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Safety, tolerability and pharmacokinetics of eteplirsen in young boys aged 6–48 months with Duchenne muscular dystrophy amenable to exon 51 skipping

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ABSTRACT

Eteplirsen is FDA-approved for the treatment of Duchenne muscular dystrophy (DMD) in exon 51 skip-amenable patients. Previous studies in boys > 4 years of age indicate eteplirsen is well tolerated and attenuates pulmonary and ambulatory decline compared with matched natural history cohorts. Here the safety, tolerability and pharmacokinetics of eteplirsen in boys aged 6–48 months is evaluated. In this open-label, multicenter, dose-escalation study (NCT03218995), boys with a confirmed mutation of the *DMD* gene amenable to exon 51 skipping (Cohort 1: aged 24–48 months, $n = 9$; Cohort 2: aged 6 to < 24 months, $n = 6$) received ascending doses (2, 4, 10, 20, 30 mg/kg) of once-weekly eteplirsen intravenously over 10 weeks, continuing at 30 mg/kg up to 96 weeks. Endpoints included safety (primary) and pharmacokinetics (secondary). All 15 participants completed the study. Eteplirsen was well tolerated with no treatment-related discontinuations, deaths or evidence of kidney toxicity. Most treatment-emergent adverse events were mild; most common were pyrexia, cough, nasopharyngitis, vomiting, and diarrhea. Eteplirsen pharmacokinetics were consistent between both cohorts and with previous clinical experience in boys with DMD > 4 years of age. These data support the safety and tolerability of eteplirsen at the approved 30-mg/kg dose in boys as young as 6 months old.

1. Introduction

Progressive and irreversible muscle damage begins at birth in boys with Duchenne muscular dystrophy (DMD) due to the absence of functional dystrophin protein [1,2]. As dystrophin-deficient muscle is mechanically unstable, it undergoes focal degeneration, stimulating release of cytokines and infiltration of immune cells. These processes lead to muscle necrosis as the regenerative capacity of muscle is overtaken and replaced with fatty and fibrotic tissue [3].

Muscle weakness, gross motor delay and difficulty in walking or running are the most common first signs and symptoms of DMD in boys < 5 years old; however, developmental delay can be evident as early as 2–3 months of age [4,5]. The current mean age of diagnosis remains delayed at 4.9 years with an average 2.5-year

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delay between first signs of DMD and diagnostic testing as a result of boys seemingly meeting developmental milestones up to the age of ≤ 7 years, albeit delayed compared to unaffected peers [6–10]. Substantial functional decline, however, may still occur even before 7 years of age in individuals with DMD, especially in those with exon 51 skip-amenable mutations [11,12]. Clinical outcomes may be improved by initiating treatment early before extensive muscle degeneration has occurred; this would allow treatments targeting unaffected muscle to have greater impact compared with later stages in the disease [13–15].

Targeted skipping of exons within the *DMD* gene using phosphorodiamidate morpholino oligomers (PMOs) can be an effective treatment approach for boys with DMD [16–18]. PMOs' strong sequence-specific binding to RNA targets alter pre-mRNA splicing to restore the reading frame and allow for production of an internally shortened but functional dystrophin protein. Pre-clinical and clinical evaluations of PMOs have demonstrated favorable, consistent and predictable safety profiles [9,16,19,20].

Eteplirsen is a PMO treatment designed to skip exon 51 of the *DMD* gene [21] and is currently approved in the United States for boys with DMD who have a confirmed genetic mutation amenable to exon 51 skipping [9,19,22]. Previous studies of eteplirsen in boys > 4 years of age indicate that it is well tolerated, has a well characterized pharmacokinetic profile and attenuates pulmonary and ambulatory decline compared with mutation-matched natural history cohorts [9,19,23–27]. Study 4658-102 (NCT03218995) is a phase 2, multicenter, open-label, dose-escalation clinical trial designed to assess safety, tolerability and pharmacokinetics of eteplirsen in boys with mutations amenable to exon 51 skipping who are 6–48 months of age. To date, this is the youngest population of boys with DMD evaluated in a clinical trial.

2. Methods

2.1. Study design

This phase 2, multicenter, open-label, dose-escalation study was conducted across 5 sites in the European Union and the United Kingdom (NCT03218995). An institutional review board or independent ethics committee at each site approved the study protocol and informed consent form prior to enrollment. This study was conducted in accordance with the ethical principles of Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained for each participant from the parent/legal guardian(s) before beginning any study-related procedures.

