 6-minute walk test was the primary functional outcome measure and was performed pretreatment and post-treatment through week 48 (every 4 weeks through week 36 and then week 48).

cardiac failure in their late teens or early 20s.[2–5] DMD is a universally fatal disease.

Exon skipping,[6–9] a promising disease-modifying approach for DMD, is induced by eteplirsen, a charge-neutral, phosphorodiamidate morpholino oligomer (PMO) that selectively binds to exon 51 of dystrophin pre-mRNA, restoring the open reading frame and enabling production of functional dystrophin. In patients with exon 51 amenable deletions (approximately 13% of DMD patients[10]), eteplirsen yields a truncated in-frame dystrophin protein, like those found in a less severe form of dystrophinopathy, Becker muscular dystrophy.[11,12]

In previous open-label trials, eteplirsen was given as a single intramuscular dose (Study 33[7]) or systemically (Study 28[9]) at doses up to 20 mg/kg/wk for 12 weeks. Although novel dystrophin was identified at the sarcolemma in both studies, Study 28 was not of sufficient duration to assess clinical outcomes. In the current trial, we employed a double-blind placebo-controlled protocol that has not been previously used for testing exon skipping. Regarding outcome measures, we assessed eteplirsen-induced dystrophin production, comparing treated to placebo cohorts; asked whether dystrophin production was affected by longer duration or a higher dose of treatment; and determined whether the observed percentage of muscle fibers expressing dystrophin was sufficient to result in functional effects on the distance walked in the 6-minute walk test (6MWT).

Patients and Methods

Patients

Boys aged 7 to 13 years with confirmed out-of-frame DMD deletions potentially correctable by skipping exon 51 and the ability to walk 200 to 400 m on the 6MWT were eligible for this study. Patients had to have been on stable glucocorticoids (prednisone or deflazacort) for ≥24 weeks to be enrolled, including: 8 DMD boys taking 18–25 mg/day deflazacort; 1 boy on 20 mg/day prednisone, and another 2 boys taking 25 mg/day prednisone. One boy was taking a prednisone weekend dosing regimen of 75 mg on Fridays and 75 mg on Saturdays. Cardiac and pulmonary functions were stable.

Study Design

This trial (Fig 1) was a 24-week, randomized, double-blind, placebo-controlled study (Study 201) consisting of 3 cohorts (placebo, 30 mg/kg/wk, 50 mg/kg/wk) conducted at Nationwide Children's Hospital (NCH), followed by a long-term

open-label extension study (Study 202) with 2 cohorts (30 or 50 mg/kg/wk) conducted at 10 additional sites throughout the United States through week 48, with the exclusive role of eteplirsen administration. After 24 weeks of double-blind dosing, the placebo-treated patients were crossed over to weekly dosing with eteplirsen and received 30 (n = 2) or 50 mg/kg (n = 2), and were thereafter identified as "placebo/delayed" cohorts.

Biopsies at all time points were done at NCH. Biceps muscle was biopsied from all patients at baseline to evaluate pretreatment dystrophin-positive fibers. To evaluate the effect of eteplirsen dose and treatment duration on dystrophin production, a second biopsy from the opposite biceps was collected at week 12 from the 4 patients in the 50 mg/kg cohort and 2 placebo-treated patients, and at week 24 from the 4 patients in the 30 mg/kg cohort and 2 placebo-treated patients. To evaluate the effect of continued exposure to eteplirsen on dystrophin production, a third biopsy was done on the left deltoid in all 12 patients at week 48.

All functional assessments (Studies 201 and 202), including the 6MWT, were performed at NCH (by the same clinical evaluators, L.P.L. and L.A.). The 6MWT was assessed on 2 consecutive days when muscle biopsies were scheduled (ie, pretreatment and weeks 12, 24, and 48). Single-day assessments were performed on weeks 4, 8, 16, 20, 32, and 36.

Study Drug

Single-use vials of either phosphate-buffered saline (PBS) or eteplirsen[13] (100 mg/ml, PBS) were diluted in 150 ml normal saline and infused over 60 minutes.

Safety and Pharmacokinetic Assessments

Vital signs, physical examinations, monitoring for injection site reactions, electrocardiograms, echocardiograms, and clinical laboratory testing, including serum and urine cystatin C and kidney injury molecule 1, were performed. T-cell responses to novel dystrophin protein were assessed via ELISpots.[14]

Eteplirsen's pharmacokinetic (PK) profile was estimated from plasma and urine collected postinfusion at week 12.

