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RESEARCH ARTICLE

Placebo-controlled Phase 2 Trial of Drisapersen for Duchenne Muscular Dystrophy

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The members of the DEMAND V Study Group are presented in Appendix.

Abstract

Objective: This double-blind, randomized, placebo-controlled Phase 2 study (NCT01462292) assessed the 24-week efficacy, safety, tolerability, and pharmacokinetics of two different subcutaneous drisapersen doses, and the 24-week off-dose persistent effect, in ambulant Duchenne muscular dystrophy (DMD) patients. **Methods**: Male DMD patients (≥5 years; time to rise from floor ≤15 s) were randomized to drisapersen 3 mg/kg/week, 6 mg/kg/week or placebo. The primary efficacy endpoint was change from baseline in 6-minute walking distance (6MWD) at week 24. Secondary endpoints included changes in timed function tests, muscle strength, and pulmonary function tests. **Results**: Fifty-one patients were randomized to placebo (N = 16), drisapersen 3 mg/kg/week (N = 17) or 6 mg/kg/week (N = 18). All but 2 patients had baseline rise from floor time <7 s. This study was exploratory and not prospectively powered; however, a difference in mean 6MWD versus placebo in favor of drisapersen 6 mg/kg/week was observed at week 24 (27.1 m; P = 0.069) and maintained 24 weeks off-treatment (27.9 m; P = 0.177). The 3 mg/kg/week group showed no statistically significant difference in mean 6MWD versus placebo. For some secondary endpoints, a more positive response in favor of drisapersen 6 mg/kg/week compared to placebo was shown. Drisapersen had a long half-life with steady state reached after approximately 36 weeks. Most common adverse events in both drisapersen groups were related to injection site reactions and subclinical proteinuria. **Interpretation**: Drisapersen 6 mg/kg/week for 24 weeks resulted in a treatment benefit in 6MWD, largely maintained 24 weeks off-treatment. This study provided insights for further studies to optimize dosage regimen.

Introduction

Duchenne muscular dystrophy (DMD) is a neuromuscular disorder that leads to progressive muscle degeneration and early death. This recessive X-linked disease affects about 1 in 3,500–5,500 live male births worldwide, with onset of symptoms typically occurring in early childhood.[1–3] While steroids can delay disease progression,[4,5] to date there is no treatment to cure the disease.[6] DMD is caused by mutations in the dystrophin-encoding *DMD* gene that lead to disruption of the mRNA open reading frame.[7] The resulting loss of functional dystrophin protein causes muscle fiber deterioration and muscle weakness.[8] The disease is characterized by a severe progressive decrease in muscle function with loss of ambulation as a key milestone. Subsequently, with increasing age, respiratory failure and cardiomyopathy also emerge.[9] As a result, and despite steroid treatment, most patients become wheelchair-bound by their mid-teens and often die in their late 20s.[1,10,11] Mutations in the *DMD* gene that preserve the open reading frame result in Becker muscular dystrophy, in which dystrophin is at least partly

functional and as a consequence these patients have a typically milder phenotype.[7,12]

Antisense oligonucleotide-induced exon skipping is a promising therapeutic strategy for treatment of DMD.[13] Drisapersen is a 2′-*O*-methyl-phosphorothioate antisense oligonucleotide that induces skipping of exon 51 in the dystrophin pre-mRNA, which restores the disrupted open reading frame of a mutation amenable by exon 51 skipping. As a result, a shorter but largely functional dystrophin protein is translated.[13] Approximately 13–14% of the boys with DMD have mutations amenable to treatment with exon 51 skipping.[6,14,15]

In a proof-of-concept study (PRO051-01), four patients with DMD who received a single 0.8 mg dose of drisapersen into the tibialis anterior muscle showed exon 51 skipping in a biopsy taken 28 days later and the presence of dystrophin in total protein extracts.[16] A dose-escalation study (PRO051-02) of drisapersen was conducted in 12 ambulant patients using doses up to 6 mg/kg/week subcutaneously, initially for 5 weeks.[17] Results of a European open-label extension study (DMD114673), in which 10 patients were still able to perform the 6-minute walk distance (6MWD) at the start have recently been published. Patients in that study initially received, after the dose-escalation part, drisapersen 6 mg/kg/week for approximately 72 weeks, followed by an 8-week treatment interruption and subsequent 12-weekly cycles of 8 weeks on- and 4 weeks off-treatment with drisapersen. Eight out of 10 patients remained ambulatory after 177 weeks of follow-up, with the two patients losing ambulation having a baseline 6MWD <330 m.[18] In addition, an exploratory, double-blind, randomized, placebo-controlled Phase 2 study (DMD114117) showed improvements in 6MWD (treatment effect >30 m) with continuous drisapersen treatment (6 mg/kg/week) after 24 ($P = 0.014$) and 48 weeks of treatment ($P = 0.051$).[19]

The aim of the Phase 2 study reported here was to assess the efficacy, safety, tolerability, and pharmacokinetics of two different doses of weekly subcutaneous administered drisapersen (3 mg/kg and 6 mg/kg) versus placebo, during a 24-week treatment phase in ambulant DMD patients. In addition, the persistence of efficacy and safety of both drisapersen doses was evaluated in a 24-week off-treatment observational phase.

Materials and Methods

Ethical conduct of the study

The study was sponsored by GlaxoSmithKline and performed in accordance with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice and applicable country-specific and subject privacy requirements, and the ethical principles outlined in the Declaration of Helsinki (2008).

This study was approved by a national, regional, or investigational ethics committee, or an institutional review board, as appropriate. All patients and their parents/legal guardian provided written informed consent prior to any study-related procedures.

Study design and patients

Study DMD114876 was an exploratory, double-blind, randomized, parallel-group, placebo-controlled Phase 2 study (NCT01462292) to investigate the efficacy, safety, tolerability, and pharmacokinetics of two different doses of drisapersen, 3 and 6 mg/kg/week administered subcutaneously, in ambulant patients with DMD. The patients were recruited at 13 centers across the United States between October 26, 2011 and November 4, 2013.

Male patients aged ≥5 years with DMD resulting from a mutation amenable by exon 51 skipping and a life expectancy of at least 1 year were eligible for inclusion. In addition, patients were required to rise from the floor unaided in ≤15 s (protocol amendment; original protocol had criterion of ≤7 s) and walk ≥75 m in 6 minutes, had received oral glucocorticoids for ≥6 months prior to screening and were at a stable dose (with the exception of weight adjustments) ≥3 months prior to screening. Any additional mutation for DMD that is not amenable by exon 51 skipping was the key exclusion criteria.

Randomization and blinding

Following screening, eligible patients were centrally randomized (2:2:1:1 ratio) to one of four treatment groups: drisapersen 3 mg/kg, drisapersen 6 mg/kg, 3 mg/kg volume-matched placebo, or 6 mg/kg volume-matched placebo.

Treatment was administered subcutaneously once-weekly during 24 weeks, followed by a 24 weeks off-treatment observational phase. To minimize skin reactions at the injection site, weekly injection site rotation was recommended. At the end of the off-treatment phase, patients that completed the study had the option to enter an open-label extension study (if eligible).

