PERIPHERAL AND CENTRAL NERVOUS SYSTEM DRUGS
ADVISORY COMMITTEE

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LIST OF ABBREVIATIONS

Abbreviation/Term	Definition
6MWT	6-Minute Walk Test
AA	Accelerated Approval
ADR	adverse drug reaction
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AUC	area under the concentration time curve
AST	aspartate aminotransferase
aPTT	activated partial thromboplastin time
AVI-4658	eteplirsen
BL	Baseline
BLQ	below the limit of quantification
BMD	Becker muscular dystrophy
BMI	body mass index
BUN	blood urea nitrogen
CDC	Centers for Disease Control
CK	creatine kinase
CLPL	plasma clearance
Cmax	maximum concentration
CYP	cytochrome P450 enzymes
CXMD	canine X-linked muscular dystrophy
DAPC	dystrophin-associated protein complex
DMD	Duchenne muscular dystrophy
DMD gene	dystrophin gene
ECG	electrocardiogram
ECHO	echocardiogram
EDB	extensor digitorum brevis
EF	ejection fraction
EK	Egen Klassifikation
ELISPOT	enzyme-linked immunosorbent spot assay

FDA	Food and Drug Administration
FVC	forced vital capacity
GGT	gamma-glutamyltransferase
GLP	Good Laboratory Practices
hDMD	humanized DMD mouse model
HR	heart rate
ICC	interclass correlation coefficient
ICH	International Conference on Harmonisation
IHC	immunohistochemistry
IM	intramuscular, intramuscularly
IND	Investigational New Drug
INR	international normalized ratio
IV	intravenous, intravenously
LOCF	last observation carried forward
LVEF	left ventricular ejection fraction
Max	maximum
mdx	dystrophic mouse model
MEP	maximum expiratory pressure
Min	minimum
MIP	maximum inspiratory pressure
MMRM	mixed model repeated measure
MVICT	maximum voluntary isometric contraction testing
NA	not applicable
NDA	New Drug Application
NHP	non-human primates
NMRC	Neuromuscular Reference Center
N/n	number
nNOS	nitric oxide synthase
NOAEL	no observed adverse effect level
NSAA	North Star Ambulatory Assessment
PDPF	Percentage of dystrophic positive fibers
PFTs	pulmonary function tests
PK	pharmacokinetic

PMO	Phosphorodiamidate morpholino oligomer
PODCI	Pediatric Outcomes Data Collection Instrument
PPMD	Parent Project Muscular Dystrophy
PT	prothrombin time
PUL	Performance Upper Limb Scale
QMT	quantitative muscle testing
RT-PCR	reverse transcription polymerase chain reaction
SAE	serious adverse event
SAM	step activity monitor
SD	standard deviation
SOC	System Organ Class
t1/2	elimination half life
TA	tibialis anterior
TEAE	treatment emergent adverse event
ULN	upper limit of normal
United Kingdom	UK
US	United States
Vss	apparent volume of distribution at steady-state
WB	Western blot

1.   EXECUTIVE SUMMARY

Sarepta Therapeutics (Sarepta) is seeking accelerated approval (AA) for eteplirsen (administered as weekly 30 mg/kg IV infusions) for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the dystrophin gene amenable to exon 51 skipping therapy. This executive summary provides an overview of the attributes of the eteplirsen development program that specifically meet the criteria listed below as requirements for accelerated approval. A complete description of each of the clinical and nonclinical results of the development program for eteplirsen is provided in respective sections of this briefing document that follow this executive summary.

Regulatory Framework

The Food and Drug Administration Safety and Innovation Act of 2012 (FDASIA) codified FDA's accelerated approval authority. The statute provides that FDA may grant AA of a product:

- *"for a serious or life-threatening disease or condition"*

- that *"has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit, or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality, that is reasonably likely to predict an effect on clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments."*

The AA pathway means that there will be an acceptable degree of uncertainty about whether the therapy will actually result in the anticipated clinical benefit. This uncertainty is addressed by the requirement that "appropriate post-approval studies to verify and describe the predicted effect" would usually be underway at the time of approval.

This uncertainty about whether the ultimate clinical benefit will be achieved is accounted for by the requirement that a product approved under the accelerated approval program have:

- *"appropriate post-approval studies to verify and describe the predicted effect,"* which are generally referred to as confirmatory postmarketing studies. FD&C Act §506(c)(2)(A). FDA's regulations explain that at the time of accelerated approval, the *"[p]ostmarketing studies would usually be studies already underway." 21 C.F.R. §314.510.*

<u>Examples of FDA's Flexibility</u>

Historically, FDA has exercised some form of regulatory flexibility in the approval of new drugs for serious and rare conditions with unmet medical needs (Sasinowski 2015). Reports of adequate and well-controlled investigations provide the primary basis for determining whether there is "substantial evidence" to support the claims of effectiveness for new drugs. "Adequate and well-controlled studies" must have "*a design that permits a valid comparison with a control to provide a quantitative assessment of drug effect." 21 C.F.R. §314.126(b)(2)*. FDA recognizes historically controlled studies, where "*[t]he results of treatment with the test drug are compared with experience historically derived from the adequately documented natural history of the disease or condition...,"* to be "adequate and well-controlled." *21 C.F.R. §314.126(b)(2)(v)*. In

prior drug approvals, FDA has determined that studies with small numbers of patients, as well as comparison to untreated historical controls, were adequately and well-controlled and thus met the "substantial evidence" standard of effectiveness.

Examples of such regulatory precedents are as follows:

- Myozyme (alglucosidase alfa) was approved on April 28, 2006 for the treatment of patients with infantile onset Pompe disease. The approval precedent reflects the use of a natural history database to create a subgroup-matched historical control, selecting patients from the broader population with efficacy that meet certain prognostic factors (e.g., age, age of onset, documented phenotype).

- Cresemba (isavuconazonium) was approved on March 6, 2015 for the treatment of adults with invasive aspergillosis and invasive mucormycosis.

- Cholbam (cholic acid) was approved on March 17, 2015 for the treatment of bile acid synthesis disorders due to single enzyme defects (SEDs), and as adjunctive treatment of peroxisomal disorders (PDs), including Zellweger spectrum disorders.

Regulatory History: Brief Summary

The regulatory history of eteplirsen is non-traditional, in that the primary efficacy outcome results are based on a small Phase 2 study which was compared to natural history control data obtained from 2 observational studies. After multiple discussions and interactions during 2013 to 2015, FDA provided a pathway for a "fileable" NDA for accelerated approval of eteplirsen and also agreed on the design of two confirmatory studies. The flexibility demonstrated by FDA was due to the serious, fatal nature of DMD, the absence of approved therapies, evidence that the drug induces the production of de novo dystrophin and has a measurable effect on functional outcomes. Additional detail is provided in Section 3.4 and Appendix 1.

Eteplirsen Is Intended to Treat a Rare Serious Medical Condition

Duchenne Muscular Dystrophy (DMD) is a serious, progressively debilitating, and ultimately fatal inherited X-linked neuromuscular disease. DMD is caused by mutations in the dystrophin gene that disrupt the mRNA reading frame, resulting in a lack of dystrophin, a critically important part of the protein complex that connects the cytoskeletal actin of a muscle fiber to the extracellular matrix. In the absence of dystrophin, patients with DMD follow a predictable disease course. Affected boys develop muscle weakness in the first few years of life, lose the ability to walk during childhood, and usually require respiratory support by their late teens. Loss of functional abilities leads to loss of independence and increasing caregiver burden. Once lost, these abilities cannot be recovered. Despite improvements in the standard of care, such as the use of glucocorticoids, DMD is an irreversible fatal disease and patients usually die of respiratory or cardiac failure in their mid to late 20s.

The prevalence of DMD in the US is approximately 9,000 to 12,000. Approximately 13% of DMD patients have mutations of the dystrophin gene that are amenable to therapies that skip exon 51, which corresponds to ~1,300-1,900 patients in the US. Genetic testing for DMD can readily and reliably identify patients amenable to exon 51 skipping therapy.

High Unmet Medical Need in DMD despite Current Standard of Care

There are no approved therapies in the US for DMD. Current standard of care guidelines for the treatment of DMD include the administration of glucocorticoids in conjunction with palliative interventions. While glucocorticoids can delay the loss of ambulation and other correlates of disease progression, they do not sufficiently ameliorate symptoms, modify the underlying genetic defect or address the absence of functional dystrophin characteristic of DMD. Moreover, glucocorticoid use is often limited by numerous side effects. Most importantly, glucocorticoids do not alter the ultimate fatal nature of the disease course.

Eteplirsen is a Targeted Therapy Specifically Designed to Treat DMD Patients Amenable to Exon 51 Skipping

The direct cause of DMD is an absence or near absence of functional dystrophin, a critical structural protein that protects muscle from injury. Normal dystrophin links intracellular actin filaments of a muscle fiber (via N-terminus) to the cell membrane and extracellular matrix (via C-terminus) acting as a "molecular shock absorber". DMD mutations amenable to exon 51 skipping therapies impair the reading of pre-mRNA resulting in an inability to assemble the correct amino acids for construction of the C-terminus of the dystrophin protein. Eteplirsen's mechanism of action is to remove exon 51 of the pre-mRNA, thereby shifting the pre-mRNA "reading frame", enabling the reading of the remaining protein including assembly of the C-terminus. This mechanism is similar to a clinically milder form of dystrophinopathy, Becker's muscular dystrophy (BMD), where the naturally occurring mutation(s) results in a shortened central domain (missing amino acids corresponding to deleted exons), but correct in-frame reading of the pre-mRNA distal to the deleted exon(s), thus enabling assembly of the correct amino acids for construction of the C-terminus and dystrophin functionality is retained.

Eteplirsen is a Structurally Unique Phosphorodiamidate Morpholino Oligomer

Eteplirsen belongs to a distinct class of novel synthetic antisense RNA therapeutics known as Phosphorodiamidate Morpholino Oligomers (PMO). Eteplirsen's sequence of 30 nucleobases is complementary to a specific target sequence within exon 51 of dystrophin pre-mRNA. It is structurally distinct from RNA and other RNA analogues (such as 2'-O-methyl phosphorothioates) through its specific use of 6-membered morpholino rings and <u>uncharged</u> phosphorodiamidate linkages throughout the oligomer. PMOs are distinct from other classes of clinical-stage synthetic antisense oligonucleotides, which typically have a 5-membered ribose ring and <u>negatively charged</u> phosphorothioate linkages.

- The PMO backbone was designed to resist enzymatic degradation and provide stability *in vivo*.

- In nonclinical studies, minimal non-specific protein binding, likely due to the uncharged phosphorodiamidate linkages, is observed.

Pivotal Study 201/202 Design

Study 201 is a completed double-blind, placebo-controlled study of eteplirsen in 12 ambulatory boys with DMD mutations amenable to exon 51 skipping. Eligible patients were randomized to receive weekly IV infusions of 30 mg/kg (N = 4), 50 mg/kg eteplirsen (N = 4) or placebo (N = 4) for the first 24 weeks.

- Following completion of the 24-week double-blind, placebo-controlled treatment period, the 4 patients originally randomized to placebo rolled over to open-label eteplirsen of 30 mg/kg (N = 2) or 50 mg/kg (N = 2) at Week 25. All 12 patients continued receiving weekly eteplirsen in the ongoing extension Study 202 for up to 3 years.

- The primary clinical endpoint of the single arm 202 study was the 6MWT, and % dystrophin-positive fibers was the primary pharmacodynamic endpoint. Clinical outcomes of North Star Ambulatory Assessment (NSAA), pulmonary function tests (PFT), loss of ambulation (LOA), and other functional measures were supportive. Clinical outcomes have been collected through Week 168 for the 12 patients enrolled in Studies 201/202.

- Given the relatively short 24-week placebo control period, the FDA requested that Sarepta obtain external control data from DMD registries with longitudinal 6MWT data. After partnering with leading DMD experts, Sarepta identified 12 international DMD registries with extensive clinical data. Of these, 2 registries were selected based on the availability of longitudinal 6MWT data for external control comparisons.

- The external control groups were comparable to the eteplirsen treated boys in terms of the key prognostic factors for DMD progression used as inclusion criteria for Study 201/202, baseline age, 6MWT and representation of genetic subtype; 2 external control groups were identified:

  - A highly comparable group with DMD mutations amenable to exon 51 skipping therapy (N = 13) (primary external control group)

  - A larger group of boys with DMD mutations amenable to any kind of exon skipping therapy (N = 50) (secondary external control group)

The comparison of clinical outcomes versus untreated External Control data on 6MWT and additional supportive endpoints is the basis for eteplirsen's submission to the FDA.

Eteplirsen Treatment Demonstrates an Effect on the "Intermediate" Clinical Endpoint 6MWT – That Is Reasonably Likely to Predict a Clinical Benefit

<u>**6 Minute Walk Test**</u>

Given the pivotal role of ambulation in daily human function and the impact of its inevitable loss in DMD, the 6MWT was agreed upon with FDA as the "intermediate" clinical efficacy outcome for Accelerated Approval. As the majority of the 6MWT data from eteplirsen was from a single arm study, FDA requested that data from external DMD registries be obtained for comparison of the long-term clinical outcomes; primarily the 168-week longitudinal 6MWT data from the eteplirsen 202 Phase 2b study. The pre-specified criteria for external control selection paralleled the Study 201/202 inclusion criteria which selected for a relatively homogeneous population that would be expected to decline on the 6MWT. Of note, in both Study 201/202 and the external registries, the 6MWT was conducted in a standardized manner according to international guidelines.

