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AAV gene therapy for Duchenne muscular dystrophy: the EMBARK phase 3 randomized trial

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Duchenne muscular dystrophy (DMD) is a rare, X-linked neuromuscular disease caused by pathogenic variants in the *DMD* gene that result in the absence of functional dystrophin, beginning at birth and leading to progressive impaired motor function, loss of ambulation and life-threatening cardiorespiratory complications. Delandistrogene moxeparvovec, an adeno-associated rh74-viral vector-based gene therapy, addresses absent functional dystrophin in DMD. Here the phase 3 EMBARK study aimed to assess the efficacy and safety of delandistrogene moxeparvovec in patients with DMD. Ambulatory males with DMD, ≥4 years to <8 years of age, were randomized and stratified by age group and North Star Ambulatory Assessment (NSAA) score to single-administration intravenous delandistrogene moxeparvovec (1.33×10^{14} vector genomes per kilogram; $n = 63$) or placebo ($n = 62$). At week 52, the primary endpoint, change from baseline in NSAA score, was not met (least squares mean 2.57 (delandistrogene moxeparvovec) versus 1.92 (placebo) points; between-group difference, 0.65; 95% confidence interval (CI), −0.45, 1.74; $P = 0.2441$). Secondary efficacy endpoints included mean micro-dystrophin expression at week 12: 34.29% (treated) versus 0.00% (placebo). Other secondary efficacy endpoints at week 52 (between-group differences (95% CI)) included: Time to Rise (−0.64 (−1.06, −0.23)), 10-meter Walk/Run (−0.42 (−0.71, −0.13)), stride velocity 95th centile (0.10 (0.00, 0.19)), 100-meter Walk/Run (−3.29 (−8.28, 1.70)), time to ascend 4 steps (−0.36 (−0.71, −0.01)), PROMIS Mobility and Upper Extremity (0.05 (−0.08, 0.19); −0.04 (−0.24, 0.17)) and number of NSAA skills gained/improved (0.19 (−0.67, 1.06)). In total, 674 adverse events were recorded with delandistrogene moxeparvovec and 514 with placebo. There were no deaths, discontinuations or clinically significant complement-mediated adverse events; 7 patients (11.1%) experienced 10 treatment-related serious adverse events. Delandistrogene moxeparvovec did not lead to a significant improvement in NSAA score at week 52. Some of the secondary endpoints numerically favored treatment, although no statistical significance can be claimed. Safety was manageable and consistent with previous delandistrogene moxeparvovec trials. ClinicalTrials.gov: NCT05096221

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Duchenne muscular dystrophy (DMD) is caused by pathogenic variants in the X-linked *DMD* gene, leading to an absence of functional dystrophin and continuous muscle damage, beginning from birth[1]. Impaired motor function can be observed by the age of 3 years and typically progresses to loss of ambulation during adolescence with standard-of-care corticosteroid treatment[1–3]. Current approved treatments, including therapies designed to produce low-level dystrophin expression, may provide benefit for a minority of patients with specific pathogenic variants, but there is an unmet need for therapies that can more effectively stabilize or slow disease progression and that could be applicable to most of the DMD patient population, which sparked the research of new innovative therapies, including gene therapy[4–10].

Delandistrogene moxeparvovec is a single-administration recombinant adeno-associated virus rhesus isolate serotype 74 (rAAVrh74) vector-based gene transfer therapy, approved in the United States for the treatment of patients with DMD at least 4 years of age with a confirmed mutation in the *DMD* gene, regardless of ambulatory status[11,12]. It is also approved in other select countries[13–18]. Delandistrogene moxeparvovec is designed to address the absence of functional dystrophin in DMD by delivering a transgene encoding delandistrogene moxeparvovec micro-dystrophin, an engineered protein retaining key functional domains of dystrophin[19].

Early-phase clinical studies demonstrated a manageable safety profile for delandistrogene moxeparvovec. In these studies, delandistrogene moxeparvovec micro-dystrophin expression was robust with sarcolemmal localization up to 60 weeks after treatment and demonstrated a sustained functional stabilization through 4 years in four males with DMD (mean age at treatment, 5.1 years; mean age at 4-year follow-up, 9.2 years)[20–22]. Here we report results from Part 1 (52 weeks) of EMBARK (ClinicalTrials.gov: NCT05096221), a large, phase 3, two-part, multinational, randomized, double-blind, placebo-controlled trial assessing delandistrogene moxeparvovec safety and efficacy in patients with DMD aged ≥4 years to <8 years[23].

Results

Patient disposition

Between October 2021 and September 2022, 173 patients were screened, 131 were randomized and 125 patients were treated (delandistrogene moxeparvovec, $n = 63$; placebo, $n = 62$; Fig. 1). Of the 173 patients screened, 13.3% were excluded due to elevated antibody titers to rAAVrh74. Analysis was by original assigned group (modified intent-to-treat population). The mean (s.d.) age at randomization was 6.03 (1.05) years, and the mean (s.d.) baseline North Star Ambulatory Assessment (NSAA) total score was 22.96 (3.75). Baseline clinical characteristics were balanced between groups (Table 1, Extended Data Table 1 and Supplementary Table 1). The week 52 cutoff date was 13 September 2023.

Primary outcome

The primary outcome was change in NSAA total score from baseline to week 52 (Part 1). The NSAA is a categorical assessment of motor function in ambulatory patients with DMD, consisting of 17 items scored with a 0, 1 or 2 based on the patient's ability to complete the task. At week 52 in the overall population, the least squares mean (LSM) change (95% confidence interval (CI)) from baseline in NSAA total score was 2.57 (1.80, 3.34) versus 1.92 (1.14, 2.70) points with delandistrogene moxeparvovec and placebo, respectively. The between-group difference was not statistically significant (0.65 (s.e. = 0.55) points; 95% CI, −0.45, 1.74; $P = 0.2441$; Fig. 2a,b). Results were consistent across pre-specified age subgroups and baseline NSAA total score subgroups (Supplementary Table 2).

Secondary outcomes

As defined per protocol, key secondary functional endpoints were Time to Rise (TTR) from the floor and 10-meter Walk/Run (10MWR) at week 52. The LSM change (95% CI) from baseline to week 52 on the TTR was −0.27 s (−0.56, 0.02) for delandistrogene moxeparvovec versus 0.37 s (0.08, 0.67) for placebo, with a between-group difference of −0.64 s (95% CI, −1.06, −0.23). Similarly, the LSM change (95% CI) from baseline to week 52 on the 10MWR was −0.34 s (−0.55, −0.14) for delandistrogene moxeparvovec versus 0.08 s (−0.13, 0.29) for placebo, with a between-group difference of −0.42 s (95% CI, −0.71, −0.13) (Fig. 2a,c,d). Subgroup analysis data are provided in Supplementary Table 2.

Other secondary functional endpoints assessed were stride velocity 95th centile (SV95C), 100-meter Walk/Run (100MWR) and time to ascend 4 steps. The LSM change (95% CI) from baseline to week 52 on SV95C was 0.06 meters per second (0.00, 0.13) for delandistrogene moxeparvovec versus −0.03 meters per second (−0.09, 0.03) for placebo, with a between-group difference of 0.10 meters per second (95% CI, 0.00, 0.19). The LSM change (95% CI) from baseline to week 52 on the 100MWR was −6.57 s (−10.05, −3.09) for delandistrogene moxeparvovec versus −3.28 s (−6.86, 0.29) for placebo, with a between-group difference of −3.29 s (95% CI, −8.28, 1.70). Analysis of time to ascend 4 steps showed LSM change (95% CI) from baseline to week 52 of −0.44 s (−0.69, −0.20) for delandistrogene moxeparvovec versus −0.08 s (−0.33, 0.17) for placebo, with a between-group difference of −0.36 s (95% CI, −0.71, −0.01) (Fig. 3). Subgroup analyses by age and baseline NSAA total scores are presented in Supplementary Table 2.

The LSM change (95% CI) from baseline to week 52 in the number of skills gained or improved as measured by the NSAA was 4.18 (3.58, 4.79) in the delandistrogene moxeparvovec group and 3.99 (3.37, 4.60) in the placebo group, with a between-group difference of 0.19 (−0.67, 1.06) (Supplementary Table 2 and Extended Data Table 2).

Analysis of Patient-Reported Outcomes Measurement Information System (PROMIS) Mobility showed an LSM (95% CI) change from baseline to week 52 of 0.05 (−0.05, 0.14) for delandistrogene moxeparvovec versus −0.01 (−0.10, 0.09) for placebo, with a between-group difference of 0.05 (−0.08, 0.19) (Supplementary Table 2). The LSM change (95% CI) from baseline for PROMIS Upper Extremity was 0.19 (0.05, 0.34) for delandistrogene moxeparvovec versus 0.23 (0.08, 0.37) for placebo, with a between-group difference of −0.04 (−0.24, 0.17).

Western blot analysis of week 12 biopsies in a subset of patients ($n = 31$) treated in trial sites where biopsies could be performed showed delandistrogene moxeparvovec micro-dystrophin expression in the treated group (mean (s.d.), 34.29% (41.04)) versus placebo (0.00% (0.00)) (Fig. 4 and Supplementary Fig. 1).

Safety

Overall, 1,188 adverse events (AEs) were reported: 674 with delandistrogene moxeparvovec and 514 with placebo (Fig. 5). AEs are described as reported by the principal investigator at each study site. In the delandistrogene moxeparvovec group, 48 patients (76.2%) experienced 235 treatment-related treatment-emergent AEs (TR-TEAEs), with most occurring within the first 90 d of infusion; 83.3% were mild to moderate in severity, 98.3% of which resolved; and the events that were assessed as unresolved by the investigator are irritability ($n = 2$), decreased appetite ($n = 1$) and an erroneous laboratory value that was normal upon repeat ($n = 1$). Fourteen patients (22.2%) experienced 21 serious AEs (SAEs), and seven patients (11.1%) experienced 10 treatment-related SAEs (TR-SAEs) (Fig. 5 and Extended Data Table 3). There were no clinically significant complement-mediated AEs that triggered medical intervention as measured by C3, C4 and 50% hemolytic complement levels, and there were no cases of thrombotic microangiopathy. There were no AEs leading to study discontinuation or death. AEs of special interest are reported in Extended Data Table 4. A full list of TEAEs is reported in Supplementary Table 3. Post-baseline changes on electrocardiogram parameters and selected echocardiogram parameters were either normal or not clinically significant, and there were no remarkable findings in vital signs.