The study was fully blinded with respect to the study drug and placebo. However, it was not blinded for dose as patients in the 3 mg/kg/week dosing groups (either drisapersen or placebo) received a lower injection volume than those in the higher dosing group.

Endpoints and assessments

The primary efficacy endpoint was change from baseline in 6MWD at week 24. The 6MWD was assessed at baseline (randomization) and every 12 weeks until week 48, using a previously described methodology.[20,21]

Secondary endpoints included assessment of ambulatory gross motor and muscle function (rise from floor, 10-m walk/run and 4-stair climb [ascent and descent] and North Star Ambulatory Assessment [NSAA]),[11,22,23] muscle strength (myometry), pulmonary function (spirometry), serum creatine kinase and lactate dehydrogenase, molecular efficacy (exon 51 skipping at mRNA level and dystrophin expression), and functional outcome assessments. Other timed function tests (rise from floor, 10-m walk/run and 4-stair climb [ascent and descent]) and NSAA were assessed at baseline and week 24. Muscle strength was recorded at baseline and week 24 by handheld myometry using a microFET2 dynamometer. Respiratory function was measured at baseline and week 24, using a non-invasive spirometer. Creatine kinase and lactate dehydrogenase serum concentrations were determined in blood samples taken at baseline and weeks 2, 4, 8, 12, 16, 20, 24, 30, 36, and 48. Muscle biopsies taken from the tibialis anterior at baseline and week 24, and additionally at either week 12 or 36, were used to assess dystrophin expression using an immunofluorescence assay or by Western blot. The biopsies were also used to determine the degree of exon 51 skipping by nested qualitative reverse-transcription polymerase chain reaction (RT-PCR)[16] and to measure drisapersen drug levels in muscle tissue. At baseline and weeks 12, 24 and 48, the Clinician Global Impression of Improvement,[24] the Physician Assessment Of Daily Living (which documents observations from the patient or people involved in his daily life of changes in the ability to perform usual day-to-day activities) and the Functional Outcomes Survey (which documents family/caregiver observations in changes in the ability of the patient to perform usual day-to-day activities) were assessed.

Safety and tolerability endpoints included AEs, physical examination, vital signs, electrocardiogram, laboratory parameters and echocardiography. The AEs were assessed at baseline, on a weekly base until week 24, and at week 30, 36, and 48. Physical examination was performed at baseline and at weeks 4, 8, 16, 24 and 48. Vital signs were measured at baseline and at weeks 4, 8, 12, 16, 20, 24, 30, 36, and 48. An electrocardiogram was recorded at baseline and weeks 4, 8, 12, and 24. The echocardiogram was taken at screening and weeks 12 and 24. Different laboratory assessments included hematology, clinical chemistry, biomarkers, urinalysis, coagulation, and immunology tests. Assessments were performed at baseline and, depending on the test, every other week thereafter until week 24, and at weeks 30, 36, and 48. Only tests with relevant outcomes will be reported in the results section.

Pharmacokinetic evaluations included maximum plasma concentration (C_{max}), time of occurrence of C_{max} (T_{max}), area under concentration-time curve from time zero (pre-dose) to 24 h post-dose ($AUC_{[0–24\ h]}$) and to last time of quantifiable concentration ($AUC_{[0\text{-}t]}$), using the pharmacokinetic-data obtained at week 23.

Statistical methods

It was planned to randomize approximately 54 patients; assuming a drop-out rate of approximately 10% over the 24-week treatment phase, to have 48 patients to evaluate. This sample size was not based on statistical considerations, but was considered to provide adequate pharmacokinetic data for each dosing regimen, and to provide information on trends with respect to efficacy and safety. Statistical analyses were performed using SAS. A two-sided test at the 0.05 significance level was employed, unless otherwise specified. Descriptive statistics are provided for the primary and secondary endpoints.

The two main analyses conducted were the original primary preplanned week 24 (on-treatment) and the week 48 (off-treatment) analysis. It was pre-planned that the two placebo were combined for analysis and reporting purposes.

The primary efficacy analysis was conducted when all patients had completed the week 24 assessments. Percent-predicted 6MWD was calculated using the Geiger equation.[25] At week 24, 6MWD was also assessed in a post hoc analysis by dichotomized age group (≤7 years, >7 years). Populations that were analyzed included: intent-to-treat (ITT; all patients who were randomized, received at least one dose of study medication and had at least one post-baseline efficacy assessment); per-protocol (PP; all ITT patients who had no major protocol deviations); and safety (all patients who received at least one dose of study medication).

Both drisapersen doses were compared separately to placebo, and a hierarchical approach was applied for the assessments. The primary efficacy endpoint was analyzed using a mixed model for repeated measures (MMRM), including fixed categorical terms for treatment, visit, treatment by visit interaction, center grouping, and continuous fixed covariates of baseline 6MWD and baseline 6MWD by visit. Secondary continuous efficacy endpoints were analyzed using analysis of covariance (ANCOVA), including fixed terms for treatment, center group and baseline score.

Results

A total of 56 patients were screened and 51 patients were randomized; all randomized patients completed

the study (Fig. 1). All patients were included in the ITT population, and 47 patients were included in the PP population. Demographic characteristics were similar across treatment groups (Table 1). The mean (SD [standard deviation]; range) age for the study population was 7.8 (2.2; 5 to 13) years. In terms of baseline characteristics (6MWD, rise from floor time and NSAA), patients on placebo had progressed slightly less than those on drisapersen 6 mg/kg/week (Table 1). In addition, they had a longer mean duration of steroid treatment: 37.1 months versus 26.8 months, respectively. Despite amending rise from floor criteria from ≤7 s to ≤15 s, only two patients had a rise from floor >7 s.