Efficacy Assessments

To evaluate dystrophin-positive fiber levels, immunohistochemical staining was done on 10 μm frozen sections of 3 separate blocks of biopsy tissue separated by at least 200 μm and evaluated by blinded expert muscle pathologists. For dystrophin localization, sections were stained with MANDYS106 NCL, a mouse monoclonal antibody to amino acids 1749–2248,[15,16] using standard immunofluorescence methodology.[9,14] Dystrophin-positive fibers and total fibers were counted, and the percentage of dystrophin-positive fibers was calculated across all samples. Pretreatment dystrophin values, composed of revertant fibers (representing somatic mutations that result in removal of additional exons from mRNA[17,18]), were subtracted from on-treatment values to determine the percentage of dystrophin-positive fibers resulting from eteplirsen treatment.

Dystrophin expression was quantified using BIOQUANT (Nashville, TN) image analysis software on MANDYS106-stained sections. Intensity of fibers expressing dystrophin from eteplirsen-treated patient biopsies was compared to normal control biopsies. Supportive measures were further provided by Western blots prepared from serial sections from the same blocks stained using NCL-Dys1 (Novacastra Laboratories, Newcastle Upon Tyne, UK) mouse monoclonal to amino acids 1181–1388.[17] Immunohistochemical analyses of β- and γ-sarcoglycan and neuronal nitric oxide synthase (nNOS) were also done. Exon skipping was confirmed by reverse transcriptase polymerase chain reaction (RT-PCR).[7]

The 6MWT was administered using the protocol established for DMD.[19] Additional functional measures were assessed at various visits and included the North Star Ambulatory Assessment, quantitative muscle testing, the 9-Hole Peg Test, pulmonary function testing (PFT), timed function tests, and quality of life (data not presented here).

Statistical Analysis

Dystrophin production was the primary endpoint at week 12 for the 50 mg/kg cohort, at week 24 for the 30 mg/kg cohort, and at week 48 for all patients. This design specifically compared dose versus duration of eteplirsen treatment. Changes from baseline at weeks 12 and 24 were compared separately from change from baseline for the combined placebo cohorts using analysis of covariance (ANCOVA) for ranked data, with baseline values and duration of DMD as covariates. Change from baseline at week 48 used a mixed model with treatment as fixed effect, and subject nested within treatment as random effect, with baseline value and time since DMD diagnosis as covariates. Additionally, paired t test was used to compare the on-treatment value with the baseline value.

Mixed model repeated measures (MMRM) was used to evaluate change from baseline in the 6MWT data (the critical functional endpoint) to week 24 and to week 48. Treatment, time, and treatment-by-time interaction terms were used as fixed effects, subject nested within treatment as random effect, and baseline value and time since DMD diagnosis as covariates. If there was strong evidence of deviation from normality, then ANCOVA for ranked data was used. Additionally, data distribution and the impact of outliers on the results were assessed by analyzing the modified intent-to-treat (mITT) population, which excluded ambulatory nonevaluable patients from the intent-to-treat (ITT) population. Type I error correction was not employed for any of these analyses.

SAS version 9.3 (Cary, NC) was used for all statistical analyses.

Results

Patient Characteristics

The patient characteristics are summarized in the Table. Patients in the 30 mg/kg cohort were older, taller, and heavier than the other 2 cohorts. The 12 enrolled patients represented 5 different genotypes, resulting in out-of-frame deletions correctable by exon 51 skipping.

Mean baseline 6MWT distance for all subjects was 381.9m (range = 261–456). The mean distance walked

A

Cohort (n)	Week 12 Mean Change from BL, SE (p-value) *	Week 24 Mean Change from BL, SE (p-value) *	Week 48 Mean Change from BL, SE (p-value) †
All Eteplirsen (8)	NA	NA	47.3, 3.89 (≤0.001)
Eteplirsen 30mg/kg (4)	ND	22.9, 2.90 (≤0.002)	51.7, 3.54 (≤0.001)
Eteplirsen 50 mg/kg (4)	0.8, 3.55 (NS)	ND	42.9, 6.72 (≤0.008)
Placebo/Delayed Eteplirsen (4) ‡	-4.0, 2.92	-4.0, 2.92	37.7, 6.30 (≤0.009) §
Eteplirsen 30 mg/kg (2)	ND	-7.48, 1.00	33.6, 5.23
Eteplirsen 50 mg/kg (2)	-0.6, 5.16	ND	41.8, 13.30