In the placebo group, 17 patients (27.4%) experienced 43 TR-TEAEs; five patients (8.1%) experienced nine SAEs (coronavirus disease 2019, anal abscess, influenza, toxic shock syndrome, vomiting, arterial injury,

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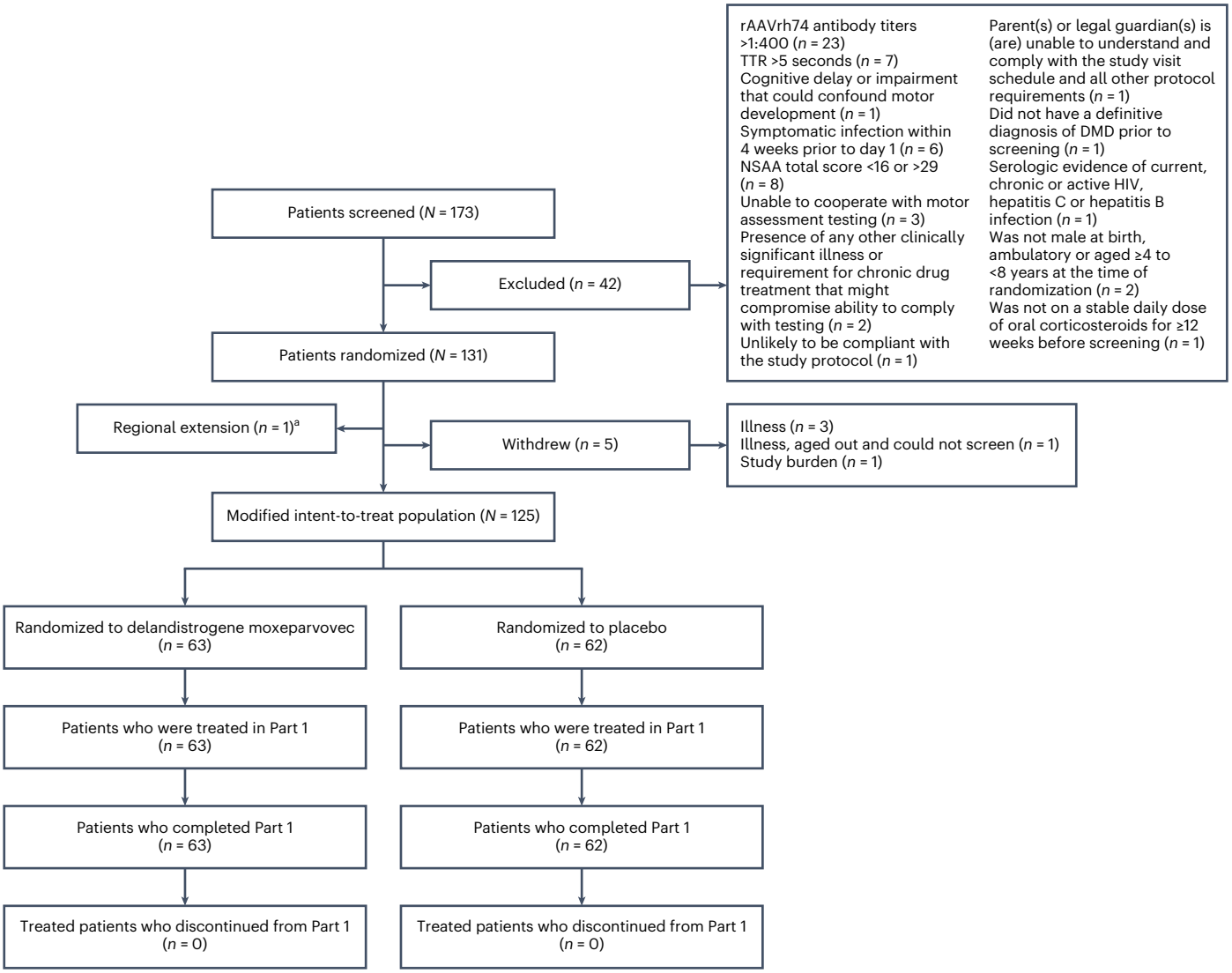

Fig. 1 | Patient disposition. [a]One patient was enrolled in Japan as part of a regional extension and was too late for inclusion in the primary analysis.

upper limb fracture, left ventricular dysfunction and pyrexia); and there were no TR-SAEs.

The most common TR-TEAEs with delandistrogene moxeparvovec were vomiting (54.0%), nausea (31.7%) and decreased appetite (27.0%). TR-TEAEs of transient liver enzyme elevations (glutamate dehydrogenase (GLDH), gamma-glutamyl transferase, alanine transaminase and/or aspartate transaminase increases; total, 41.3%) occurred within the first 90 d after infusion (median, 42 d), resolved spontaneously or after an increase in peri-infusion corticosteroid treatment (10/26 (38.5%) patients with a liver event), and none progressed to liver failure. Post-infusion-added corticosteroid treatment for immunosuppression was increased if gamma-glutamyl transferase levels were confirmed to be ≥150 U L^{-1} or if there were any other clinically significant liver function abnormalities after infusion. The investigator may have made subsequent adjustments to immunosuppressive therapy in reaction to the subsequent course of acute liver injury or other AEs.

In the delandistrogene moxeparvovec group, seven patients (11.1%) experienced 10 TR-SAEs as reported by the principal investigator: acute liver injury (terms selected by investigators that refer to similar clinical patterns of liver biochemical markers included transient liver enzyme elevations (three events), hepatotoxicity and liver injury (one event of each)), myocarditis, nausea, vomiting, pyrexia and rhabdomyolysis (one event of each) (Fig. 5). The onset of the TR-SAEs were days 30–51 for acute liver injury, day 1 for myocarditis, nausea, vomiting and pyrexia and day 2 for rhabdomyolysis. All have resolved. For detailed narratives on TR-SAEs, see Extended Data Table 3.

There was a single event (1.6%) of myositis (TR-TEAE, separate from the event of rhabdomyolysis) reported in the delandistrogene moxeparvovec group that occurred on day 92 after infusion. The event occurred in a patient with a deletion of exons 46–50. There were no concurrent or recent illnesses or increased activity reported. Upon presentation, the patient was asymptomatic with a creatine kinase (CK) of 40,360 U L^{-1} (1.2× baseline) and an abnormal urinalysis (protein 1+, ketones 1+, hemoglobin 2+). The patient received intravenous (IV) fluids. By day 94, CK was down to 22,872 U L^{-1}; however, CK increased to more than 40,000 U L^{-1} on day 96. The patient received a single IV dose of 18.125 mg of methylprednisolone and oral corticosteroid increase for 3 d. CK decreased to 19,315 U L^{-1} on day 99. The patient remained asymptomatic without any muscle tenderness, weakness or pain, and the mild event of myositis was assessed as recovered on day 108 after infusion. Myositis was reported by the investigator due to increased CK levels that were measured per protocol. The timing, severity and clinical course of this event differentiates it from previously observed events of immune-mediated myositis[24].

Table 1 | Demographics and baseline clinical characteristics (modified intent-to-treat population)[a]

Characteristic	Delandistrogene moxeparvovec (*n*=63)	Placebo (*n*=62)	All (*N*=125)
Age, mean (s.d.), years	5.98 (1.06)	6.08 (1.05)	6.03 (1.05)
4–5 years, *n* (%)	30 (47.6)	29 (46.8)	59 (47.2)
6–7 years, *n* (%)	33 (52.4)	33 (53.2)	66 (52.8)
Race group, *n* (%)			
Asian	8 (12.7)	11 (17.7)	19 (15.2)
Black or African American	0 (0)	2 (3.2)	2 (1.6)
White	49 (77.8)	46 (74.2)	95 (76.0)
Multiple	1 (1.6)	0 (0)	1 (0.8)
Other	2 (3.2)	1 (1.6)	3 (2.4)
Not reported	3 (4.8)	2 (3.2)	5 (4.0)
Ethnicity, *n* (%)			
Hispanic or Latino	15 (23.8)	8 (12.9)	23 (18.4)
Not Hispanic or Latino	47 (74.6)	53 (85.5)	100 (80.0)
Not reported/unknown	1 (1.6)	1 (1.6)	2 (1.6)
Dosing weight, mean (s.d.), kg	21.29 (4.62)	22.37 (6.42)	21.83 (5.59)
Time since corticosteroid treatment started, mean (s.d.), years	1.07 (0.92)	0.97 (0.83)	1.02 (0.88)
Steroid type, *n* (%), at baseline			
Any use of deflazacort	43 (68.3)	28 (45.2)	71 (56.8)
Any use of prednisone/prednisolone	63 (100)	62 (100)	125 (100)
Other	0 (0)	0 (0)	0 (0)
Pathogenic variant, *n* (%)[b]			
Large deletion	45 (71.4)	41 (66.1)	86 (68.8)
Large duplication	3 (4.8)	3 (4.8)	6 (4.8)
Small variant	15 (23.8)	18 (29.0)	33 (26.4)
Nonsense variant	8 (12.7)	7 (11.3)	15 (12.0)
Frameshift variant	4 (6.3)	5 (8.1)	9 (7.2)
Intron variant	3 (4.8)	6 (9.7)	9 (7.2)
NSAA total score, mean (s.d.), points	23.10 (3.75)	22.82 (3.78)	22.96 (3.75)
TTR, mean (s.d.), seconds	3.52 (0.81)	3.60 (0.68)	3.56 (0.75)
10MWR, mean (s.d.), seconds	4.82 (0.79)	4.92 (0.73)	4.87 (0.76)
SV95C, mean (s.d.), meters per second[c]	1.82 (0.30)	1.77 (0.29)	1.79 (0.30)
100MWR, mean (s.d.), seconds[d]	60.67 (15.55)	63.01 (17.01)	61.80 (16.25)
Time to ascend 4 steps, mean (s.d.), seconds[e]	3.17 (1.01)	3.37 (1.09)	3.27 (1.05)
CK, mean (s.d.), UL^{-1} [f]	18,143.42 (8016.26)	18,188.89 (6521.12)	N/A

[a]Sex and racial and ethnic demographic answers were self-reported. Sex is not shown in this table; per inclusion criterion 1, patients must be male at birth to be eligible to participate in this study. [b]Large deletions and large duplications are the two types of larger structural variants, which extend one or more exons and/or are 50 nucleotides or more in length, inclusive of any nucleotides affected in intronic regions. Small variants include single-nucleotide variants, small insertions and small deletions. One patient had two variants (a large duplication and a large inversion), and it is listed in the large duplication variant category. [c]SV95C: delandistrogene moxeparvovec *n*=61, placebo *n*=62, total *N*=123. [d]100MWR: delandistrogene moxeparvovec *n*=63, placebo *n*=59, total *N*=122. [e]Time to ascend 4 steps: delandistrogene moxeparvovec *n*=63, placebo *n*=61, total *N*=124. [f]CK: delandistrogene moxeparvovec *n*=62, placebo *n*=62. Mean (s.d.) is provided for continuous variables. Frequency (%) is provided for categorical variables. N/A, not applicable.

Exploratory outcomes

Mean CK levels decreased with delandistrogene moxeparvovec versus placebo, with an LSM between-group difference in change from baseline to week 52 of −4,343.59 U L^{-1} (95% CI, −6,616.04, −2,071.15) (Extended Data Table 5 and Supplementary Fig. 2).

Sensitivity analyses

The pre-specified global statistical test on a composite of six functional endpoints (NSAA total score, TTR, 10MWR, SV95C, 100MWR and time to ascend 4 steps) conducted to analyze the totality of evidence for the treatment effect showed a difference (P = 0.0044) between delandistrogene moxeparvovec and placebo.

Post hoc analyses

A post hoc analysis showed that 3.2% of patients in the delandistrogene moxeparvovec group versus 16.4% of patients in the placebo group (odds ratio = 0.091; 95% CI, 0.01, 0.61) progressed to a TTR of over 5 s at week 52, a threshold of prognostic significance for loss of ambulation[3,25].