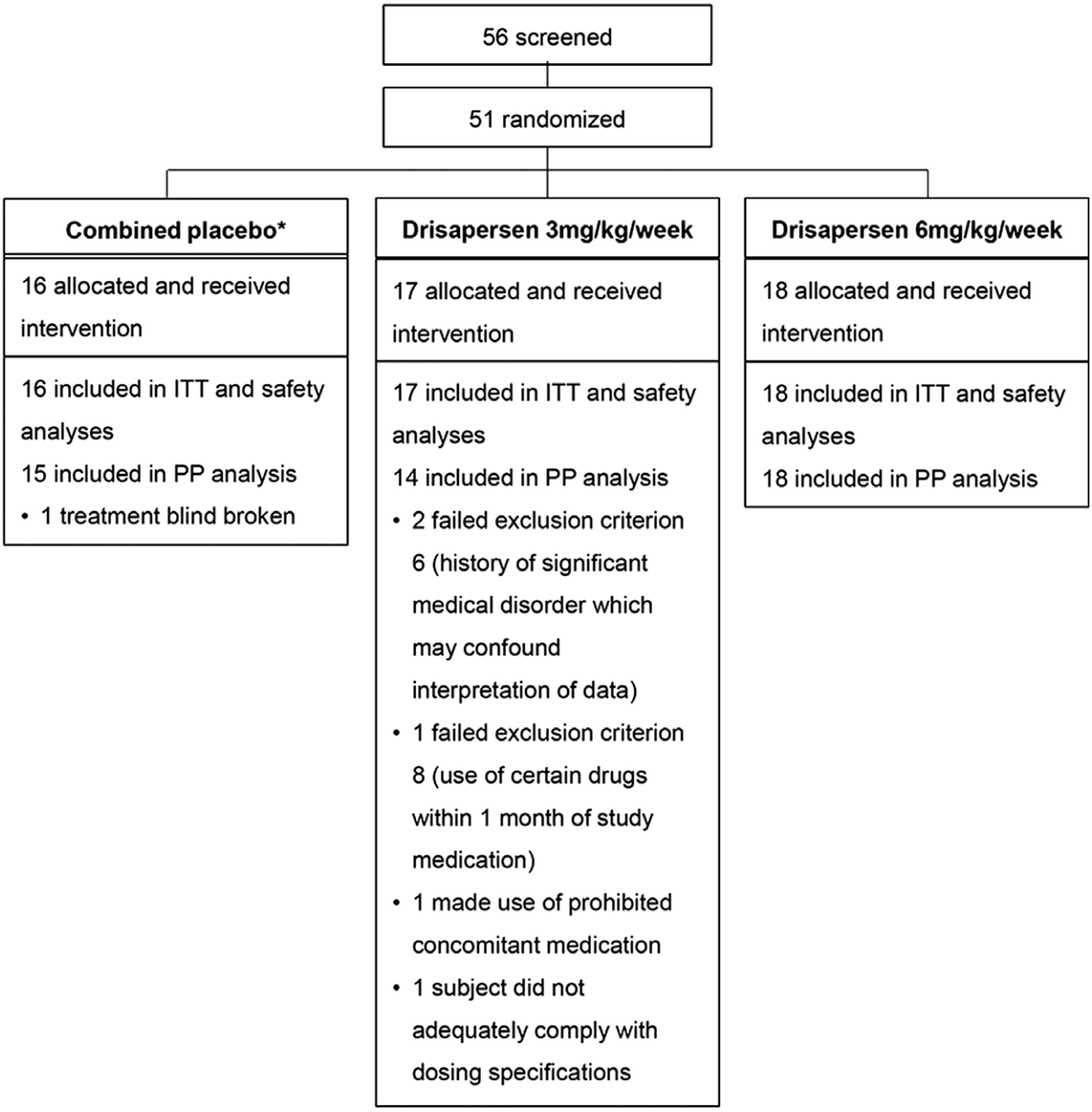


Figure 1. Summary of Patient Disposition. *The two placebo treatment groups (drisapersen 3 and 6 mg/kg/week volume-matched) were combined for analysis and reporting purposes. ITT, intent-to-treat; PP, per-protocol. One patient could have failed more than one exclusion criterion.

Table 1. Patient Baseline Demographics and Clinical Characteristics – Safety Population.

	Placebo (N = 16)	Drisapersen 3 mg/kg/week (N = 17)	Drisapersen 6 mg/kg/week (N = 18)
Age, years	8.0 (1.8)	7.8 (1.9)	7.6 (2.7)
Height, cm	122.7 (6.4)	119.8 (8.1)	120.4 (13.5)
Weight, kg	30.2 (8.5)	28.9 (6.5)	29.5 (12.5)
Body Mass Index, kg/m²	19.9 (4.5)	19.9 (3.0)	19.6 (4.6)
Time since first symptoms, months	57.3 (29.7)	67.3 (27.1)	59.0 (29.5)
Time since diagnosis, months	45.5 (29.7)	47.1 (26.4)	46.5 (26.8)
Time since first corticosteroids, months	37.1 (24.3)	33.3 (16.0)	26.8 (22.5)
Corticosteroid regimen, N (%)			
Continuous	15 (94)	15 (88)	18 (100)
Intermittent	1 (6)	2 (12)	0
6MWD[1], m	416.4 (57.0)	415.2 (58.1)	396.2 (60.7)
% Predicted 6MWD, %	71.3 (11.0)	72.4 (12.9)	69.9 (11.4)
Rise from floor[1], s	4.5 (1.6)	5.0 (2.2)	5.2 (2.5)

All values are mean (SD) unless stated otherwise.
[1]Shown for the ITT population.
6MWD, 6-min walk distance; ITT, intent-to-treat; SD, standard deviation.

Primary efficacy endpoint

6MWD

At week 24, a treatment difference in 6MWD of 27.1 m was observed for the drisapersen 6 mg/kg/week group compared with placebo (Table 2). The results showed a trend (P = 0.069) in favor for drisapersen 6 mg/kg/week. For the drisapersen 3 mg/kg/week treatment group, no statistically significant treatment difference over placebo was observed (−8.9 m; P = 0.554). The adjusted mean change (standard error) from baseline in 6MWD at week 24 for the three treatment groups is shown in Table 2 and the mean change over time in Figure 2A. Figure 2B shows that for each specific change in 6MWD from baseline, the proportion of patients having a better change outcome was greater for the group of patients receiving drisapersen 6 mg/kg/week than for those receiving placebo.

The PP population analysis results were broadly supportive of the ITT analysis. For drisapersen 6 mg/kg/week and 3 mg/kg/week, the mean (95% confidence intervals [CI]) treatment differences over placebo were 19.4 m (−11.6, 50.4) and −16.4 m (−49.4, 16.5), respectively. The ITT analysis is considered the primary analysis for interpretation.

Analysis of 6MWD by age group at week 24 revealed a mean (95% CI) treatment difference versus placebo for the ≤7 years age group of 30.7 m (−28.6, 90.1) for the drisapersen 6 mg/kg/week group (N = 10, P = 0.291) and −7.9 m (−64.7, 48.9) for the 3 mg/kg/week group (N = 8, P = 0.772). The mean (95% CI) treatment difference in patients >7 years was 27.8 m (−9.0, 64.6) for the drisapersen 6 mg/kg/week group (N = 8, P = 0.131) and −0.1 m (−38.9, 38.6) for the 3 mg/kg/week group (N = 9, P = 0.994).

During the off-treatment period (Fig. 2A), the drisapersen 6 mg/kg/week group maintained the benefit (27.9 m, P = 0.177) over placebo that was observed at week 24. The drisapersen 3 mg/kg/week group 6MWD declined further, with a mean treatment difference over placebo of −24.8 m at week 48 (P = 0.238). The week 48 analysis of the drisapersen 3 mg/kg/week group included one outlier who lost 200 m in 6MWD in the off-treatment phase, who also had a foot fracture incurred during this phase.

Change in percent-predicted 6MWD[5,22,25,26] was analyzed to take the maturational influence on distance walked into account. At week 24, a treatment difference of 5.2% (P = 0.051) was observed in favor of drisapersen 6 mg/kg/week compared with placebo for percent-predicted 6MWD (Table 2). This was still observed at week 48, although slightly smaller (4.8%, P = 0.154). For the drisapersen 3 mg/kg/week group, the treatment difference versus placebo was −1.5% (P = 0.584) and −4.1% (P = 0.240) at weeks 24 and 48, respectively. The analysis at week 48 of this group included the one outlier that lost 200 m in 6MWD in the off-treatment phase.