2.2. Participants

Eligible participants were male, 6–48 months of age, with an established clinical diagnosis of DMD and a confirmed genetic mutation amenable to exon 51 skipping. Key exclusion criteria were the use of any pharmacologic treatment that might affect muscle strength or function within 12 weeks prior to initial dosing, including growth hormone, and use of anabolic steroids or any previous or current experimental treatment.

2.3. Treatment cohorts

Participants were enrolled into 2 cohorts based on their age. Boys 24–48 months comprised Cohort 1 and those 6 to < 24 months comprised Cohort 2. Cohort 2 was enrolled only after the first 3 participants in Cohort 1 completed at least 12 intravenous (IV) infusions of eteplirsen and all available safety data were reviewed. An external Data Monitoring Committee reviewed safety data at least weekly through the first 12 weeks of dosing for the first 3 participants of each cohort and then at least quarterly thereafter.

Eligible boys received a weekly IV infusion of eteplirsen for up to 96 weeks (Fig. 1). Dose titration lasted 10 weeks overall (1 infusion/week) to slowly achieve the target dose of 30 mg/kg. Eteplirsen dosing began at 2 mg/kg for 2 weeks and was escalated to 4 mg/kg, 10 mg/kg, 20 mg/kg and 30 mg/kg. Participants were to continue to receive weekly 30 mg/kg IV infusions of eteplirsen for the duration of the study. After completing the treatment period, eligible participants were invited to consent into an open-label extension (OLE) study.

2.4. Study endpoints and assessments

The primary objective was to evaluate safety. The primary endpoints assessed were incidence of adverse events (AEs), incidence of adverse events of special interest (including infusion-related reactions [IRRs]), abnormal changes from baseline or clinically significant worsening of clinical safety laboratory abnormalities (hematology, chemistry, coagulation and urinalysis) and abnormal changes from baseline or worsening of vital signs, physical examination findings, electrocardiograms (ECGs) and echocardiograms (ECHOs). IRRs were defined as events reported with a start during or within 24 h after an infusion that were medically reviewed by a pharmacovigilance specialist and physician to determine whether they met the criteria for IRR. Renal function blood tests included creatinine, blood urea nitrogen and serum cystatin C. Abnormal renal function blood test results requiring repetition were: serum creatinine ≥ 0.3 mg/dL above baseline, urine protein to creatinine ratio ≥ 150 mg/g, urine albumin to creatinine ratio ≥ 30 mg/g, estimated glomerular filtration rate ≤ 60 mL/min/1.73 m^2, serum creatinine ≥ 1.5× upper limit of normal [ULN], elevated cystatin C > ULN, red blood cells > 1/hpf and elevated kidney injury molecule 1> ULN. A value of > 2+ for urine protein on 2 consecutive dipstick tests was considered markedly abnormal, and a 24-h urine collection needed to be undertaken to confirm any abnormal results. The criterion for proteinuria was > 500 mg/24 h. Safety endpoints were assessed regularly throughout the duration of the study.

Pharmacokinetic (PK) parameters of eteplirsen at the 2, 10, 20 and 30 mg/kg dose by population PK methods were evaluated as a secondary endpoint. Maximum plasma concentration (C_{max}), time of C_{max} (T_{max}), area under the concentration-time curve (AUC), apparent volume of distribution at steady state, clearance, elimination half-life and percentage of dose excreted in urine (Ae%) were assessed. Blood and urine sampling occurred at Weeks 2, 6, 8, 10 and 24. Serial blood samples were collected immediately prior to the end of infusion and 1–3 and 6–8 h after infusion. Urine samples were collected over the first 4 h after the start of infusion.

Of note, muscle biopsies were not part of the study protocol and, therefore, the percent of exon skipping and change in dystrophin expression could not be assessed.

2.5. Statistical analyses

There was no formal sample size calculation. The sample size was based on qualitative considerations and was sufficient to allow evaluation of safety and the estimation of PK parameters for eteplirsen in the studied population. The study was not powered to evaluate efficacy in this population. AEs, vital signs, height/length, weight, clinical chemistry, hematology, renal function, coagulation, urinalysis and ECGs were analyzed using descriptive statistics.

