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Abstract

We report results from a phase 2, randomized, double-blind, 2-period trial (48 weeks each) of domagrozumab and its open-label extension in patients with Duchenne muscular dystrophy (DMD). Of 120 ambulatory boys (aged 6 to <16 years) with DMD, 80 were treated with multiple ascending doses (5, 20, and 40 mg/kg) of domagrozumab and 40 treated with placebo. The primary endpoints were safety and mean change in 4-stair climb (4SC) time at week 49. Secondary endpoints included other functional tests, pharmacokinetics, and pharmacodynamics. Mean (SD) age was 8.4 (1.7) and 9.3 (2.3) years in domagrozumab- and placebo-treated patients, respectively. Difference in mean (95% CI) change from baseline in 4SC at week 49 for domagrozumab vs placebo was 0.27 (−7.4 to 7.9) seconds (p = 0.94). There were no significant between-group differences in any secondary clinical endpoints. Most patients had ≥1 adverse event in the first 48 weeks; most were mild and not treatment-related. Median serum concentrations of domagrozumab increased with administered dose within each dose level. Non-significant increases in muscle volume were observed in domagrozumab- vs placebo-treated patients. Domagrozumab was generally safe and well tolerated in patients with DMD. Efficacy measures did not support a significant treatment effect.

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Keywords: Duchenne muscular dystrophy; myostatin inhibitor; domagrozumab; 4-stair climb.

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1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked, progressive, neuromuscular disorder caused by mutations in
the DMD gene that result in a lack of dystrophin protein. Dystrophin is critical for membrane integrity in skeletal and cardiac muscle cells; its loss leads to skeletal and cardiac muscle degeneration [1].

Myostatin (growth and differentiation factor 8) is a member of the transforming growth factor-β superfamily and a negative regulator of skeletal muscle mass [2,3]. Loss or inhibition of myostatin in mdx mouse DMD models leads to increased muscle mass, increased strength, and decreased fat substitution and fibrosis [4-11]. This identifies myostatin inhibition as a therapeutic target in multiple neuromuscular disorders, including dystrophinopathies. Various strategies to inhibit myostatin pharmacologically have been employed, including neutralizing antibodies and adnexins, inhibitory myostatin propeptides, myostatin receptor-Fc fusion protein, follistatin the endogenous inhibitor of myostatin and myostatin receptor blocking antibodies [12].

Domagrozumab, a humanized recombinant immunoglobulin (Ig)G1 antibody that binds to myostatin, and inhibits its activity, was evaluated for treatment of DMD. Wild-type and mdx mice treated with the murine equivalent of domagrozumab RK35 had significant increases in body weight, lean body mass, and grip strength. Cynomolgus monkeys treated with domagrozumab showed significant dose-dependent increases in lean muscle mass and muscle volume, but no functional test was conducted [9].

A randomized phase 2 trial of domagrozumab evaluated safety, efficacy, pharmacokinetics (PK), and pharmacodynamics (PD) of multiple ascending doses of domagrozumab in ambulatory boys with DMD. Patients who completed the phase 2 trial were invited to participate in an open-label extension (OLE) trial to evaluate the long-term safety, efficacy, PK, and PD of domagrozumab. We report results from both trials.

2. Materials and methods

2.1. Patients

Ambulatory boys aged 6 to <16 years with genetically confirmed DMD were enrolled if they were able to perform the 4-stair climb (4SC) in ≥2.5 but ≤12 seconds at screening, and were receiving glucocorticosteroids for ≥6 months, with a stable regimen for ≥3 months prior to guardians signing the informed consent. Other inclusion criteria included adequate hepatic and renal function.

Exclusion criteria included: underlying disposition for iron accumulation or bleeding disorder; cognitive impairment or behavioral issues that would have affected the conduct of the study; history of surgery within 6 weeks, or planned surgery during the study; any injury that may impact function testing; compromised cardiac function (left-ventricular ejection fraction [LVEF] <55% as determined on cardiac MRI or echocardiogram [ECHO] screening); current or prior treatment with anti-myostatin; exon skipping; nonsense mutation–targeted therapies; or treatment with utrophin modifiers within the preceding 30 days or for >30 days in total.

Patients were included in the OLE if they completed the phase 2 trial through week 97, had adequate hepatic function, glutamate dehydrogenase (GLDH) ≤20 units/L, iron content estimate within the normal range on a liver MRI, and provided consent.