> **There was a high degree of homogeneity and comparability between eteplirsen and
> untreated external control patients for baseline characteristics including age, 6MWT and
> representation of DMD genetic subtypes. Moreover, uniformity of DMD care between the
> 2 patient groups was shown with high compliance to international DMD care guidelines,
> including the longitudinal use of steroids, physical therapy and orthotic devices.**

Eteplirsen treated patients from pivotal Study 201/202 (N = 12) demonstrated a durable and large
magnitude of effect, a 151 meter advantage, over the course of 3 years, when compared to a
highly comparable external control (EC) of similarly aged untreated boys with DMD mutations
amenable to exon 51 skipping (N = 13). This treatment effect manifested in a divergence of the
trajectory of disease following the first year of eteplirsen therapy.
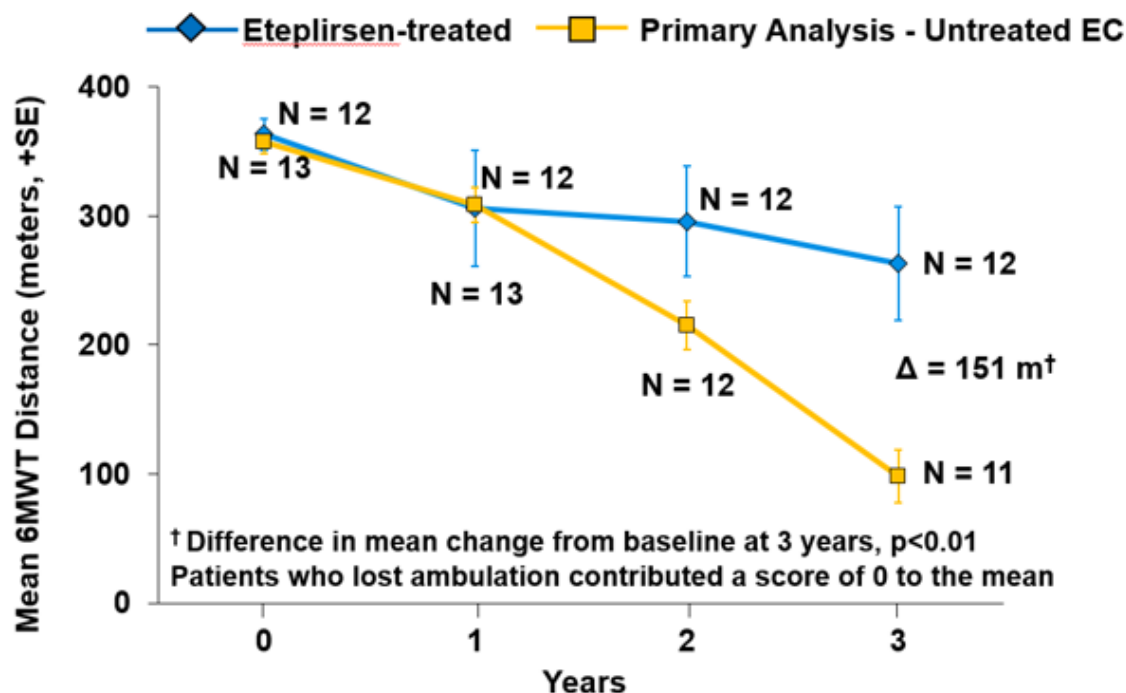
When compared to a larger external control group of DMD patients amenable to any exon
skipping therapy (N = 50), there was a substantive advantage of 79 meters for patients treated
with eteplirsen at Year 3. This larger group of DMD patients provides a more conservative
comparison, which is representative of the broader DMD patient population amenable to exon
skipping.

A series of sensitivity analyses of the 6MWT results accounting for covariates of age and
baseline 6MWT consistently demonstrated a clinically relevant advantage for the eteplirsen
treated group compared to the external control patients with exon 51 skippable mutations.
Nominal p values associated with the sensitivity analyses continued to be significant.

> **The 151 meter difference between eteplirsen and external control exon 51 patients
> (N = 13) is clinically significant and statistically persuasive.**

Eteplirsen Treatment Demonstrates an Effect on a Potential Surrogate Endpoint (dystrophin) - That Is Reasonably Likely to Predict a Clinical Benefit

Novel Dystrophin Production

Eteplirsen mechanism of action has been established via RT-PCR testing and sequencing which showed exon skipping and also the presence of internally shortened dystrophin mRNA in all eteplirsen-treated patients evaluated to date.

Significant dystrophin production was demonstrated as early as Week 24 of pivotal Study 201 with increased levels of percent dystrophin positive muscle fibers (PDPF) and dystrophin intensity, compared to pre-treatment values. Moreover, the 24-week findings were confirmed by an independent review by 3 pathologists.

Sustained dystrophin production was shown through Week 180 by three assays, with complementary detection techniques each showing significant increases in dystrophin including percent dystrophin positive fibers, dystrophin intensity by fluorescence and Western Blot. The methodology for dystrophin testing was developed in consultation with FDA.

Week 180 Dystrophin Assays	Untreated (Mean % Dystrophin of Normal)	Treated (Mean % Dystrophin of Normal)	Difference of Means (Treated vs. Untreated)	P-Value
PDPF	1.12%	17.39%	+16.27%	<0.001
Intensity	9.41%	22.61%	+13.20%	<0.001
Western Blot	0.08%	0.93%	+0.85%	<0.007

Supportive Endpoints of Loss of Ambulation, NSAA and PFTs Consistent with 6MWT

Loss of Ambulation

Ambulatory compromise and loss of ambulation are hallmarks of the progressive muscle degeneration characteristic of DMD. Once confined to a wheelchair, other symptoms tend to follow in rapid succession. Consistent with results of the 6MWT, fewer eteplirsen treated boys lost ambulation (2/12; 16.7%) compared to the external control patients who were amenable to exon 51 skipping therapy (6/13; 46.2%) or compared to the external cohort of boys with mutations amenable to any exon 51 skipping (18/50; 36%).

Northstar Ambulatory Assessment (NSAA)

The NSAA is a clinician-reported outcome instrument specifically designed to measure function in ambulatory patients with DMD. The 17-items are scored on an ordinal scale and include assessments of abilities such as rising from the floor, climbing and descending a step, 10-meter walk/run and lifting the head. In both Studies 201/202 and the external Italian Telethon registry, the NSAA was performed according to standardized published methods.

In the comparison of baseline NSAA, both the eteplirsen treated patients and untreated external control patients (exon 51) had impaired and/or lost functional abilities with a total score of 24.9 and 22.0 out of a possible 34 total score. Over the first year, both the eteplirsen treated boys and the Italian Telethon group declined in NSAA function. However, following Year 1, the decline in function for the eteplirsen group became slower and by the end of Year 3 there was a

~3 point greater decline for the untreated boys. The 3-point difference is of clinical relevance and may represent loss or impairment of up to 3 activities.

Rise Time

The ability to rise from supine is a critical activity for DMD patients and it is one of the early abilities to be lost in DMD and is predictive of loss of ambulation. In the analysis comparing the ability to rise from a supine position (without external support) 92% vs 88% of eteplirsen treated vs Italian Telethon patients had this ability at baseline. However at the end of Year 3, 55% of eteplirsen treated boys had maintained the ability to rise whereas only 14% of the Italian Telethon patients maintained this ability.

Pulmonary Function Tests

Respiratory function in DMD is progressively impaired over time as the dystrophic process affects respiratory muscles, including the diaphragm leading to significant morbidity and mortality. Pulmonary function data from DMD patients who received eteplirsen for approximately 3 years were compared to patient level data. Eteplirsen treated boys had slower deterioration of respiratory muscle function as measured by FVC %predicted (annual decrease of 3.2%) when compared to a cohort of patients in the same age range (annual decrease of 5.8%). Additionally, based on review of published literature MEP %predicted and MIP %predicted may also decline more slowly with eteplirsen treatment, although the comparison is limited.

Safety

The safety profile of eteplirsen has been characterized in 114 patients with DMD amenable to exon 51 skipping therapies, including 88 patients who have received doses of 30 mg/kg or higher. Safety monitoring has included frequently scheduled clinical and/or laboratory assessments for infusion reactions, renal toxicity, hepatotoxicity, coagulopathy, and cutaneous and cardiac-related events with no apparent signal for significant safety risks.

The drug is well tolerated as evidenced by the low rates of serious or severe or adverse events leading to discontinuation of study drug.

- The favorable tolerability of eteplirsen is demonstrated by low rates of treatment emergent SAEs (N = 2; 1.8%) and AEs resulting in study drug discontinuation (N = 1; 0.9%).

- The most common (≥10% of patients) TEAEs occurring in patients treated with eteplirsen at the clinical dose of ≥30 mg/kg and observed at higher rates than reported for placebo were headache, vomiting, cough, procedural pain, upper respiratory infection, arthralgia, contusion, excoriation, nasopharyngitis, and nasal congestion.

  – The majority of events were mild and resolved with ongoing study drug.

  – Many events may be reflective of the types of conditions that occur in a pediatric population with DMD.

- Three mild events were considered potential adverse drug reactions due to the temporal relationship with eteplirsen administration: erythema, flushing, and mild temperature elevation.

- Adverse Events of Special Interest (i.e., cardiac, renal, hepatic, coagulopathy, infusion and cutaneous reactions, and leukopenia) and related safety laboratory parameters were reviewed and no apparent safety signal was detected.

Favorable Benefit Risk Profile of Eteplirsen

The favorable benefit of eteplirsen is demonstrated by multiple endpoints presenting both clinical and pharmacodynamics evidence showing that eteplirsen offers boys with DMD (who are amenable to exon 51 skipping) an effective treatment.

- Eteplirsen mechanism of action and production of novel dystrophin has been established by pharmacodynamic data from 36 patients across 4 studies;
  - Significant differences in dystrophin production when compared to baseline or untreated controls as early as Week 24 and sustained through Week 180 using methodology developed in collaboration with FDA
- Primary endpoint of 6MWT (intermediate endpoint for accelerated approval) demonstrates that eteplirsen offers boys a significantly slower rate of decline in ambulation, endurance, and muscle function as measured by the 6MWT over a 3-year treatment period compared to highly comparable untreated external controls.
  - Large and significant effect compared to external control of 151 meters
  - Importantly, fewer eteplirsen treated boys experienced loss of ambulation
- Other supportive endpoints are directionally consistent with results of the 6MWT, including NSAA and pulmonary function test.

This clinical benefit is accompanied by a safety profile that indicates that eteplirsen is well tolerated with no apparent signal of significant safety risks. Although the safety dataset of 114 patients may not detect very rare events and therefore carries the potential risk of uncertainty, this needs to be weighed against the certainty of relentless disease progression and premature death for boys with DMD. Continued safety surveillance of ongoing clinical trials, post-marketing pharmacovigilance, along with a planned DMD registry, will provide an evolving understanding of the safety profile of eteplirsen.

Confirmatory studies to verify the clinical benefit of eteplirsen are underway

Sarepta is committed to the completion of confirmatory trials that will not only verify the clinical benefit of eteplirsen using the 6MWT and other functional endpoints, but will also contribute to the safety profile of eteplirsen.

Furthermore, Sarepta is not only committed to confirming the efficacy of eteplirsen but also understanding its PMOs, and expanding the development of the PMO platform to address other less common types of genetic variants of DMD, with a planned study in boys with DMD with genetic mutations amenable to exon 45 and exon 53 skipping therapies.

2.   OVERVIEW OF DUCHENNE MUSCULAR DYSTROPHY (DMD)

2.1.   Onset and Progression

Duchenne muscular dystrophy (DMD) is a rare, serious, life threatening, degenerative neuromuscular disease with a recessive X-linked inheritance. Caused by mutations in the dystrophin gene, DMD is characterized by the absence, or near absence, of functional dystrophin protein, leading to relentlessly progressive deterioration of skeletal muscle function from early childhood, and premature death, usually by 30 years of age.

The progression of DMD follows a predictable course. Biochemical and molecular evidence of myofiber membrane instability are typically evident from shortly after birth (Chen 2005); however, clinical manifestations of ongoing muscle damage are usually obscured by otherwise normal growth and maturation during infancy. In fact, initial symptoms of DMD are often not reported until 2-3 years of age, with patients being diagnosed, on average at approximately 4 to 5 years of age (Bushby 2010a; Ciafaloni 2009; van Ruiten 2014). Initial symptoms of DMD most often include waddling gait, toe walking, falls, and delayed speech (Ciafaloni 2009; van Ruiten 2014). Compared with healthy, same-age peers, the achievement of motor milestones in patients with DMD is delayed, and performance on tests of motor function, such as timed function tests, is markedly impaired (Beenakker 2005; McDonald 2010a; McDonald 2010b).

Functional improvements due to natural growth are observed heterogeneously in boys younger than age 7, until the characteristic degeneration and loss of muscle tissue outpaces maturational development and physical growth (McDonald 2010b, Mazzone 2013). At 7 years of age the disease trajectory for DMD has been observed to decline in a relentless and progressively precipitous fashion. Once this threshold is crossed, disease trajectory is predictively negative and 6MWD decreases more rapidly each year (Mendell 2015; Mazzone 2013; Pane 2014b). At this time, DMD boys who were steadily gaining in physical function, albeit at a slower rate than their healthy age-matched peers, begin a progressive decline. This age dichotomy is supported by literature; in a 3 year longitudinal dataset boys who entered into observation prior to age 7 demonstrated improvement for the first two years, with decline observed by the third year when the mean age of the cohort was over 8 years (Pane 2014b; Mendell 2015).

By 8 years of age, most DMD patients lose the ability to rise from the floor and climb stairs, have an increasingly labored gait, and often fall while walking. By 10 to 14 years of age, most have become wheelchair dependent. Once confined to a wheelchair, other symptoms tend to follow in rapid succession. There is gradual loss of upper limb, trunk, and neck function, such that self- grooming, toileting, bathing, dressing, unsupported sitting, and eating become impaired or impossible, severely affecting patient quality of life, as well as that of caregiver and families (Bendixen 2012; Bendixen 2014; Buyse 2012; Buyse 2015; Hahn 1997; Magliano 2014; Uzark 2012).

While few, if any, respiratory symptoms have been reported in the earliest stages of DMD, data from recent natural history studies in patients with DMD suggest that from the time pulmonary function testing (PFT) is first performed, usually at the ages of 4 or 5 years, percent predicted values for forced vital capacity (FVC) and maximum inspiratory (MIP) and expiratory (MEP) pressures decline (Khirani 2014; Mayer 2015). Diaphragmatic muscles progressively weaken

during adolescence, and patients often require ventilation support in their mid to late teens (Bushby 2010a; Bushby 2010b).

There is also an increased risk of cardiomyopathy with DMD (Thomas 2012), which usually manifests after 10 years of age as dilated cardiomyopathy with reduced left ventricular ejection fraction. The prevalence of cardiomyopathy has been shown to increase with age and disease progression, with 10% to 20% of patients affected between 6 and 13 years of age and over 60% of patients ≥18 years affected (Spurney 2014). Historically, patients with DMD died from respiratory or cardiac failure in their late teens or early 20s (Brooke 1989; Eagle 2002). Although recent studies suggest that use of ventilation support, steroids, surgery, diet and other supportive measures may increase life span by several years (Kohler 2009; Bushby 2010a; Bushby 2010b; Moxley 2010), DMD remains fatal by early adulthood.

2.2. Diagnosis and Determination of Mutation

Historically, diagnosis of DMD had to be confirmed by muscle biopsy; however, genetic testing for DMD has become a common part of the diagnostic process in treatment centers in the US and Europe, thereby reducing the need for muscle biopsies. The use of newer methods of testing, such as next generation sequencing, has greatly improved the sensitivity and accuracy of genetic testing for DMD and ensures that patients amenable to exon 51 skipping can be readily and reliably identified (Wei 2014; Bovolenta 2012). Importantly, in the US, even patients and families lacking or having insufficient insurance coverage are able to access genetic testing for DMD at no cost through the "Decode Duchenne" program, which was launched by Parent Project Muscular Dystrophy (PPMD).