B

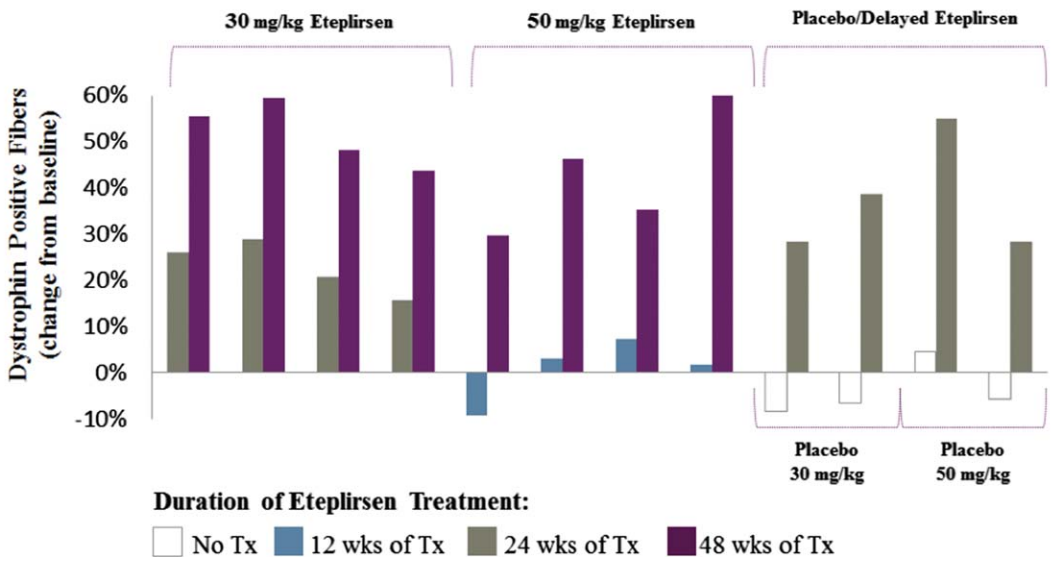

FIGURE 2: Dystrophin-positive muscle fibers after 12, 24, and 48 weeks of eteplirsen. (A) Mean absolute change from baseline in the percentage of dystrophin-positive fibers by treatment cohort; *p* values are for comparison between eteplirsen and placebo using the combined results from weeks 12 and 24, and are based on an analysis of covariance model for ranked data, with treatment as a fixed effect and baseline value and time since Duchenne muscular dystrophy diagnosis as covariates. Mean changes shown are based on descriptive statistics. †Probability values are from a paired *t* test comparing the week 48 value to baseline. ‡Results from the placebo-treated patients biopsied at weeks 12 and 24 are combined. *Placebo/delayed eteplirsen patients began receiving eteplirsen at week 25 and had received a total of 24 doses at week 48. BL = Baseline; NA = not applicable; ND = not done; NS = not significant; SE = standard error. (B) Change from baseline in dystrophin-positive fibers are shown by cohort and by duration of treatment with eteplirsen (Tx). The first biopsies (blue) at 12 weeks (50 mg/kg cohort) showed little or no dystrophin beyond expected numbers from revertant fibers. At 24 weeks (army green), there was a definite increase in dystrophin positive fibers. At 48 weeks (purple) the 30 and 50 mg/kg cohorts both showed significant dystrophin production. The placebo/delayed group biopsied at 12 weeks (blanks) showed only minimal fibers (could be fewer than baseline depending on the number of revertant fibers). By 48 weeks, the placebo/delayed group had been treated for 24 weeks and now showed significant dystrophin-positive fibers.

by the 30 mg/kg cohort was approximately 40 m less than that of the 2 other cohorts. Mean distances on the 6MWT at baseline were similar to those in other DMD studies.[20,21]

Safety and Pharmacokinetic Profile

Through week 48, eteplirsen was well tolerated, with no treatment-related adverse events (Supplementary Materials). No changes were observed in vital signs or physical examination, including injection site reactions. Electrocardiograms, echocardiograms, and PFTs remained stable, and no changes were observed in chemistries, hematology, coagulation, or renal or liver function. Mild and transient proteinuria was observed in a single placebo-treated patient. Dystrophin-induced T-cell responses were not observed.

Week 12 PK parameters revealed dose-proportional exposure and similar plasma clearance. The mean half-