2.2. Standard protocol approvals, registrations, and participant consent

A phase 2 trial (B5161002; ClinicalTrials.gov: NCT02310763) and an OLE trial (Study B5161004; ClinicalTrials.gov: NCT02907619) were investigated. Both studies were conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects, guidelines for Good Clinical Practices, and Declaration of Helsinki. The protocols, any amendments, and informed consent/assent documents were approved by the institutional review board or ethics committee at each study center. Parent or legal guardians provided written, informed consent prior to any study-specific activity being performed.

2.3. Study design and treatment

The phase 2 trial was a randomized, 2-period, double-blind, placebo-controlled, multiple ascending dose (5, 20, and 40 mg/kg) study of domagrozumab vs placebo in ambulatory boys with DMD. The trial was conducted between November 24, 2014 and November 23, 2018, in patients from 31 sites in 8 countries.

Patients were stratified by 4SC time (≤ or >8 seconds) at baseline and randomly assigned to 1 of 3 sequence groups. Randomization into sequence groups (treatment assignment) was generated using an Interactive Response Technology System by unblinded, dispensing personnel. The first exposure to domagrozumab was a within-patient dose escalation (5, 20, and 40 mg/kg) for 48 weeks; subsequent exposure continued at 40 mg/kg, determined to be the maximum tolerated dose in the dose escalation period, in both the phase 2 and OLE trials. In Sequence 1, domagrozumab within-patient dose escalation (period 1) was followed by domagrozumab in weeks 49–96 (period 2). In Sequence 2, domagrozumab within-patient dose escalation in the first 48 weeks was followed by placebo in weeks 49–96. In Sequence 3, placebo in the first 48 weeks was followed by domagrozumab within-patient dose escalation in weeks 49–96 (Fig. 1). Patients received treatment for 96 weeks, i.e., two treatment periods of 48 weeks each, without pause between periods.

At each dose level, the study drug was administered over 2 hours by IV infusion every 4 weeks for a total of 16 weeks (4 doses; Fig. 1). At the initiation of the phase 2 trial, individual dose escalation for each patient occurred after review of all available safety data through the planned fourth dose within each dose level until the External-Data Monitoring Committee agreed, after a number of patients had completed the first 48 weeks of the phase 2 trial, that dose escalation could...
The phase 2 trial and the OLE trial were terminated in August 2018 after the analysis of phase 2 trial data (described below) showed there was no significant treatment effect vs placebo in the primary efficacy endpoint (4SC) and other functional endpoints.

2.4. Procedures and outcomes

The primary objectives of the phase 2 trial were safety and tolerability of multiple ascending, repeat IV doses of domagrozumab and efficacy at 49 weeks based on an observed mean change from baseline in 4SC time (assessed every 8 weeks) in patients treated with domagrozumab vs placebo. The primary objective of the OLE trial was long-term safety of domagrozumab in boys with DMD.

Safety assessments in the two trials included: frequency and severity of incidence of abnormal and clinically relevant laboratory findings; physical examinations; vital signs; EKG, LVEF by cardiac MRI or ECHO (determined by site preference); liver MRI R2* monitoring iron accumulation [13]; and the Columbia Suicide Severity Rating Scale. AEs of special interest included, liver iron accumulation, precocious puberty and epistaxis (seen with ACE-031, a less selective myostatin inhibitor) [14]. GLDH, a liver-specific injury biomarker was added to monitor for drug-induced liver injury in addition to transaminases, which are elevated from dystrophic muscle in DMD.

The North Star Ambulatory Assessment (NSAA) [15-17], range of motion using goniometry [18], Performance of Upper Limb (PUL) [19,20], 6-minute-walk distance (6MWD) [21], pulmonary function based on forced vital capacity (FVC) using spirometry [17,22], and strength assessed by hand-held myometry were conducted every 8 weeks in the phase 2 trial and every 24 weeks in the OLE. Secondary functional endpoints were assessed as mean change from baseline vs placebo during the first 49 weeks in the phase 2 trial. In the OLE trial, all functional assessments, including 4SC time, were assessed as change from overall baseline (beginning of phase 2) and change from OLE baseline. In both studies, change from baseline for all functional assessments was also analyzed by sequence group.