2.3. Current Treatments for DMD and Unmet Medical Need

There is no approved therapy for DMD in the US. Currently, uniform standard of care guidelines for treatment of patients with DMD in the US and Europe include the administration of glucocorticoids in conjunction with nutritional, orthopedic, respiratory, cardiac, pain, psychosocial, and other palliative interventions (Bushby 2010a; Bushby 2010b). Aside from glucocorticoids, none of these interventions have been shown to impact loss of ambulation. Although glucocorticoids can delay the loss of ambulation as well as the onset of respiratory dependence, scoliosis, and cardiomyopathy (Beenakker 2005; Biggar 2006; Pradhan 2006; Manzur 2009; Schram 2013; Henricson 2013a), they do not sufficiently ameliorate symptoms or address the underlying genetic mutation and lack of functional dystrophin. Moreover, glucocorticoid use is often limited by numerous side effects, including weight gain, behavioral changes, hypertension, hyperglycemia and osteoporosis. Thus, there remains a high unmet medical need for treatments in patients with DMD.

2.4. Epidemiology

The worldwide incidence of DMD is 1 in 3,500-5,000 newborn boys, irrespective of geographical region, race, or population density (Zaharieva 2013; Mendell 2012; Moat 2013). The prevalence of DMD in the United States (US) is estimated to be approximately 9,000 to 12,000. The most common cause of DMD is deletion mutations of one or more DMD exons, accounting for approximately two-thirds of DMD cases (Aartsma-Rus 2009; Bladen 2015). Approximately 13% of all DMD patients have mutations amenable to therapies that skip

exon 51, corresponding to approximately 1,300-1,900 patients in the US who would potentially benefit from exon 51 skipping therapy (Figure 1). Another 16% percent have mutations amenable to treatment by skipping exons 45 (8%) and 53 (8%), and an additional 51% have mutations amenable to treatment by skipping other exons. Thus, hypothetically, exon skipping PMO therapies could potentially address treatment needs for approximately 80% of all DMD mutations.

Figure 1:   **US Prevalence of Patients with Exon 51 Skippable Deletions and other DMD Mutations**

- ◆ 9,000 to 12,000 boys with DMD

- ◆ Exon 51 skipping amenable DMD patients: ~1,300 to 1,900 boys

Exon 51 ~13%
Non-skippable Mutations ~20%
Exon 45 ~8%
Exon 53 ~8%
Other Skippable Exons ~51%
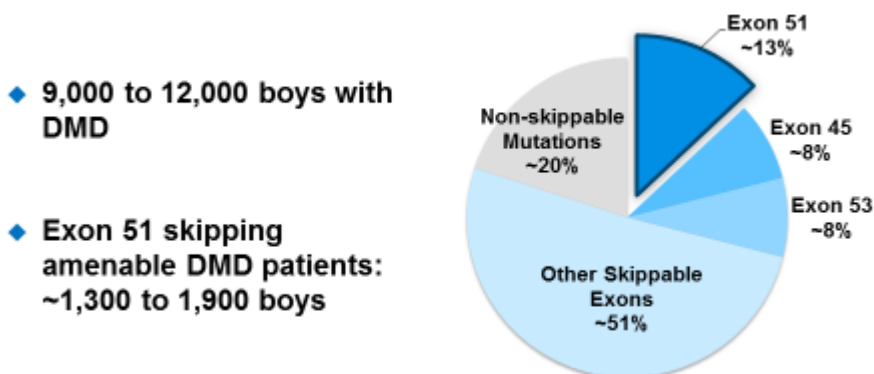
Source: Adapted from Aartsma-Rus 2009.

2.5.   Pathophysiology and Role of Dystrophin

2.5.1.   Dystrophin in Normal Muscle

Dystrophin is a low abundance (<0.1%) protein in muscle tissue with a slow translation time (~16h) and low turnover (half-life of ~2 months) (Wu 2012; Tennyson 1995; Hoffman 1987).

Dystrophin is a critical structural protein that protects muscle from strain-induced injury. Often referred to as a "molecular shock absorber", dystrophin links the intracellular actin filaments of a muscle fiber to the cell membrane and surrounding extracellular matrix through its interaction with the dystrophin-associated protein complex (DAPC). Dystrophin binds directly to cytoplasmic actin through its *N-terminal actin-binding domain* and localizes to the sarcolemma and the DAPC via its *C-terminal dystroglycan binding domain* (Figure 2). Together, dystrophin and the other components of the DAPC protect muscle from the forces of repeated contraction and relaxation (Kobayashi 2012).

Figure 2:   The DAPC in Normal Muscle
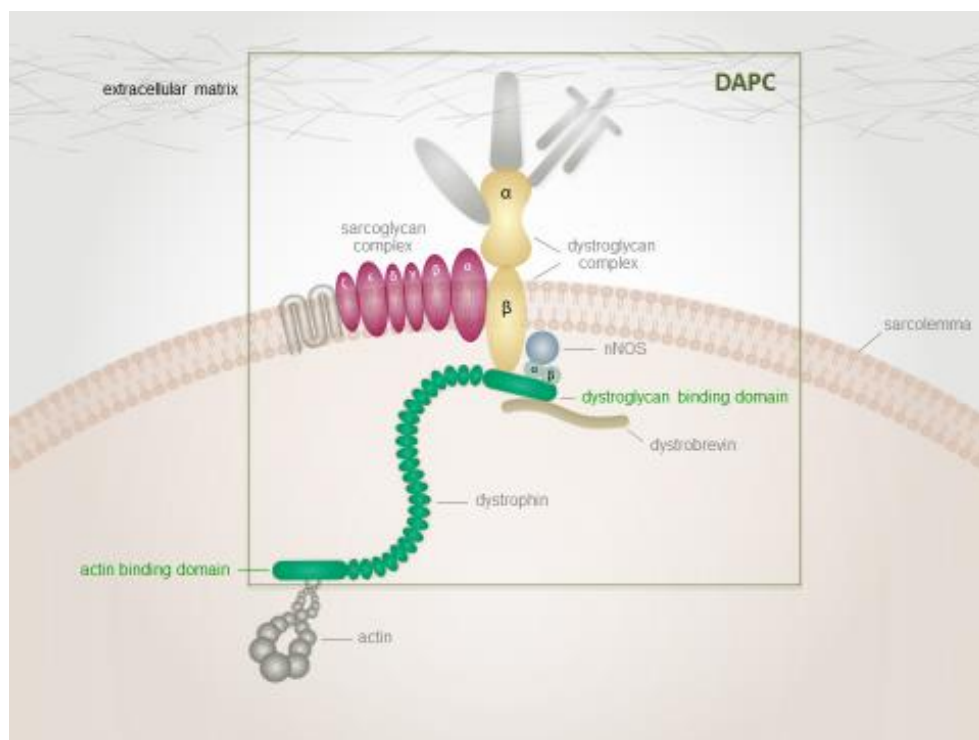
Abbreviations: DAPC = dystrophin associated protein complex.
Adapted from Kobayashi 2012.

2.5.2.   Dystrophin Protein in DMD and BMD

Mutations in the gene encoding dystrophin give rise to a spectrum of neuromuscular disorders called dystrophinopathies. The most common mutations are whole exon deletions, which, depending on the exon(s) deleted, result in severe and fatal DMD or the significantly milder dystrophinopathy, Becker muscular dystrophy (BMD).

Whole exon deletions that disrupt the mRNA reading frame, also referred to as "out-of-frame deletions" are the primary cause of DMD. Out-of-frame mutations prevent translation of functional dystrophin protein downstream of the mutation, creating an unstable protein lacking a *C-terminal dystroglycan binding domain* (Figure 3A). The absence of functional dystrophin prevents the connection between the intracellular cytoskeleton and the cell membrane leading to repeated cycles of cellular inflammation, degeneration, and cumulative damage to muscle. Over the clinical course of DMD, the inherent ability of muscle cells to repair and regenerate is exhausted and muscle is progressively replaced by fibrotic tissue and fat (Blake 2002; Emery 2002).

In contrast, whole exon deletions that <u>do not</u> disrupt the mRNA reading frame, also referred to as "in-frame deletions", are usually associated with BMD. Such mutations result in a dystrophin protein missing amino acids in the central domain of the dystrophin protein; however, the C- and N-terminal binding domains are retained. (Figure 3B). Due to this preservation of functional dystrophin, BMD patients generally have a much later onset of symptoms, a milder and slower

disease course, and a near normal life expectancy (McDonald 1995; Bushby 1993a; Bushby 1993b; Kaspar 2009).

Figure 3:   The DAPC in DMD (3a) and BMD (3b) Muscle

3a
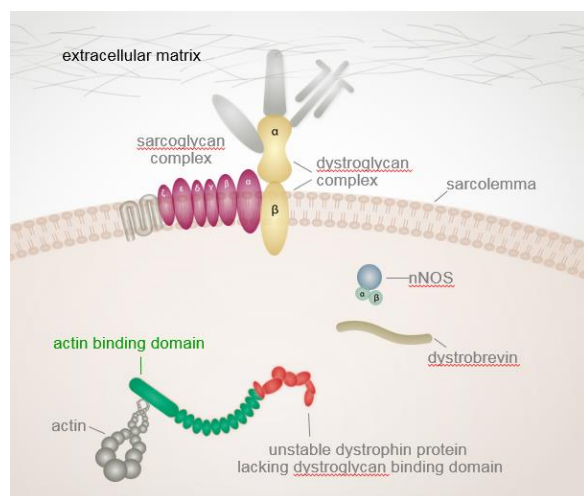
3b
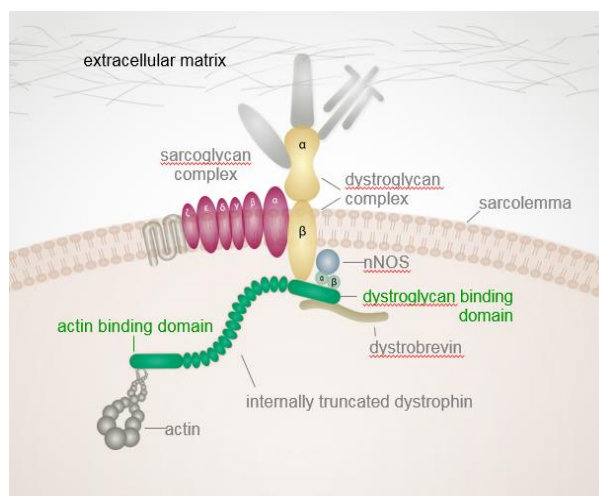
Adapted from Kobayashi 2012.

2.5.3.   Relationship of Dystrophin to DMD and BMD Severity

The clinical literature has not established a linear relationship between dystrophin expression and clinical course of dystrophinopathies (Hoffman 1988; Bushby 1993b; Bushby 1992; Taylor 2012; Nicholson 1993; Hoffman 1989; Anthony 2014; van den Bergen 2014; Goldberg 1998; Lenk 1996). A review of publications identified 82 DMD and 137 BMD patients with sufficient clinical detail to categorize their disease course and dystrophin levels (Western blot). In BMD expression of dystrophin by Western Blot has been variable with estimates from the literature ranging from 3% to 78% of normal muscle levels (van den Bergen 2014). Dystrophin levels, while lower overall in patients with DMD, were highly variable among both patient groups, and did not appear to correlate linearly with disease severity.

One of these studies (van den Bergen 2014) evaluated 33 BMD patients whose dystrophin levels were based on biopsies of the anterior tibialis muscle. In this individual study, there was also no linear relation found between dystrophin levels and muscle strength or age at different disease milestones. This finding led the authors to posit that the presence of a relatively small amount of dystrophin may be sufficient to result in a disease course that is milder than DMD.

An example of the presence of low levels of dystrophin resulting in a milder disease course is also demonstrated by genetic variations of DMD. Many DMD patients express very low levels of dystrophin attributable to spontaneous exon skipping that occurs naturally. The sporadic muscle fibers expressing dystrophin are referred to as "revertant" fibers. In particular, patients amenable to exon 44 skipping have been shown to express higher levels of dystrophin than are typically seen in DMD patients due to the phenomenon of naturally occurring revertant muscle fibers (Anthony 2014).

Corresponding to the presence of some dystrophin, patients with DMD amenable to skipping exon 44 experience a milder disease course compared to other types of DMD (Anthony 2014). A recent study conducted in a cohort of 191 similarly aged boys with DMD (Pane 2014a) examined the relationship between DMD genotypes and distance walked on the 6-Minute Walk Test (6MWT). Patients amenable to exon 44 skipping walked further (a mean distance of 398 meters) compared to boys with DMD amenable to skipping exons 45, 51, and 53 who walked mean distances of 334, 362, and 344 meters, respectively (Figure 4). A recent study of 513 steroid treated boys with DMD also demonstrated that Exon 44 amenable patients declined at a slower rate than the overall DMD population over 24-months on the North Star Ambulatory Assessment (NSAA) (p <0.01) (Ricotti 2015).

Figure 4:   Comparison of 6MWT Performance in Patients with DMD Mutations Amenable to Exon Skipping
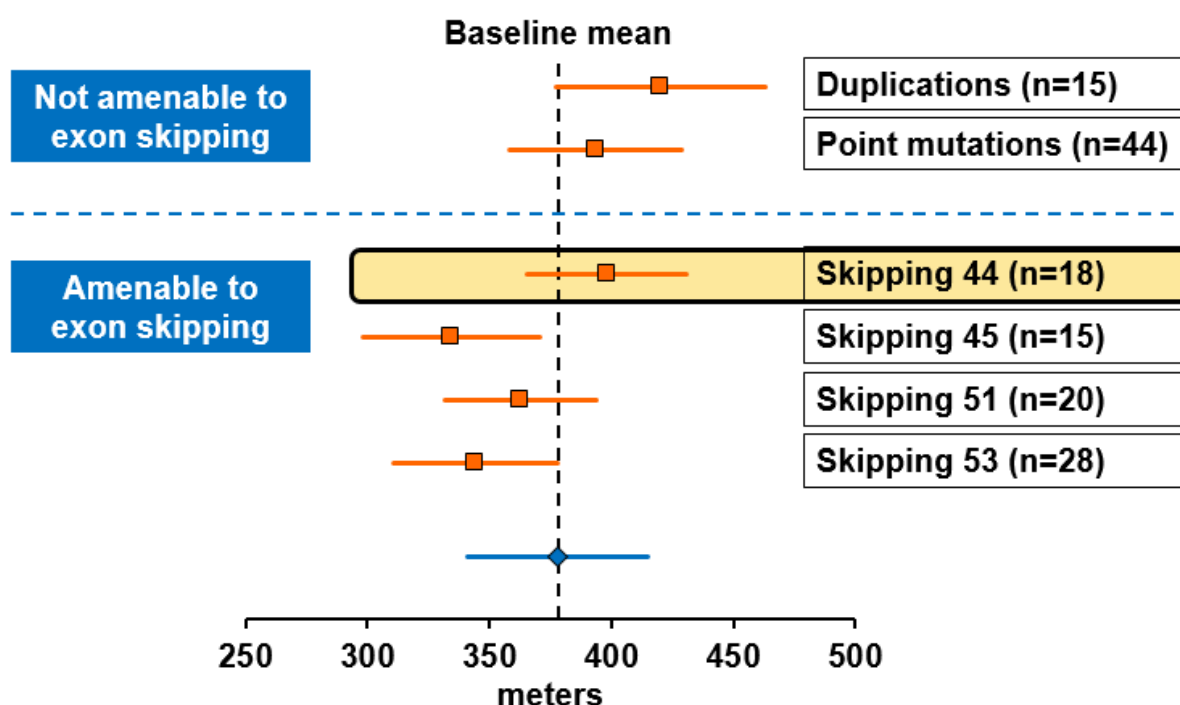
Source: Adapted from: Pane 2014a. Figure shows mean distance walked on the 6MWT in patients with different DMD mutations relative to the mean distance walked by the whole cohort.