Secondary efficacy endpoints

The results of the other timed function tests did not show a statistically significant difference for either of the treatment

Table 2. Summary of the primary efficacy endpoints and timed function tests – ITT population.

Endpoint	Placebo (*N* = 16)	Drisapersen 3 mg/kg/week (*N* = 17)	Drisapersen 6 mg/kg/week (*N* = 18)
6-minute walk distance, m			
Baseline (SD)	416.4 (56.99)	415.2 (58.05)	396.2 (60.66)
Week 24 adjusted mean change (SE)	−11.0 (10.7)	−19.9 (10.0)	16.1 (9.9)
Week 24 treatment difference (95% CI);		−8.9 (−39.1,21.2);	27.1 (−2.2, 56.4);
P-value		0.554	0.069
6-minute walk distance, % predicted			
Baseline (SD)	71.3 (11.0)	72.4 (12.9)	69.9 (11.4)
Week 24 adjusted mean change (SE)	−3.5 (1.9)	−5.0 (1.8)	−1.7 (1.8)
Week 24 treatment difference (95% CI);		−1.5 (−7.0,4.0);	5.2 (−0.0,10.5);
P-value		0.584	0.051
Rise from floor time, s			
Baseline (SD)	4.49 (1.62)	4.96 (2.21)	5.19 (2.47)
Week 24 adjusted mean change (SE)	1.12 (0.7)	1.50 (0.66)	1.95 (0.66)
Week 24 treatment difference (95% CI);		0.39 (−1.62, 2.39);	0.83 (−1.07, 2.73);
P-value		0.699	0.384
10-m walk/run, s			
Baseline (SD)	5.12 (1.35)	4.97 (1.17)	5.38 (1.35)
Week 24 adjusted mean change (SE)	−0.04 (0.20)	0.52 (0.19)	−0.01 (0.19)
Week 24 treatment difference (95% CI);		0.56 (0.00, 1.13);	0.04 (−0.50, 0.57);
P-value		0.050	0.890
4-stair climb – ascent time, s			
Baseline (SD)	3.53 (1.80)	3.14 (1.29)	4.60 (3.18)
Week 24 adjusted mean change (SE)	0.59 (0.31)	0.59 (0.30)	−0.22 (0.30)
Week 24 treatment difference (95% CI);		0.00 (−0.88, 0.89);	−0.80 (−1.66, 0.05);
P-value		0.997	0.064
4-stair climb – descent time, s			
Baseline (SD)	2.94 (1.17)	3.34 (2.17)	4.05 (2.28)
Week 24 adjusted mean change (SE)	0.60 (0.47)	−0.09 (0.44)	0.19 (0.44)
Week 24 treatment difference (95% CI);		−0.69 (−2.03, 0.66);	−0.41 (−1.70, 0.88);
P-value		0.311	0.523

The reported *P*-values are drisapersen versus placebo.

SD, standard deviation; SE, standard error.

A negative difference compared to placebo represents benefit over placebo.

groups compared with placebo. There were small differences between both drisapersen groups and placebo after 24 week of treatment (Table 2 and Fig. 3). The largest treatment benefit on timed function tests was seen in the 4-stair ascent test. This treatment effect was evident despite the higher baseline stair ascent time observed in the 6 mg/kg/week group compared to placebo. The NSAA, the pulmonary function tests and total muscle strength revealed variable changes from placebo for both treatment groups after 24 weeks of treatment (Table S1). Baseline forced vital capacity was near normal in all groups and remained in the normal ranges over 24 weeks. Of note is that the knee extensor strength showed a numerical improvement from baseline to week 24 in the drisapersen 6 mg/kg/week group (Table S1). The totality of the data, represented by a forest plot of the treatment effect of these efficacy endpoints, indicates that patients treated with drisapersen 6 mg/kg/week had more positive responses than patients receiving placebo (Fig. 3).

The serum level of two markers for muscle damage in DMD,[1,27,28] creatine kinase and lactate dehydrogenase, decreased from baseline at week 24 in all three groups. The change was numerically larger in the treatment groups compared to placebo (Table S1).

In muscle biopsies ($n = 49$) from the tibialis anterior, the levels of exon 51 skipping assessed by nested RT-PCR were found to be higher in the drisapersen treatment groups. The mean intensity of exon 51 skipped dystrophin mRNA product was 4.37 and 4.44 arbitrary units in the 3 and 6 mg/kg/week groups, respectively, versus 1.53 arbitrary units on placebo. Immunofluorescence analysis ($n = 34$) or Western blot ($n = 11$) analysis revealed no consistent treatment-related increase in dystrophin at week 24 (data not shown).

The majority of patients in all three treatment groups had minimal or no change in Clinician Global Impression of Improvement at week 24, with no difference between treatment groups. Most patients had some improvement on the Physician Assessment of Daily Living (defined as 'any improvement') at week 24. These findings were similar for all three treatment groups (data not shown). Results of the Functional Outcomes Survey individual scores (including general health, mobility, physical activities, hand dexterity, and use of assistive devices) showed that patients' function was similar in the three treatment groups at both weeks 12 and 24, with some trends in favor of the 6 mg/kg/week treatment group. For example, for 'mobility' and 'hand dexterity' there was numerically a higher number of patients that improved on all questions at week 24 in the 6 mg/kg/week group versus placebo. The same was true for 5 out of 6 questions on physical activity. In addition, there was a trend in favor of the drisapersen 6 mg/kg/week group compared with placebo and the drisapersen 3 mg/kg group for 'general health'.

None of the patients, in any of the groups, lost ambulation during the course of the study.

Safety endpoints

Most patients in each group reported an AE during the treatment phase (Table 3); however, the majority of these AEs were only mild to moderate in intensity. The incidence of drug-related AEs during the treatment phase was higher in the two drisapersen treatment groups compared with placebo. There were no deaths and no AEs led to permanent discontinuation of study drug or withdrawal from the study. Two serious AEs were reported; both occurred in the placebo group.

The most commonly reported AEs of special interest during the treatment phase were related to injection site reactions; these occurred more frequently in both drisapersen groups (65% and 72% in the 3 and 6 mg/kg/week group, respectively) than in the placebo group (31%). Most frequently reported were injection site erythema, discoloration and bruising (Table S2) but none of these events were severe or classified as serious AE. Renal AEs were the second most common AEs occurring in 31% of placebo, 12% of drisapersen 3 mg/kg/week and 28% of drisapersen 6 mg/kg/week treated patients. Subclinical proteinuria (placebo 13%, 3 mg/kg/week 6% and 6 mg/kg/week 17%) and chromaturia (placebo 13%, 3 mg/kg/week 6% and 6 mg/kg/week 6%) were the most frequently reported renal AEs. Among the key renal laboratory parameters, a higher proportion of patients in both drisapersen treatment groups (53% and 78% of the patients in the 3 and 6 mg/kg/week group, respectively) had elevated α_1-microglobulin levels compared to placebo (19% of the patients), with the 6 mg/kg/week group having the highest levels. This is consistent with interference with protein uptake in the proximal tubule leading to mild subclinical proteinuria. During the off-treatment phase, the most common AEs were influenza and nasopharyngitis (Table S2); α_1-microglobulin levels were not measured during the off-treatment period. Renal AEs (chromaturia) were only reported in the 6 mg/kg/week group and chromaturia was the only reported event.