To characterize long-term effect following 96 weeks treatment with domagrozumab, data were compared with a historical control group derived from the Cooperative International Neuromuscular Research Group (CINRG) DMD natural history database [23,24]. The control cohort from CINRG was selected to closely match the population for age, glucocorticosteroid usage, baseline 4SC time, ambulatory status, and baseline LVEF ≥55%. The historical control group was first compared with Sequence 3 (placebo group in the phase 2 trial) by evaluating the mean change from baseline at week 49 to assess similarities between the two untreated populations. The historical control group was then compared to Sequence 1 to evaluate the mean change from baseline at week 97. Matched control data were selected by comparing the baseline criteria to all available visits in the CINRG database. Some patients in the historical control group had multiple eligible baseline visits eligible for comparison matching enrollment criteria (with a follow-up visit 96 ± 9 weeks later), and in these cases their baseline visit was chosen at random for the week 97 analysis. The same approach was used for the week 49 analysis for historical control patients with eligible baseline visits and follow-up visits 48 ± 4 weeks later.

Secondary PD endpoints specific to the phase 2 trial included changes in thigh muscle volume on MRI (every 12 weeks) [25,26] and modulation of myostatin in serum (every 4 weeks). A prespecified subgroup analysis was performed to assess treatment effects in a subgroup of patients with baseline 4SC ≥3.5 to ≤8 seconds who may be more rapidly declining with lower variability over a 1-year period [27].

Figure 1. Phase 2 trial (Study B5161002) design. OLE, open-label extension (B5161004).
In the OLE trial, pulmonary function tests also included forced expiratory volume in 1 second and peak expiratory flow rate. Ambulatory status was reviewed at each visit in the OLE trial. Loss of ambulation in OLE was defined as the inability to walk ≥10 meters unassisted and without braces, and in the historical control group as daily wheelchair use.

The functional health status as assessed by the Pediatric Outcomes Data Collection Instrument (PODCI) was an exploratory endpoint, which was completed by parents or caregivers of children aged ≤10 years; the adolescent self-report version was completed by patients ≥11 years old [28].

Other clinical outcomes assessments in the OLE assessed the long-term effects of domagrozumab on: (i) health-related quality of life using the EuroQol-5 Dimensions 3 Levels (EQ-5D-3L) and healthcare resource utilization in boys with DMD; and (ii) caregiver burden due to child’s DMD, using the Zarit Burden Interview (ZBI) and work productivity and activity impairment using the Work Productivity and Activity Impairment questionnaire adapted for the caregiver (WPAL:CG) [29].

Blood samples for PK, anti-drug antibody (ADA), and neutralizing antibody analyses were collected during the 96 weeks of the study and were analyzed using validated analytical methods. PK parameters included maximum serum concentration (Cmax); time to Cmax (Tmax); trough (predose) serum concentration (Ctrough); serum concentration at the end of planned 2-hour infusion (C1); area under the serum concentration–time curve over the dosing interval τ (AUCτ), where τ = 4 weeks (672 hours); and the average serum concentration over the dosing interval (Cav). Cmax, Tmax, and Ctrough were derived from serum of all patients treated with domagrozumab. AUCτ, Cav, and drug clearance were derived from patients with additional PK sampling who received domagrozumab in the first 48 weeks.

2.5. Statistical analysis

The planned sample size of 105 patients for the phase 2 trial was designed to detect a 2.5-second difference in change from baseline to week 49, in 4SC time, between domagrozumab and placebo treatment, assuming a common SD of 4.0 seconds with 80% power at α = 0.05 (2-sided), a 2:1 treatment allocation, 1 interim analysis for futility prior to the primary analysis, and 10% attrition rate. All analyses were based on the full analysis set, which included all randomized patients who received ≥1 dose of study drug. Patients assigned to Sequence 1 and Sequence 2 (i.e., treated with domagrozumab through week 48) were analyzed together and compared with patients in Sequence 3 (i.e., treated with placebo through week 48).

In the phase 2 trial, the primary efficacy endpoint, change from baseline in 4SC time, was analyzed using a longitudinal mixed-effects model for repeated measures (MMRM). The baseline result, treatment, time, and treatment by time interaction, were included as fixed effects in the model. Patients were included as a random effect and the model was fit with an unstructured covariance for the repeated measures. The distribution of the 4SC time was assumed to be right-skewed. Negative values in 4SC mean improvement. Transformations (including log transformation) of the 4SC time were evaluated to ensure the normality assumption was met. Contrasts were created to estimate the differences in change from baseline, in 4SC time, at the end of each dose-treatment level for the first 48 weeks (weeks 17, 33, and 49). The final analysis of the primary endpoint was performed at week 49, though data continued to be collected through week 97.

Missing data were handled using maximum likelihood techniques for MMRM. This analysis is unbiased under the assumption of missing at random when the model assumptions hold (i.e., missingness is unrelated to the missing values, had they been observed). Patients who lost the ability to complete a functional assessment and/or ambulate were assumed to be missing not at random. Additional imputation methods to assess the sensitivity of the analysis to missing not at random data were also performed and included completer analyses (i.e., patients who reached week 49), and transforming time to complete a functional assessment to velocity, so that patients with a missing time were assumed to have a velocity of zero.