In summary, published literature indicates that the presence of functional dystrophin results in milder disease course in patients with BMD and DMD mutations amenable to exon 44 skipping. Although a linear correlation between the amount of dystrophin and clinical course has not been established, it is clear that the presence of some dystrophin results in disease amelioration.

3.   ETEPLIRSEN DEVELOPMENT

3.1.   Background Information on Eteplirsen Injection

Eteplirsen belongs to a distinct class of novel synthetic antisense RNA therapeutics called Phosphorodiamidate Morpholino Oligomers (PMO), which are a redesign of the natural nucleic acid structure (Figure 5). PMOs offer specific clinical potential advantages that have been documented in vivo.

- PMOs incorporate modifications to the sugar ring of RNA that protect it from enzymatic degradation by nucleases in order to ensure stability in vivo. PMOs are distinguished from natural nucleic acids and other antisense oligonucleotide classes in part through the use of 6-membered synthetic morpholino rings, which replace the 5-membered ribofuranosyl rings found in RNA, DNA and many other synthetic antisense RNA oligonucleotides.

- The uncharged phosphorodiamidate linkages specific to PMOs are considered to potentially confer reduced off-target binding to proteins. PMOs have a phosphorodiamidate linkage that links each morpholino ring instead of the <u>negatively charged</u> phosphorothioate linkage used in other clinical-stage synthetic antisense RNA oligonucleotides.

- The sequence of eteplirsen's 30 nucleobases is designed to be complementary to a specific target sequence within exon 51 of dystrophin pre-mRNA. Each morpholino ring in eteplirsen is linked to one of four heterocyclic nucleobases found in DNA (adenine, cytosine, guanine, and thymine).

Figure 5:   Phosphorodiamidate Morpholino Oligomer Structure (vs Phosphorothioate)
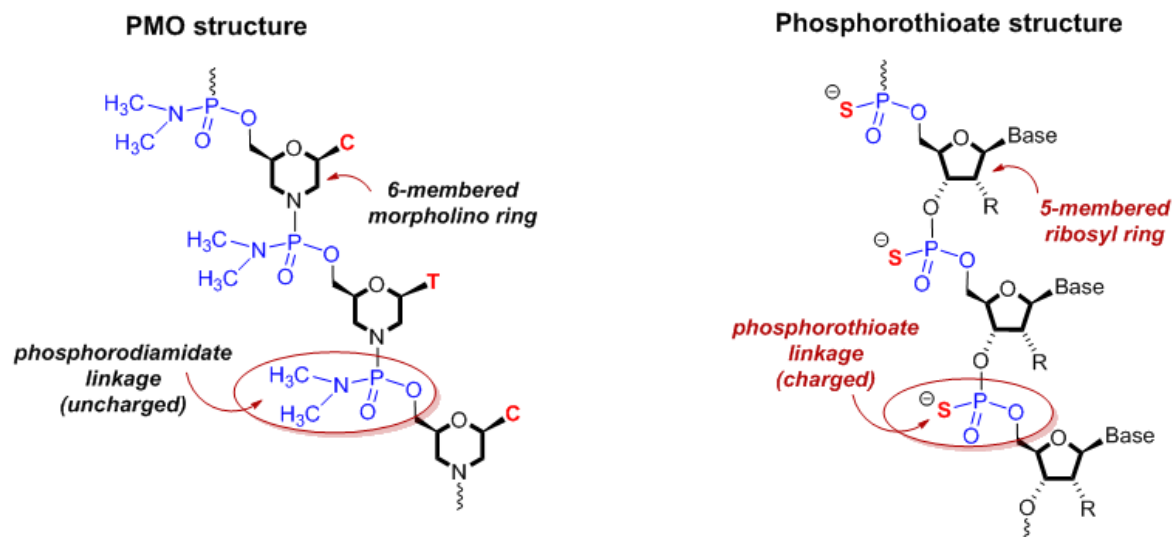
The chemical name for eteplirsen is:

RNA, [P-deoxy-P-(dimethylamino)] (2′,3′-dideoxy-2′,3′-imino-2′,3′-seco) (2′a→ 5′) (C-m^{5}U-C-C-A-A-C-A- m^{5}U-C-A-A-G-G-A-A-G-A- m^{5}U-G-G-C-A- m^{5}U- m^{5}U- m^{5}U-C- m^{5}U-A-G), 5′-[P-[4-[[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]carbonyl]-1-piperazinyl]-N,N-dimethylaminophosphonamidate]

Note "m^{5}U" stands for 5-methyluracil (i.e., thymine).

3.2.   Rationale for Development and Mechanism of Action

A potential therapeutic approach to the treatment of DMD caused by out-of-frame mutations in the *DMD* gene is suggested by the milder form of dystrophinopathy known as BMD, which is caused by in-frame mutations. The ability to convert an out-of-frame mutation to an in-frame mutation would hypothetically preserve the mRNA reading frame and produce an internally shortened yet functional dystrophin protein. Eteplirsen was designed to accomplish this.

Eteplirsen targets dystrophin pre-mRNA and induces skipping of exon 51, so it is excluded or skipped from the mature, spliced mRNA transcript. By skipping exon 51, the disrupted reading frame is restored to an in-frame mutation. While DMD is comprised of various genetic subtypes, eteplirsen was specifically designed to skip exon 51 of dystrophin pre-mRNA. DMD mutations amenable to skipping exon 51 include deletions of exons contiguous to exon 50 or exon 52, and comprise the largest subgroup of DMD patients (13%).

Eteplirsen is an antisense RNA therapeutic-targeted with a nucleobase sequence that is complementary to a specific sequence contained within exon 51 of dystrophin pre-mRNA. Hybridization of eteplirsen with the targeted pre-mRNA sequence interferes with formation of the pre-mRNA splicing complex and deletes exon 51 from the mature mRNA. The structure and conformation of eteplirsen allows for sequence-specific base pairing to the complementary sequence contained in exon 51 of dystrophin pre-mRNA as illustrated by Figure 6.

Figure 6:   Eteplirsen binding to Dystrophin pre-mRNA via Watson-Crick Base Pairing
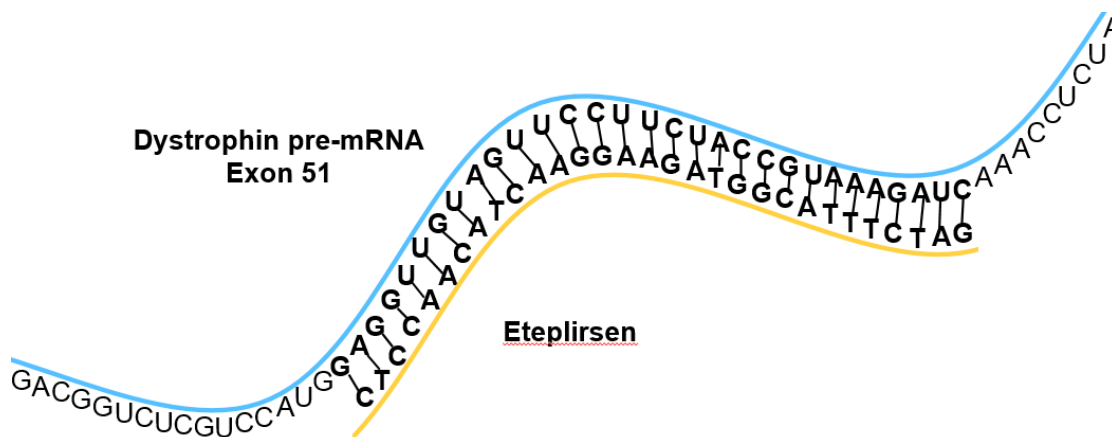
Restoration of the Dystrophin Reading Frame Using Exon Skipping

Normal dystrophin mRNA containing all 79 exons will produce normal dystrophin protein. The graphic in Figure 7 depicts a small section of the dystrophin pre-mRNA and mature mRNA, from exon 47 to exon 53. The shape of each exon depicts how codons are split between exons. Rectangular shaped exons start and end with a complete codon. Arrow shaped exons start with a complete codon but end with a split codon, with only the first nucleotide of a codon. The chevron shaped exons start with an incomplete codon, with the second two nucleotides of the codon it splits with the previous, arrow shaped, exon (see Appendix 17 for additional detail).

Figure 7:   **Depiction of Section of Normal Dystrophin Pre-mRNA**

Dystrophin mRNA missing whole exons from the dystrophin gene typically result in DMD. The graphic in Figure 8 illustrates a type of genetic mutation (deletion of exon 50) that is known to result in DMD. Since exon 49 ends in a complete codon and exon 51 begins with the second nucleotide of a codon, the reading frame after exon 49 is shifted, resulting in out-of-frame mRNA reading frame and incorporation of incorrect amino acids downstream from the mutation. The subsequent absence of a functional C-terminal dystroglycan binding domain results in production of an unstable dystrophin protein.

Figure 8:   **Depiction of section of Abnormal Dystrophin pre-mRNA (example of DMD)**

Eteplirsen skips exon 51 to restore the mRNA reading frame. Since exon 49 ends in a complete codon and exon 52 begins with the first nucleotide of a codon, deletion of exon 51 restores the reading frame, resulting in production of an internally-shortened dystrophin protein with an intact dystroglycan binding site, similar to an "in-frame" BMD mutation; Figure 9.

Figure 9:   **Depiction of Eteplirsen Restoration of "In-frame" reading of pre-mRNA**

Source: Adapted from Kole 2012.

3.3.   Proposed Indication, Dosing and Administration

The proposed prescribing information for eteplirsen includes the following indication:

Eteplirsen injection is indicated for the treatment of DMD in patients who have a confirmed mutation of the dystrophin gene amenable to exon 51 skipping therapy. This indication is approved based on an intermediate endpoint demonstrating delayed disease progression as measured by the 6MWT. Continued clinical benefit will be evaluated through confirmatory trials.

Eteplirsen injection is supplied in single-use, 2- and 10-mL glass vials containing 100 or 500 mg eteplirsen, respectively. The concentrated drug product is provided as a 50 mg/mL sterile, isotonic, phosphate-buffered (pH 7.5) solution without preservatives. Eteplirsen injection is diluted to 100 to 150 mL with normal saline prior to administration via intravenous (IV) infusion.

Eteplirsen at 30 mg/kg will be administered chronically (i.e., lifetime dosing) by once-weekly IV infusions between 35 to 60 minutes in duration.

3.4.   Regulatory History and Framework

Framework for Accelerated Approval of a Rare Disease or Condition

There is a new emphasis on broader use of accelerated approval to expedite patients' access to important treatments for serious and life-threatening disease conditions. This was endorsed in the September 2012 President's Council of Advisors on Science and Technology (PCAST) *Report to the President on Propelling Innovation in Drug Discovery, Development, and Evaluation*. The Report noted that "*[h]istorically, the use of Accelerated Approval has been primarily used in a limited number of therapeutic areas—principally, HIV/AIDS, cancer, and inhalation anthrax (87 percent of cases)...*" As such, the Report concluded that "*...the Nation would benefit if the FDA were to expand the use in practice of acceptable indications to other serious or life-threatening diseases*" and "*...FDA should expand the use in practice of its existing authority for Accelerated Approval.*"

Meanwhile, the Food and Drug Administration Safety and Innovation Act of 2012 (FDASIA) codified and expanded FDA's accelerated approval authority. The statute provides that FDA may grant accelerated approval of:

> …a product for a serious or life-threatening disease or condition . . . upon a determination that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit, or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality, that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments.

Federal Food, Drug, & Cosmetic Act (FD&C Act) § 506(c)(1)(A).

FDASIA clarified FDA's flexibility in administering the accelerated approval program, especially for rare, serious conditions with a high unmet medical need. The 2012 law makes clear that FDA has always had in its regulations the authority to consider all types of evidence: *"The evidence to support that an endpoint is reasonably likely to predict clinical benefit…may include epidemiological, pathophysiological, therapeutic, pharmacologic, or other evidence developed using biomarkers…or other scientific methods or tools."* FD&C Act § 506(c)(1)(B)

In addition, the new law acknowledged that fewer, smaller trials may be the basis for accelerated approval:

> …the FDA should be encouraged to implement more broadly effective processes for the expedited development and review of innovative new medicines intended to address unmet medical needs for serious or life-threatening diseases or conditions, including those for rare diseases or conditions, using a broad range of surrogate or clinical endpoints and modern scientific tools earlier in the drug development cycle when appropriate. *This may result in fewer, smaller, or shorter clinical trials* for the intended patient population or targeted subpopulation without compromising or altering the high standards of the FDA for the approval of drugs.

FDASIA § 901(a)(1)(C) (emphasis added).

Finally, the accelerated approval pathway means that there will be, at the time of accelerated approval, an acceptable degree of uncertainty about whether the therapy will actually result in the anticipated clinical benefit. This uncertainty about whether the ultimate clinical benefit will be achieved is accounted for by the requirement that a product approved under the accelerated approval program have *"appropriate postapproval studies to verify and describe the predicted effect,"* which are generally referred to as confirmatory postmarketing studies. FD&C Act § 506(c)(2)(A). FDA's regulations explain that at the time of accelerated approval, the *"[p]ostmarketing studies would usually be studies already underway."* 21 C.F.R. § 314.510.

Accelerated approval is highly relevant to eteplirsen for the treatment of Duchenne Muscular Dystrophy (DMD) because the totality of available evidence from a variety of sources allow, at the time of granting accelerated approval, "*experts qualified by scientific training and experience"* to conclude that the drug has an effect on either dystrophin (a surrogate endpoint) or 6MWT (an intermediate clinical endpoint) that is reasonably likely to be confirmed on a clinical endpoint in either one of the two planned confirmatory postmarketing studies. FDA described these two potential pathways under accelerated approval in the April 2014 meeting minutes outlined below.

Eteplirsen Regulatory History

The eteplirsen IND was submitted in August 2007 for the treatment of DMD patients with mutations amenable to exon 51 skipping therapy. Orphan Drug and Fast Track designations were also granted for eteplirsen for this indication in October and November 2007, respectively. Based on promising results observed in the Phase 1 proof of concept study (Study 33) and a 12-week dose-ranging study (Study 28) conducted in the United Kingdom from 2007 to 2010, Sarepta conducted a 28-week double-blind, placebo-controlled Phase 2 study (Study 201) in July 2011. In February 2012, an ongoing long-term Phase 2b open-label eteplirsen extension study (Study202) was initiated where all 12 patients who participated in Study 201 received eteplirsen. Study 202 has now been ongoing for approximately 4 years.