Discussion

Results from EMBARK Part 1 confirmed that, at week 52, the safety profile of delandistrogene moxeparvovec is consistent with prior experience, and AEs were medically manageable with appropriate monitoring and treatment[20–22]. Immune reactions stimulated by the

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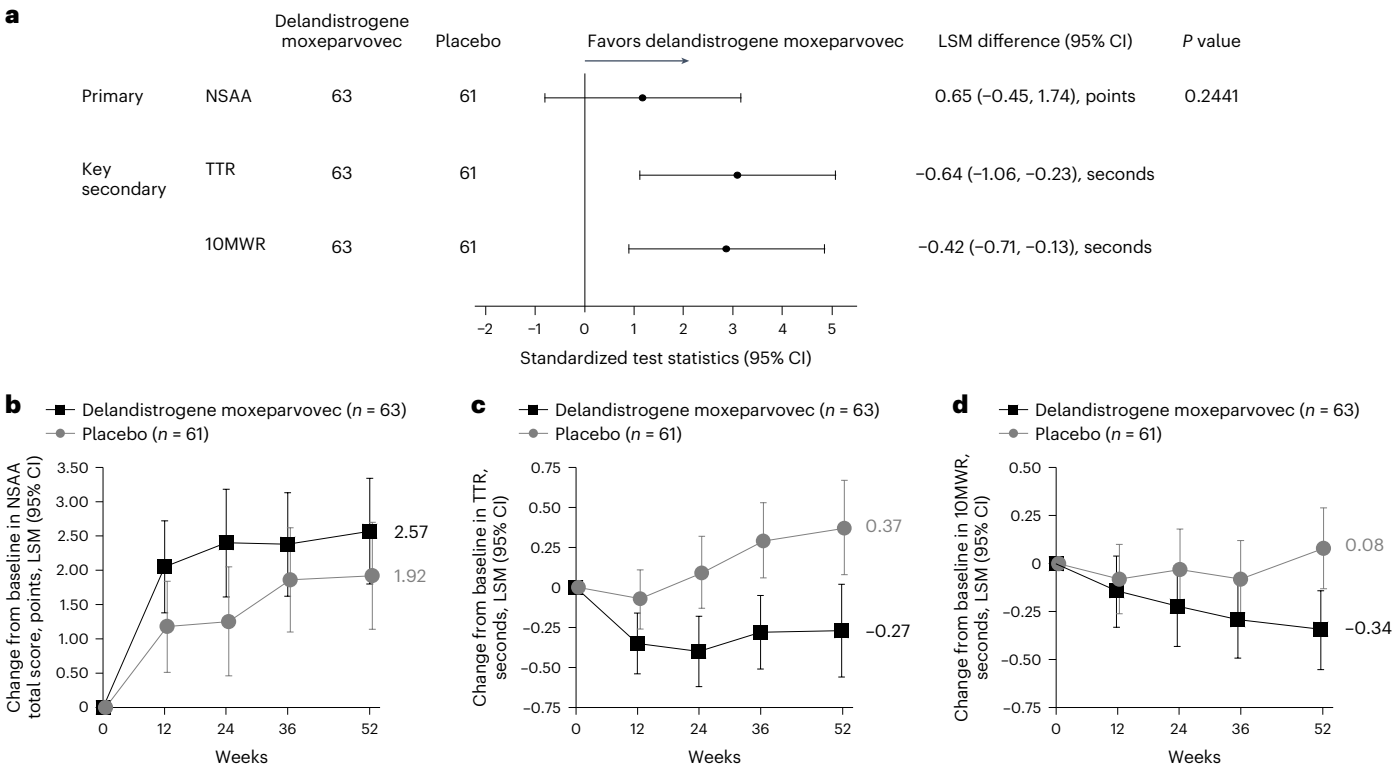

Fig. 2 | Primary endpoint and key functional secondary endpoints. a, Forest plot showing the primary endpoint (change from baseline to week 52 in NSAA total score, points) and key functional secondary endpoints (change from baseline to week 52 in TTR, seconds, and change from baseline to week 52 in 10MWR, seconds) for delandistrogene moxeparvovec and placebo groups in the modified intent-to-treat population. LSMs (of change from baseline) and CIs were standardized by dividing by the s.e. LSM differences are on original scale (without s.e. adjustment). TTR and 10MWR signs were reversed in the forest plot to align favorable directions among endpoints. Numerical results of LSM difference kept the original signs. One patient in the placebo group had missing data at week 52; functional tests were marked as invalid by the clinical evaluator due to back pain from compression fractures. **b**, Line graph showing LSM change from baseline to week 52 in NSAA total score, points, for delandistrogene moxeparvovec ($n = 63$) and placebo ($n = 61$) groups in the modified intent-to-treat population. Data are presented as LSM values ± 95% CI. **c**, Line graph showing LSM change from baseline to week 52 in TTR, seconds, for delandistrogene moxeparvovec ($n = 63$) and placebo ($n = 61$) groups in the modified intent-to-treat population. Data are presented as LSM values ± 95% CI. **d**, Line graph showing LSM change from baseline to week 52 in 10MWR, seconds, for delandistrogene moxeparvovec ($n = 63$) and placebo ($n = 61$) groups in the modified intent-to-treat population. Data are presented as LSM values ± 95% CI. **a**–**d**, The widths of the CIs have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects. Negative values for TFTs (TTR and 10MWR) show an improvement in the time taken to achieve these endpoints.

AAV vector are thought to be the primary cause of AEs in systemic AAV gene therapy, and each vector serotype may have a distinctive safety profile[26,27]. Delandistrogene moxeparvovec uses the rAAVrh74 vector, a clade E AAV[28], distinct from the AAV9 clade F vector used in some DMD clinical trials and an approved gene therapy for spinal muscular atrophy[29,30] (a clade being a phylogenetic group whose members share similarities in both function and serology)[28,31,32]. One of the challenges posed by the presence of pre-existing anti-AAV antibodies is the potential for activation of the complement system, which may lead to inflammation and safety concerns[33,34]. The particular characteristics of rAAVrh74 as well as the trial design may contribute to the absence of clinically significant complement-mediated AEs observed in the delandistrogene moxeparvovec clinical trials[31,35]. The rationale behind selection of the rAAVrh74 vector was that the non-human primate origin would decrease the likelihood of pre-existing immunity[19,34]. Seroprevalence analyses of patients with DMD in a previous study suggested that the presence of pre-existing antibodies against AAVrh74 was lower compared to AAV2, AAV8 and AAV9 seroprevalence[36]. Patients with elevated rAAVrh74 antibody titers (>1:400) were not eligible for the delandistrogene moxeparvovec clinical trials (excluding ongoing and upcoming trials designed to assess ways to overcome pre-existing immunity)[34,37]. Additionally, the use of MHCK7 as the promoter, associated with high levels of expression in skeletal muscles, and the inclusion of an enhancer to drive expression in the heart results in minimal off-target expression[10,19]. The safety profile observed thus far for delandistrogene moxeparvovec has supported the use of pre-infusion and post-infusion corticosteroid rather than a more intense prophylactic regimen of immunosuppressive drugs[35].

Delandistrogene moxeparvovec did not show a statistically significant difference in the primary endpoint at week 52 versus placebo. Some of the key secondary and other functional endpoints that consisted of well-validated measures of ambulatory function in DMD numerically favored treatment, although no statistical significance can be drawn. Furthermore, the separation on TTR and 10MWR were consistent and similar in magnitude across the age groups.

The heterogeneity of disease progression is a challenge when designing DMD clinical trials, specifically trials of short duration[38]. Particularly, during the ages of 4–7 years, motor function and coordination, including ambulation, may be still improving, maintaining or starting to decline from peak function as patients may be in the maturational or the plateau/early-decline phase[39]. During the maturational phase, ambulatory function is still improving due to developmental changes in coordination and muscle growth and regeneration. Throughout the plateau and early-decline phase, peak motor function has generally been achieved with an onset of functional decline[39]. Furthermore, treatment with standard-of-care daily steroids may improve muscle strength and function over the short term in patients with DMD aged 4–7 years[40], making demonstration

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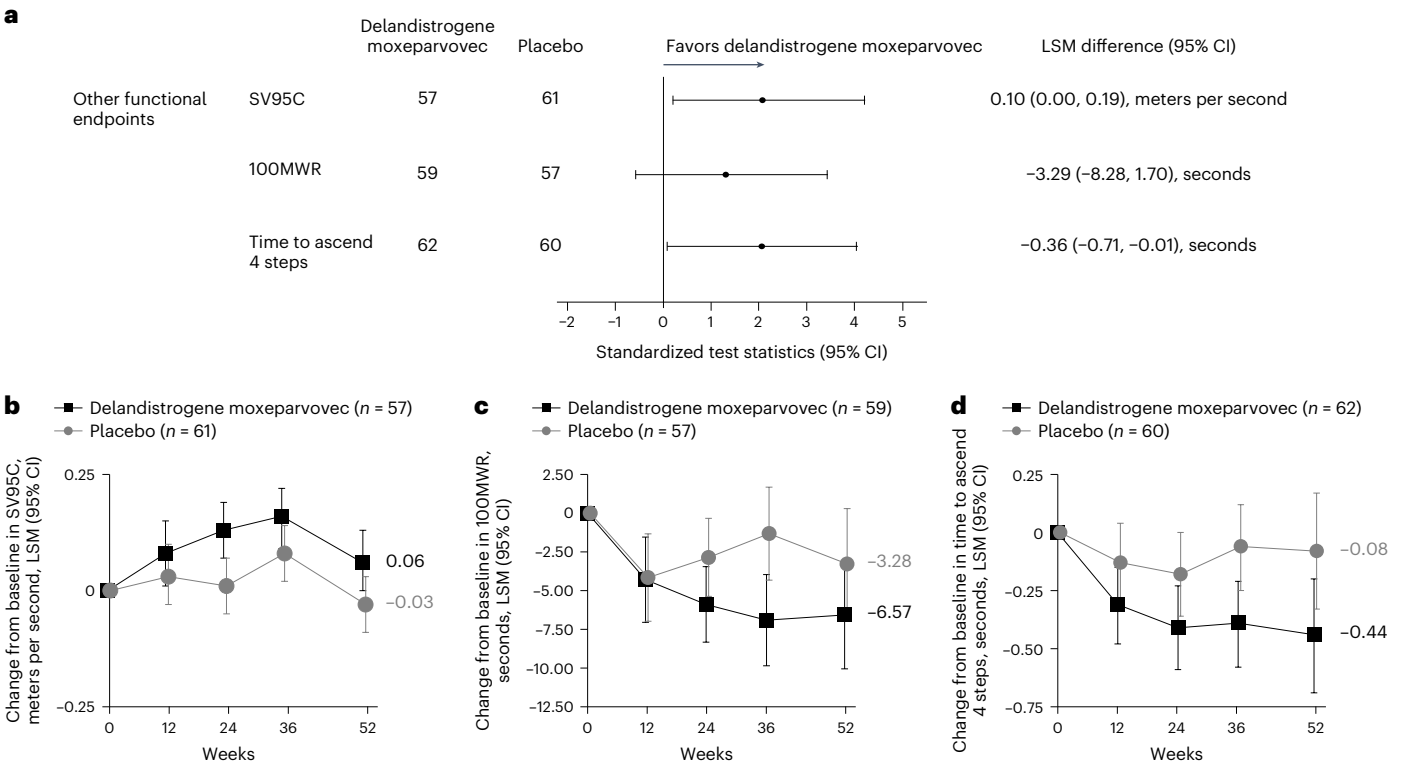

Fig. 3 | Other functional endpoints—SV95C, 100MWR and time to ascend 4 steps. a, Forest plot showing other functional endpoints (change from baseline to week 52 in SV95C, meters per second; 100MWR, seconds; and time to ascend 4 steps, seconds) for delandistrogene moxeparvovec and placebo groups in the modified intent-to-treat population. LSMs (of change from baseline) and CIs were standardized by dividing by the s.e. Numerical results of the LSMs are on original scale (without s.e. adjustment). Signs of TFTs (100MWR and time to ascend 4 steps) were reversed in the forest plot to align favorable directions among endpoints. Numerical results of LSM difference kept the original signs. SV95C: a small number of patients did not have sufficient recorded hours at week 52 for analysis; 100MWR and time to ascend 4 steps: a small number of tests at either baseline or week 52 were marked as invalid by the clinical investigator; the most common reason was due to behavior. **b**, Line graph showing LSM change from baseline to week 52 in SV95C, meters per second, for delandistrogene moxeparvovec ($n = 57$) and placebo ($n = 61$) groups in the modified intent-to-treat population. Data are presented as LSM values ± 95% CI. **c**, Line graph showing LSM change from baseline to week 52 in 100MWR, seconds, for delandistrogene moxeparvovec ($n = 59$) and placebo ($n = 57$) groups in the modified intent-to-treat population. Data are presented as LSM values ± 95% CI. **d**, Line graph showing LSM change from baseline to week 52 in time to ascend 4 steps, seconds, for delandistrogene moxeparvovec ($n = 62$) and placebo ($n = 60$) groups in the modified intent-to-treat population. Data are presented as LSM values ± 95% CI. **a**–**d**, The widths of the CIs have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects. Negative values for TFTs (100MWR and time to ascend 4 steps) show an improvement in the time taken to achieve these endpoints.

of incremental short-term treatment benefit or further functional improvement in this patient population particularly challenging, especially in patients who had initiated steroids shortly before screening. In EMBARK, all patients were treated with daily corticosteroids, and baseline characteristics were well balanced across important prognostic variables, such as age, duration of steroid use, NSAA total score and timed function tests (TFTs), predicting similar disease progression between cohorts[41].