Secondary endpoints were analyzed using the same longitudinal mixed model as described for the primary endpoint. In the prespecified subset of patients with an anticipated rapid disease decline (>1 year), the mean change on functional tests from baseline vs placebo was evaluated. MRI of the right thigh was acquired without the use of contrast, using a proton density weighted spin-echo sequence with 5 mm slices and 0 mm gap to cover the entire thigh (knee-to-hip). Imaging data were sent to BioTelemetry Research (Cardiocore & VirtualScopics, Rochester, NY) for quality evaluation and analysis. In addition to thigh muscle volume measurements, muscle volume index measurements were calculated based on whole thigh MRI images. Muscle volume index is a measure of the fraction of the total thigh tissue that is lean muscle[30] and was calculated as follows: (muscle volume / [muscle volume + inter/intra-muscular fat volume]) × 100.

PK population for serum concentration included all patients who received ≥1 dose of domagrozumab and had ≥1 concentration value reported. The population for PK parameters included all patients who received ≥1 dose of domagrozumab and had ≥1 PK parameter calculated. The population for myostatin serum concentration included all patients who had ≥1 concentration value reported.

3. Results

3.1. Patients

Of 162 patients with DMD who were screened for study entry, 121 enrolled and 120 were treated (Supplementary material – Fig. S1). Key reasons for screening failure were 4SC <2.5 seconds and LVEF <55%. Mean age at baseline was 8.7 (range, 6–15) years. The mean (SD) weight in
Table 1
Patient demographics and baseline characteristics by sequence group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sequence 1 n = 41</th>
<th>Sequence 2 n = 39</th>
<th>Sequence 3 n = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), year</td>
<td>8.3 (1.9)</td>
<td>8.5 (1.5)</td>
<td>9.3 (2.3)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>33 (80.5)</td>
<td>33 (84.6)</td>
<td>35 (87.5)</td>
</tr>
<tr>
<td>Black</td>
<td>1 (2.4)</td>
<td>0</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Asian</td>
<td>6 (14.6)</td>
<td>5 (12.8)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (2.4)</td>
<td>1 (2.6)</td>
<td>0</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>29.9 (8.5)</td>
<td>30.3 (8.8)</td>
<td>35.3 (14.4)</td>
</tr>
<tr>
<td>Range</td>
<td>14.8–48.8</td>
<td>16.4–50.1</td>
<td>19.0–86.4</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>19.4 (3.9)</td>
<td>19.5 (3.8)</td>
<td>20.7 (5.8)</td>
</tr>
<tr>
<td>Height, mean (SD), cm</td>
<td>123.2 (8.0)</td>
<td>123.6 (8.9)</td>
<td>128.9 (9.3)</td>
</tr>
<tr>
<td>Glucocorticosteroids use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deflazacort</td>
<td>25 (61.0)</td>
<td>20 (51.3)</td>
<td>25 (62.5)</td>
</tr>
<tr>
<td>Prednisone or prednisolone</td>
<td>16 (39.0)</td>
<td>19 (48.7)</td>
<td>15 (37.5)</td>
</tr>
<tr>
<td>6MWD, meters</td>
<td>5.2 (2.5)</td>
<td>5.0 (3.3)</td>
<td>5.3 (2.8)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>357.9 (99.0)</td>
<td>374.0 (99.2)</td>
<td>350.8 (84.9)</td>
</tr>
<tr>
<td>NSAA: Time to complete 10-m run/walk, s</td>
<td>6.5 (1.9)</td>
<td>5.7 (1.3)</td>
<td>6.4 (1.8)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAA: Time to stand from supine, s</td>
<td>7.1 (5.1)</td>
<td>7.7 (5.9)</td>
<td>7.7 (3.8)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAA score, s</td>
<td>19.5 (6.7)</td>
<td>21.5 (7.1)</td>
<td>20.2 (7.2)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUL: overall score</td>
<td>66.8 (4.0)</td>
<td>67.0 (5.2)</td>
<td>67.1 (3.2)</td>
</tr>
</tbody>
</table>

4SC = 4-stair climb; BMI = body mass index, NSAA = North Star Ambulatory Assessment; PUL = performance of upper limb.