Pharmacokinetic endpoints

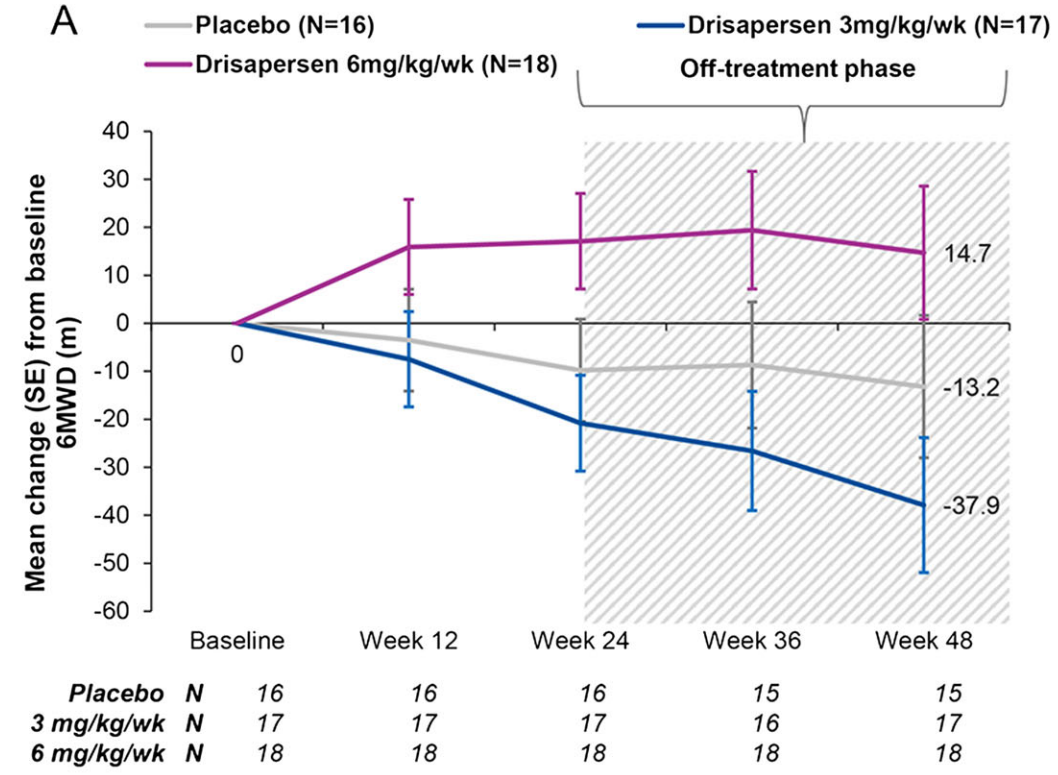
The pharmacokinetic parameters, measured in the plasma, are summarized in Table 4. The plasma exposure, determined by C_{max}, $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}24)}$, increased dose proportionally between the drisapersen 3 and 6 mg/kg/week groups; the T_{max} was comparable for both doses.

The muscle tissue exposure increased more than dose proportional, that is, fourfold (from 2.7 μg/g on 3 mg/kg to 10.8 μg/g on 6 mg/kg, 17 biopsies were analyzed in each treatment group) for a twofold increase in dose from 3 to 6 mg/kg at week 24 (Table 5, and Figure S1). Drisapersen was eliminated slowly from muscle tissue; 12 weeks after drisapersen treatment was stopped, muscle tissue exposure levels were only reduced by approximately 40%, suggesting an initial muscle tissue elimination half-life of approximately 16 weeks. The long tissue elimination half-life of drisapersen suggests a slow accumulation of muscle tissue drisapersen, which was reflected in the low levels after 12 weeks of treatment (2.1 μg/g and 5.0 μg/g in the 3 mg/kg and 6 mg/kg group, respectively). As a result, tissue steady state levels had not been reached after 24 weeks of treatment.

Discussion

This exploratory Phase 2 study assessed the efficacy, safety, tolerability, and pharmacokinetics of two weekly subcutaneous drisapersen doses (3 mg/kg and 6 mg/kg) versus placebo during a 24-week treatment phase and evaluated the persistence of efficacy and safety of both doses in a 24-week off-treatment observational phase.

The 6-minute walk test has been used in clinical trials to evaluate endurance and muscle function in neuromuscular diseases and has been validated as a clinically meaningful endpoint in ambulant DMD patients with population changes already observed over a short period of time (24–52 weeks).[5,21–23,26,29] In this ambulant DMD population, drisapersen 6 mg/kg/week resulted in an improvement from baseline in 6MWD (16.1 m) at week 24. Even though the patients in the placebo group were