Based on the regulatory framework of FDASIA and after several meetings with the Division of Neurology Products, agreement was reached on the content of an NDA submission, primarily based on the Phase 2b dataset, under the provisions of 21 CFR §314.510 (Subpart H) regulations established for *accelerated approval* of new drugs for serious or life-threatening illnesses, and confirmatory study design.

Of note, the FDA requirements for Accelerated Approval (AA) are detailed in Table 1. In addition, "*the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments need to be taken into account. Post marketing confirmatory trials are required to verify and describe the anticipated effect on irreversible morbidity or mortality or other clinical benefit."* The eteplirsen NDA submission meets the criteria for AA.

Table 1:   Eteplirsen Meets Accelerated Approval Requirements

Characteristics	Accelerated approval Section 506 (c)	Eteplirsen pathway
Disease	Serious and life threatening Severe or rare Lack of alternative treatments	Duchenne Muscular Dystrophy
Surrogate or clinical "intermediate" endpoint	*Reasonably* likely to predict an effect on irreversible morbidity or mortality (IMM), or other clinical benefit	Intermediate primary endpoint: Six Minute Walk Test (6MWT)
Post-marketing studies	Post-marketing (PM) confirmatory trials required to verify and describe anticipated effect	Two studies agreed upon

FDA Meeting Minutes Received April 2014 (Culmination of 4 meetings held between November 8, 2013 and March 2014)

The FDA provided the following guidance:

- The FDA outlined 2 potential pathways to accelerated approval:

  1. *"Considering the 201/202 6MWT data as a finding on an "intermediate" clinical endpoint, or"*

  2. *"Using the dystrophin biomarker data as surrogate endpoint(s)"*

- For option (1), Sarepta 201/202 6MWT data would need to be compared to a matched historically-controlled DMD population similar to the eteplirsen treated patients; patient-level data for would need to be submitted for both groups. In order to minimize bias, the supportive care, such as steroid use and physical therapy, for both groups would need to be similar.

- The FDA remained "skeptical" about the persuasiveness of the existing biomarker data as it had been analyzed by a single pathologist and therefore potentially open to bias. FDA proposed a collaborative effort to better understand the methods and analyses used for these data, with the goal of applying suitable, consistent, and objective methods for measuring increases in functional dystrophin protein, which would be amenable to independent verification.

- The FDA also proposed obtaining a fourth biopsy and comparing these samples, in blinded fashion, to samples obtained from a group of treatment-naïve patients with exon 51 DMD, as a source of additional biomarker data.

Confirmatory Studies

To enable accelerated approval, the *"FDA envisioned 2 approaches to confirmatory trials:"*

  1. *"A historically-controlled trial of eteplirsen, and*

2. *A randomized, placebo-controlled trial of another PMO with the same mechanism of action, with demonstration of a correlation between dystrophin production and definitive clinical benefit on the 6MWT or another clinical measure."*

September 2014: Type B Pre-NDA Meeting

In addition to the previously agreed upon NDA content described above, the FDA required the following additional information to be included in the NDA submission:

- 3-month data from at least 12 to 24 newly exposed patients

- Individual patient-level data for the historical control patients, including rise time or similar timed function tests, baseline factors including steroids, and any ancillary care that affects physical function

- Dystrophin source images, and key analyses

- Study 201/202 Week 168 efficacy data

May 2015: Type C Pre-NDA Follow Up Meeting

In the September 2014 Pre-NDA meeting, FDA considered Sarepta's proposal for the NDA to be acceptable. FDA also noted the following points:

- Data from the Fourth Biopsy, taken at Week 180, while not required in the initial NDA, was also required to be submitted to the NDA post submission.

- To aid comparison of the Study 201/202 6MWT and NSAA data to historical controls, details of care such as steroid use, other medication use, physical therapy and pulmonary therapy needed to be obtained.

- Independent assessment of the percentage of dystrophin-positive fibers (PDPF) from Studies 201/202 and Study 28

- Review of available historical data regarding dystrophin expression and phenotype in BMD focusing on the natural history of Becker genotypes that would be created by skipping exon 51

Based on the FDA guidance received during the September 2014 and the May 2015 Pre-NDA meetings, the eteplirsen NDA was submitted on June 26, 2015 and filed on August 25, 2015. Priority Review status was also granted, requiring a 6-month review period compared to the standard 10-month review.

In addition, both confirmatory studies (Table 2) will be underway by Q1 of 2016, with the PROMOVI study almost fully enrolled.

Table 2:   **Confirmatory Studies to Support Eteplirsen Accelerated Approval**

Acronym (Protocol No.)	Study Design	Treatment	Duration
PROMOVI (4568-301)	Open-label versus concurrent untreated control	Eteplirsen	96 weeks
ESSENCE (4045-301)	Randomized, double-blind, placebo-controlled	SRP-4045 SRP-4053	48 weeks with planned open label extension

4. NONCLINICAL STUDIES

4.1. Exon Skipping Increases Dystrophin and Improves Function in Dystrophic Animals

The feasibility of ameliorating the DMD phenotype using exon skipping to restore the dystrophin mRNA open reading frame is supported by nonclinical research. Numerous studies in dystrophic animal models of DMD have shown that restoration of dystrophin by exon skipping leads to reliable improvements in muscle strength and function (Sharp 2011; Yokota 2009; Wu 2008; Wu 2011; Barton-Davis 1999; Goyenvalle 2004; Gregorevic 2006; Yue 2006; Welch 2007; Kawano 2008; Reay 2008; van Putten 2012). A compelling example of this comes from a study in which dystrophin levels following exon skipping (using a PMO) therapy were compared with muscle function in the same tissue. In dystrophic *mdx* mice, tibialis anterior (TA) muscles treated with a mouse-specific PMO maintained ~75% of their maximum force capacity after stress-inducing contractions, whereas untreated contralateral TA muscles maintained only ~25% of their maximum force capacity (p <0.05) (Sharp 2011). In another study, 3 dystrophic *CXMD* dogs received at (2-5 months of age) exon-skipping therapy using a PMO-specific for their genetic mutation once a week for 5 to 7 weeks or every other week for 22 weeks. Following exon-skipping therapy, all 3 dogs demonstrated extensive, body-wide expression of dystrophin in skeletal muscle, as well as maintained or improved ambulation (15 m running test) relative to baseline. In contrast, untreated age-matched *CXMD* dogs showed a marked decrease in ambulation over the course of the study (Yokota 2009).

PMOs were shown to have more exon skipping activity at equimolar concentrations than phosphorothioates in both mdx mice and in the humanized DMD (hDMD) mouse model, which expresses the entire human DMD transcript (Heemskirk 2009). In vitro experiments using reverse transcription polymerase chain reaction (RT-PCR) and Western blot (WB) in normal human skeletal muscle cells or muscle cells from DMD patients with different mutations amenable to exon 51 skipping identified eteplirsen as a potent inducer of exon 51 skipping. Eteplirsen-induced exon 51 skipping has been confirmed in vivo in the hDMD mouse model (Arechavala-Gomeza 2007).

4.2. Nonclinical Development of Eteplirsen

A comprehensive set of nonclinical pharmacokinetic (PK), safety pharmacology, and toxicity studies has been performed as part of eteplirsen's development.

4.2.1. Nonclinical Pharmacokinetics

Key nonclinical PK study findings include the following:

- An in vivo PK study in *mdx* mice demonstrated an apparent plasma half-life of approximately 6 hours, widespread distribution to skeletal, cardiac, and diaphragm muscles, high concentrations in kidneys and urine, and predominantly renal excretion

- Combined in vitro protein binding, cytochrome P450 enzymes (CYP) or drug transporter interactions, and hepatic microsomal metabolism study results demonstrated a low potential for drug-drug interactions for eteplirsen in humans

    – Low in vitro binding of ^{14}C-eteplirsen to human plasma proteins (6.1% to 16.5%)

    – No in vitro inhibition of the major human CYP isoenzymes tested (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) at biologically relevant concentrations (i.e., <1 mg/mL)

    – No induction of CYP2B6 or CYP3A4 and minimal induction of CYP1A2 only at high concentrations (>1 mg/mL) in human primary hepatocyte cultures

    – No interactions as either a substrate or inhibitor of key human drug transporters OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, and BSEP at biologically relevant concentrations (i.e., <1 mg/mL)

4.2.2. Renal Toxicity of Eteplirsen in Animals

Good Laboratory Practice (GLP) repeat-dose toxicity studies of eteplirsen, administered by IV injection once weekly for 12 weeks in dystrophic (*mdx*) and non-dystrophic mice, for 10 weeks in juvenile rats, and for 12 and 39 weeks in non-human primates (NHPs), demonstrated that the kidney was the main target organ. Renal findings for eteplirsen in mice and NHPs consisted of non-adverse morphological changes of multifocal, renal tubular basophilia/vacuolation, with minimal-to-slight tubular degeneration. These findings were not associated with significant changes in renal-related clinical pathology parameters (e.g., serum creatinine or urea nitrogen, urine chemistries) and the no observed adverse effect level (NOAEL) was the highest dose level tested in these species (960 mg/kg in mice and 320 mg/kg in NHPs). In juvenile rats, renal histopathology findings of marked tubular dilatation, vacuolation, and basophilia were accompanied by minimal to slight necrosis, minimal hemorrhage/interstitial inflammation, increased renal weights, increased serum creatinine/urea nitrogen, and decreased creatinine clearance at the highest dose level tested (900 mg/kg) and were considered adverse. Due to the adverse renal effects at 900 mg/kg, the NOAEL in juvenile rats was 300 mg/kg. Nevertheless, renal effects of eteplirsen in animals were less severe than those reported at lower doses for the phosphorothioate antisense oligonucleotide, drisapersen (Frazier 2014). Eteplirsen plasma exposures assessed in the juvenile rat and NHP toxicity studies were high, increased in a nearly dose-dependent manner, and were 8-fold (juvenile rats) and 28-fold (NHPs) higher than human exposures, based on plasma AUC at the NOAEL versus mean human AUC at 30 mg/kg.

4.2.3. Other Nonclinical Findings for Eteplirsen

Phosphorothioates are known to cause a number of other target organ toxicities in animals, including complement activation and pro-inflammatory effects, coagulopathies, thrombocytopenia, vascular injury, and hepatic Kuppfer cell basophilia (Levin 1998; Monteith 1999; Levin 2001; Henry 2008; Frazier 2014; Engelhardt 2015; Frazier 2015). Thorough evaluations of the developing immune system in juvenile rats, which included T cell-dependent antibody responses and immunophenotyping of peripheral blood T- and B-cell subpopulations (total/helper/cytotoxic T-cells, B-cells, and NK cells), demonstrated that eteplirsen had no adverse effect on the immune response. In addition, quantitation of the Bb, C3a, and C5a fragments of the complement

alternative pathway on Day 8 and at Weeks 13 and 39 in the chronic study showed that eteplirsen did not cause complement activation at the highest dose level tested in NHPs (320 mg/kg). Injection site reactions in repeat-dose toxicity studies were infrequent, non-adverse, and showed evidence of reversibility. There was no evidence of eteplirsen-induced thrombocytopenia or vascular injury observed in mice, juvenile rats, or NHPs after repeated high weekly doses of 960 mg/kg, 900 mg/kg, or 320 mg/kg, respectively.

A safety pharmacology study in NHPs showed that single IV injections of eteplirsen at doses up to 320 mg/kg had no adverse effects on cardiovascular, respiratory, renal, hepatic, or global neurological functional assessments. In repeat-dose studies, no morphological changes were observed in the heart, no effects on electrocardiogram parameters, including heart rate (HR) and PR, RR, QT, and QTc intervals, and no effects on coagulation parameters were detected after once-weekly IV injections of eteplirsen in NHPs at doses up to 320 mg/kg for 39 weeks.

No effects were detected on the male reproductive system in mice, juvenile rats, and NHPs in repeat-dose toxicity studies. Eteplirsen did not affect neuromuscular development in juvenile rat pups, including performance in forced swim tests (Cincinnati water maze), grip strength measurements, hind-limb splay, and motor activity, after once-weekly IV injections of doses up to 900 mg/kg. This dose level produced exposures approximately 32-fold higher than human exposures based on plasma AUC. No pathological changes in skeletal muscles or histopathological evidence of hepatic Kuppfer cell basophilia were observed in any of the repeat-dose toxicity studies conducted in mice, juvenile rats, or NHPs. Finally, there was no evidence of eteplirsen-associated mutations, chromosomal aberrations, or clastogenic potential in the International Conference on Harmonisation (ICH) standard battery of genotoxicity tests.

5.   CLINICAL PHARMACOLOGY

The eteplirsen clinical pharmacology program is currently composed of the human pharmacodynamic and pharmacokinetic data generated from 4 clinical trials (Studies 33, 28, 201, and 202) conducted in patients with DMD. In addition, nonclinical studies conducted with human biomaterials are described in Section 4.2.1.

5.1.   Pharmacokinetics

Plasma and urine samples were collected from eteplirsen-treated patients for analysis of eteplirsen levels in order to estimate PK parameters in the pivotal Studies 201/202 and supportive Study 28. Overall, the PK profile of eteplirsen was predictable and demonstrated a half-life of between 3-4 hours with the majority of eteplirsen eliminated within 24 hours and no significant accumulation in plasma observed following once weekly dosing.

In Studies 201/202 (in which patients received IV eteplirsen at doses of 30 or 50 mg/kg), the PK profile of eteplirsen was consistent across the time points assessed, and there were no notable differences in C_{max} (maximum concentration), AUC (area under the concentration time curve), $t_{1/2}$, and CL at Weeks 12 and 152, indicating no accumulation (Table 3). Overall, the 30 and 50 mg/kg/week dose levels resulted in dose proportional C_{max} and AUC. Plasma clearance (CL_{PL}), V_{ss} (apparent volume of distribution at steady state), and $t_{1/2}$ (half-life) were similar at both dose levels. Results of 24-hour urine collections in eteplirsen-treated patients showed that approximately 64% of the dose was excreted as unchanged drug at the 30 mg/kg dose level and approximately 69% at the 50 mg/kg dose level.