Sequence 3 (35.3 [14.4] kg) was higher than that in Sequence 1 (29.9 [8.5] kg) and Sequence 2 (30.3 [8.8] kg), which was driven by two patients with weights of 86.4 kg and 81.8 kg each. Most patients were on a high daily dose of glucocorticosteroids. Other demographics and baseline characteristics were comparable across all sequence groups (Table 1).

By week 49, seven patients discontinued from the phase 2 trial because of: unwillingness to participate in study (n = 3), lost to follow-up (n = 1), AE (n = 1), and other reason (n = 2). By week 97, 48 patients discontinued due to early termination of the trial, 65 patients completed 96 weeks of the phase 2 trial, and 59 were treated in the OLE. All 59 patients discontinued the OLE: 55 because of early termination of the trial, 1 death (unrelated to study drug), 1 moved away from the site, and 2 no longer willing to participate.

3.2. Safety

In the first 48 weeks of the phase 2 trial, 115 patients experienced all-causality, treatment-emergent AEs (TEAEs; Table 2). Five patients had 6 serious AEs (appendicitis and anxiety, superior sagittal sinus thrombosis, femur fracture, troponin increased, and femoral neck fracture); none were considered drug-related. TEAEs by sequence in the phase 2 weeks 49–96 and OLE were similar to those in the first 48 weeks.

3.3. Efficacy

The primary endpoint of mean change in 4SC time at week 49 was not met in the phase 2 trial (Fig. 2). Based on the MMRM analysis, the difference in mean change in 4SC time from baseline to week 49 for domagrozumab vs placebo was 0.27 seconds (95% CI: −7.4, 7.9; p = 0.94). All
sensitivity analyses showed directionally favorable but non-statistically significant results with domagrozumab (Table 3). There were no significant differences in the mean change from baseline on the 4SC between the domagrozumab and placebo in the prespecified subgroup, based on baseline 4SC time at all measurements (Table 3).

The mean change in 4SC at week 49 in the placebo group was similar to the historical control group (difference, 0.211; 95% CI: −2.835, 3.257; \( p = 0.8908 \)). These findings suggest the historical control dataset can serve as an adequate control group for the analysis of 4SC at week 97. There was no significant difference in mean change in 4SC time from baseline to week 97 between Sequence 1 and the historical control group; the difference using MMRM was 0.819 seconds (95% CI: −1.431, 3.090; \( p = 0.4748 \)). Also, there was no significant change in 4SC time between Sequence 1 and the historical control group from overall baseline to week 170 in the OLE trial (difference, 10.57; 95% CI: −1.313, 22.463; \( p = 0.0811 \)). In the OLE, there was a mean increase from overall baseline to week 146 of 6.784 seconds (95% CI: 0.749, 12.819) observed in the 4SC time among 15/59 evaluable patients across the 3 treatment sequences.

No significant treatment differences were found for the analysis of FVC, percent predicted FVC, NSAA, 6MWD, myometry-based muscle strength, or PUL (Table 4). There was no significant difference in time to loss of ambulation

---

Table 3

<table>
<thead>
<tr>
<th>Mean Change From Baseline to Week 49</th>
<th>Domagrozumab, Sequence 1&amp;2 Combined, Mean (SE)</th>
<th>Placebo, Mean (SE)</th>
<th>Difference, Domagrozumab vs Placebo, Mean (95% CI)</th>
<th>( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4SC, MMRM, s</td>
<td>8.28 (2.15)</td>
<td>8.01 (3.03)</td>
<td>0.27 (−7.40, 7.90)</td>
<td>0.94</td>
</tr>
<tr>
<td>Log-transformed 4SC, MMRM, log s</td>
<td>0.30 (0.05)</td>
<td>0.40 (0.08)</td>
<td>−0.10 (−0.28, 0.09)</td>
<td>0.29</td>
</tr>
<tr>
<td>Log-transformed 4SC changes, MMRM, %</td>
<td>−</td>
<td>−</td>
<td>−9.4 (−24.7, 8.9)</td>
<td>0.29</td>
</tr>
<tr>
<td>Completer(^a) analysis 4SC, MMRM, s</td>
<td>1.88 (0.74)</td>
<td>2.75 (1.04)</td>
<td>−0.87 (−3.42, 1.67)</td>
<td>0.50</td>
</tr>
<tr>
<td>4SC velocity, MMRM, 4 stairs/s</td>
<td>−0.05 (0.01)</td>
<td>−0.06 (0.01)</td>
<td>0.02 (−0.01, 0.05)</td>
<td>0.30</td>
</tr>
<tr>
<td>4SC subgroup: baseline 4SC ≥3.5 &amp; ≤8 s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full analysis set</td>
<td>3.62 (0.94)</td>
<td>3.53 (1.16)</td>
<td>0.094 (−3.08, −3.27)</td>
<td>0.95</td>
</tr>
<tr>
<td>Completer(^a) analysis</td>
<td>1.53 (0.62)</td>
<td>2.49 (0.76)</td>
<td>−0.97 (−2.9, 1.0)</td>
<td>0.33</td>
</tr>
<tr>
<td>PPAS of 4SC, s</td>
<td>7.03 (2.02)</td>
<td>7.77 (2.79)</td>
<td>−0.75 (−7.98, 6.49)</td>
<td>0.83</td>
</tr>
<tr>
<td>Wilcoxon test of 4SC, rank score</td>
<td>45.53</td>
<td>52.86</td>
<td>−7.33</td>
<td>0.22</td>
</tr>
<tr>
<td>Wilcoxon test of velocity 4SC, rank score</td>
<td>59.23</td>
<td>52.59</td>
<td>6.64</td>
<td>0.31</td>
</tr>
</tbody>
</table>