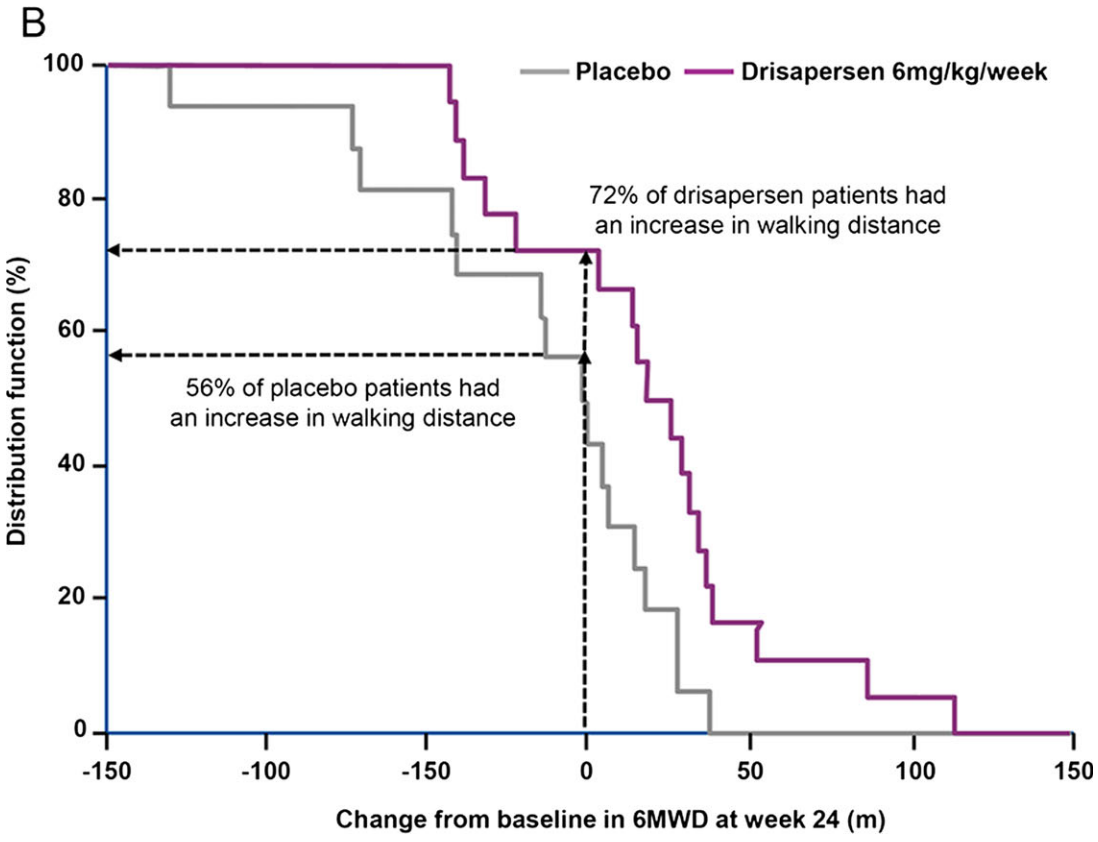


Figure 2. (A) Adjusted Mean Change From Baseline in 6MWD (m) at Week 24 (End of Treatment Phase) and Week 48 (End of Off-Treatment Phase of 24 Weeks) – MMRM Analysis, ITT Population and (B) Proportion of patients with a specific change in 6MWD at week 24. In the primary efficacy MMRM analysis of change from baseline in 6MWD at week 24, a treatment difference over the placebo was only observed for the drisapersen 6 mg/kg/week group. The increase in 6MWD was maintained at week 48 for the drisapersen 6 mg/kg/week group, while the other groups showed a continuous decline in 6MWD. The results presented in part A are from the statistical model fitted at the end of the study, including all data up to week 48. The inclusion of data from visits post week 24, therefore, leads to slightly different results at week 24 to those obtained from the primary analysis as presented in the text. Conclusions remain unchanged. The proportion of patients that maintained or improved in 6MWD from baseline was larger in the drisapersen 6 mg/kg/week group than in the placebo group. Shading corresponds to the off-treatment phase. 6MWD, 6-min walk distance; ITT, intent-to-treat; MMRM, mixed model for repeated measures.

slightly less progressed than those in the 6 mg/kg/week group, there was an improvement of 27.1 m in the drisapersen 6 mg/kg/week group compared to placebo, that was largely maintained (27.9 m) 24 weeks off-treatment. After 24 weeks of treatment, the difference in mean change from baseline 6MWD in favor of drisapersen 6 mg/kg/week of 27.1 m ($P = 0.069$) was similar to that observed in DMD114117 (35.1 m, $P = 0.014$).[19] Due to great heterogeneity of disease progression, it can be difficult to observe significant treatment effects in DMD trials of ≤1 year duration.[22,30] In addition, treatment duration was 24 weeks, while steady state drisapersen tissue levels are only reached after approximately 36 weeks (BioMarin Pharmaceutical Inc. data on file).

Drisapersen had a positive effect on the results of the 4-stair climb test, which has been recently employed as a primary endpoint in ambulatory DMD trials. Muscle strength has been shown to remain relatively stable over a 1-year course in ambulatory DMD boys, with the exception of knee extension which shows a decline in most patients.[19] In this study, knee extensor strength increased after 24-week treatment only in the drisapersen 6 mg/kg/week group.

The drisapersen 3 mg/kg/week group showed no statistically significant difference in mean change from baseline 6MWD compared to placebo. The marked decline at week 48 was substantially impacted by one outlier and probably does not reflect a general trend for the entire 3 mg/kg/week group. Differences in clinical response with the 3 mg/kg and 6 mg/kg dose levels can be explained by low tissue levels with the 3 mg/kg, which were only 25% of the levels reached in the 6 mg/kg group after 24 weeks. It is therefore likely that the muscle exposure in the 3 mg/kg group never reached a minimal level required for a clinical effect. In addition, the long estimated tissue elimination half-life and the low tissue drisapersen levels that were reached after 12 weeks during the treatment period suggest that steady state had not yet been reached after the 24 week treatment period.

To account for the influence of growth and maturational effects on the 6MWD, the percent-predicted 6MWD was investigated.[5,22,25,26] These results were similar to those

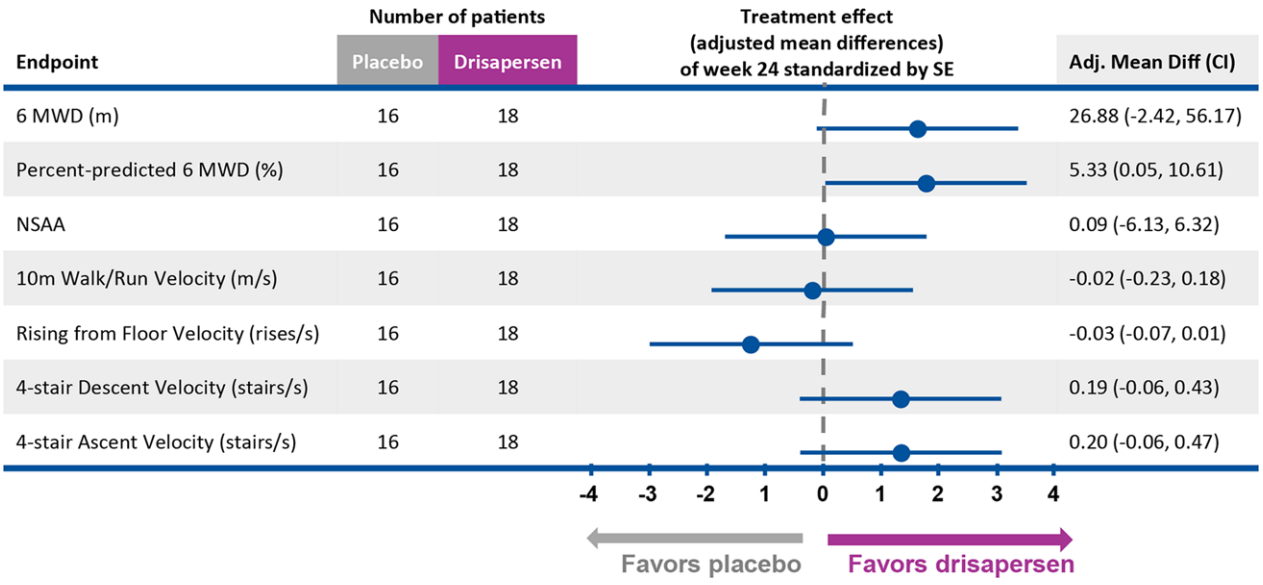


Figure 3. Summary of Treatment Effect of Selected Efficacy Outcomes. Forest plot of the adjusted mean difference (MMRM analysis) at week 24 indicates that most treatment effects are in favor of drisapersen treatment. Drisapersen includes the patients of the 6 mg/kg/week group only. CI, confidence interval; MMRM, mixed model for repeated measures; NSAA, North Star Ambulatory Assessment.