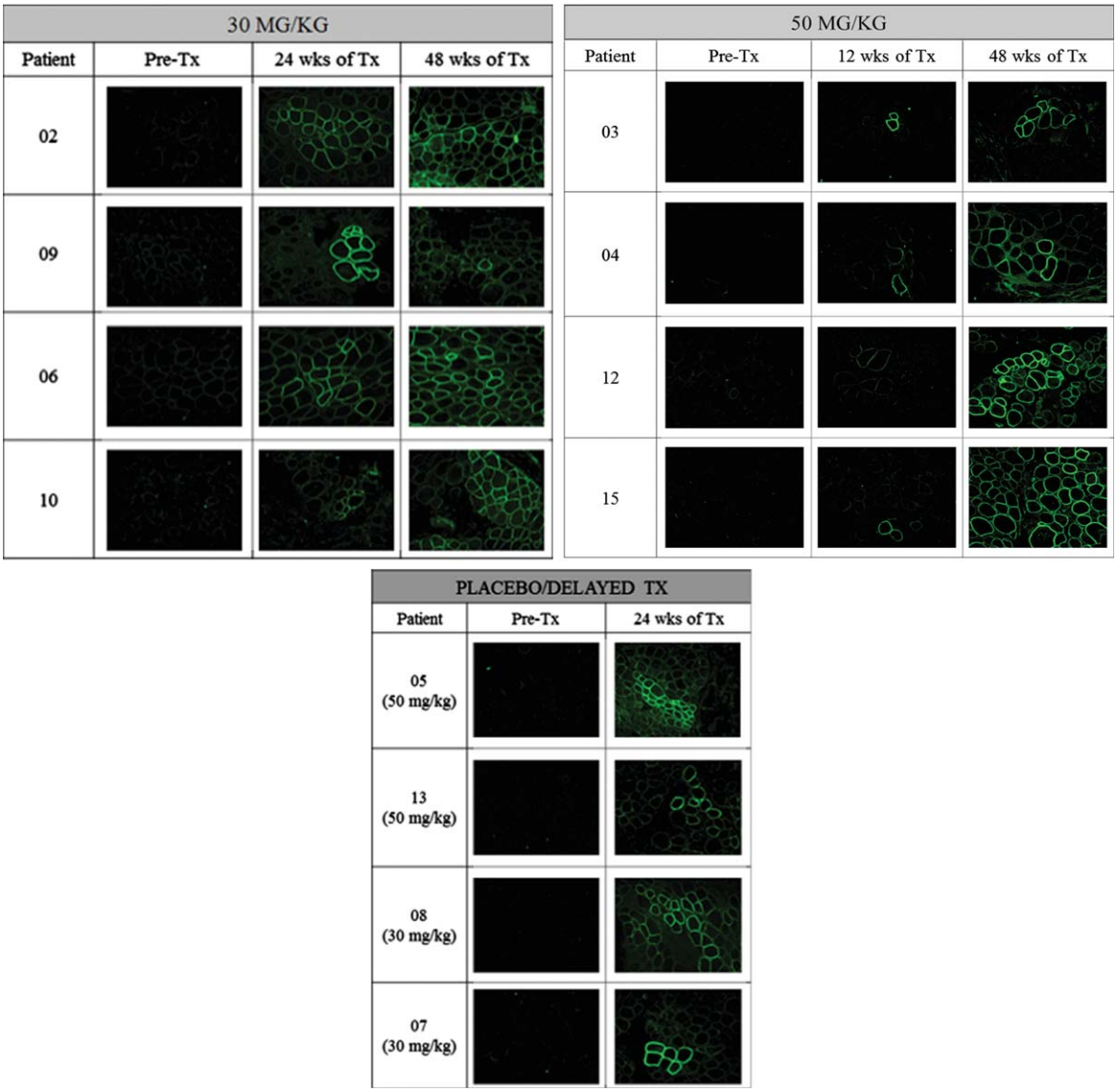

FIGURE 3: Effects of eteplirsen on the dystrophin-associated glycoprotein complex. Representative examples of time-dependent increases in dystrophin-positive fibers can be seen in relation to treatment for all participating study patients.

life was 3.3 and 3.2 hours for the 30 and 50 mg/kg cohorts, respectively. Renal clearance accounted for approximately 65 to 70% of total systemic clearance.

Dystrophin

Treatment with 50 mg/kg for 12 weeks resulted in no increases in percentage of dystrophin-positive fibers compared to pretreatment, and the change from baseline (mean = 0.8%, range = −9.3 to 7.4%) was not statistically different compared to the placebo cohorts (Fig 2). At week 24 (12 additional weeks of treatment with eteplirsen), the 30 mg/kg dose resulted in a 22.9% (range = 15.9–29.0%) increase from pretreatment ($p \leq 0.002$) compared to the combined placebo group. These data suggest that at least 12 weeks of treatment with eteplirsen is needed to produce perceptible increases in dystrophin production in muscle biopsies.

The within-cohort comparison of the percentage of dystrophin-positive fibers (eg, week 12 vs baseline for the 50 mg/kg cohort, and week 24 vs baseline for the 30mg/kg cohort) resulted in a statistically significant difference for the 30 mg/kg cohort ($p \leq 0.004$) but not for the week 12, 50 mg/kg, or combined placebo cohorts.