This study highlights the value of objective and quantitative functional measures, such as TFTs and SV95C, for short-duration trials in younger patients with DMD treated with corticosteroids. The responsiveness of NSAA and TFTs, particularly in the younger population, is an area of recent investigation that will inform future trials in DMD[3,42,43]. In the present study, key secondary and other functional endpoints appeared to be more sensitive measures for this age group and study duration, with the ability to detect functional decline earlier, as previously shown[39]. Based on the broad scoring intervals for each functional assessment, NSAA scores of 1 (performance of tasks with difficulty or compensation) can only decline to 0 if functions are completely lost and can only improve to 2 if compensations are eliminated[39]. A score of 1 represents a broad range of abilities: a patient performing a task with slight difficulty may score a 1, whereas a patient performing a task with great difficulty but still able to complete the task may also score a 1. Therefore, in this early ambulatory patient population, the NSAA may not have been sensitive enough to detect a difference that was statistically significant at 52 weeks. First, there was a greater proportion of patients in the placebo group who progressed past the key prognostic threshold of 5 s on the TTR, which represents an earlier loss of ambulation. This indicates that delandistrogene moxeparvovec may reduce the odds of progressing to a TTR of more than 5 s by up to 91% and has the potential to modify the course of the disease. Second, the functional endpoint SV95C is a novel digital objective measure of ambulatory performance of daily activities in patients' normal daily environment that is qualified for use by the European Medicines Agency as a primary endpoint in clinical trials of DMD[44–46]. Finally, although the primary endpoint did not show a statistically significant difference at week 52 versus placebo, the global statistical test, a composite measure of efficacy, supported the totality of evidence of treatment effect with delandistrogene moxeparvovec and indicated the presence of a functional treatment effect after accounting for multiple hypotheses tested across the primary and secondary study endpoints. The pre-specified global statistical test combines information from multiple endpoints and reduces multiple testing problems into a single test against the global null hypothesis of no treatment effect on all endpoints. Although the TTR and 10MWR assessments are included in the NSAA as 'rise from floor' and 'run', the assessment of

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a

	Delandistrogene moxeparvovec (*n* = 17)	Placebo (*n* = 14)
Key secondary endpoint		
Western blot (adjusted for muscle content), percent normal		
Mean (s.d.)	34.29 (41.04)	0.00 (0.00)
Median	19.11	0.00

b

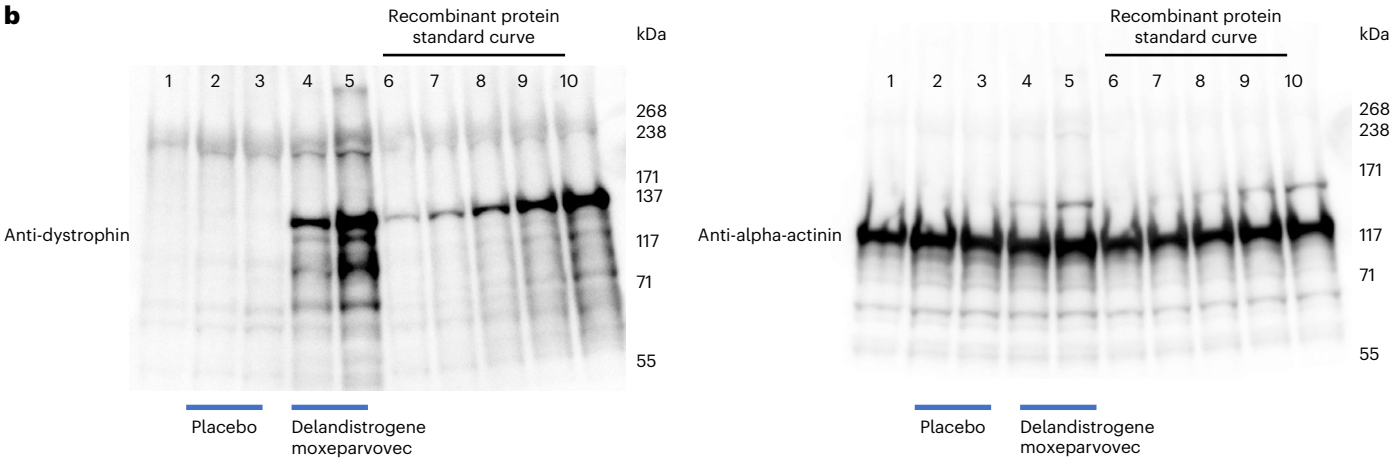

Fig. 4 | Delandistrogene moxeparvovec micro-dystrophin expression at 12 weeks after infusion in a subset of patients. a, Delandistrogene moxeparvovec micro-dystrophin expression at week 12 as measured by western blot, percent normal (*n* = 17) and placebo (*n* = 14) groups in patients who had a muscle biopsy. Baseline data were not available as muscle biopsies were performed only at week 12. Each patient had two samples of biopsies taken, and all samples were analyzed. **b**, Representative western blots for delandistrogene moxeparvovec micro-dystrophin (left) and loading controls (right) from week 12 biopsies. Lane 1: DMD pool (negative control); Lanes 2–3: samples from placebo-treated patients; Lanes 4–5: samples from delandistrogene moxeparvovec–treated patients; Lanes 6–10: recombinant micro-dystrophin protein standard curve (21.85, 43.70, 87.39, 174.79 and 349.58 fmol mg^{-1}). The faint upper lower molecular weight bands are non-specific. The 137-kDa band denotes the presence of delandistrogene moxeparvovec micro-dystrophin and was quantified. Each patient had two samples of biopsies taken, and all samples were analyzed.

these items in the NSAA is categorical and scored with a 0, 1 or 2 based on the patient's ability to complete the task. The TTR and 10MWR key secondary endpoints are quantitative and assess the time it takes for the patient to complete the assessment.

No significant differences were observed between delandistrogene moxeparvovec and placebo groups for either PROMIS measure at week 52. This may have been related to a ceiling effect, as evidenced by the high baseline scores, rendering the overall score insensitive to capturing potential improvements or differences over 52 weeks. Furthermore, given that the studied population was still in the maturational phase, this likely reduced the potential to observe differences between groups over the 52-week timeframe.

In earlier studies with long-term follow-up, ambulatory patients treated with delandistrogene moxeparvovec at age 5.1 years (mean) showed stabilization of NSAA total scores over 4 years in four males with DMD[22]. Notably, the mean patient age at 4 years after treatment (9.2 years) surpassed the mean age at which NSAA total score has been shown to peak and subsequently decline (6.3 years) by approximately 3 years[22,39]. Furthermore, delandistrogene moxeparvovec may confer benefit to patients at various stages of disease progression by resulting in greater improvements versus natural history in the maturational phase of the disease and prevention of decline in older patients[39]. Delandistrogene moxeparvovec aims to protect muscles against further damage and stabilize or slow the decline of function; therefore, treatment may result in a higher natural peak of motor function for patients treated in the maturational phase compared to stabilization of motor function in those treated in the plateau/early-decline phase, with divergence from the natural disease course expected to widen over time.

Potential study limitations include the placebo group being limited to 1 year, due to ethical concerns of withholding disease-modifying treatment from patients in need of treatment. Although the study was blinded, because vomiting and nausea were the most common TR-TEAEs shortly after infusion, patients or caregivers may have become aware of treatment allocation. In addition, TTR is assessed in the primary endpoint of NSAA total score as the item 'rise from floor' and separately as a key secondary endpoint, which may be perceived as an overlap of outcome measures. However, it is important to note that the assessment of items in the NSAA is categorical and scored with a 0, 1 or 2 based on the patient's ability to complete the task, whereas the TTR endpoint is quantitative and evaluates the time it takes for the patient to complete the assessment.

In conclusion, delandistrogene moxeparvovec did not show a statistically significant difference compared to placebo in the primary endpoint at week 52. Key secondary endpoints and other functional endpoints numerically favored delandistrogene moxeparvovec in the overall population and age subgroups, although no statistical significance can be claimed. This is consistent with long-term results from earlier delandistrogene moxeparvovec trials and the essential myoprotective role of functional dystrophin[5,20,22,47]. No new safety signals were identified in EMBARK, supporting a manageable safety profile of delandistrogene moxeparvovec. Among the TR-SAEs, no life-threatening events, deaths or study discontinuations were reported, and all have resolved. Collectively, the delandistrogene moxeparvovec safety profile observed in EMBARK was consistent with that observed in other trials in the clinical development program[19–22]. Of note, no clinically significant complement-mediated AEs were observed in this study, consistent with other clinical studies that have used the rAAVrh74 vector.

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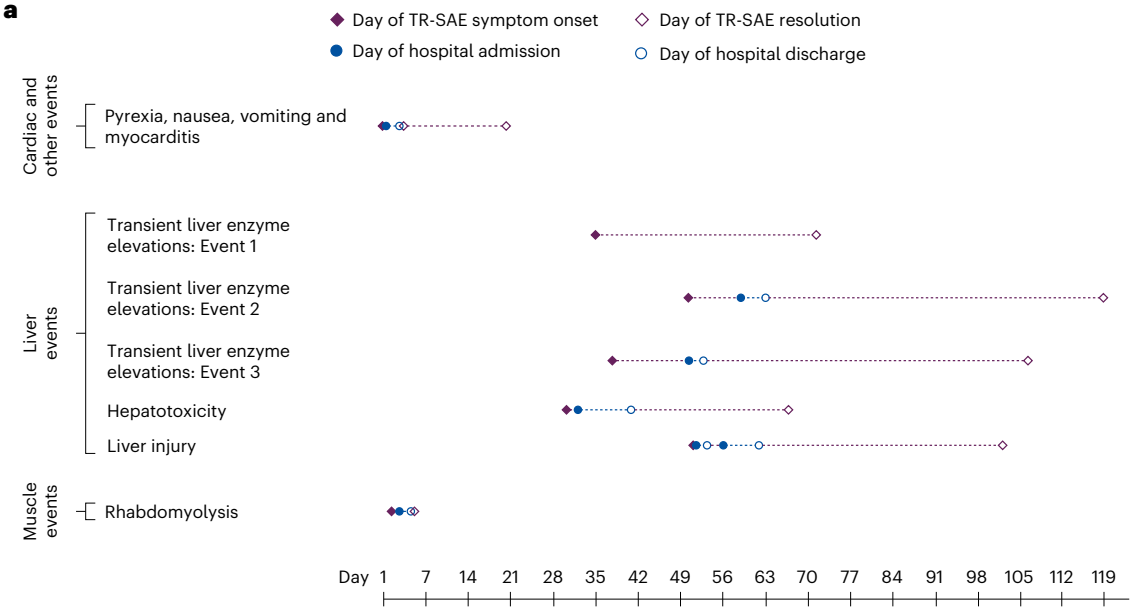
a



b

	Delandistrogene moxeparvovec (*n* = 63)	Placebo (*n* = 62)
Patients with at least one AE, *n* (%)	62 (98.4)	57 (91.9)
Number of AEs, *n*	674	514
Patients with at least one SAE, *n* (%)	14 (22.2)	5 (8.1)
Number of SAEs, *n*	21	9
Patients with any TEAE, *n* (%)	62 (98.4)	57 (91.9)
TEAEs, *n*	664	502
Patients with any TR-TEAE, *n* (%)	48 (76.2)	17 (27.4)
Number of TR-TEAEs, *n*	235	43
Patients with any TR-SAE, *n* (%)	7 (11.1)	0
Number of TR-SAEs, *n*	10	0
Patients with an AE leading to study discontinuation, *n* (%)	0	0
Number of deaths, *n* (%)	0	0
TR-TEAEs occurring in >10% of patients, *n* (%)		
Vomiting	34 (54.0)	0
Nausea	20 (31.7)	5 (8.1)
Decreased appetite	17 (27.0)	1 (1.6)
GLDH increased[a]	15 (23.8)	2 (3.2)
Pyrexia	10 (15.9)	0
Abdominal pain upper	8 (12.7)	1 (1.6)

Fig. 5 | Timeline of TR-SAEs in delandistrogene moxeparvovec–treated patients. a, The timeline of events for the 10 TR-SAEs experienced by seven patients treated with delandistrogene moxeparvovec, detailing SAE symptom onset, hospital admission, hospital discharge and SAE resolution. See Extended Data Table 3 for a complete TR-SAE safety narrative. **b**, [a]GLDH increases were based on investigator assessment and their institution's normal range. Shown are summaries of AEs, SAEs, TEAEs, TR-TEAEs, TR-SAEs, AEs leading to study discontinuation, deaths and TR-TEAEs occurring in more than 10% of patients. The safety population included all patients who received study treatment (excluding one patient enrolled under a regional addendum). Events are listed in descending order of frequency in the delandistrogene moxeparvovec group. AEs were classified according to the *Medical Dictionary for Regulatory Activities*.