Table 3:   Plasma Pharmacokinetics at Weeks 12 and 152 in Studies 201/202

Study 201/202		Mean Plasma Pharmacokinetic Parameters						
Treatment Group (n)	Time Point	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-24} (hr*ng/mL)	$AUC_{0-\infty}$ (hr*ng/mL)	CL_{PL} (mL/hr/kg)	V_{ss} (mL/kg)	$t_{1/2}$ (hr)
30 mg/kg (4)	Week 12	1.08	77,200	91,040	91,170	339	601	3.30
50 mg/kg (4)		1.14	124,600	180,825	181,162	319	638	3.17
30 mg/kg (6)[*]	Week 152	1.12	85,067	127,457	127,810	244	526	3.54
50 mg/kg (6)[*]		1.11	125,750	192,618	193,181	322	690	3.78

AUC_{0-24}=area under the plasma concentration-time curve from time 0 to 24 hours; $AUC_{0-\infty}$=area under the plasma concentration-time curve from time 0 to infinite time; CL_{PL}=total clearance of drug after extravascular administration; C_{max}=observed maximum plasma concentration; $t_{1/2}$=elimination half-life; T_{max}=time to the observed maximum plasma concentration; V_{ss}=apparent volume of distribution at steady-state
[*] Includes 2 placebo subjects who began eteplirsen dosing at Week 25

In Study 28 (in which patients received IV eteplirsen at doses of 0.5, 1, 2, 4, 10, or 20 mg/kg/wk for 12 weeks), results for the plasma half-life was short, ranging between 1.6 and 3.6 hours, indicating rapid elimination. At the 2 highest dose levels (10 and 20 mg/kg), renal clearance accounted for 63.8% and 60.5% of total clearance, respectively, and was approximately the same as glomerular filtration rate in healthy boys between 5 to 15 years of age (Harriet Lane Handbook, 2015). Renal clearance ranged from 116 to 229 mL/hr/kg across dose levels. In units of mL/min, renal clearance across dose levels ranged from 62.6 mL/min to 119.4 mL/min, which spans the range of glomerular filtration rate in healthy boys age 5 to 15 (approximately

44 to 125 mL/min). This estimate was made via a commonly used pediatric glomerular filtration rate formula (Schwartz and Gauthier, 1985) along with Centers for Disease Control (CDC) growth charts from 2000 (MedCalc.com) and estimates of plasma creatinine concentration in children.

Analyses of PK characteristics in subgroups (demographic or disease characteristics) were not performed due to the uniformity of the patient populations in the clinical studies and the relatively small sample size. However, no significant differences in PK characteristics were observed in nonclinical studies between juvenile and adult rats, suggesting that eteplirsen human PK is not likely to be age dependent.

5.2.   Pharmacodynamic Effects

The pharmacodynamic effects of eteplirsen administration were evaluated in patients with DMD by examination of muscle biopsy tissue samples obtained during clinical trials.

- The mechanism of action of eteplirsen is exon 51 skipping during mRNA processing, which results in the production of internally shortened dystrophin mRNA and ultimately dystrophin protein. Exon 51 skipping was confirmed by RT-PCR analysis of dystrophin mRNA extracted from muscle tissue samples following eteplirsen administration in all patients who were treated with eteplirsen.

- The molecular goal of eteplirsen therapy is induction of dystrophin production which was demonstrated by 3 complementary methods; determination of percent dystrophin positive fibers (PDPF), testing for dystrophin intensity and Western Blot. Evaluation of PDPF dystrophin positive fibers not only demonstrate dystrophin production, but also correct localization of the newly formed dystrophin at the sarcolemma membrane.

5.3.   Drug-Drug Interactions

As noted in Section 4, results of in vitro studies indicated that eteplirsen was not metabolized by hepatic microsomes, was not a potent inducer or inhibitor of the major human CYP enzymes, and was not a substrate, nor did it have any major inhibitory potential for any of the key human drug transporters. Furthermore, published data show that corticosteroid medications used in the treatment of DMD are not expected to alter the pharmacokinetics or efficacy of exon skipping therapy (Verhaart 2012).

6.   ETEPLIRSEN CLINICAL STUDIES CONTRIBUTING TO EVALUATION OF EFFICACY

Across the clinical studies the efficacy of eteplirsen has been evaluated by a continuum of study endpoints that reflect the mechanism of action, pharmacodynamics effects and clinical outcomes relevant to DMD.

6.1.   Endpoints Evaluating Efficacy in Eteplirsen Clinical Studies
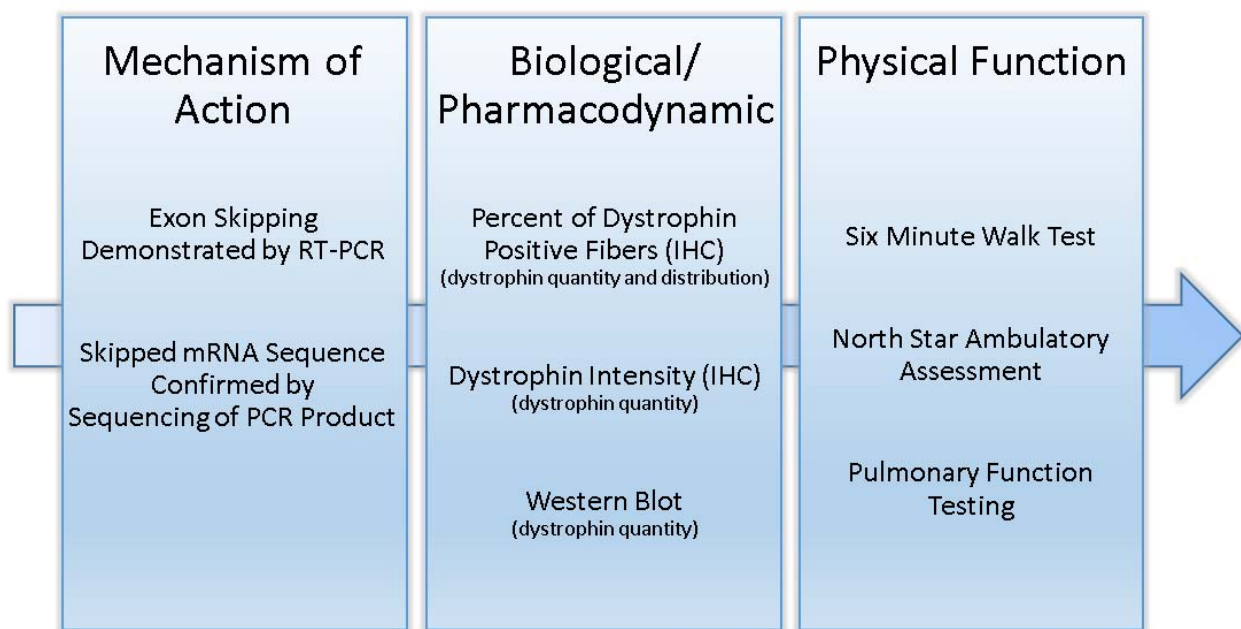
The eteplirsen clinical development program was initiated in pediatric DMD patients with a mutation amenable to exon 51 skipping who received eteplirsen intramuscularly at very low doses in Study 33 in order to demonstrate proof of principle for exon 51 skipping.

- **Proof of Concept Study 33** was the first study, conducted in boys with DMD (primarily non-ambulatory [N = 7]). A single- IM dose of either 0.09 or 0.9 mg/kg was injected into the extensor digitorum brevis muscle of one foot with placebo injected into the other foot. RNA analyses demonstrated that eteplirsen resulted in exon 51 skipping and immunohistochemistry showed production of novel dystrophin.

After demonstrating the mechanism of action and production of dystrophin in Study 33, a dose-ranging study (Study 28) administered eteplirsen at dose levels up to 20 mg/kg.

- **Dose-Ranging Study 28** is a completed 12-week study administering 0.5 to 20 mg/kg of eteplirsen by weekly IV infusion to ambulatory pediatric DMD patients (N = 19). Exon skipping and induction of dystrophin protein expression by eteplirsen was shown with most consistent results observed for the higher dose levels of 10 and 20 mg/kg. No dose limiting toxicities were identified.

Based on these encouraging initial data, a double-blind, placebo-controlled Phase 2 study (Study 201) was designed. Since the maximum tolerated dose had not been reached in Study 28, higher doses of eteplirsen, 30 and 50 mg/kg weekly IV infusion, were selected for Study 201.

Study 202 was an open-label extension study evaluating eteplirsen for a period of over 3 years including 6MWT, NSAA and PFTs.

- **Pivotal Study 201 and its ongoing extension, Study 202** are conducted in ambulatory DMD boys 7 to 13 years of age (N=12) administering 30 or 50 mg/kg by IV infusion. These studies have been ongoing for over 3 years with collection of clinical and biologic outcome data. No dose limiting toxicities were identified.

Key aspects of the studies contributing to evaluation of biologic and functional efficacy are summarized below in Table 4.

Table 4:   Clinical Studies Contributing to Pharmacodynamic and Functional Efficacy

Descriptor	Study Number			
	Pivotal		Supportive	
	Study 201	**Study 202**	**Study 28**	**Study 33**
Study Design	Randomized, double-blind, placebo-controlled, multiple-dose, single-center (US) study	Multi-center (US), open-label, multiple-dose extension study	**Dose-ranging study** Open-label, multiple-dose, (UK)	**Proof of concept** Single-blind, placebo-controlled, single-dose, investigator-sponsored, (UK)
Dosing Regimen	Eteplirsen 30 or 50 mg/kg/week, or placebo (IV) Weeks 1-24, then eteplirsen 30 or 50 mg/kg Weeks 25-28	Eteplirsen 30 or 50 mg/kg/week (IV)	Eteplirsen 0.5, 1.0, 2.0, 4.0, 10.0 or 20.0 mg/kg/week (IV)	Eteplirsen 0.09 or 0.9 mg IM in the EDB of 1 foot and placebo (IM) in the EDB of the opposite foot
Endpoints	**Primary** = Change from BL in PDPF at Week 12 (50 mg/kg group and 2 placebo patients), and at Week 24 (30 mg/kg group and 2 placebo patients). **Other** = 6MWT, Timed 4-step Test, and NSAA; PFTs; Exon skipping (RT-PCR) and dystrophin PDPF and intensity in biopsied muscle	**Primary Functional** = Change from BL in the 6MWT **Primary Biological** = Change from BL (of Study 201) to Week 48 in PDPF **Other =** Exon skipping (RT-PCR) and change from BL in dystrophin intensity, NSAA, PFTs, and Timed 4-Step Test	**Primary** = safety and tolerability **Primary Exploratory** = Change from BL to Week 14 in dystrophin PDPF **Other** = Change from BL to Week 14 in dystrophin intensity and protein levels (Western blot)	**Primary** = safety **Key Secondary** = Exon Skipping (RT-PCR); Restoration of dystrophin protein expression and the DAPC
Required Age at Entry (yrs)	7-13		5-15	10-17
Study Status	Completed	Ongoing	Completed	Completed
No. Enrolled	12		19	7
No. Completed	12	NA	18[a]	7
Study Period	July 2011 – Feb 2012	Feb 2012 – Nov 2014 for Efficacy Aug 2015 for Safety	Jan 2009 – June 2010	Oct 2007 – April 2009
Study Duration	28 Weeks	Week 168 efficacy; Week 208 safety	12 Weeks	Single Dose

Abbreviations: BL=Baseline; yrs=years; EDB=extensor digitorum brevis muscle; IM=intramuscular;
 IV=intravenous; No.=number; NSAA=North Star Ambulatory Assessment; PDPF=percentage of dystrophin
 positive fibers; PFT=pulmonary function testing; RT-PCR=reverse transcriptase-polymerase chain reaction;
 US=United States; UK=United Kingdom; Wk=week.
[a] One patient in the 4.0 mg/kg/wk group discontinued treatment due to an adverse event.

6.2.   Pivotal Studies 201/202

Study 201 is a completed, 28-week double-blind, placebo-controlled study of eteplirsen in 12 ambulatory boys with DMD mutations amenable to exon 51 skipping. Eligible patients were randomized to receive weekly IV infusions of 30 (N = 4) or 50 mg/kg eteplirsen (N = 4) or placebo (N = 4) for the first 24 weeks. Afterwards, the 4 patients originally randomized to placebo, rolled over to open label eteplirsen of 30 mg/kg (N=2) or 50 mg/kg (N=2). Figure 10 presents a schematic for Studies 201/202.

- Following completion of Study 201, all 12 patients continued receiving weekly eteplirsen in the ongoing extension Study 202 for up to 3 years.

- During both studies, muscle biopsies of upper arms were obtained for assessment of exon skipping and dystrophin production for all patients at Baseline and study Weeks 48, and 180. In order to minimize the overall number of muscle biopsies, half of the study patients also underwent biopsies at Week 12 (50 mg/kg) and the other half of patients had an additional muscle biopsy at Week 24 (30 mg/kg).

- Clinical outcomes of 6MWT, NSAA, rise time, pulmonary function tests and other functional measures were performed to assess changes in muscle function over time and have been collected through Week 168 for the 12 patients enrolled in Study 201/202.

Figure 10:   Schematic of Study Flow for Pivotal Studies 201/ 202
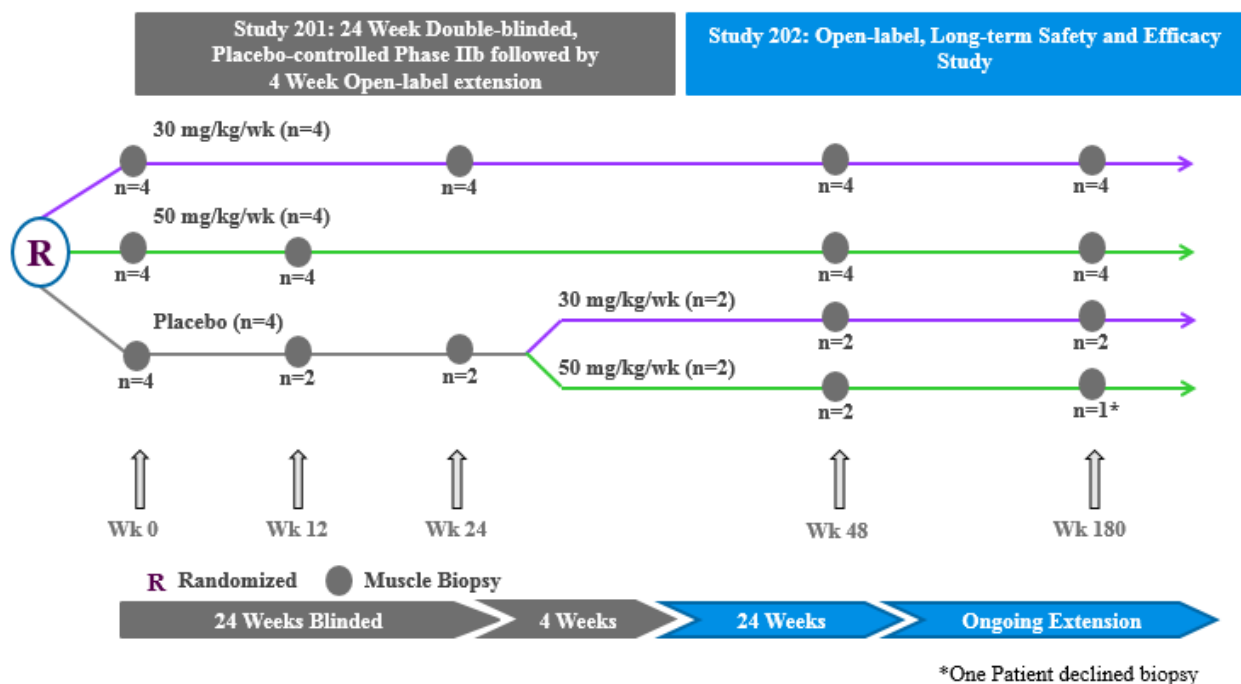
Inclusion Criteria

Study 201 inclusion criteria were designed to select a homogeneous population of DMD boys that would be expected to experience a predictable decline in 6MWT over the course of the study. Selection of this narrow population was considered the best group to evaluate whether stabilization of function would occur with eteplirsen intervention. Accordingly, the inclusion

criteria specified boys aged 7 to 13 with baseline 6MWT between 180 and 440 meters. The age of 7 years was selected as this was the time-point in the course of DMD when progressive muscle degeneration and loss of function begin to outpace natural growth and maturation such that DMD patients functionally decline. The impact of age and baseline 6MWT on performance on 6MWT are described in the current DMD literature (Henricson 2013a; Pane 2014a; McDonald 2010b; Mazzone 2013; Ricotti 2013; Ricotti 2015).