At week 48 (see Fig 2), the 30 and 50 mg/kg cohorts showed significant increases ($p \leq 0.001$) in percentage of dystrophin-positive fibers (mean = 47.3%, range = 29.8–60.3%). The 4 patients in the placebo/delayed cohort taking 30 mg/kg (n = 2) and 50 mg/kg (n = 2) of eteplirsen also showed significant increases ($p \leq 0.008$, mean = 37.7%, range = 28.4–55.1%). The

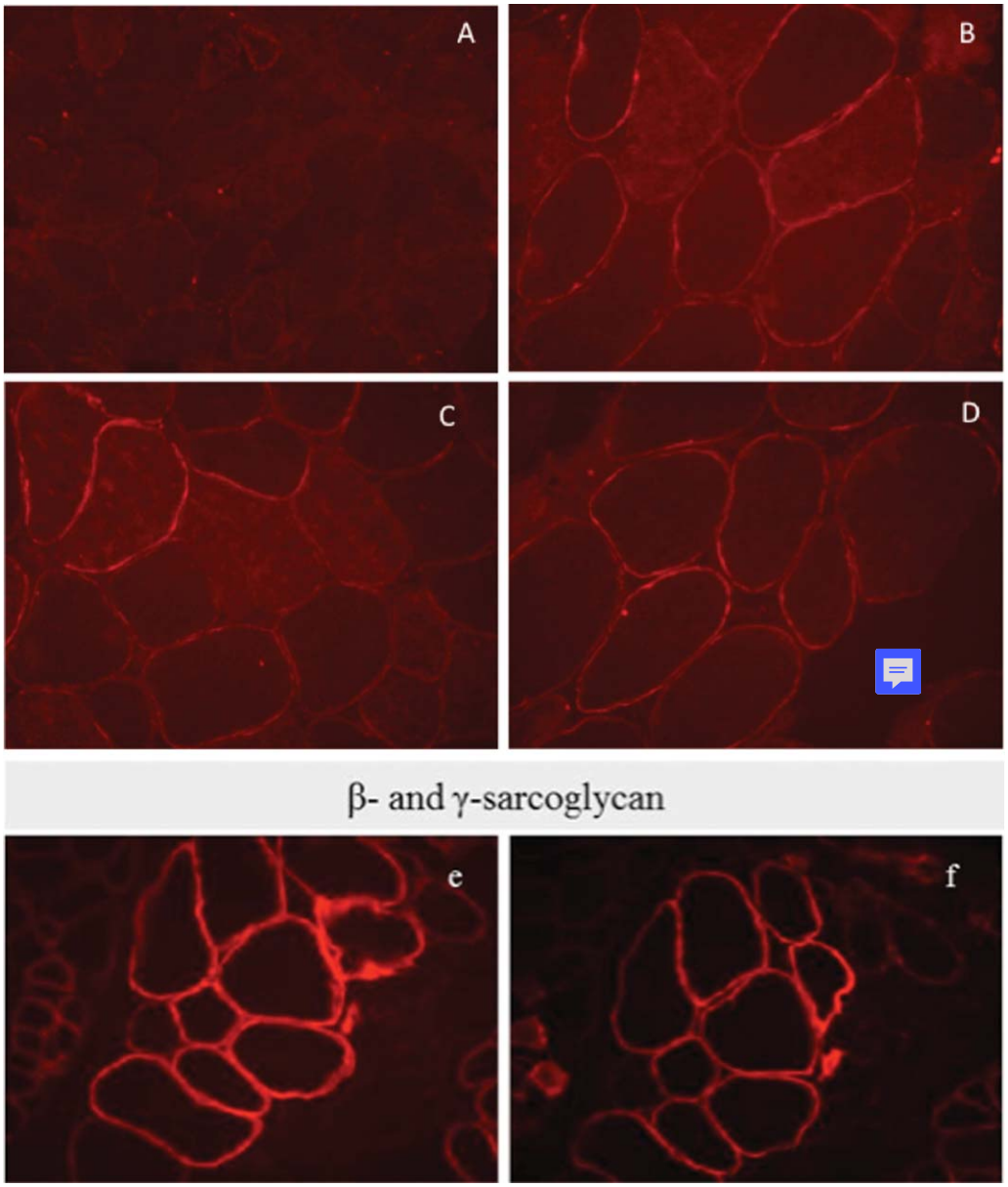

FIGURE 4: (A) Neuronal nitric oxide synthase (nNOS) staining in muscle from Duchenne muscular dystrophy with deletion in exon 52. (B, D) Restoration of nNOS binding site following eteplirsen treatment from Patient 006 at week 48. (C) Normal muscle showing nNOS binding (no treatment). Notice the nNOS binding in normal control is the same intensity as with eteplirsen. (E, F) β-Sarcoglycan (E) and γ-sarcoglycan (F) staining at week 48 (Patient 006), demonstrating restoration of the sarcoglycan complex with eteplirsen.

between-treatment comparisons of all cohorts, including between the combined eteplirsen and the placebo/delayed cohorts, resulted in no significant differences.