ECG parameters, including left ventricular ejection fraction (LVEF) and change from baseline in LVEF to each visit, were summarized by visit for each age cohort and overall. PK parameters

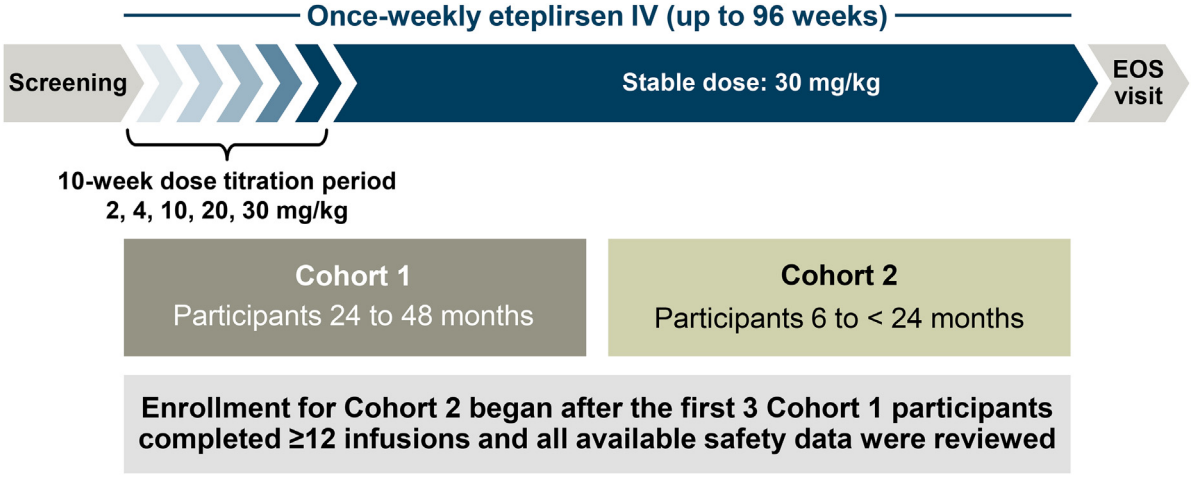

Fig. 1. Study design.

Table 1
Baseline characteristics.

Characteristic	Cohort 1 Age 24 to 48 months (n = 9)	Cohort 2 Age 6 to < 24 months (n = 6)	Total (N = 15)
Age, months	36.8 (8.2)	16.0 (7.1)	28.5 (12.9)
Height/length, cm	96.6 (6.4)	77.1 (6.1)	88.8 (11.6)
Weight, kg	16.3 (2.7)	10.6 (2.4)	14.0 (3.8)
BMI, kg/m^2	17.4 (1.8)	17.5 (1.7)	17.4 (1.7)
Mutation, n (%)			
45–50	3 (33.3)	1 (16.7)	4 (26.7)
48–50	0	1 (16.7)	1 (16.7)
49–50	2 (22.2)	2 (33.3)	4 (26.7)
50	0	1 (16.7)	1 (6.7)
52	4 (44.4)	1 (16.7)	5 (33.3)
Time since DMD diagnosis, months	12.3 (6.7)	7.8 (8.0)	10.5 (7.3)
Duration of corticosteroid use at baseline, months[a]	2.5 (1.7)[b]	0	2.5 (1.7)
Corticosteroid type, n (%)			
Deflazacort	2 (22.2)	0	2 (13.3)
Prednisone	0	0	0
No corticosteroids taken	7 (77.8)	6 (100)	13 (86.7)
Corticosteroid frequency, n (%)			
Continuous	2 (22.2)	0	2 (13.3)
Intermittent	0	0	0

Values are mean (SD) unless otherwise noted.
[a] Baseline was defined as the last value prior to the first dose of eteplirsen administration.
[b] n = 2.
BMI, body mass index. DMD, Duchenne muscular dystrophy.

for eteplirsen were calculated from plasma and urine concentration data using non-compartmental analysis, as appropriate.

For all hypothesis testing, 2-sided significance level was 0.05 with no formal adjustment for multiplicity.

3. Results

3.1. Participants and treatment exposure

There were 15 participants enrolled in the study: 9 boys in Cohort 1 and 6 boys in Cohort 2. All participants completed the study through Week 96 (Fig. 2). No participants discontinued the study. Demographic and baseline characteristics are summarized in Table 1. At baseline, median age was 28.5 months (N = 15) and mean weight was 14.0 kg. Mean time from DMD diagnosis to baseline was 10.53 months (12.33 months in Cohort 1 and 7.83 months in Cohort 2). Most boys (13/15, 86.7%) were not taking corticosteroids at baseline. For the 2 boys taking corticosteroids at baseline (both in Cohort 1), mean duration of corticosteroid use was 2.5 months. Two boys (both in Cohort 1) started steroids ~14 and 21 months after study entry, respectively.