Other clinical studies of this gene therapy in patients younger and older than those studied in EMBARK are ongoing[48–50], and open-label extension data from studies in process should provide a better understanding of the long-term effects of delandistrogene moxeparvovec[51,52].

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions

and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-03304-z.

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Methods

Trial oversight

This trial was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines[23]. The trial protocol and all amendments were approved by an institutional review board and ethics committee at each site. The full list of institutional review boards and ethics committees is available in the Supplementary Information. The protocol is available upon reasonable request. Here we report results from a planned analysis, per protocol, of Part 1 (52 weeks) of EMBARK (SRP-9001-301; ClinicalTrials.gov: NCT05096221), a large, phase 3, two-part, multinational, randomized, double-blind, placebo-controlled trial assessing delandistrogene moxeparvovec safety and efficacy in patients with DMD aged ≥4 years to <8 years[23]. The primary analysis of the study was performed after all patients completed Part 1. No interim analysis was planned before the completion of Part 1. EMBARK was conducted at 42 sites in the United States, Europe and Asia[23]. The first patient was enrolled on 8 November 2021, and the last patient was enrolled on 14 September 2022. Informed consent was obtained from parent(s)/legal guardian(s), and patients' assent was obtained when indicated. No compensation was offered for participation in the study other than covering for meals and travel-related expenses. All authors contributed to the design of the study, data collection, analyses, interpretation, manuscript writing, reviewing and approval and the decision to publish. The sponsor had final responsibility for the design of the trial, protocol, database maintenance, trial conduct, data analyses and confirmation of the accuracy of the data. All authors gathered the data, had access to the data and vouch for its accuracy and completeness for fidelity to the trial protocol. All authors contributed to data analysis and interpretation as well as manuscript writing, reviewing and approval. All authors jointly decided to publish the manuscript. An independent data monitoring committee continues to monitor safety, efficacy, data quality and study integrity.

Trial design

For all patients, the day before infusion, and, in addition to baseline stable oral corticosteroids, standard-of-care corticosteroid dosage was continued, and prednisone 1 mg kg^{-1} d^{-1} was added for suppression of potential AEs caused by immune response to the AAV vector, continuing for at least 60 d to a maximum total dose of 60 mg d^{-1} and then tapered to pre-infusion dosing. Patients were randomized (1:1 ratio) by interactive response technology to either a single IV administration of commercial process delandistrogene moxeparvovec material (1.33×10^{14} vector genomes per kilogram (vg/kg), linear standard quantitative polymerase chain reaction (PCR)), or placebo (0.9% sodium chloride solution) through a peripheral limb vein and stratified by age group (≥4 years to <6 years or ≥6 years to <8 years) at randomization and by NSAA total score (≤22 or >22) at screening. The random allocation sequence was saved in the interactive response technology system, which automatically assigned treatment based on sequence. All patients, parents/caregivers, investigators and site staff were blinded, except for the unblinded site pharmacist.

The crossover study consists of Part 1 (52 weeks (complete)) and Part 2 (52 weeks) followed by an open-label, follow-up study of at least 5 years (Supplementary Fig. 3). In Part 2, patients who received placebo in Part 1 received delandistrogene moxeparvovec, whereas those treated with delandistrogene moxeparvovec in Part 1 received placebo.

Between November 2020 and August 2022, the protocol was updated three times. The updates to the protocol are summarized below.

- Version 1 (17 November 2020) to Version 2 (2 August 2021)
  - The primary reasons necessitating updates to the protocol were to add a blinded crossover design, so that patients randomized to placebo in Part 1 of the study had the opportunity to receive delandistrogene moxeparvovec in Part 2, and patients randomized to delandistrogene moxeparvovec in Part 1 received placebo in Part 2 to maintain the blind; to further clarify and refine the inclusion and exclusion criteria as well as stratification factors; to adjust the sample size; and to add a transgene ELISA endpoint.
- Version 2 (2 August 2021) to Version 3 (30 August 2021)
  - The primary reasons necessitating updates to the protocol were to update exon language for inclusion criterion 2 and to update safety monitoring and AESI language.
- Version 3 (August 2021) to Version 4 (August 2022)
  - The primary reasons necessitating updates to the protocol were to update the randomization language to allow approximately 50% of patients to be randomized in the ≥4-year to <6-year age group and to update safety monitoring language.

Gene therapy description

Delandistrogene moxeparvovec uses the rAAVrh74 vector due to its transduction efficiency and relatively low seroprevalence in patients with DMD compared to other AAV serotypes. The muscle-specific MHCK7 promoter and cardiac enhancer region drives expression in cardiac and skeletal muscles, including the diaphragm, with minimal off-target expression, and the delandistrogene moxeparvovec transgene encodes the key functional domains of full-length dystrophin, including anchor regions at the N-terminus and cysteine-rich (CR) region for actin and the dystrophin-associated protein complex, respectively, spectrin repeats 1–3 and 24 and hinge domains 1, 2 and 4 to maintain molecular flexibility[53]. The delandistrogene moxeparvovec inverted terminal repeat (ITR) to ITR sequence is available in the Supplementary Information.

Patients

A patient must meet all of the following criteria to be eligible to participate in this study:

1. Is male at birth (self-reported), ambulatory and ≥4 years to <8 years of age at the time of randomization.
2. Has a definitive diagnosis of DMD before screening based on documentation of clinical findings and prior confirmatory genetic testing using a clinical diagnostic genetic test. The genetic report must describe a frameshift deletion, frameshift duplication, premature stop ('nonsense'), canonical splice site mutation or other pathogenic variant in the *DMD* gene fully contained between exons 18 and 79 (inclusive) that is expected to lead to absence of dystrophin protein.
3. Is able to cooperate with motor assessment testing.
4. Has an NSAA total score >16 and <29 at the screening visit.
5. Has a TTR from the floor of <5 s at the screening visit.
6. Stable daily dose of oral corticosteroids for at least 12 weeks before screening, and the dose and regimen are expected to remain constant (except for modifications to accommodate changes in weight) throughout the study.
7. Has rAAVrh74 antibody titers of less than 1:400 (that is, not elevated) as determined by an ELISA.
8. Patients who are sexually active must agree to use, for the entire duration of the study, a condom, and the female sexual partner must also use a medically acceptable form of birth control (for example, oral contraceptive).
9. Has (a) parent(s) or legal guardian(s) who is/are able to understand and comply with the study visit schedule and all other protocol requirements.
10. Is willing to provide informed assent (if applicable) and has (a) parent(s) or legal guardian(s) who is/are willing to provide informed consent for the patient to participate in the study.

A patient who met any of the following criteria was excluded from this study:

1. Has *DMD* gene:
   a. Pathogenic variants between or including exons 1–17
   b. In-frame deletions, in-frame duplications and variants of uncertain significance
   c. Pathogenic variants fully contained within exon 45 (inclusive).
2. Has a left ventricular ejection fraction of less than 40% on the screening echocardiogram or clinical signs and/or symptoms of cardiomyopathy.
3. Major surgery within 3 months before day 1 or planned surgery or procedures that would interfere with the conduct of the study for any time during this study.
4. Presence of any other clinically significant illness (including cardiac, pulmonary, hepatic, renal, hematologic and immunologic), behavioral disease, infection, malignancy, concomitant illness or requirement for chronic drug treatment that, in the opinion of the investigator, creates unnecessary risks for gene transfer, medical condition or extenuating circumstance that, in the opinion of the investigator, might compromise the patient's ability to comply with the protocol-required testing or procedures or compromise the patient's well-being, safety or clinical interpretability.
5. Has serologic evidence of current, chronic or active HIV, hepatitis C or hepatitis B infection.
6. Has a symptomatic infection (for example, upper respiratory tract infection, pneumonia, pyelonephritis and meningitis) within 4 weeks before day 1.
7. Demonstrates cognitive delay or impairment that could confound motor development in the opinion of the investigator.
8. Treatment with any of the following therapies according to the timeframes specified:
   a. Any time:
      – Gene therapy
      – Cell-based therapy (for example, stem cell transplantation)
      – CRISPR–Cas9 or any other form of gene editing
   b. Within 12 weeks of day 1 and any time during the study:
      – Use of human growth factor or vamorolone
   c. Within 6 months of day 1 and any time during the study:
      – Any investigational medication
      – Any treatment designed to increase dystrophin expression (for example, Translarna, EXONDYS 51, VILTEPSO, VYONDYS 53 and AMONDYS 45)
9. Has received a live virus vaccine within 4 weeks or inactive vaccine within 2 weeks of the day 1 visit or expects to receive a vaccination during the first 3 months after day 1.
10. Has abnormal laboratory values considered clinically significant, including, but not limited to:
    a. Gamma-glutamyl transferase >2× the upper limit of normal
    b. Glutamate dehydrogenase >15 U L^{-1}
    c. Total bilirubin > upper limit of normal (elevations in total bilirubin confirmed to be due to Gilbert's syndrome are not exclusionary)
    d. White blood cell count >18,500 per microliter
    e. Platelets ≤150,000 per microliter
11. Family does not want to disclose patient's study participation with general practitioner/primary care physician and other medical providers.
12. In the opinion of the investigator, the patient is not likely to be compliant with the study protocol.

Race and ethnicity were self-reported, determined by a two-question format and categories consistent with US Food and Drug Administration guidance[54]. Sex was self-reported by the patient or the parent/guardian. Per disease etiology, only males were enrolled.

Patient withdrawal criteria

A patient can withdraw from study participation at any time for any reason. A patient who withdraws before dosing may be replaced at the discretion of the sponsor. In addition, the sponsor may decide to stop the study participation of any patient as deemed necessary. The investigator may also stop the study participation of any patient at any time. Reasons for withdrawal from the study include, but are not limited to:

- The patient or parent(s)/legal guardian(s) withdraw(s) consent.
- Before randomization and dosing, it is determined that the patient was erroneously included in the study (that is, was found to not have met the eligibility criteria).

The investigator or study staff will document the reason(s) for withdrawal on the electronic case report form. If withdrawn patients received the study drug, every effort should be made to request that the patient allows follow-up for safety purposes.

Patients who withdraw from the study must return the wearable device.

Patients who have been dosed and withdraw from the study but do not withdraw consent will be asked to continue telephone calls to collect AEs and concomitant medication information and have blood collected for laboratory assessments per protocol every week for the first 12 weeks (±3 d) after infusion (if patients withdraw within this window) and then for safety laboratory assessments approximately every 6 months (±1 month) starting from the date of the last safety laboratory assessment before withdrawal. For this study, safety laboratory assessments include the following: electrolytes, troponin, liver function, hematology, high-sensitivity C-reactive protein and complement, renal function and urinalysis.