In agreement with these findings, eteplirsen significantly increased mean fluorescence signal intensity of muscle fibers expressing MANDYS106 at week 48 in the 30 mg/kg cohort ($p \leq 0.023$), the 50 mg/kg cohort ($p \leq 0.005$), the combined eteplirsen cohort ($p \leq 0.001$), and the placebo/delayed cohort ($p \leq 0.006$).

Figure 3 illustrates eteplirsen's time-dependent effect on the percentage of dystrophin-positive fibers, and Figure 4 illustrates the associated restoration of sarcolemmal nNOS and β- and γ-sarcoglycan. Dystrophin expression and exon skipping were confirmed by RT-PCR and Western blot (representative patient results are shown in Fig 5).

6MWT

The adjusted mean change using the MMRM model for the 6 MWT from baseline to week 24 was −25.8 m (±30.6) for the placebo cohort, and −128.2 m (±31.6) and −0.3 m (±31.2) for the 30 and 50 mg/kg cohorts,

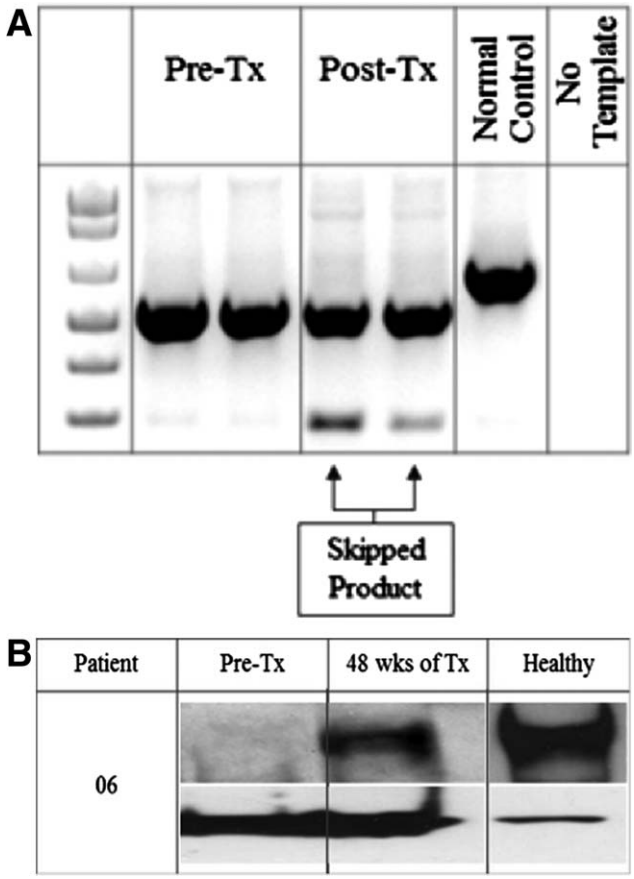

FIGURE 5: (A) Reverse transcriptase polymerase chain reaction shows skipped product (289bp) post-treatment (Tx) in the muscle of Patient 012 (deletions in exons 49–50). (B) Western blot results at baseline and at week 48 (deletion in exon 52) and from healthy control.