\( 4SC = 4\)-stair climb; MMRM = mixed model for repeated measures; PPAS = per protocol analysis set.

\(^a\) Completer is defined as patient with 4SC data at week 49. Patients with missing 4SC values at week 49 have been excluded.
from the OLE baseline throughout the OLE trial between Sequence 1 and the historical control group (p = 0.3011). In the OLE, 17 (of 59) patients experienced loss of ambulation (Sequence 1: n = 3, Sequence 2: n = 7, Sequence 3: n = 7).

### 3.4. Clinical outcomes assessments

Decreases from overall baseline for the PODCI parent-reported scores were observed in all 5 items and the Global Functioning Scale over the course of both trials (Table 4). The least decrease from overall baseline was seen for the pain/comfort core scale (mean change at week 146, −6.3; 95% CI: −13.6, 1.1). The greatest decrease from overall baseline was for transfer and basic mobility core scale (mean change at week 146, −21.1; 95% CI: −32.6, −9.7). A change from baseline in the adolescent self-report measure was not performed due to the limited number of baseline visit assessments.

There was no change in general health status (EQ-5D-3L, EQ-5D-Youth) and health resource utilization from baseline of the OLE trial throughout its course. There was no change in caregiver burden from OLE baseline through the end of the OLE (mean change in ZBI total score, −0.6; 95% CI: −3.4, 2.3, among 18/59 evaluable patients at week 49). Impact on work productivity and activity impairment was observed from OLE trial baseline throughout the course of the OLE trial in all 4 items of the WPAI:CG: mean change at week 49 was 2.3 (95% CI: −2.63, 7.224) for percent worktime missed, 7.1 (95% CI: 0.2, 14.1) for percent impairment while working, 9.2 (95% CI: 2.496, 15.871) for percent overall work impairment, and 8.9 (95% CI: 1.1, 16.7) for percent activity impairment.

### 3.5. Pharmacokinetics

Median serum concentrations of domagrozumab increased with increasing dose level for patients within each dose level (first 48 weeks and weeks 49–96) following domagrozumab IV administration at 5, 20, and 40 mg/kg. Following repeated administration at 40 mg/kg, median serum concentrations remained generally constant for patients during weeks 49–96 in Sequence 1. C\textsubscript{trough} values generally increased from the first dose of domagrozumab through the last dose, within each dose level during escalation. The C\textsubscript{trough} values appeared in steady-state by the last dose of within-patient dose escalation in all sequences and remained at steady-state with domagrozumab 40 mg/kg in Sequence 1, weeks 49–96. PK parameters for domagrozumab following the first and last dose administration at each dose level for patients, with additional PK sampling in the first 48 weeks, (Sequences 1 and 2) are described in Table 5.
Table 5
PK parameters of domagrozumab for patients with additional PK sampling in the first 48 weeks (Sequences 1 and 2).