Table 3. Summary of AEs During the On-treatment and Off-treatment Phases – Safety Population.

	Placebo (*N* = 16)	Drisapersen 3 mg/kg/week (*N* = 17)	Drisapersen 6 mg/kg/week (*N* = 18)
Treatment phase, *N* (%)			
Any AEs[1]	13 (81)	16 (94)	17 (94)
AEs related to study treatment	4 (25)	10 (59)	14 (78)
Serious AEs[2]	1 (6)	0	0
Any AE of special interest[4]	9 (56)	11 (65)	16 (89)
Injection site reaction	5 (31)	11 (65)	13 (72)
Renal abnormalities	5 (31)	2 (12)	5 (28)
Inflammation	1 (6)	1 (6)	5 (28)
Coagulation abnormalities	2 (13)	0	1 (6)
Hepatic abnormalities	1 (6)	0	1 (6)
AEs leading to permanent discontinuation of study treatment	0	0	0
Off-treatment phase, *N* (%)			
AEs	9 (56)	8 (47)	14 (78)
AEs related to study treatment	1 (6)	2 (12)	3 (17)
Serious AEs[3]	1 (6)	0	0
Any AE of special interest[4]	1 (6)	2 (12)	6 (33)
Injection site reaction	0	2 (12)	3 (17)
Renal abnormalities	0	0	1 (6)
Inflammation	0	0	2 (11)
Coagulation abnormalities	1 (6)	0	0
Hepatic abnormalities	0	0	1 (6)

[1]Of which only 2 severe AEs – 1 on placebo: thermal burn, not considered drug-related; and 1 on drisapersen 6 mg/kg/week: urticaria, considered drug-related.
[2]Placebo: wound infection staphylococcal, not considered drug-related.
[3]Placebo: atypical pneumonia, not considered drug-related.
[4]No 'AE of special interest: Thrombocytopaenia' was observed during the study.
AE, adverse event.

Table 4. Pharmacokinetic Parameters Following Administration of Drisapersen 3 or 6 mg/kg/week at Week 23 – Full Pharmacokinetic Population.

Parameter	Drisapersen 3 mg/kg/week (*N* = 8)	Drisapersen 6 mg/kg/week (*N* = 8)
C_{max} (μg/mL)[1]	2.8 (46%)	6.2 (28%)
$AUC_{(0\text{-}t)}$ (μg h/mL)[1]	37.7 (42%)	71.0 (48%)
$AUC_{(0\text{–}24\ h)}$ (μg h/mL)[1]	30.2 (39%)	57.3 (42%)
T_{max} (h)[2]	2.0 (1.8–9.3)	2.0 (1.8–9.0)

[1]Geometric mean (CV%).
[2]Median (range).

Table 5. Drisapersen tissue level (μg/g) in tibialis anterior muscle biopsies from DMD patients treated with drisapersen 3 or 6 mg/kg/week or placebo, at 12, 24 and 36 weeks.

Time point	Placebo (*N* = 16)	Drisapersen 3 mg/kg/week (*N* = 17)	Drisapersen 6 mg/kg/week (*N* = 18)
Week 12	0.0 (0.0); 7	2.1 (1.6); 6	5.0 (3.4); 8
Week 24	0.0 (0.0); 16	2.7 (2.0); 17	10.8 (7.4); 17
Week 36	0.0 (0.0); 7	1.6 (0.9); 7	6.4 (3.4); 7

All values are mean (SD); number of biopsies available and analyzed. Time point week 36 reflects 12 weeks off-treatment.

from the absolute 6MWD data. The longitudinal change in 6MWD in untreated boys with DMD is influenced by age, with those ≤7 years showing increased 6MWD, but at a reduced rate relative to typically developing children and those >7 years generally showing a decline in 6MWD.[5,21–23] No significant treatment differences were observed between either drisapersen group compared to placebo when 6MWD was analyzed by age group.

The 6MWD outcomes of the present US study are consistent with the results that were previously reported for the mainly European DMD114117 study.[19] That study had a similar early ambulant patient population, although slightly younger, and reported a statistically significant improvement in 6MWD of 35.1 m over placebo after 24 weeks of drisapersen 6 mg/kg/week. Pharmacokinetic modeling suggests that, with a loading regimen of twice-weekly drisapersen dosing for the first 3 weeks, comparable drisapersen tissue concentrations are achieved 4 weeks earlier than with weekly drisapersen administration (BioMarin Pharmaceutical Inc. data on file) and could, as a result, lead to faster therapeutic response and greater (and statistically significant) clinical benefit measured at 24 weeks of treatment. It may also have contributed to the greater consistency in treatment benefit across secondary endpoints.

The results of the 6 mg/kg/week group on the secondary endpoints muscle function/strength, pulmonary function and ambulation were in favor of drisapersen, though no significant differences between the two treatment groups compared with placebo were observed. These results are consistent with those of the DMD114117 and DMD114044 studies.[19,31] Data from natural history studies demonstrated that it can be difficult to observe significant treatment effects on the measures that were used as secondary outcome in this study after a period of only 48 weeks,[22,30,32,33] while the current study only had a 24 week treatment period. In addition, the 24 week treatment period was not sufficient to reach steady state drisapersen tissue levels. Nevertheless, there was a trend towards a benefit of drisapersen.

The serum levels of markers for muscle damage in DMD,[1,27,28] were reduced after 24 weeks of drisapersen

treatment, supporting the effect of drisapersen on muscle membrane integrity and damage.[1]

Drisapersen at doses of 3 and 6 mg/kg/week administered subcutaneously was generally well tolerated, with all randomized patients completing the study. The majority of patients reported injection site reactions during the treatment phase. However, during the study, none of the reactions was severe or classified as a serious AE. There is a theoretical concern that these reactions could have unblinded the patients receiving drisapersen and contributed to motivational differences in the performance of the 6MWD. However, two lines of evidence mitigate this concern. First, the 3 mg/kg/week treatment group had a comparable rate of injection site reactions. Secondly, analysis of the treatment effect versus severity of injection site reactions in the two separate dose cohorts showed no relationship between severity of injection site reaction and change in 6MWD over 24 and 48 weeks.

The subcutaneous administration of antisense oligonucleotides is known to lead to local skin reactions nearby the injection site. Persistent injection site reaction could potentially progress over time.[34] At the end of this study, none of the injection site reactions in this study was severe or classified as a serious AE, similar to the results in the recently published DMD114044 study. However, in the phase 3 study, 16% of the injection site reactions remained unresolved upon study close.[31] The progressing injection site reactions were one of the reasons that drisapersen did not reach approval.