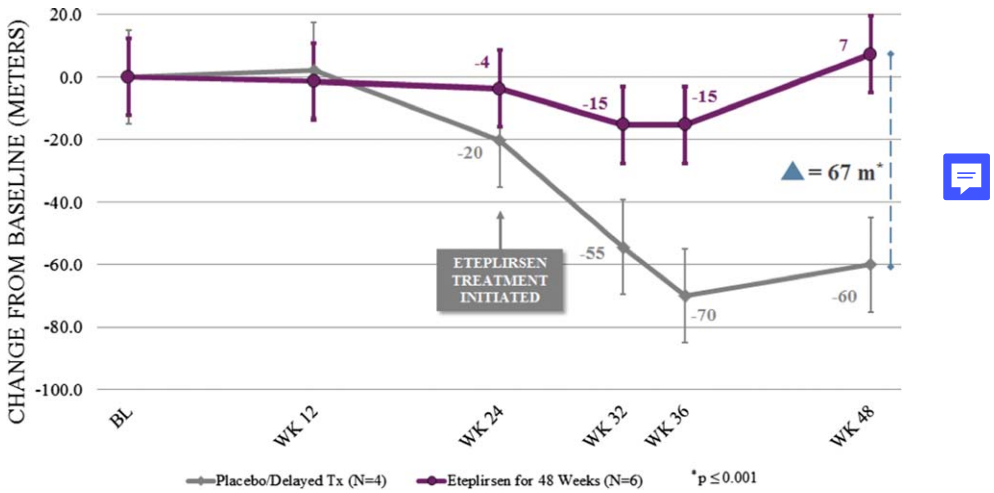

FIGURE 6: Functional efficacy of eteplirsen. The dark purple line shows the change from baseline (BL) in distance walked on the 6-minute walk test (6MWT) over time for the 6 evaluable patients who received eteplirsen from the start of Study 201 (the 2 boys who lost ambulation at or beyond week 24 were excluded from this analysis). The dark gray line shows change from baseline in distance walked on the 6MWT for the 4 patients who received placebo for the first 24 weeks and eteplirsen for the last 24 weeks. It is estimated from dystrophin expression studies and multiple biopsies that significant dystrophin was produced after 12 weeks. This is clearly indicated in the placebo/delayed cohort starting on eteplirsen at week 25 and stabilizing in function at week 36. The eteplirsen-treated cohorts diverged from placebo/delayed patients at week 12, after which they no longer showed significant decline.

respectively. The large decline in the 30 mg/kg cohort was directly attributable to Patients 009 and 010, who showed rapid disease progression immediately after enrollment. When these 2 patients were excluded from the cohort, the adjusted mean change from baseline was 14.2m (±14.4). As data distribution analysis revealed severe violation of normality assumption, the analysis was repeated using ANCOVA for ranked data, showing no significant differences between the 2 eteplirsen and placebo cohorts.

The adjusted mean change from baseline to week 48 on the 6MWT was −68.4 m (±37.6) for the placebo/delayed cohort, and −153.4 m (±38.7) and +21 m (±38.2) for the 30 and 50 mg/kg eteplirsen-treated cohorts, respectively (Fig 6). The large decline in the 30 mg/kg cohort was again due to Patients 009 and 010, and when they were excluded from the 30 mg/kg cohort, the adjusted mean change from baseline was 31.5m (±19.9). As data distribution analysis revealed severe violation of normality assumption, the analysis was repeated using ANCOVA for ranked data, showing no significant differences between the 30 mg/kg and placebo cohorts but a statistically significant difference between the 50 mg/kg and placebo/delayed cohorts ($p \leq 0.016$).

The progressive loss of ambulation is the hallmark of DMD, with most patients becoming wheelchair-dependent by age 10 to 14 years.[2,3] The 6MWT results for the 4 patients in the placebo/delayed cohort was consistent with natural history studies,[21–23] culminating in a loss of almost 70m by week 48. The previously mentioned 2 patients (009 and 010) in the 30 mg/kg cohort showed rapid disease progression evident in more advanced stages of the disease. Patient 009 walked 346m at baseline, but declined by 112m at week 4. When consistent increases in dystrophin-positive fibers were observed, he declined an additional 184m, and became nonambulatory. His identical twin (010) walked 261m at baseline but declined by 213m at week 24. These 2 patients were the tallest and among the oldest of the 12 patients, and had the lowest baseline 6MWT values, placing them at increased risk for imminent loss of ambulation.[21–23] The inclusion of their data in the statistical analysis resulted in severe deviation from normality due to being extreme values (outliers), requiring the use of data transformation for the ITT population (n = 12), which justifies further evaluation of the results after excluding them from the mITT population (n = 10). Both boys remained on eteplirsen with no treatment-related adverse events, maintained stable pulmonary and upper limb function, and showed increases in dystrophin-positive fibers at weeks 24 (20.8% and 15.9%) and 48 (48.3% and 43.6%).

The remaining 6 ambulation-evaluable patients in the 30 (n = 2) and 50 mg/kg (n = 4) cohorts who received eteplirsen for 48 weeks demonstrated stable performance on the 6MWT from baseline to week 48, which was significantly different (67.3m difference; $p \leq 0.001$) compared to the placebo/delayed cohort (see Fig 6). The 50 mg/kg cohort was also significantly different (87.4m difference; $p \leq 0.001$) compared to the placebo/delayed cohort.

Discussion

The data from this trial suggest the potential for eteplirsen as a safe and effective disease-modifying therapy for DMD, whose current management consists primarily of treatment with glucocorticoids.[24]

Eteplirsen resulted in increased dystrophin production in all patients treated for >12 weeks. Increases in dystrophin were observed in all patients following at least 24 weeks of treatment, including those who started eteplirsen at week 25 (placebo/delayed). Dystrophin intensity was accurately measured using BIOQUANT image analysis on the same fibers expressing dystrophin. Confirmation was further demonstrated by Western blots and by the restoration of components of the dystrophin-associated protein complex (eg, sarcoglycans) and the presence of the nNOS-binding site, localized to dystrophin spectrinlike repeats 16/17 of exon 45.