Steroid use may also influence performance on the 6MWT; (Pane 2014a; Henricson 2013a; McDonald 2010b; McDonald 2013b; Ricotti 2013; Ricotti 2015). DMD clinical trial design guidelines, recommend selection of a population of patients having similar anticipated disease trajectories. (FDA Draft Guidance for Industry: Duchenne Muscular Dystrophy and Related Dystrophinopathies: Developing Drugs for Treatment, 2015) Therefore, Study 201 required that patients received a stable dose of steroids for ≥24 weeks prior to enrollment. Study 201 Key Inclusion criteria are provided in Table 5. Appendix 2 provides a complete list of Study 201 inclusion and exclusion criteria.

Table 5:   Key Entry Criteria for Pivotal Study 201

Population	Male with DMD
	Genetically confirmed deletion mutation amenable to exon 51 skipping
	Aged 7-13 years
	Intact L/R biceps or alternative upper arm muscle group
Disease characteristics	Ambulatory with baseline 6MWT 180-440 meters
	Stable cardiac function with LVEF >40% on screening echocardiogram
	Stable pulmonary function with FVC ≥50% predicted; supplemental oxygen not required
	Stable dose of oral corticosteroids ≥24 weeks before study
	No cognitive or behavioral disorder that would impair ability to perform on 6MWT

6.2.1.   Study 201/202 Pre-specified Endpoint Results

6.2.1.1.   Primary endpoint of Study 201

The primary endpoint of Study 201 was dystrophin production at Weeks 12 and 24. This was achieved at Week 24 for the 30 mg/kg dose group with demonstration of a significant increase of dystrophin in eteplirsen treated patients in comparison to both baseline pre-treatment values and a significant increase relative to placebo treated patients.

6.2.1.2.   Primary endpoint of Study 202

The primary functional endpoint of Study 202 was comparison of Week 48 6MWT results for boys originally randomized to eteplirsen vs placebo separating out boys who were able and unable to complete the 6MWT at Week 48. A co-primary endpoint was Week 48 data for dystrophin which was analyzed for dose selection.

In the analysis of 6MWT in Study 202, there were 2 eteplirsen treated boys who lost ambulation early in the course of the study. In retrospect, eteplirsen treatment may have been introduced too late in the course of their disease to evaluate benefit on ambulation. These 2 eteplirsen boys had the lowest 6MWT at the time of entry into Study 201 and it may be considered that they were at high risk for loss of ambulation. To evaluate for potential treatment effect of eteplirsen, an analysis of the 6MWT of the eteplirsen boys who maintained ambulation compared to the boys originally randomized to placebo, showed a mean difference of 67 meters at the end of 48 weeks as shown in Figure 11.

Figure 11:   **Study 201/202: Analysis of 6MWT by Treatment Group and Completion of 6MWT**
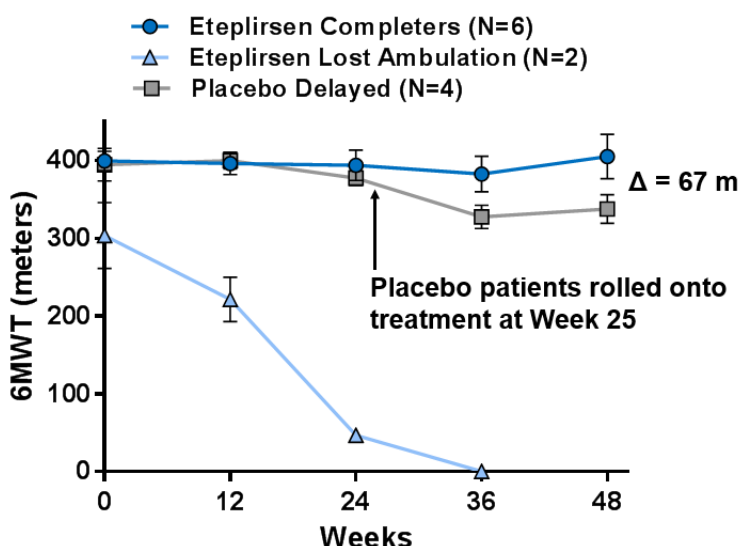
Based on these encouraging but limited results, the hypothesis of eteplirsen efficacy was strengthened and it was decided that Study 202 be further extended to continue observation and collection of longitudinal clinical outcomes. However, given the relatively short duration of 24 weeks for the placebo-controlled portion of Study 201, there was an absence of long-term concurrent placebo controlled data. As recommended by the FDA, Sarepta obtained individual patient data from untreated DMD patients enrolled in observational registries who were highly comparable to the eteplirsen treated patients.

The basis of the eteplirsen application is the analysis of the clinical outcomes of 6MWT, Loss of ambulation, NSAA compared to external control cohorts used for comparison of long-term outcomes.

6.2.2.   External Control Cohort Used For Comparison of Long Term Efficacy Data

In the absence of long-term, concurrent, placebo-controlled data from Study 201/202, Sarepta obtained individual patient data from untreated DMD patients enrolled in observational registries who were comparable to the eteplirsen-treated patients. The selection process for both registries, as well as individual patients was conducted in a manner to minimize the potential for selection bias. The clinical outcome experience from the external cohorts was used for comparison of 2 clinical efficacy endpoints; the 6MWT and NSAA.

Sarepta consulted with external DMD experts and reviewed findings from international DMD groups to identify potential registries that could provide individual patient 6MWT data including at least a baseline and post-baseline value for 6MWT. Twelve candidate external DMD registries with clinical outcome data were identified; however, only 2 databases had available, prospectively collected, 6MWT data including a baseline and at least 1 post-baseline value:

- Italian Telethon DMD Registry database (N = 97); Professor Eugenio Mercuri, MD, PhD (Catholic University in Rome); 11 participating tertiary care centers

- Leuven Neuromuscular Reference Center (NMRC) database (N = 89); Professor Nathalie Goemans, MD (University Hospitals in Leuven, Belgium); single site

Registry Characteristics Similar to Eteplirsen Studies 201/202

Although the registries were chosen primarily based on availability of 6MWT outcomes, both registries had characteristics including entry criteria and DMD standards of care comparable to Study 201/202. These registries include the requirement for a genetically confirmed diagnosis of DMD as was the case in Studies 201/202. Both registries followed patients over comparable time periods (2007 – present for the Leuven NMRC and 2008 – present for the Italian Telethon registries) with Studies 201/202 (2011 – present). Finally, Studies 201/202 and both registries excluded patients with known cognitive or behavioral disorders that would be likely to impair compliance with the functional assessments.

In addition, as with Studies 201/202, both registries follow international DMD patient care guidelines (Bushby 2010a, Bushby 2010b) used to set standards for the use of steroids as well as the use of physical therapy and orthotic devices to support continued ambulation.

- Italian Telethon investigators, as well as the principal investigator of the Leuven NMRC, are members of TREAT-NMD, a European organization of neuromuscular experts promote the use of these international treatment guidelines for DMD.

- While guidelines were published in 2010, clinics participating in both registries had been adhering to the standards at the time the registry studies were initiated including the use of steroids.

- The 2 lead physical therapists for the Italian Telethon Registry and Studies 201/202 were part of an international group which trained physical therapists on the 6MWT, further ensuring consistency and standardization of collection of 6MWT data.

Criteria for External Control Cohort was Based on Study 201 Inclusion Criteria

From the combined data set of 186 patients provided by the 2 registries, individuals were identified for inclusion in the external control group(s) using the key prognostic entry criteria for Study 201. Of note, *all patients* who met these criteria were included in the external control cohorts:

- They were required to be ≥7 years of age,

- Ambulatory with a baseline and at least 1 post baseline 6MWT result

- Receiving glucocorticoid therapy per treatment guidelines

- Genetically confirmed DMD amenable to exon 51 skipping therapy.

The primary external control group for comparison was comprised of boys with a mutation that was amenable to exon 51 skipping therapy (N=13). A secondary external control group was also identified which included patients with a DMD mutation amenable to any type of exon skipping therapy (N=50). This secondary group provided a larger sized group for comparison, albeit in a population of DMD with a mutation amenable to any kind of exon skipping.

Schematic for Selection of External Control Groups

The schematic below (Figure 12) illustrates the application of the selection filters used to identify external control patients, which were based on Study 201 inclusion criteria.

- From the 186 untreated DMD patients included in the registries, 116 received glucocorticoids at Baseline and had Baseline and at least one post-Baseline 6MWT

- 91 of the 116 were ≥7 years of age

- 50 of the 91 had DMD genetic mutations amenable to any exon skipping therapy

- 13 of the 50 had DMD specifically amenable to exon 51 skipping therapy

Thus, 2 analysis groups were identified:

- Primary Analysis Group – includes the 13 patients with _DMD_ mutations amenable to exon 51 skipping and is the most relevant comparator for the 3-year 6MWT data from Study 201/202 eteplirsen-treated patients. A subset of 10 external control patients from the Italian Telethon registry had longitudinal NSAA data; NSAA data were not provided for patients in the Leuven NMRC.

- Secondary Analysis Group – includes the 50 patients with _DMD_ mutations amenable to any exon skipping and thus provides a larger group for comparison of 3 years of 6MWT from Eteplirsen Studies 201/202. A subset of 34 patients from the Italian Telethon registry had longitudinal NSAA data; NSAA data were not provided for patients from the Leuven NMRC.

Figure 12:   Selection of External Control Groups for 6MWT and NSAA Comparison
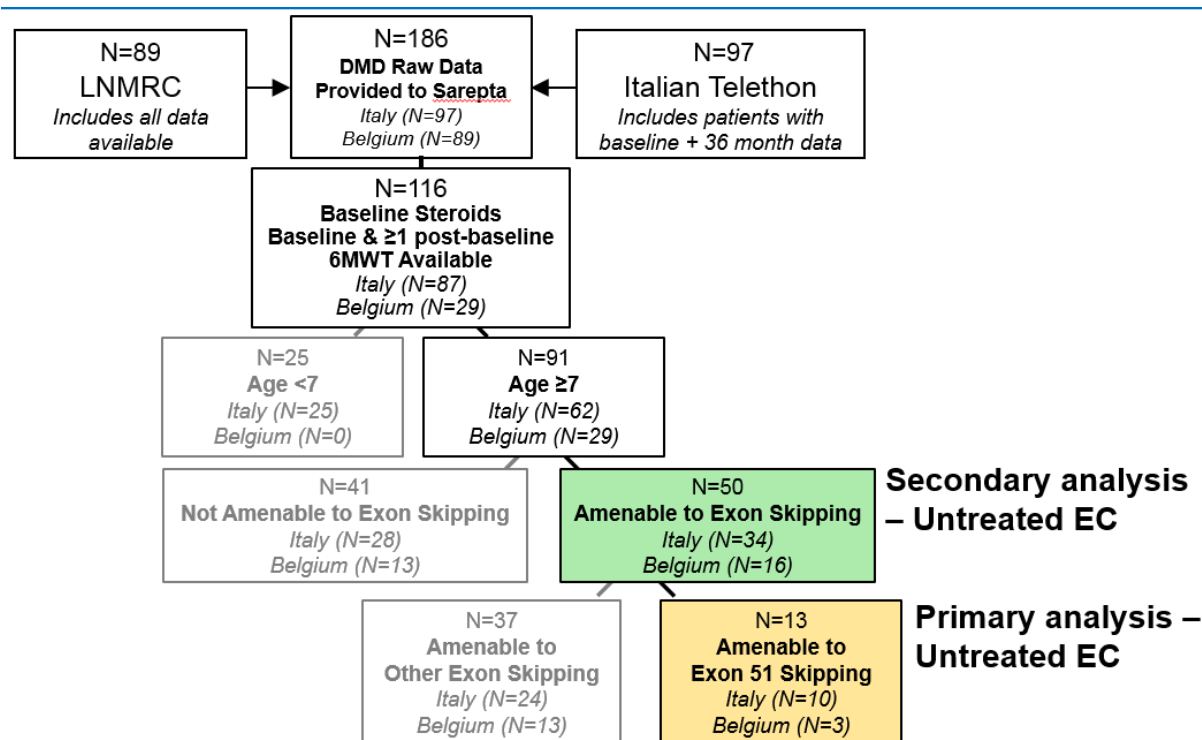
6.3.   Comparable Baseline Demographic and Disease Characteristics between Study 201/202 and External Control Cohorts

Study 201/202 had only 24 weeks of randomized placebo-controlled data, and as such use of external control cohorts was necessary in order to compare long-term clinical outcome data. Sarepta recognizes the potential limitations (ICH E 10) of using external control groups. The approach to identification of the external control groups was conducted in a manner to minimize these potential limitations. Table 6 summarizes the potential issues associated with the use of an external control cohort and approaches that Sarepta used in order to address or evaluate the potential for bias.

Table 6:   Approaches or Analyses to Address Potential limitations of Use of an External Control Group ICH E10*

Issue	Approach to Address Limitation	Relevant Section in Briefing Document
Inability to control bias in selection of external control	Selected all registries with available baseline and post-baseline 6MWT data, without regard to 6MWT results	Section 6.2.2
	Sensitivity analyses of the 6MWT with covariates of baseline 6MWT and age continue to demonstrate significant benefit for 6MWT for eteplirsen	Section 6.5.1.1
An external control group is often identified retrospectively leading to potential selection bias	Although identification was retrospective, ***all patients meeting criteria based on inclusion for Study 201/202 were in cohort***	Section 6.2.2
Difficulty in establishing comparability of eteplirsen and external control groups	High degree of comparability between eteplirsen and EC on baseline characteristics was established	Section 6.3
	High compliance rates with standard of care guidelines for DMD was shown	Section 6.4
Historical control groups have worse outcomes than apparently similar control group in a randomized study	The EC groups had similar or slightly better 6MWT performance compared to the placebo arm of a published randomized drisapersen clinical trial	Section 6.5.1.2
	The EC groups experienced loss of ambulation at an age comparable to published DMD literature	Section 6.5.2

* Choice of Control Group and Related Issues in Clinical Trials

Demographic and key Baseline characteristics for the 12 patients treated with eteplirsen in the pivotal 201/202 studies and the primary (exon 51 skipping, N = 13) and secondary (any exon skipping, N = 50) external control groups used for comparison on the 6MWT are presented in Table 7. Demographic and Baseline characteristics for the external control groups used for comparison on the NSAA are not presented as they are a subset of the 6MWT control groups.