<table>
<thead>
<tr>
<th>Parameter, units</th>
<th>Domagrozumab Parameter Summary Statisticsa by Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mg/kg IV</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
</tr>
<tr>
<td>C0, μg/mL</td>
<td>2</td>
</tr>
<tr>
<td>AUC0–t, μg·h/mL</td>
<td>21700, 31300</td>
</tr>
<tr>
<td>Cmax, μg/mL</td>
<td>119, 147</td>
</tr>
<tr>
<td>Ctrough, μg/mL</td>
<td>32.3, 46.6</td>
</tr>
<tr>
<td>Tmax, hr</td>
<td>2.08, 2.25</td>
</tr>
<tr>
<td>CL, mL/hr/kg</td>
<td>0.126, 0.17</td>
</tr>
<tr>
<td>N</td>
<td>5, 5, 5, 5</td>
</tr>
<tr>
<td>AUC0–t, μg·h/mL</td>
<td>246700 (31)</td>
</tr>
<tr>
<td>C0, μg/mL</td>
<td>126.2 (12)</td>
</tr>
<tr>
<td>Ctrough, μg/mL</td>
<td>36.72 (31)</td>
</tr>
<tr>
<td>Tmax, hr</td>
<td>2.3 (2.03–2.42)</td>
</tr>
<tr>
<td>CL, mL/hr/kg</td>
<td>0.1247 (21)</td>
</tr>
</tbody>
</table>

AUC0–t area under the serum concentration–time curve during a dosing interval; C0 = serum concentration at the end of planned 2 hr infusion; Cmax = average drug concentration; CL = drug clearance; Cmax = maximum serum concentration; Ctrough = trough serum concentration; CV = coefficient of variation; NC = not calculated; NR = not reported; Tmax = time to reach maximum serum concentration.

a Summary statistics were not presented if fewer than 3 patients have reportable parameter values. The minimum and maximum were reported.

b N = number of patients in the treatment group in the indicated statistics; n1 = number of patients contributing to the summary statistics; n2 = number of patients contributing to the summary statistics for AUC0–t, CL, and C0; n2 = number of patients contributing to the summary statistics for Cmax and Tmax.

c Individual patient values were presented for n<3.

d Geometric mean (geometric %CV) for all except: median (range) for Tmax. Geometric means not presented for Ctrough when individual values include 0.

3.6. Serum myostatin pharmacodynamics and imaging biomarkers

An increase in baseline total serum myostatin (domagrozumab-bound and free myostatin) post domagrozumab treatment was observed for all the treatment sequences. Total serum myostatin Ctrough levels were slightly higher for all dose levels compared with placebo. There were no apparent dose-specific trends observed for total myostatin Ctrough levels.

The MMRM analysis showed no significant differences between domagrozumab and placebo in mean percent change from baseline on thigh muscle volume measures on MRI at weeks 17, 33, and 49. This analysis includes measurement of whole thigh muscle volume and whole thigh inter/intramuscular fat volume. Although there were no significant differences in mean percent change from baseline, for both muscle volume and muscle volume index, there were directionally favorable differences between domagrozumab and placebo; the differences (domagrozumab to placebo) at weeks 17, 33, and 49, respectively, were 2.19%, 2.11%, and 2.86% for whole thigh muscle volume and 0.76%, 1.76%, and 1.98% for thigh whole muscle volume index.

3.7. Immunogenicity

Based on all immunogenicity samples tested in the phase 2 trial, only 1 sample in one patient in Sequence 3 had a positive ADA titer (≥1.88), which was at week 65; the patient’s subsequent samples were negative for ADA. No participants in the OLE trial tested positive for ADA.

4. Discussion

Multiple ascending, monthly doses of domagrozumab at 5, 20, and 40 mg/kg were generally safe and well tolerated. The phase 2 trial did not meet its primary efficacy endpoint of mean change from baseline in 4SC time at week 49. Sensitivity analyses all showed directionally favorable, but non-statistically significant and not clinically meaningful treatment effects with domagrozumab vs placebo.

Potential etiologies for the disappointing outcome in this trial include different PD of domagrozumab in humans versus mice, recent finding of marked reduced baseline myostatin levels in patients with DMD suggesting a more severe downregulation of the myostatin pathway in patients with DMD compared with mdx mice, and/or other ligands not
impacted by domagrozumab playing an important role in primate muscle mass regulation [31]. Another potential reason for lack of efficacy relates to the recent finding, identified after the protocol initiation, that in the mdx mice, daily glucocorticosteroids administration were shown to interfere with potential benefits associated with myostatin inhibition [32]; however, the murine homolog of domagrozumab mRK35, produced similar increases in body weight and lean muscle mass in control and mdx mice with and without prednisolone treatment [33]. With the more recent negative clinical results from other myostatin inhibitors (e.g., bimagrumab, ACE-083, RG6206) in multiple muscle wasting diseases, a comprehensive review of myostatin inhibition as a target for human muscle wasting diseases is warranted.