Treatment with eteplirsen for 48 weeks resulted in a 67.3m advantage compared to placebo/delayed treatment on the 6MWT in ambulation-evaluable patients. This difference exceeds the 28 to 44m treatment effects reported in several registration-directed studies,[25–28] as well as in DMD natural history studies.[20–22] Unfortunately, 2 boys (twins) randomized to the 30 mg/kg cohort rapidly lost ambulation and were excluded from the mITT analysis. This is justified on the basis of these patients experiencing a more advanced stage of the disease than the rest of the enrolled patients, although with a small sample size this could indicate variability in response. In a model developed by McDonald et al,[19] height was noted as a key criterion along with age and baseline 6MWT in predicting a more rapid progression to loss of ambulation. That is, the taller and older the subjects are, along with lower baseline 6MWT distance, the higher the predicted percentage of loss of ambulation. These 2 patients were among the oldest (9.8 years old), were the 2 tallest, and had the lowest baseline 6MWT distance among the entire study population. We also have magnetic resonance imaging confirmation of advanced disease in a separate study with DMD patients at a time point between weeks 24 and 28, revealing a severe loss of muscle tissue with replacement of fat and

TABLE : Baseline Demography and Disease Characteristics

Treatment Arm	Placebo/Delayed Eteplirsen, n = 4	Eteplirsen, 30 mg/kg, n = 4	Eteplirsen, 30 mg/kg, n = 2[a]	Eteplirsen, 50 mg/kg, n = 4
Mutation, No. (%)				
45–50	0	2 (50)	0	1 (25)
48–50	0	1 (25)	1 (50)	0
49–50	3 (75)	0	0	2 (50)
50	1 (25)	0	0	0
52	0	1 (25)	1 (50)	1 (25)
Male gender, No. (%)	4 (100)	4 (100)	2 (100)	4 (100)
Age, yr				
Mean	8.5	9.3	9.5	8.5
SD	1.73	0.50	0.71	1.29
min, max	7, 10	9, 10	9, 10	7, 10
Height, cm				
Mean	119.3	130.5	124.0	121.3
SD	3.40	9.47	9.90	7.85
min, max	116, 124	117, 138	117, 131	117, 133
Weight, kg				
Mean	30.6	34.8	30.0	29.0
SD	6.04	7.05	6.38	6.38
min, max	22.1, 36.2	24.8, 39.8	24.8, 35.1	23.7, 38.3
Race, No. (%)				
Asian	0	1 (25)	1 (50)	0
White	4 (100)	3 (75)	1 (50)	4 (100)
6MWT, m[b]				
Mean	394.5	355.3	407.0	396.0
SD	42.25	74.78	49.50	26.61
min, max	364, 456	261, 442	372, 442	365, 429

[a]Ambulation-evaluable patients.
[b]6MWT results are the maximum observed value of 2 tests administered on 2 consecutive days during screening.
6MWT = 6-minute walk test; min = minimum; max = maximum; SD = standard deviation.

connective tissue that was much more extensive than in all of the other patients (manuscript in preparation: Krista H. Elvire Vandenborne).

The data presented here show a clear relationship between systemic treatment with eteplirsen (an exon-skipping drug; proof of mechanism of action by RT-PCR) and dystrophin production in DMD muscle as demonstrated by percentage of positive fibers, followed by a statistically significant difference in walking ability in treated versus placebo/delayed ambulation-evaluable patients. Eteplirsen's clinical benefit mirrored the time required to produce consistent increases in dystrophin. The majority of the patients in the 30 and 50 mg/kg cohorts maintained a stable walking distance over 48 weeks, whereas the patients in the placebo/delayed cohort stabilized around week 36 (12 weeks after eteplirsen exposure). This observed delay between initiation of eteplirsen and detectable dystrophin production and stabilization in clinical function likely results from an accumulation of novel dystrophin over time, possibly due

to its long half-life[29–32] and the need to achieve a steady state of dystrophin to optimize diffusion across the muscle. Furthermore, because splice switching oligomers do not target skeletal muscle specifically, their uptake is partly dependent on local events such as muscle perfusion, damage, and inflammation.[33] Because dystrophic muscle undergoes accelerated cycles of degeneration and regeneration, with some cells having less stable membranes than others at any time point, it is likely that some muscle fibers take up more drug than others, allowing them to respond more robustly. Over repeated doses, eteplirsen accesses more muscle fibers, thus allowing for more dystrophin-positive fiber production over time.

The benefits seen with eteplirsen in treating the underlying cause of DMD in patients amenable to skipping exon 51 should encourage the development of PMO technology to treat other genotypes amenable to exon skipping to potentially treat an estimated 70 to 80% of all DMD patients with dystrophin gene deletions.[10,33]

APPENDIX

Members of the Eteplirsen Study Group

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Potential Conflicts of Interest

Kate Melia, Peter Sazani, Jay B. Saoud, and Ed Kaye are employed at Sarepta Therapeutics. The study data was generated at Nationwide Children's Hospital and they were not involved in that process.

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