Assessments and endpoints

Patients were monitored weekly for 12 weeks after infusion and at weeks 24, 36 and 52. The NSAA and TFTs (TTR, time to ascend 4 steps, 10MWR and 100MWR)[21] were performed at baseline and at weeks 12, 24, 36 and 52. The NSAA is a categorical assessment, and items are scored with a 0, 1 or 2 based on the patient's ability to complete the task. The TFTs are quantitative and assess the time it takes for the patient to complete the assessment. For SV95C assessments, a wearable device (Syde) was worn daily for 3 weeks before infusion and then for 3 weeks before week 12, 24, 36 and 52 clinic visits. Week 12 biopsies from the medial gastrocnemius muscle in a subset of patients ($n = 31$), performed at sites pre-selected based on experience in performing muscle biopsies as routine in their diagnostic repertoire, were collected using open or core biopsies; each patient had two samples of biopsies taken, and all samples were processed for western blot[20,21]. Baseline biopsy data were not available for comparison as muscle biopsies were performed only at week 12. AE reporting was continuous, beginning at informed consent/assent.

The modified intent-to-treat population (all randomized patients who received study treatment (excluding one patient enrolled under a regional addendum), $N = 125$) was the analysis population for efficacy endpoints (Supplementary Table 4). The primary endpoint was change from baseline to week 52 in NSAA total score. The three pre-specified key secondary endpoints (in rank order) were quantity of delandistrogene moxeparvovec micro-dystrophin expression at week 12 (western blot) and change from baseline to week 52 in TTR and 10MWR. Other secondary endpoints were change from baseline to week 52 in: SV95C[44], 100MWR and time to ascend 4 steps; change from baseline to week 52 in PROMIS scores in the Mobility and Upper Extremity Function domains; and number of skills gained or improved at week 52 as measured by the NSAA.

Safety assessments in the safety population (all patients who received study treatment (excluding one patient enrolled under a regional addendum)) included TEAEs, SAEs, AEs of special interest,

clinically significant changes in vital signs and physical examination findings and clinically relevant changes in safety laboratory assessments, electrocardiograms and echocardiograms. The exploratory endpoint in Part 1 included change in CK levels in blood.

Methodology for processing and analyzing biologic samples

Week 12 biopsies collected at study sites were from the lower extremities of the medial gastrocnemius muscle, or alternatively allowed muscle groups, in a subset of patients using open or probe biopsies in accordance with allocation protocols and as previously described[20,21]. Samples were mounted, frozen in 2-methylbutane (isopentane) cooled in liquid nitrogen, stored at −80 °C and transferred in dry ice to the sponsor laboratory and transferred frozen to −80 °C freezer storage.

Western blot analyses were performed following Good Clinical Laboratory Practice standards, in accordance with validated methodology adapted from Charleston et al.[55]. Homogenized biopsy samples were assayed for total protein. Negative controls and total protein samples (20 µg per sample) as well as a five-point standard curve (recombinant micro-dystrophin (Curia) ranging from 21.85 to 349.58 fmol mg^{-1} protein) were resolved using SDS-PAGE (Invitrogen). Membranes with transferred proteins were probed using an anti-dystrophin primary antibody (DYS3, 1:20; Leica Biosystems) and then anti-mouse immunoglobulin G-conjugated horseradish peroxidase (Amersham ECL anti-mouse immunoglobulin G peroxidase-linked species-specific whole antibody (from sheep)) (NA931V, 1:1,000; Cytiva). A chemiluminescence imaging system (Alliance Q9 Advanced Imager, UVITEC) was used to visualize bound enzyme activity, and ImageQuant TL version 8.2 software (Cytiva) was used to analyze the bands. Contrast was automatically adjusted in the entire image by ImageQuant TL software; quantitative value remained the same as the original untuned image. For the loading control, membranes were probed with anti-alpha actinin antibody (A7811, 1:100,000; Sigma-Aldrich) and then the same secondary antibody and imaging procedure as described above. In each sample, delandistrogene moxeparvovec micro-dystrophin was quantified using data that were normalized to each patient's muscle content. Control samples used in western blot assays were kindly provided by Steven A. Moore (Wellstone Center, University of Iowa). As the muscle biopsy samples being tested are from patients with varying conditions of muscle degeneration, it is necessary to normalize delandistrogene moxeparvovec micro-dystrophin expression data generated by western blot to muscle content. Protein expression data generated by western blot are expressed as percent of normal control samples derived from a pool of normal control muscle biopsied. Muscle content is then determined using Masson's trichrome histological stain paired with digital image analysis on a serial section within the same biopsy. The algorithm quantifies the area of muscle as a percentage of total area, generating percent muscle content. The adjusted values represent the percent normal delandistrogene moxeparvovec micro-dystrophin expression normalized to the percent muscle content. Thus, the resulting muscle content adjusted expression values provide meaningful measurement of micro-dystrophin expression in tissues with progressive muscle degeneration, as present in the DMD patient population.

Statistical analysis

Assuming an s.d. of 3.5 estimated based on previous delandistrogene moxeparvovec clinical studies[19–22] and a 10% dropout rate at week 52, with a type 1 error of 0.05 (two-sided), a sample size of 120 with 1:1 randomization provided approximately 90% power to detect a mean difference of 2.2 in change in NSAA total score from baseline to week 52 between the delandistrogene moxeparvovec and placebo groups under the two-sample *t*-test. Estimate of effect size for difference between mean was equal to the ratio of expected difference and s.d. (2.2/3.5).

A restricted maximum likelihood-based mixed model for repeated measures (MMRM) analysis was used to compare delandistrogene moxeparvovec with placebo from baseline to week 52, with 95% CIs for the difference in LSM between treatment groups. SAS software version 9.4 was used to perform the statistical analysis for the primary endpoint. In this model, the response vector consisted of the change from baseline in NSAA total score at each post-baseline visit in Part 1. The model included the covariates of treatment group (categorical), visit (categorical), treatment group by visit interaction, age group at the time of randomization (categorical), baseline NSAA total score, age group at the time of randomization by visit interaction and baseline NSAA total score by visit interaction. All covariates were fixed effects in this analysis. An unstructured covariance matrix was used to model the within-patient variance–covariance errors. If the unstructured covariance structure resulted in a lack of convergence, the heterogeneous first-order autoregressive covariance structure was used. The Kenward–Roger approximation was used to estimate the denominator degrees of freedom. In the primary analysis, missing data were assumed to be missing at random. An MMRM analysis similar to the one for the primary endpoint was performed to compare the two treatment groups for each of the secondary endpoints, with baseline NSAA raw total score replaced with the corresponding baseline for the secondary endpoint, as well as NSAA group at the time of screening (≤22 versus >22) added as a covariate.

For the primary endpoint, a subgroup analysis was conducted with respect to all subgroup variables (≥4 years to <6 years or ≥6 years to <8 years) and NSAA total scores (≤22 versus >22). For each category of a subgroup variable, an MMRM similar to the primary analysis model was fitted using subset data. For age group subgroup analysis, age group and age group by visit interaction were removed from the MMRM model as a covariate. For the secondary endpoints, subgroup analysis was conducted with respect to the age and NSAA group (at the time of screening), using an analysis method similar to the subgroup analyses for the primary endpoint (with baseline NSAA total score being replaced with the baseline value for the corresponding endpoint in the MMRM model, as well as NSAA group at the time of screening (≤22 versus >22) added as a covariate, if applicable).

Because the primary endpoint did not meet statistical significance, and because the statistical analysis plan did not include a provision for correcting for multiplicity beyond the planned hierarchical testing procedure, results are reported as point estimates with between-group differences in LSM changes and 95% CIs. The widths of the CIs have not been adjusted for multiplicity and should not be used to infer definitive treatment effects for secondary outcomes or in subgroups.

To assess the totality of evidence wholistically and address the concern of multiple hypothesis testing, an additional pre-specified efficacy exploratory analysis that was not controlled for multiplicity within the hierarchical testing procedure was performed using the Wei–Lachin procedure[56]. The test was performed as a global statistical test on a composite of multiple endpoints (as pre-specified as a sensitivity analysis), assessing overall treatment effects among the primary, key secondary and other functional efficacy endpoints (NSAA total score, TTR, 10MWR, SV95C, 100MWR and time to ascend 4 steps). The global statistical test combines information from multiple endpoints and reduces multiple testing problems into a single test against the global null hypothesis of no treatment effect on all endpoints. The global statistical test was implemented by comparing the sum of observed *t*-statistics from multiple endpoints against the null distribution induced by 10,000 permutations[57].

Hierarchical statistical testing (at completion of Part 1)

This analysis included the analyses of all data through the completion of Part 1 for the following endpoints:

- Change in NSAA total score from baseline to week 52 (Part 1)
- Quantity of delandistrogene moxeparvovec micro-dystrophin expression at week 12 (Part 1) as measured by western blot[a]
- Change in TTR from the floor from baseline to week 52 (Part 1)[a]

- Change in time of 10MWR from baseline to week 52 (Part 1)[a]
- Change in SV95C from baseline to week 52 (Part 1)
- Change in time of 100MWR from baseline to week 52 (Part 1)
- Change in time to ascend 4 steps from baseline to week 52 (Part 1)
- Change in PROMIS Mobility score from baseline to week 52 (Part 1)
- Change in PROMIS Upper Extremity score from baseline to week 52 (Part 1)
- Number of skills gained or improved at week 52 (Part 1) as measured by the NSAA

[a]Key secondary efficacy endpoints.

Additional statistical considerations

Analyses of exploratory endpoints defined for Part 1 of the study were performed as follow-on analyses of the above endpoints. The Part 1 analysis also included disposition, demographics and baseline characteristics, medical history, concomitant medications, treatment exposure and compliance, baseline and post-baseline corticosteroids and protocol deviations.

The initial power analysis relied on data from the phase 1 study[22]. Subsequent adjustments to the power analysis assumptions were made in response to new findings from the additional phase 2 and phase 1b studies[20,21].

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The datasets presented in this article are not readily available as this study is ongoing, and access to the data is limited to those that support the findings of this study. De-identified patient-level data cannot be disclosed due to confidentiality agreements and the risk of re-identification. Qualified researchers may request access to the data that support the findings of Part 1 of this study and clinical study documents from Sarepta Therapeutics, Inc. by contacting medinfo@sarepta.com, subject to review by the study sponsors on a case-by-case basis. Data requests will be fulfilled within 90 d, and a data transfer agreement may be required.

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Author contributions

J.R.M., F.M., E.M.M., H.K., U.S.-S.: conception or design of the work; acquisition of data; interpretation of data; drafted the work or critically reviewed it; approved the submitted version; and agreed to be accountable for the accuracy and integrity of all aspects of the work. C.M.M.: acquisition of data; analysis of data; interpretation of data; drafted the work or critically reviewed it; approved the submitted version; and agreed to be accountable for the accuracy and integrity of all aspects of the work. E.C., C.L.-A., A.N., C.P., A.V. and C.M.Z.: acquisition of data; interpretation of data; drafted the work or critically reviewed it; approved the submitted version; and agreed to be accountable for the accuracy and integrity of all aspects of the work. M.G., A.P.M., C.R., C.W., D.R.A., E.D., S.M., R.A.P., W.Z. and P.F.: analysis of data; interpretation of data; drafted the work or critically reviewed it; approved the submitted version; and agreed to be accountable for the accuracy and integrity of all aspects of the work. T.S., J.S.E. and L.R.R.-K.: conception or design of the work; analysis of data; interpretation of data; drafted the work or critically reviewed it; approved the submitted version; and agreed to be accountable for the accuracy and integrity of all aspects of the work.