Overall, the mean number of infusions administered was 93 (Cohort 1, 95; Cohort 2, 91). Mean number of infusions at the 30-mg/kg dose was 85. Over half of the participants, 9/15 (60.0%), had an implantable venous access device (IVAD) placed during the study (Cohort 1, 4; Cohort 2, 5). In the 4 boys from Cohort 1, the port was placed on study days 5, 65, 135 and 259. In the 5 boys from Cohort 2, the port was placed on study days 5, 13, 16 (port then removed on day 415 due to physician and family preference), 104 and 209. Mean time on eteplirsen was 96.5 weeks for both cohorts, representing a mean number of 1.85 patient-years overall.

3.2. Safety

Eteplirsen was well tolerated in boys as young as 6 months of age, with no new safety signals after 96 weeks of treatment and no discernable difference between Cohort 1 and 2. All treatment-emergent AEs (TEAEs) were mild or moderate in severity with the majority of TEAEs occurring in Cohort 1 (Table 2). All participants experienced at least 1 TEAE, with the most common (≥ 50% of participants) consistent with those commonly seen in pediatric populations: pyrexia, cough, nasopharyngitis, vomiting and diarrhea (Table 3). Treatment-related TEAEs (vomiting, localized edema, flushing), all mild in severity, were reported in 3 participants (20.0%) overall: 2 boys in Cohort 1 and 1 boy in Cohort 2. Only 1 serious TEAE, mild bronchiolitis, was reported in Cohort 2

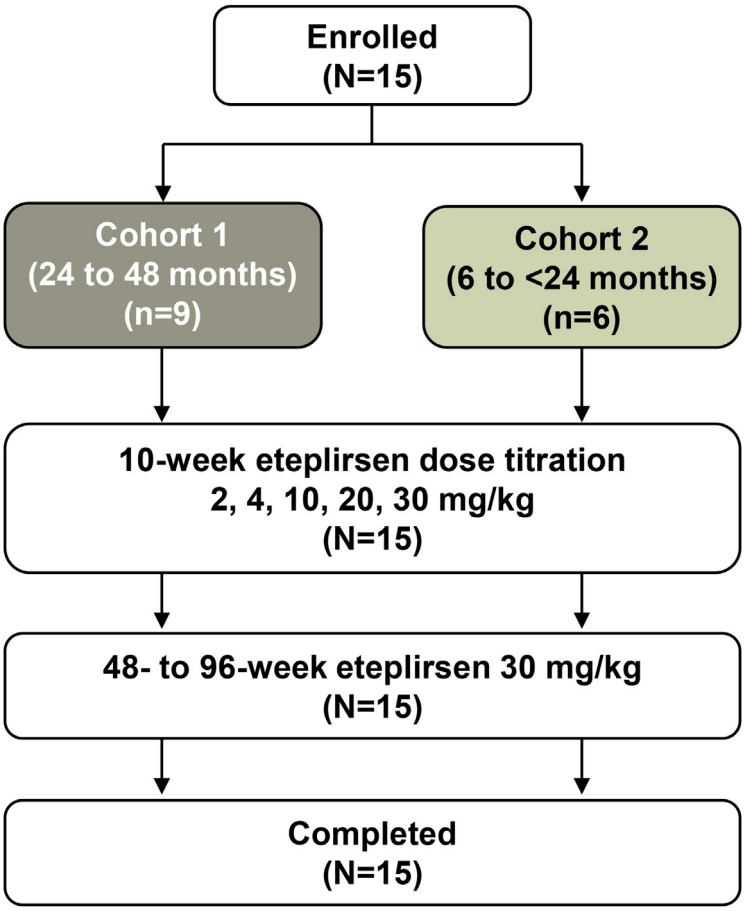

Fig. 2. Participant disposition.

Table 2
Summary of TEAEs.

Participants with ≥ 1 TEAE, n (%)	Cohort 1 Age 24 to 48 months (n = 9)	Cohort 2 Age 6 to < 24 months (n = 6)	Total (N = 15)
Any TEAE	9 (100)	6 (100)	15 (100)
TEAE related to study drug	2 (22.2)	1 (16.7)	3 (20.0)
Serious TEAE	0	1 (16.7)	1 (6.7)
Serious TEAE related to study drug	0	0	0
TEAE leading to discontinuation	0	0	0
TEAE leading to death	0	0	0
Number of TEAEs by severity			
Mild	234	165	399
Moderate	5	12	17
Severe	0	0	0

TEAE, treatment-emergent adverse event.

and was unrelated to treatment. There were no treatment-related discontinuations or deaths.

Most participants (80.0%) experienced at least 1 adjudicated IRR. All were mild in severity and most (43/44) were assessed as unrelated to study drug by the investigator. The most common IRRs (≥ 20% of participants) were rhinorrhea, diarrhea, cough, vomiting and pyrexia. Of the boys who received an IVAD port, there were no IVAD-related serious bloodstream infections reported. Four events associated with IVADs were recorded: catheter site eczema, catheter site swelling, procedural pain and dermatitis contact; all were mild in severity and unrelated to treatment and 1 was reported on the day of study infusion.