The majority of patients experienced ≥1 TEAE. The incidence of AEs was comparable between domagrozumab and placebo, and among the 3 dose levels of domagrozumab. AEs were mostly mild and moderate and consistent with those reported previously in patients with DMD [14,34-36]. None of the reported serious AEs were considered drug-related.

Preliminary data showed numerically greater LVEF decline with domagrozumab exposure at weeks 97 vs 49. The final data showed decrease in risk, but although the quantitative difference in LVEF was neither statistically different nor considered clinically meaningful, the event was classified as a potential risk, with the implication for additional monitoring.

The 4SC functional test was selected as the primary efficacy endpoint for this trial based on results of prior glucocorticosteroid trials [37-39], and because it is considered a reliable measure of motor function [27,40]. A mean change of 4.7 ± 7.5 seconds in 4SC time at week 48 was demonstrated in patients aged ≥7 years treated with glucocorticosteroids (placebo arm) [27]. This difference is greater than the minimal clinically important difference, i.e., clinically meaningful value of 2.1–2.2 seconds for 4SC calculated by McDonald et al. [41]. An assumed change of 2.5 seconds (as determined a priori) from baseline in the 4SC time in patients treated with domagrozumab vs placebo may suggest both a statistically significant and clinically meaningful change.

Although neither muscle volume nor muscle volume index measures were statistically significant in this study, they are both consistent with a potential anabolic effect. The measure of muscle volume index is of particular interest in patients with DMD, because as the disease progresses, the percent of total muscle volume decreases, whereas fatty replacement of the muscle increases [30]. This study confirms the ability to obtain reproducible and valid thigh muscle volume and muscle volume index measures in an international multicenter trial in a DMD population.

Among PODCI subscales, mobility and function scores can distinguish between functional milestones in patients with DMD [23,42,43]. A difference was seen in the domains of upper extremity and physical function core scale, transfer and basic mobility core scale, pain/comfort core scale, and global functioning scale. Although natural history data suggest PODCI subscales scores, especially mobility-related, can distinguish between functional milestones representative of disease progression in patients with DMD [23,42], little data exist with respect to responsiveness of PODCI scores to treatment.

Total serum myostatin levels were increased with domagrozumab treatment as the binding of myostatin to domagrozumab serves to sequester myostatin. The binding of an antibody to its ligand increases the total plasma levels of the ligand; because the ligand takes on the properties (distribution and clearance) of the free antibody [44]. Total myostatin in the serum accounts for myostatin bound to domagrozumab and free myostatin. Based on the concentrations of domagrozumab in the serum, >95% of serum myostatin was bound (derived from a PK/PD analysis to be published elsewhere) and unable to interact with its target receptor. The systemic exposures increased with each domagrozumab dose escalation and systemic exposures were consistent with the predictions in healthy volunteers [45,46].

Several points may be useful for interpretation of our findings and for design of future studies. This was the first trial of domagrozumab in patients, and the lack of data in patients led to a complicated study design, which was difficult to execute. The 4SC measure may not be an optimal primary efficacy endpoint to assess treatment effect of myostatin inhibition; as true for other functional measures in DMD, 4SC is affected clinically by other confounders (e.g., ankle contractures and weight gain) and not solely related to muscle strength. It does, however, reflect an activity important for daily living. There is an emerging consensus that NSAA may be the best outcome measure for ambulatory patients with DMD [47-50]. However, the lack of efficacy in several secondary function and biomarker endpoints is consistent with the lack of efficacy in the 4SC measurement and suggests that the selection of 4SC as the primary endpoint was not the sole cause for the negative outcome in this trial. The 2-year outcomes were compared with historical control group as there was no true 2-year placebo group in this study, but this allowed all patients with rare disease the opportunity to receive an active drug. Also, despite using some functional criteria to exclude outliers, a number of outliers were still present. This, together with the non-random distribution of missing the primary endpoint data, posed challenges for the MMRM analysis.

In conclusion, domagrozumab was generally safe and well tolerated in this population of patients with DMD. The phase 2 trial did not meet its primary efficacy endpoint. In addition, the totality of evidence, including secondary endpoints, did not support a significant treatment effect with domagrozumab, which led to termination of both trials. The studies were not terminated due to safety reasons.
Acknowledgments

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Data Sharing Statement

Upon request, and subject to certain criteria, conditions, and exceptions (see https://www.pfizer.com/science/science-clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual de-identified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines and medical devices (1) for indications that have been approved in the US and/or EU or (2) in programs that have been terminated (i.e., development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The de-identified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

Supplementary materials

Supplementary material associated with this article can be found in the online version, at doi:10.1016/j.nmd.2020.05.002.

References