Competing interests

J.R.M. received study funding from Sarepta Therapeutics while at Nationwide Children's Hospital at the time of the study and is currently an employee of Sarepta Therapeutics. J.R.M. is a co-inventor of AAVrh74.MHCK7.micro-dys technology. F.M. has received honoraria and grants from Sarepta Therapeutics for participating at symposia and advisory boards and is involved as an investigator in Sarepta Therapeutics clinical trials. He reports participation in advisory boards for Novartis, F. Hoffmann-La Roche, Ltd., Edgewise Therapeutics, Dyne Therapeutics, Pfizer, PTC Therapeutics and Italfarmaco. C.M.M. reports grants from Capricor Therapeutics, Catabasis, Edgewise Therapeutics, Epirium Bio, Italfarmaco, Pfizer, PTC Therapeutics, Santhera Pharmaceuticals and Sarepta Therapeutics and has a consultancy/advisory role with Biomarin, Capricor Therapeutics, Catalyst, Edgewise Therapeutics, Italfarmaco, PTC Therapeutics, F. Hoffmann-La Roche, Ltd., Santhera Pharmaceuticals and Sarepta Therapeutics. He has received honoraria from PTC Therapeutics and Sarepta Therapeutics. E.M.M. has received fees from AveXis, Biogen and F. Hoffmann-La Roche, Ltd. E.C. has received honoraria from Sarepta Therapeutics for participating in advisory boards and research and/or grant support from the Centers for Disease Control and Prevention, CureSMA, the Muscular Dystrophy Association, the National Institutes of Health, Orphazyme, the Patient-Centered Outcomes Research Institute, Parent Project Muscular Dystrophy, PTC Therapeutics, Santhera, Sarepta Therapeutics and the US Food and Drug Administration. H.K. has received grants from Sarepta

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Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-024-03304-z.

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Extended Data Table 1 | Summary of location of genetic pathogenic variants (modified intent-to-treat population)

Genetic pathogenic variant, *n* (%)	Delandistrogene moxeparvovec (*n* = 63)	Placebo (*n* = 62)	Total (*N* = 125)
Exons 1–17	0	0	0
Exons 18–58	55 (87.3)	53 (85.5)	108 (86.4)
Exons 59–71	5 (7.9)	3 (4.8)	8 (6.4)
Exons 72–79	0	0	0
Intron[a] Exons 18–58 Exons 59–71	3 (4.8) 1 (1.6) 2 (3.2)	6 (9.7) 4 (6.5) 2 (3.2)	9 (7.2) 5 (4.0) 4 (3.2)

[a]Introns are grouped based on the nearest exon category.

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Extended Data Table 2 | Number of skills gained, improved and maintained at week 52 as measured by the NSAA (modified intent-to-treat population)

Characteristic	Delandistrogene moxeparvovec (*n* = 63)	Placebo (*n* = 62)
Number of skills gained as measured by the NSAA		
Number of skills gained at week 52, mean (SD)	0.71 (1.02)	0.52 (1.01)
Number of skills improved as measured by the NSAA		
Number of skills improved at week 52, mean (SD)	3.44 (2.31)	3.44 (2.50)
Number of skills maintained as measured by the NSAA		
Number of skills maintained at week 52, mean (SD)	10.75 (2.61)	10.61 (2.28)

Skills are gained when the average item score is 0 at baseline and >0 at Part 1 week 52; skills are improved when the average item score at baseline is >0 but less than the average item score at Part 1 week 52; and skills are maintained when the average item scores at baseline and Part 1 week 52 are the same and >0.

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Extended Data Table 3 | Treatment-related SAE narrative table

Transient liver enzyme elevations (three events)	Event 1: On the day of infusion, a patient experienced intermittent nausea and vomiting which was treated as an outpatient. Relevant baseline values were: ALT = 449 U/L and AST = 261 U/L. On day 35, the patient had elevated liver enzymes (ALT 939 u/l, AST 667 u/l, GGT 25 u/l and total bilirubin 0.2 mg/dl with no associated signs or symptoms). On day 37, the patient reported nausea for 6 days and the peri-infusion steroid immunosuppression was doubled. On day 42, the patient developed vomiting. Liver enzymes remained elevated (ALT 875, AST 531, GGT 26 and total bilirubin 0.3). The prednisolone daily dose was continued at 2 mg/kg/day. On day 49, the patient reported decreased appetite and rash (resolved the same day). Decreased energy level and lethargy were reported on day 49. On day 56, IV methylprednisolone (17 mg/kg/day) was administered for 3 days. The liver enzyme elevation was resolved on day 71, 36 days after onset. Event 2: A patient was diagnosed with elevated liver enzymes on day 50 (ALT 843 u/l, AST 818 u/l and GGT 98 u/l). The dose of prednisone was doubled on day 54. On day 59, he was hospitalized (ALT 861, AST 559 and GGT 253). IV methylprednisolone (22.34 mg/kg/day) was given for 5 days, which decreased the liver enzymes. On day 63, the patient was discharged on prednisolone (10.53 mg/kg/day) for 3 days. As the liver enzymes continued to improve, the prednisone dose was tapered down. The liver enzyme elevations were resolved on day 119, 69 days after their onset. Event 3: On day 37 following infusion, a patient had GGT (55 u/l) and GLDH (48 u/l) elevations. On day 41, the daily dose of prednisolone was doubled. On day 50, the patient, while asymptomatic, was hospitalized to start IV methylprednisolone (38 mg/kg/day) for 3 days due to GGT elevated to 192 u/l. On day 52, the last dose of IV methylprednisolone was given, and the patient was discharged on prednisolone (2 mg/kg/day). On day 106, GGT and GLDH levels were within normal limits. The peri-infusion prednisolone was reduced weekly by 0.25 mg/kg until it was fully stopped on day 127. The GLDH increase resolved on day 71, 34 days after its onset. The GGT increase resolved on day 106, 69 days after its onset.
Hepatotoxicity	On day 30 following infusion, a patient developed constipation, nausea and right-sided abdominal pain with right upper quadrant tenderness to palpation. Liver enzymes were elevated: ALT 1,868 u/l, AST 2,667 u/l, GGT 107 u/l and total bilirubin 2.3 mg/dl. The patient reported not eating or drinking for 6 days prior to noticing yellowish pigmentation of the sclerae (possible icterus). The patient was admitted to the PICU for treatment with high-dose pulse IV methylprednisolone (20 mg/kg daily) and monitoring. On day 33, an abdominal ultrasound revealed mildly echogenic liver suggestive of diffuse hepatocellular disease/fatty
	infiltration without focal abnormality. Methylprednisolone IV was increased to 40 mg/kg daily every 12 hours due to continued jaundice and elevated liver enzymes (ALT 2,525, AST 3,197 and total bilirubin of 4.2 mg/dl). The patient was transferred out of the PICU on day 36. Hepatology/GI consultation noted scleral icterus and jaundice, tenderness to percussion over right upper quadrant, generalized abdominal tenderness to deep palpation without hepatomegaly. Overall, the patient was clinically stable without evidence of fulminant liver failure. Drug-induced liver injury possibly related to gene therapy was suspected. On day 39, the patient reported mildly increased abdominal pain (ALT 1,933, AST 1,515, GGT 272 and total bilirubin 6.5). Repeat abdominal ultrasound showed severe gallbladder wall edema with pericholecystic fluid and edema, likely due to hepatic dysfunction; no gallstones; diffuse hyper-echogenicity throughout the liver parenchyma consistent with severe transaminitis. On day 41, the patient had improved (ALT 1,434, AST 1,087 and total bilirubin 4.0) and was discharged on high-dose oral prednisone starting at 60 mg daily and tapering by 10 mg every week. The patient was restarted on oral deflazacort (20 mg daily) the day after discharge. Hepatotoxicity was resolved on day 67, 37 days after its onset.
Liver injury	On day 51 following infusion, a patient was hospitalized for GI consultation and treatment with IV methylprednisolone (2.56 mg/kg/day) due to a significant increase in liver enzymes (ALT 2,897 u/l, AST 1,878 u/l, GGT 182 u/l and total bilirubin of 0.7 mg/dl). The patient was found to be an alpha-1 antitrypsin carrier during hospitalization. On day 53, the patient was doing well without significant symptoms, with improved liver enzymes (ALT 1,806, AST 856 and total bilirubin 0.3) and was discharged. On day 54, liver enzymes started to increase again (ALT 2,409, AST 1,620 and total bilirubin 0.5). The dose of oral prednisolone was increased to 3 mg/kg/day, then tapered down to 2.75 mg/kg/day on day 55. The patient was re-admitted for additional work-up and IV methylprednisolone (2.56 mg/kg/day) in addition to the oral prednisolone of 55 mg daily was given. On day 57, a liver biopsy result was consistent with chronic hepatitis with neutrophilic and lymphocytic portal infiltrate interface hepatitis and bile ductular proliferation, as well as bridging fibrosis with focal nodule formation (Grade 3, Stage 3 Batt-Ludwig grading and staging system). The alpha-1 antitrypsin phenotype was M1Z, and cryptogenic cirrhosis has been reported with increased frequency in patients with this alpha-1 antitrypsin phenotype. On day 58, the dose of IV methylprednisolone was increased to 12.5 mg/kg/day. Decline in the liver enzymes (ALT 1,894, AST 743 and total bilirubin 0.3) prompted the tapering down of daily IV methylprednisolone until day 62. On day 59, an ultrasound elastography of the right liver lobe was consistent with a METAVIR fibrosis stage of F3 (severe). The patient was discharged on day 62 and prescribed a daily dose of oral prednisone (2 mg/kg/day), which was tapered down biweekly until it reached the DMD maintenance dose on day 95. Liver injury was resolved on day 102, 51 days after its onset.
Myocarditis, pyrexia, nausea and vomiting	Approximately 6 hours after infusion, a patient developed pyrexia with maximum temperature of 40°C without resolution after taking acetaminophen at home and emesis, which prompted hospitalization. During hospitalization (day 1), troponin-I was elevated (61 x ULN), peaking at 140 x ULN on day 2. Besides
	hypotension (90/47) that resolved without intervention, the patient was asymptomatic with no significant change from baseline on echocardiogram. The patient was kept in observation and administered ondansetron (for vomiting) and maintenance IV fluids. He was discharged on day 3. Pyrexia, nausea and vomiting were resolved on day 3, 3 days after their onset. Myocarditis was resolved on day 21 following infusion (ECHO was normal and troponin-I 0.3 x ULN).
Rhabdomyolysis	On day 2 following infusion, a patient developed decreased urine output with CK level of 11,078 u/l (baseline CK was 13,959 u/l). On day 3, the patient developed fever, vomiting (episode of non-bloody non-bilious emesis with decreased oral intake), abdominal pain (episodic sharp lower abdominal/suprapubic pain), constipation, intermittent bilateral upper and lower extremities pain, crankiness, body ache and decreased urinary output that prompted hospitalization. No weakness or dark urine was reported. The patient denied recent increase in physical activity. He was febrile and tachycardic; the abdomen was distended with generalized abdominal tenderness. The patient was diagnosed with rhabdomyolysis and was admitted for hydration and observation. Laboratory results showed elevated CK with normal BUN. Urinalysis showed 3+ blood (likely myoglobinuria) and 2+ protein. The patient was treated with oral paracetamol 325 mg twice for pain, maintenance IV fluids and maintenance (24 mg) doses of prednisolone. On day 5, the patient was improved, with resolution of fever, no evidence of renal impairment, and no muscle or abdominal pain. CK levels continued to improve. The patient was discharged. Rhabdomyolysis was resolved on day 5, 2 days after its onset. On day 171, the patient again experienced a second episode of severe muscle aches in his lower extremities associated with fever and sore throat. Laboratory tests included CK of 95,356 u/l with stable renal function; IV hydration resolved the myalgia. The respiratory pathogen PCR panel was positive for influenza. The cause of the rhabdomyolysis was likely multifactorial, including viral illness. This second episode was resolved on day 174, 3 days after its onset, and was assessed by the principal investigator as unrelated to delandistrogene moxeparvovec.

ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; ECHO, echocardiogram; GGT, gamma-glutamyl transferase; GI, gastrointestinal; PICU, pediatric intensive care unit; ULN, upper limit of normal.