No AEs of thrombocytopenia, hepatotoxicity or anaphylaxis were reported, and no kidney toxicity or renal AEs were observed. No participants experienced a serum creatinine or cystatin C above the upper limit of normal or met the criterion for proteinuria of > 500 mg/24 h. Markedly elevated urine protein on urine dipstick was reported in 33.3% of participants overall, consistent with dipstick results in the general pediatric population [28,29]. There were no participants who discontinued or missed a dose of drug due to proteinuria or elevated urine protein during the study.

No cases of hematuria were reported. Routine laboratory monitoring of abnormal results associated with kidney toxicity yielded a single result, deemed unrelated to treatment. A boy in Cohort 2 had a low creatine clearance (59.8 mL/min) at screening and Week 24 prior to eteplirsen administration (56.9 mL/min); clearance values were otherwise normal throughout the study, and the boy completed the study.

Shifts from baseline in serum chemistry values were not clinically significant and shifts from baseline in hematology parameters were generally minimal throughout the study with no markedly abnormal shifts in platelet counts throughout the study.

Table 3
TEAEs observed in > 50% of all participants.

Preferred Term	Cohort 1 Age 24 to 48 months (n = 9)	Cohort 2 Age 6 to < 24 months (n = 6)	Total (N = 15)
≥ 1 TEAE, n (%)	9 (100)	6 (100)	15 (100)
Pyrexia	7 (77.8)	6 (100)	13 (86.7)
Cough	7 (77.8)	5 (83.3)	12 (80.0)
Nasopharyngitis	7 (77.8)	5 (83.3)	12 (80.0)
Vomiting	8 (88.9)	4 (66.7)	12 (80.0)
Diarrhea	5 (55.6)	3 (50.0)	8 (53.3)
Rhinitis	4 (44.4)	4 (66.7)	8 (53.3)

TEAE, treatment-emergent adverse event.

Table 4
Pharmacokinetic parameters.

Cohort 1 (Age 24 to 48 months)					
Parameter	2 mg/kg (Week 2)	10 mg/kg (Week 6)	20 mg/kg (Week 8)	30 mg/kg (Week 10)	30 mg/kg (Week 24)
C_{max}, µg/mL					
geometric mean	n = 8	n = 9	n = 9	n = 9	n = 8
(GCV, %)	9.67	46.5	63.3	93.7	78.2
	(75.9%)	(72.3%)	(123%)	(55.5%)	(92.2%)
T_{max}, h					
median	n = 8	n = 9	n = 9	n = 9	n = 8
(range)	0.58	0.58	0.78	0.58	0.63
	(0.17, 2.67)	(0.47, 4.25)	(0.50, 2.75)	(0.50, 1.48)	(0.42, 6.83)
AUC_{last}, µg*h/mL					
geometric mean	n = 8	n = 9	n = 9	n = 9	n = 8
(GCV, %)	13.8	56.1	92.1	119	100
	(118%)	(57.2%)	(94.7%)	(30.8%)	(42.5%)
$Ae_{0-4\ h}$, µg					
mean	n = 3	n = 7	n = 6	n = 8	n = 7
(SD)	7720	56,000	102,000	263,000	239,000
	(9060)	(73,300)	(108,000)	(209,000)	(140,000)
$Fe_{0-4\ h}$, %					
mean	n = 3	n = 7	n = 6	n = 8	n = 7
(SD)	27.2	32.2	32.1	50.9	52.5
	(33.8)	(40.9)	(31.0)	(35.2)	(33.4)
Cohort 2 (Age 6 to < 24 months)					
Parameter	2 mg/kg (Week 2)	10 mg/kg (Week 6)	20 mg/kg (Week 8)	30 mg/kg (Week 10)	30 mg/kg (Week 24)
C_{max}, µg/mL					
geometric mean	n = 5	n = 6	n = 6	n = 6	n = 6
(GCV, %)	4.22	17.2	85.0	63.8	59.7
	(120%)	(192%)	(67.6%)	(124%)	(82.7%)
T_{max}, h					
median	n = 5	n = 6	n = 6	n = 6	n = 6
(range)	0.58	0.72	0.73	0.92	0.72
	(0.42, 0.67)	(0.58, 3.32)	(0.53, 1.17)	(0.50, 2.75)	(0.58, 1.83)
AUC_{last}, µg*h/mL					
geometric mean	n = 5	n = 6	n = 6	n = 6	n = 6
(GCV, %)	6.13	27.8	81.4	85.0	89.6
	(73.1%)	(113%)	(89.6%)	(114%)	(43.8%)
$Ae_{0-4\ h}$, µg					
mean	n = 3	n = 6	n = 5	n = 6	n = 4
(SD)	1430	28,700	65,600	94,700	147,000
	(1390)	(24,100)	(47,900)	(68,500)	(132,000)
$Fe_{0-4\ h}$, %					
mean	n = 3	n = 6	n = 5	n = 6	n = 4
(SD)	6.81	29.7	35.2	33.6	45.5
	(7.07)	(27.7)	(26.6)	(27.9)	(41.4)