Subclinical proteinuria and chromaturia as well as elevated urinary α_1-microglobulin levels were the most frequently reported renal abnormalities, which may indicate mild reversible interference with protein reabsorption in the proximal tubule. Of note is the lack of significant renal dysfunction related to proteinuria in the treatment phase of the study. Moreover, all but one renal AE resolved in the off-treatment phase. These safety results are consistent with the findings that were reported for the DMD114117 and DMD114044 studies.[19,31]

These data contributed to a better understanding on the clinical utility of drisapersen to slow disease progression across a wide range of DMD disease severity. Several key points should be considered when interpreting the findings of the present study. This study was conducted at centers based in the United States only and was only exploratory and not prospectively powered.

Corticosteroid treatment has been the standard of care for DMD as it helps to maintain muscle strength and ambulation in DMD patients.[11,21] The patients on placebo had a longer mean duration of steroid treatment than those on 6 mg/kg/week drisapersen (37.1 months versus 26.8 months, respectively), which could lead to differences in expected functional decline. In addition, the proportion of patients receiving continuous corticosteroid regimen varied across the groups. There is also variation across centers in the type of administered corticosteroid, as well as the dose and/or frequency of dosing.[4] As such, variability in corticosteroid treatment contributes to the observed heterogeneity in disease progression.[11,21,23]

A prior clinical trial (DMD114044)[31] documented trends in low level dystrophin production in patients treated with 6 mg/kg/day of drisapersen versus placebo treatment. Pre-treatment biopsies were not obtained in this study. Evaluation of week 48 high quality biopsies showed an increase of dystrophin levels in drisapersen compared to placebo subjects, which was not statistically significant. In the same subset of biopsies, low levels of dystrophin were detected by Western blot analysis (lower limit of quantification and detection approximately 1.000% and 0.125%), and no significant difference between placebo and drisapersen treatment was observed. This is a relatively similar low level of dystrophin by Western blot analysis relative to that reported with eteplirsen at 48 weeks, using different methodology. In this same study, the median trough drisapersen plasma concentration increased over time up to 48 weeks of dosing, whereas the mean drisapersen muscle tissue homogenate concentration slowly increased over time reaching steady state at approximately 36 weeks post-treatment.[31]

In the present study, drisapersen tissue concentrations close to 10 μg/g were achieved in many patients by 24 weeks (at 6 mg/kg/week). This is an important threshold level, as results from the clinical trial program have showed that drisapersen tissue concentrations above 10 μg/g show the best treatment response (Figure S1, BioMarin Pharmaceutical Inc. data on file). Because of the slow muscle clearance of drisapersen during the off-treatment period, it is expected that the clinical effects are sustained for a longer period after stopping treatment.

Summary

The data from this exploratory study suggest that treatment with drisapersen 6 mg/kg/week has shown beneficial effects in slowing disease progression in an early ambulant DMD population that is amenable to exon 51 skipping. Specifically, drisapersen 6 mg/kg/week for 24 weeks resulted in a treatment benefit in 6MWD and most secondary endpoints as patients in the drisapersen 6 mg/kg/week dosing group showed after 24 weeks either improved function or relatively less decline than patients receiving placebo. This benefit was largely maintained 24 weeks off-treatment. Drisapersen had a long half-life with steady state reached after approximately 36 weeks. The most common adverse events in both drisapersen groups were related to injection site reactions and

subclinical proteinuria. This study provided insights for further studies in the clinical program to optimize dosage regimen. Although results of the different clinical trials were promising, the clinical development of drisapersen has been ceased by the study sponsor.

Acknowledgments

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Author Contributions

C.M.M: site PI for 876 study, review of study data with BioMarin – Prosensa, development, and critical review of manuscript. B.W: site PI for 876 study, review of study data with BioMarin – Prosensa and review of manuscript. K.M.F: site PI for 876 study, review of study data with BioMarin – Prosensa and review of manuscript. R.W: conduct of statistical analyses, review of study data with BioMarin – Prosensa, and development and review of manuscript. S.K: study design, data analysis, interpretation of data analyses in results, and assisted in development and review of manuscript. A.L: data analysis, interpretation of data analyses in results, and assisted in development and review of manuscript. Z.L: contribution to conducting the statistical analyses, interpretation of the data analyses in the results, and discussion, and review of manuscript. G.C: study design, medical monitor of the study, interpretation of data analyses, and development and review of manuscript. All non-author collaborators, including the DEMAND V study group see Table S3.

Conflicts of Interest

C.M.M. acts as a consultant for BioMarin, Sarepta Therapeutics, PTC Therapeutics, Eli Lilly, Pfizer, Akashi Therapeutics, Catabassis, Marathon, Italfarmaco, Capricor, and Santhera Pharmaceuticals. B.W. received personal fees and non-financial support from the Drisapersen Expert Advisory meeting in London and Sarepta's Advisory Board meeting in Phoenix, Arizona. K.M.F. serves as a site investigator on trials supported by PTC Therapeutics and Akashi Therapeutics, and has received personal fees from Sarepta Therapeutics and a grant from CureDuchenne outside the submitted work. R.W. receives consultant fees from BioMarin. S.K. is an employee of BioMarin Pharmaceutical Inc. A.L. is an employee of BioMarin Pharmaceutical Inc. Z.L. is an employee of BioMarin Pharmaceutical Inc. G.C. is an employee of BioMarin Pharmaceutical Inc. and holder of stock and options.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. Concentration of muscle tissue drisapersen concentration following subcutaneous administration. A) Mean (± 95% CI) muscle tissue concentration in Study DMD114044 (Drisapersen 6 mg/kg/week, $N = 109$); B) Mean (± 95% CI) muscle tissue concentration in Study DMD114876 (Drisapersen 3 mg/kg/week, $N = 17$; Drisapersen 6 mg/kg/week, $N = 18$). CI=confidence interval; SD=standard deviation

Table S1. Summary of the Secondary Efficacy Endpoints: Assessment of muscle strength, ambulation, pulmonary function and muscle integrity – ITT Population

Table S2. Summary of the Most Common AEs in the On-treatment and Off-treatment Phases Occurring in ≥5% of Patients and in ≥2 Patients for ≥1 of the Treatment Groups, in Descending Frequency for Total Group – Safety Population

Table S3. List of all collaborators, from the Demand V Study that contributed to the conduct of the study and data collection.

Appendix
Demand V Study Group

Research Institute at Nationwide Children's Hospital: Kevin M. Flanigan, MD; Children's Medical Center Dallas: Susan T. Iannaccone, MD; University of Minnesota: Peter I. Karachunski, MD; University of Iowa: Katherine D. Mathews, MD; University of California Davis Medical Center: Craig M. McDonald, MD, Erik K. Henricson, PhD, MPH, Nanette C. Joyce, DO; Washington University: Alan Pestronk, MD; Northwest Florida Clinical Research Group, LLC: James B. Renfoe, MD; Shriners Hospitals For Children, Portland, Oregon health Sciences: Barry S. Russman, MD; Duke University Medical Center: Edward C. Smith, MD; Stanford University: Yuen T. So, MD, Ching H. Wang, MD; John W. Day, MD, PhD; Columbia University Medical Center: Douglas M. Sproule, MD; Kennedy Krieger Institute: Kathryn R. Wagner, MD, PhD; Cincinnati Children's Hospital Medical Center: Brenda Wong, MD.