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Extended Data Table 4 | AEs of special interest

	Delandistrogene moxeparvovec (*n* = 63)	Placebo (*n* = 62)
Patients meeting any GGT/GLDH criteria below, *n* (%)		
Time to onset: Overall	12 (19.0)	0 (0)
GGT or GLDH >8 x ULN	9 (14.3)	0 (0)
GGT or GLDH >5 x ULN and persists for ≥2 weeks	7 (11.1)	0 (0)
GGT or GLDH >3 x ULN and either total bilirubin >2x ULN or International Normalized Ratio >1.5	4 (6.3)	0 (0)
GGT or GLDH >3 x ULN and the new appearance[a]	1 (1.6)	0 (0)
Time to onset: Within 90 days since infusion	11 (17.5)	0 (0)
GGT or GLDH >8 x ULN	9 (14.3)	0 (0)
GGT or GLDH >5 x ULN and persists for ≥2 weeks	7 (11.1)	0 (0)
GGT or GLDH >3 x ULN and either total bilirubin >2 x ULN or International Normalized Ratio >1.5	3 (4.8)	0 (0)
GGT or GLDH > 3x ULN and the new appearance[a]	1 (1.6)	0 (0)
Time to onset: After 90 days since infusion	0 (0)	0 (0)
GGT or GLDH >8 x ULN	0 (0)	0 (0)
GGT or GLDH >5 x ULN and persists for ≥2 weeks	0 (0)	0 (0)
GGT or GLDH >3 x ULN and either total bilirubin >2 x ULN or International Normalized Ratio >1.5	0 (0)	0 (0)
GGT or GLDH >3 x ULN and the new appearance[a]	0 (0)	0 (0)
Patients with any TEAEs of myositis		
Dysphonia		
Time to onset: Overall	1 (1.6)	0 (0)
Time to onset: After 12 weeks since infusion	1 (1.6)	0 (0)
Patients with any TEAEs of thrombotic microangiopathy		
Acute kidney injury		
Time to onset: Overall	0 (0)	1 (1.6)
Time to onset: After 12 weeks since infusion	0 (0)	1 (1.6)
Patients with any TR-TEAEs of hypersensitivity		
Myocarditis		
Time to onset: Overall	1 (1.6)	0 (0)
Time to onset: Within 2 weeks since infusion	1 (1.6)	0 (0)
Patients with platelet count <75,000/mm^3		
Time to onset: Overall	2 (3.2)	0 (0)
Time to onset: Within 2 weeks since infusion	0 (0)	0 (0)
Time to onset: After 2 weeks since infusion	2 (3.2)	0 (0)
Patients with any TEAEs of rhabdomyolysis		
Myalgia		
Time to onset: Overall	4 (6.3)	1 (1.6)
Time to onset: Within 2 weeks since infusion	0 (0)	1 (1.6)
Time to onset: Within 2–12 weeks since infusion	2 (3.2)	0 (0)
Time to onset: After 12 weeks since infusion	2 (3.2)	0 (0)
Myoglobinuria		
Time to onset: Overall	1 (1.6)	1 (1.6)
Time to onset: Within 2 weeks since infusion	0 (0)	0 (0)
Time to onset: Within 2–12 weeks since infusion	0 (0)	1 (1.6)
Time to onset: After 12 weeks since infusion	1 (1.6)	0 (0)
Rhabdomyolysis		
Time to onset: Overall	2 (3.2)	4 (6.5)
Time to onset: Within 2 weeks since infusion	2 (3.2)	0 (0)
Time to onset: Within 2–12 weeks since infusion	0 (0)	3 (4.8)
Time to onset: After 12 weeks since infusion	1 (1.6)	1 (1.6)
Chromaturia		
Time to onset: Overall	1 (1.6)	3 (4.8)
Time to onset: Within 2 weeks since infusion	1 (1.6)	0 (0)
Time to onset: Within 2–12 weeks since infusion	0 (0)	1 (1.6)
Time to onset: After 12 weeks since infusion	0 (0)	2 (3.2)
Patients with troponin elevations[b]		
Time to onset: Overall	2 (3.2)	1 (1.6)
Time to onset: Within 4 weeks since infusion	1 (1.6)	0 (0)
Time to onset: After 4 weeks since infusion	2 (3.2)	1 (1.6)

[a]GGT or GLDH > 3× ULN and the new appearance (that is, onset coincides with the changes in hepatic enzymes) of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia (>5%) potentially related to hepatic inflammation. [b]Troponin I > 3× ULN for patients with non-elevated baseline values (baseline troponin I > ULN) or troponin I > 3× baseline for patients with elevated baseline values (baseline troponin I > ULN). [b]GGT, gamma-glutamyl transferase; ULN, upper limit of normal.

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Extended Data Table 5 | Mean CK at baseline and week 52 (UL^{-1}) (modified intent-to-treat population)

Timepoint	Delandistrogene moxeparvovec (*n* = 63)	Placebo (*n* = 62)
Baseline, mean, u/l (SD)	18,143.42 (8,016.26) (*n* = 62)	18,188.89 (6,521.12)
Week 52, mean, u/l (SD)	13,120.03 (6,088.27) (*n* = 61)	17,372.18 (6,863.95) (*n* = 61)

nature portfolio

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	The study database is in the Medidata Rave EDC System. The Clinical Ink Capture platform was used to collect parent and caregiver questionnaire data.
Data analysis	SAS® software Version 9.4 (Cary, North Carolina) was used to perform the statistical analysis for the primary endpoint. For western blot analysis, ImageQuant TL version 8.2 software (Cytiva, MA, USA) was used to analyze the bands.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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The datasets presented in this article are not readily available as this study is ongoing, and access to the data is limited to those that support the findings of this study. Deidentified patient-level data cannot be disclosed due to confidentiality agreements and the risk of reidentification. Qualified researchers may request

access to the data that support the findings of Part 1 of this study and clinical study documents from Sarepta Therapeutics, Inc., Cambridge, MA, USA, by contacting medinfo@sarepta.com, subject to review by the study sponsors. Data requests will be fulfilled within 90 days, and a data transfer agreement may be required.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex was self-reported by the patient or parent/guardian. Per disease etiology, only males were enrolled.
Reporting on race, ethnicity, or other socially relevant groupings	Reported in Table 1. Racial and ethnic demographic questions were self-reported and the two question format and categories used were consistent with the FDA guidance https://www.fda.gov/regulatory-information/search-fda-guidance-documents/collection-race-and-ethnicity-data-clinical-trials-and-clinical-studies-fda-regulated-medical
Population characteristics	Reported in Table 1 - patients with DMD aged ≥4–<8-years-old.
Recruitment	125 male patients with DMD gene mutations were enrolled at study sites for this gene transfer study. Patients could encompass any ethnic or racial background. Full inclusion criteria are provided in the main text. Investigator and recruitment sites are listed in the supplement.
Ethics oversight	The study was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. Informed consent was obtained from parent(s)/legal guardian(s), and patients' assent was obtained when indicated. There was no compensation for participation in the study other than covering for meals and travel-related expenses. Institutional review boards and ethics committees are listed here and in the Supplementary Information: ADVARRA IRB; WCG IRB; Boston Children's Hospital's Institutional Review Board; OHSU Institutional Review Board; The Institutional Review Boards of the Ann & Robert H. Lurie Children's Hospital of Chicago; University of Utah Institutional Review Board; University of California San Diego Institutional Review Board Administration; Stanford University; Columbia Research Human Research Protection Office Institutional Review Boards; University of California Los Angeles Institutional Review Board (UCLA IRB); Children's Hospital of Philadelphia's (CHOP) institutional Review Board (CHOP's IRB); Vanderbilt Human Research Protection Program (HRPP) Health Sciences Committee 1 - Institutional Review Board; Medical College of Wisconsin Institutional Review Board (MCW IRB); Johns Hopkins Medicine Institutional Review Board (JHM IRB); South Central - Oxford A Research Ethics; Institutional Review Board of Kaohsiung; Research Ethics Committee of National Taiwan University Hospital; CEIm Parc Taulí; COMITATO ETICO TERRITORIALE LAZIO AREA 3; UZ Gent Commissie voor Medische Ethiek UZ Gent; Ethikkommission der Medizinischen Fakultät der LMU München; Ethikkommission der Medizinischen Fakultät der Universität Duisburg-Essen; Central Institutional Review Board, Hong Kong; Institutional Review Board of Kobe University Hospital; National Center of Neurology and Psychiatry Institutional Review Board; Tokyo Women's Medical University Institutional Review Board; National Center for Child Health and Development Institutional Review Board.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	131 were randomized and 125 were treated. Assuming an SD of 3.5 estimated based on previous delandistrogene moxeparvovec clinical studies and a 10% dropout rate at week 52, with a type 1 error of 0.05 (2-sided), a sample size of 120 with 1:1 randomization provided approximately 90% power to detect a mean difference of 2.2 in change in NSAA total score from baseline.
Data exclusions	No data were excluded from the analyses.
Replication	Replication was not performed given that EMBARK is a clinical trial.
Randomization	Randomized 1:1 by interactive response technology.
Blinding	All patients, parents/caregivers, investigators, and site staff were blinded, except for the unblinded site pharmacist.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Membranes with transferred proteins were probed using an anti-dystrophin primary antibody (DYS3; 1:20; Leica Biosystems, Wetzlar, Germany), then anti-mouse immunoglobulin G-conjugated horseradish peroxidase (NA931V; 1:1,000; Cytiva, MA, USAGE Healthcare, Chicago, IL, USA). For the loading control, membranes were probed with anti-alpha actinin antibody, (A7811; 1:100,000; Sigma-Aldrich, MO, USA) and then the same secondary antibody as described above.
Secondary Antibody Name: Amersham™ ECL™ anti-mouse IgG peroxidase-linked species-specific whole antibody (from sheep); Supplier Name: Cytiva Catalog Number: NA931-1ML; Clone Name: N/A; Lot Number: 17358735 and 1763873; Dilution used in western blot: 1:1000.

Validation

Statements from the manufacturer, Leica Biosystems: NCL-DYS3 is intended for the qualitative identification by light microscopy of Dystrophin (Nterminus) by immunohistochemistry. Reacts strongly with the amino terminal domain (between amino acids 321 and 494) of human dystrophin. Patient immunoreactivity indicates epitope is near exons 10 to 12. Epitope mapping suggests that sequences from amino acids 308 to 351 are involved in antibody binding. This region spans the junction of exons 9 and 10 and the epitope recognized may be part of a hinge region joining the amino domain to the central rod domain. No reactivity with DMD/BMD patients deleted for exons 10 to 12. No cross-reaction is observed with mouse (high background only), rat, rabbit, dog, chicken, hamster and pig dystrophin. QC tested on frozen tissue/cells. Tested against a previous batch and passed acceptance criteria for staining pattern and intensity (sensitivity and accuracy) and reproducibility (n=3).
Qualification of primary antibody lots at Translational Biology, Sarepta: Primary antibody NCL-DYS3 lots are bridged using our validated western blot method before use in clinical testing.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Clinicaltrials.gov identifier, NCT05096221.

Study protocol

The protocol was supplied for submission and is available upon request.

Data collection

EMBARK (Part 1) was conducted between 14 October 2021 and 13 September 2023 at 42 sites in the US, Europe and Asia (sites listed in Supplement).

Outcomes

Primary endpoint (Part 1): Change in NSAA total score from baseline to week 52.
Key secondary endpoints (Part 1): Quantity of delandistrogene moxeparvovec micro-dystrophin expression at week 12 as measured by western blot; change in TTR from the floor from baseline to week 52; change in time of 10MWR from baseline to week 52.
Other secondary endpoints (Part 1): Change in SV95C from baseline to week 52; change in time of 100MWR from baseline to week 52; change in time to ascend 4 steps from baseline to week 52; change in Patient-Reported Outcomes Measurement Information System Mobility score from baseline to week 52; change in Patient-Reported Outcomes Measurement Information System Upper Extremity score from baseline to week 52; number of skills gained or improved at week 52 as measured by the NSAA; safety of delandistrogene moxeparvovec.
Exploratory endpoints (Part 1): Change in CK from baseline over 52 weeks.
The endpoints were predefined in the SAP and protocol based on clinically important endpoints for DMD.

Plants

Seed stocks	N/A.
Novel plant genotypes	N/A.
Authentication	N/A.