$Ae_{0-4\ h}$, amount of unchanged drug excreted in urine from time 0 to 4 h after completion of dosing; AUC_{last}, area under the plasma concentration-time curve from time 0 to the last quantifiable concentration, calculated by a combination of linear and logarithmic trapezoidal methods (linear up/log down method); C_{max}, maximum plasma concentration. $Fe_{0-4\ h}$, fraction of total administered drug excreted in urine as unchanged drug from time 0 to 4 h after completion of dosing, expressed as a percentage; GCV, geometric coefficient of variation; T_{max}, time of occurrence of C_{max}.

There were no trends over time of vital signs, ECGs or ECHOs that were clinically notable.

3.3. Pharmacokinetics

PK characteristics of eteplirsen were consistent between both cohorts and aligned with expectations based on clinical experience in the older population (Table 4). All 15 participants were included in the plasma PK analysis for Weeks 6, 8 and 10 (10, 20 and 30 mg/kg, respectively). Two boys were excluded from Week 2 (2 mg/kg) plasma PK analysis due to 1 sample taken from the drug infusion port instead of from the peripheral blood vessel and 1 sample being below the limit of quantification. One boy was excluded from Week 24 (30 mg/kg) plasma PK analysis due to the sample being taken out of window. T_{max} of eteplirsen was estimated to be 0.4–0.6 h post dosing, consistent across both cohorts and all dose levels. C_{max} and AUC_{last} values increased with increasing dose level through 20 mg/kg and remained similar to the PK exposure parameters at 30 mg/kg on Weeks 10 and 24. Variability was high across all dose levels, with overall geometric coefficient of variation (GCV) values ranging from 82.8% to 136% for C_{max} and 41.7% to 113% for AUC_{last}. At 30 mg/kg, eteplirsen

exposure was consistent between cohorts, with Cohort 1 C_{max} and AUC_{last} values 1.1- to 1.5-fold of those observed in Cohort 2.

All 15 participants were included in the urine PK analysis for Week 6 (10 mg/kg). PK analysis for Weeks 2, 8, 10 and 24 (2, 20, 30 and 30 mg/kg) excluded 8, 2, 1 and 3 boys, respectively, due to participants having either a missing urine volume and/or missing urine concentration value. One boy from Cohort 1 and 1 boy from Cohort 2 were excluded on Week 2 (2 mg/kg) due to incorrect timing of the sample collection and aberrant sampling that would result in deviation of PK, respectively. Urine PK parameters support that urinary excretion is time-independent and a major pathway of eteplirsen clearance. The amount excreted in the urine through 4 h post dose (Ae_{0-4h}) increased with dose, with mean percent of dose excreted (Fe_{0-4h}) ranging from 6.81% to 52.5% across all dose levels.

4. Discussion

Results of this phase 2 study provide evidence of the safety and tolerability of weekly eteplirsen 30 mg/kg infusions in boys with DMD as young as 6 months of age, with the PK profile being consistent with that seen in older boys treated with eteplirsen. A recent case report of a single 10-month-old boy treated with eteplirsen over 24 weeks also demonstrated that the drug was well tolerated with no AEs [30]. As the first study of eteplirsen treatment in boys as young as 6 months old, results contribute to the safety profile of eteplirsen across a wide range of age and disease severity [19,23,26]. Moreover, methodologic decisions that were implemented offer valuable insights for trial design in a young population of boys with DMD, and lessons learned from this trial can be optimized and applied to future trials.

There were no new safety signals up to 96 weeks following treatment initiation, and no discernable differences between age cohorts (6 to <24 months vs 24–48 months). The safety profile was consistent with that of other studies of eteplirsen [16,23,31], including no evidence of kidney toxicity. Extensive kidney testing in children in trials often yields some urine abnormalities; this testing, however, can be time consuming, resource intensive and burdensome for trial participants, and can lead to unnecessary complexity and difficulty in recruiting without adding significant value. Reassessing the need for such laborious testing may be merited for future trials. Since this study was the first to utilize a PMO in boys with DMD as young as 6 months of age, extensive kidney testing was warranted and confirmed that there was no evidence of kidney toxicity as observed in other studies of eteplirsen. Ongoing clinical trials will continue to monitor renal function with PMO treatment.

IRR is an important identified risk; most participants experienced IRRs that were non-serious, unrelated to treatment and consistent with those previously reported. Due to the nature of how IRR events were recorded, which included recording any event occurring within a 24-h window post infusion or the next calendar day if the time of AE onset or infusion start time was missing, IRR events reported in this study may be an overestimation of what can be expected in clinical practice. While the current dosing regimen of weekly IV infusions required investment in time and effort, the placement of an IVAD and the option for home dosing eased management, including during the COVID-19 pandemic. Importantly, there were no port-related serious bloodstream infections or other port-safety concerns in this very young population. As obtaining vascular access in children can be challenging [32], the favorable safety and tolerability profile surrounding the use of ports supports their utility in ensuring treatment efficiency while improving participant experience.

Assessing PK in children can be especially complicated in those < 2 years of age, who undergo the greatest changes in developmental processes (e.g., renal maturation, body weight changes) that affect drug disposition [33]. Despite this, using a sparse sampling approach from all participants, PK parameters of eteplirsen in this study were shown to be consistent with clinical studies in older boys with DMD, bridging the knowledge gap of PK in the youngest age range. A population PK model developed using plasma concentration data from eteplirsen-treated participants from this trial and previous trials in older boys with DMD (> 4 years old) further supports the comparable PK characteristics across the broad DMD population. This model demonstrated that eteplirsen exposures in the younger age groups (including ages 0.5 to < 4 years) were within the exposure range in adolescents when using the weight-based dosing regimen of 30 mg/kg that is associated with biological efficacy in older boys [34]. Of note, the data continue to support that urinary excretion is the major pathway of eteplirsen clearance.

Investigating the therapeutic effect of eteplirsen in this age group is critical given the documented early muscle damage and onset of functional delays in DMD compared with unaffected peers [5,35,36]. Treatments that target the underlying cause of the disease, such as eteplirsen, may have greater impact on preventing muscle damage early in life, resulting in less muscle mass loss. Evidence supporting this hypothesis has been gleaned from recent studies in patients with other genetic progressive muscular diseases [37], including spinal muscular atrophy, in which emerging treatments such as the splice-switching antisense oligonucleotide nusinersen, the small molecule risdiplam and the adeno-associated viral vector-based gene therapy onasemnogene abeparvovec have shown transformative therapeutic benefits when initiated early in the disease onset [38–41].

This safety study was not designed or powered to evaluate efficacy. Regardless, interpretation of any type of functional testing in this age range can be problematic due to confounding factors, including ongoing development and lack of validated efficacy assessments for this age group. In addition, specific for this trial and in line with current standard of care recommendations in this age group, the protocol did not require participants to be on standard steroid regimens prior to study entry. Furthermore, inclusion criteria allowed recruitment of children with behavioral comorbidities that may otherwise have excluded them from other studies (such as those unable to comply with functional clinical assessments). Because of this, along with the inherent challenges of conducting long assessments in a young population, there were many missing data for exploratory endpoints. Results from the OLE study and future studies can help facilitate discussions around efficacy of eteplirsen treatment for this young population.

Diagnosing young boys with DMD is challenging because clues of disease in infants and younger boys are rather unspecific, and there is insufficient awareness of the presenting symptoms of DMD among primary health care providers. Routine health checks, depending on the preventative services of each health care system, may also not be close enough in time. This results in many missed opportunities to identify early signs and symptoms of DMD. Moreover, in contrast to longitudinal studies in older children that document disease progression, the volume of natural history data for this age group is too limited to facilitate comparison with typical peer development and determination of baseline values in various assessments. Participants in this study were diagnosed at an early age because of abnormal laboratory results such as creatinine kinase elevations, family history and presence of clinical signs suggestive of muscle weakness and DMD allowing for early intervention. Timely and accurate diagnosis of DMD is a crucial aspect of care, considering the pathogenesis of DMD starts at birth [42,43]. Current recommendations emphasize the importance of an early diagnosis as it enables timely genetic counseling and assessment of carrier status [44]. Accurate diagnosis allows

mobilization of a multidisciplinary standard of care team, which has been shown to improve outcomes and survival of boys with DMD and reduce the psychological burden associated with a prolonged diagnostic odyssey [1,44-47]. As DMD is the most common childhood-onset form of muscular dystrophy, newborn screening programs have the potential to decrease the persistent diagnostic delay that is typically seen and are currently being explored [48,49]. While these programs have been limited to pilot programs, the Parent Project Muscular Dystrophy health organization in the United States submitted a nomination package to add DMD to the Recommended Uniform Screening Panel [50].

Currently, there is no other treatment for this study population that targets the underlying cause of the disease. Ataluren (PTC Therapeutics) has been approved by the European Medicines Agency but is not indicated for boys with DMD and mutations amenable to exon 51 skipping [51,52]. A recent open-label study in infants with DMD (0.4–2.4 years of age) has shown that intermittent dosing with corticosteroids (5 mg/kg per day weekends only) led to a slowing of disease progression and some reduction in safety concerns (stunting of growth, cushingoid features) compared with untreated boys [53]. Notwithstanding these findings, clear guidelines on the type, age of initiation, dose of corticosteroid treatments in DMD or their long-term benefit has yet to be established in clinical practice [1]. Moreover, their potential use relative to exon skipping therapies in this young population is also unknown. However, most participants in this study (13/15; 86.7%) were not on corticosteroids before or during the study.

The main limitation of the study is the open-label nature of the study and small sample size. Enrollment for pediatric participants is always challenging, particularly when it comes to rare diseases and in this age range; all participants enrolled, however, completed the study. The average number of infusions received was 93.1 over a 96-week period, indicating that less than 3 weekly doses were missed which bodes well for future clinical trials in this age range. Moreover, given the rarity and seriousness of the disease for which there is an urgent need to develop safe and effective therapies, the study was open label with no placebo control. This was considered the most appropriate and acceptable study design by global ethics and regulatory agencies. Of note, the unique early diagnoses of enrolled participants could also have introduced a selection bias.

This was the first clinical trial of eteplirsen in boys aged 6–48 months, the youngest population in a clinical trial to date of exon 51 skip-amenable boys with DMD. These data support the safety and tolerability of eteplirsen in boys as young as 6 months old.

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Declaration of Competing Interest

EM has received consultant fees and speaker honoraria from Sarepta Therapeutics, Inc. and, relevant for DMD, has received consultancies from Dyne Therapeutics, Sarepta, NS and PTC Therapeutics**.** **AMS** has no conflicts to disclose. **LS** has participated on advisory boards for Sarepta Therapeutics, Inc. **ND** has participated on advisory boards for Sarepta Therapeutics, Inc. **HS, WZ, XN, LE,** and **SY** are employees of Sarepta Therapeutics, Inc., and may own stock/options in the company. **FM** has received consultant fees and speaker honoraria from Sarepta Therapeutics, Inc. He is a member of the Pfizer SAB, and relevant for DMD, has received consultancies from Dyne Therapeutics, Roche and PTC Therapeutics.

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List of 4658-102 Study Sites

Country	Role	Affiliation
Belgium	**Principal Investigator**: Nicolas Deconinck **Sub Investigators:** Rudy Van Coster Arnaud Vanlander	UZ Gent NMRC - Admin Ped 3K12D Corneel Heymanslaan 10 9000 Gent
France	**08-19 to Present** **Principal Investigator**: Andreea Seferian **Sub Investigators:** Silvana De Lucia Teresa Gidaro Laura Vanden Brande **Up until 08-19** **Principal Investigator**: Laurent Servais	Hopital Trousseau, Bâtiment Lemariey, 2e étage - porte 2 i-Motion Plateforme D'Essais Cliniques Pédiatriques 26 Avenue Dr Arnold Netter, Paris, 75012
Germany	**Principal Investigator**: Janbernd Kirschner **Sub Investigator:** Sabine Borell	Universitaetsklinikum Freiburg Department of Neuropediatric and Muscular Diseases Mathildenstr. 1 Freiburg 79106
Italy	**Principal Investigator**: Eugenio Mercuri **Sub Investigators:** Claudia Brogna Marika Pane Lavinia Fanelli Giulia Norcia	Fondazione Policlinico Universitario Agostino Gemelli UOC Neuropsichiatria Infantile Largo Agostino Gemelli 8, Roma 00168
United Kingdom	**Principal Investigator**: Francesco Muntoni **Sub Investigators:** Chiara Brusa Mary Chesshyre Kate Maresh Jaqueline Pitchforth Lucia Schottlaender Mariacristina Scoto Arpana Silwal Fedrica Trucco	UCL Great Ormond Street Institute of Child Health Dubowitz Neuromuscular Centre 30 Guilford Street London WC1N 1EH

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