Study 201/202 patients were a mean of 9.41 years of age at Baseline, while patients in the primary (N = 13) and secondary (N = 50) external control groups had mean ages of 9.45 and 9.68, respectively. Genetic mutations were similar between Studies 201/202 and those in the primary external group (N=13), with each specific type of genetic mutation observed in Study 201/202 also represented among the boys in the primary external control cohort. Mean baseline height and weight were generally comparable, although patients from the Italian Telethon were slightly taller and heavier. Mean 6MWT scores across the eteplirsen treated and external control groups were within 10 meters of each other and the distribution of patients over the range of Baseline scores for both 6MWT and NSAA was similar (Figure 13).

Importantly, all patients in all groups had been on steroids for at least 6 months prior to Baseline and remained on steroids throughout the study or follow-up period.

The high comparability of the treated pivotal study and untreated control patients across these baseline parameters confirms the validity of the process used for selection of the external controls. Baseline characteristics for the individual patients in Study 201/202 (N = 12) as well as the external control of exon 51 skippable (N = 13) are provided in Appendix 2.

Table 7:   Demography and Baseline Characteristics of Eteplirsen Patients in Studies 201/202 vs External Controls

Parameter	Pivotal Study	Untreated External Control Groups	
	Study 201/202 (N = 12)	Primary Analysis Exon 51 Skipping (N = 13)	Secondary Analysis Any Exon Skipping (N = 50)
Male Gender	100%	100%	100%

Age, years
  Mean (SD)
  Median
  Min, Max
	N = 12
	9.41 (1.18)
	9.7
	7.3, 11
		N = 13
		9.45 (1.45)
		9.0
		7.3, 11.8
			N = 50
			9.68 (1.52)
			9.54
			7.0, 13.0

Height, cm
  Mean (SD)
  Median
  Min, Max
	N = 12
	123.9 (8.37)
	119
	117, 138
		N = 10
		131.3 (3.16)
		131
		126, 136
			Not available

Weight, kg
  Mean (SD)
  Median
  Min, Max
	N = 12
	33.0 (7.29)
	34.6
	23.4, 42
		N = 10
		35.5 (8.75)
		32
		26, 48.0
			Not available

Genotype (exon skippable)
  45-50
  48-50
  49-50
  50
  52
	N = 12
	3
	1
	5
	1
	2
		N = 13
		3
		2
		3
		2
		3
			Amenable to any exon skipping[a]

Steroid use ≥24 weeks prior to Baseline (% yes)	100%	100%	100%

Steroid use:
  Continuous
  Intermittent
	
	11
	1
		
		8
		5
			
			32
			18

Ambulatory (% yes)	100%	100%	100%
6MWT Assessment method	Assessor training and script per modified ATS procedure	Assessor training and script per modified ATS procedure	Assessor training and script per modified ATS procedure

6MWT Distance (m)
  Mean (SD)
  Median
  Min, Max
	N = 12
	363.2 (42.19)
	370
	256, 416
		N = 13
		357.6 (66.75)
		373
		200, 458
			N = 50
			355.7 (87.28)
			356
			100, 558

Comparison of eteplirsen (n = 12) to Italian Telethon Exon 51 Amenable (n = 10) Any Exon (n = 34)			

Total NSAA Score [c]
  Mean (SD)
  Min, Max
	N = 12
	24.9 (4.93)
	17,31
		N = 10
		22.0 (6.27)
		10,31
			N = 34
			22.7 (6.31)
			10, 32

Rise Time[d]
  Mean (SD)
  Median
  Min, Max
	N=12
	8.2 (7.57)
	5.5
	3.1, 30
		N=8
		8.4 (8.99)
		5.9
		2.37, 30
			N=28
			8.9 (9.03)
			5.7
			2, 30

Abbreviations: 6MWT = Six minute walk test; EC = external control; SD = standard deviation.

[a] Seven of the 50 (14%) had deletion mutations amenable to exon 44 skipping; this genotype may have a milder phenotype compared to other skippable mutations (Pane 2014a).

[b] Bushby 2010a; Bushby 2010b

[c] NSAA data were only available for patients in the Italian Telethon DMD Registry database.

[d] Rise Time data were only available for Italian Telethon Patients. Patients unable to rise without external support were assigned a rise time of 30 seconds (Henricson 2013)

Figure 13:   **Baseline Distribution of 6MWT and NSAA Scores for Eteplirsen-Treated Patients in 201/202 vs External Controls**

A) 6 Minute Walk Test   **B) North Star Ambulatory Assessment**
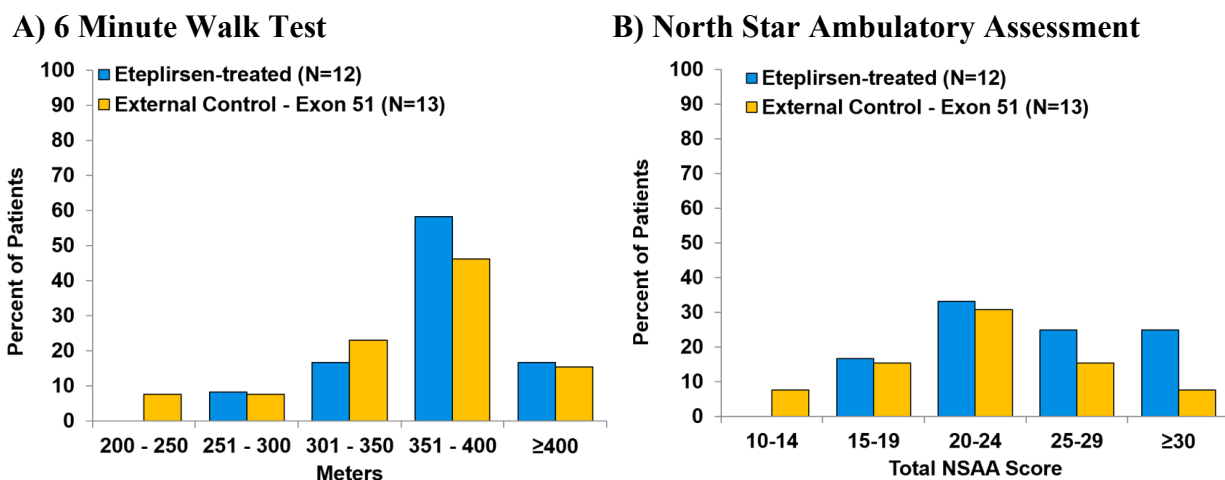
6.4.   Longitudinal and Standard of Care Parameters for Studies 201/202 and External Control Patients

In addition to the analysis of baseline parameters of age, 6MWT, NSAA score, height and weight, the trajectory of growth in terms of height and weight are depicted over the course of the 3-year follow-up period by Figure 14.

Patients from both Study 201/202 and the external control cohort received steroids for a period of at least 24 weeks prior to baseline. Furthermore all boys from both Study 201/202 and the external control cohort continued to receive steroids (including deflazacort and prednisolone) through the duration of the 3-year follow-up period. Details regarding the type of glucocorticoid therapy for individual patients are provided in Appendix 3.

Figure 14:   Mean Height and Weight over 3-Year Period for Study 201/202 (N = 12) vs. Italian Telethon (N = 10)
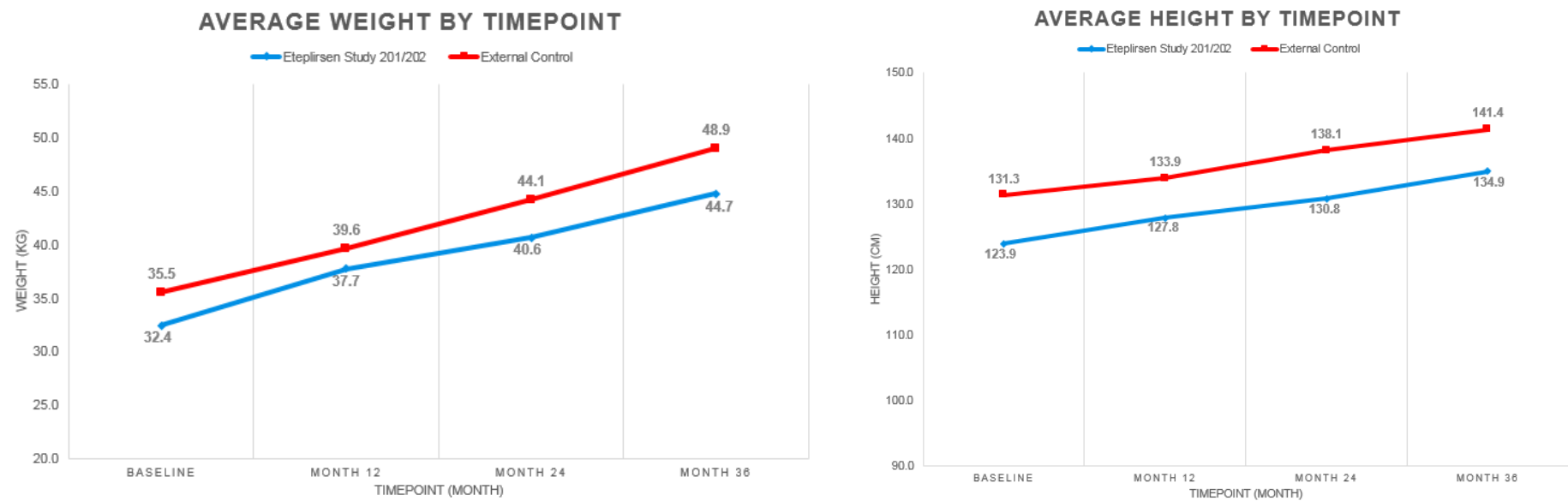
Comparison of Study 201/202 patients and the patients from the Italian Telethon Registry, demonstrated that both sets of patients received regular physical therapy and showed high compliance rates with the use of orthoses to maintain lower extremity flexibility.

Most eteplirsen-treated patients as well as those in the Italian study followed home stretching routines in addition to meeting routinely with a trained physical therapist. Patients in the Italian cohort met with a trained physical therapist at least twice and as many as 6 days a week.

In order to maintain ankle flexibility, which is important to ambulation, many boys with DMD wear night splints. The majority of patients in both cohorts wear night splints or, in the case of 2 patients in the Italian cohort, were found not to need them. Details regarding comparison of physical therapy and orthoses are provided by Table 8.

Table 8:   **Studies 201/202 Comparison of Physical Therapy and Use of Orthoses**

		Italian Telethon N = 10	Study 201/202 Eteplirsen N = 12
Physical Therapy	**PT Regimen**	**# of Patients**	**# of Patients**
Home therapy	Stretching with parents/other	6	8
Swimming	1 day/week	5	1
	2-3 days week	0	1
Frequency of appointments with trained physical therapist	4-6 days/week	5	2
	2-3 days/week	5	3
	1 day/week	0	4
	1 day/year	0	1
Use of night splints to maintain ankle flexibility	Use orthoses	8	11
	Orthoses not needed (TA<10°)	2	0
	Orthoses not used	0	1

6.5.   Functional Endpoints

6.5.1.   Six-Minute Walk Test

Given the importance of ambulatory compromise to the DMD disease process, 6MWT was chosen as the primary endpoint. The 6MWT is an integrated assessment of global muscle function and endurance that also incorporates cardiac and respiratory functions (ATS 2002) and has been established as accurate, reproducible, simple to administer, and well tolerated in ambulatory patients with DMD (McDonald 2010a). It is also clinically relevant in DMD as decline in ambulatory capacity, is associated with reductions in DMD patient- and caregiver-reported quality of life (Bendixen 2012; Bendixen 2014; Magliano 2014; Uzark 2012; Henricson 2013b). Furthermore the 6MWT is an accepted outcome measure for DMD according to the recent FDA draft guidance for industry on developing drugs for DMD (FDA 2015).

In both the eteplirsen-treated and external control patients, the 6MWT was performed according to published methods modified for DMD patients (ATS 2002; McDonald 2010a) where patients are asked to walk a pre-set course for 6 minutes during which they receive scripted

encouragement and are followed by a member of the testing staff to ensure patient safety. Furthermore, both lead physical therapists for the eteplirsen study 201/202 and the Italian Telethon have collaborated in an international initiative to train physical therapists on administration of the 6MWT in DMD.

6MWT data for eteplirsen-treated and 2 groups of external control patients (primary exon 51 skipping N = 13 and secondary any exon skipping N = 50) were compared, using the analysis of covariance (ANCOVA) model with group (e.g., genotype [exon skipping amenable vs. non-amenable], age [<7 vs. ≥7 years], treatment [eteplirsen vs. untreated]) as a fixed-effect term and Baseline 6MWT as a covariate. Change from Baseline to Years 1, 2, and 3 was also summarized.

No adjustments were made for multiple comparisons as these analyses were descriptive; p-values, when provided, are nominal and included for guidance purposes only. For eteplirsen-treated patients (N = 12), data for the 30 and 50 mg/kg cohorts (N=8) were pooled along with data for the placebo-to-eteplirsen cohort (N=4) after correcting for the 24-week placebo-period (i.e., by counting Week 24, the last week prior to receiving eteplirsen, as Baseline).

Results for 6MWT of Eteplirsen (N=12) vs External controls amenable to exon 51 (N=13)

Eteplirsen treated patients (N = 12) showed a slower rate of decline in the 6MWT, compared to primary external controls with DMD mutations amenable to exon 51 skipping (N = 13). The 2 patient groups had similar baseline and disease progression trajectories through Year 1 (12 months) supporting the comparability of the groups prior to and during initial stages of treatment with eteplirsen. This is also consistent with pharmacodynamic data that indicates it may take up to 24 weeks to establish significant dystrophin production. However, as the study progresses and the stabilization of ambulation in eteplirsen-treated patients is juxtaposed against the predictable decline of ambulation in untreated external controls, the impact of eteplirsen becomes apparent.

A large treatment effect of 150.8 meters is shown after the course of 3 years. After Year 1, the treated and untreated patients began to diverge, resulting in a 75.1-meter difference in 6MWT decline by Year 2, and a larger, statistically significant (p <0.01) difference of 150.8 meters between the groups by Year 3 (Figure 15).

Figure 15:   **Mean 6MWT Values over Time in Eteplirsen-Treated Patients (Studies 201/202) vs. Primary External Controls**
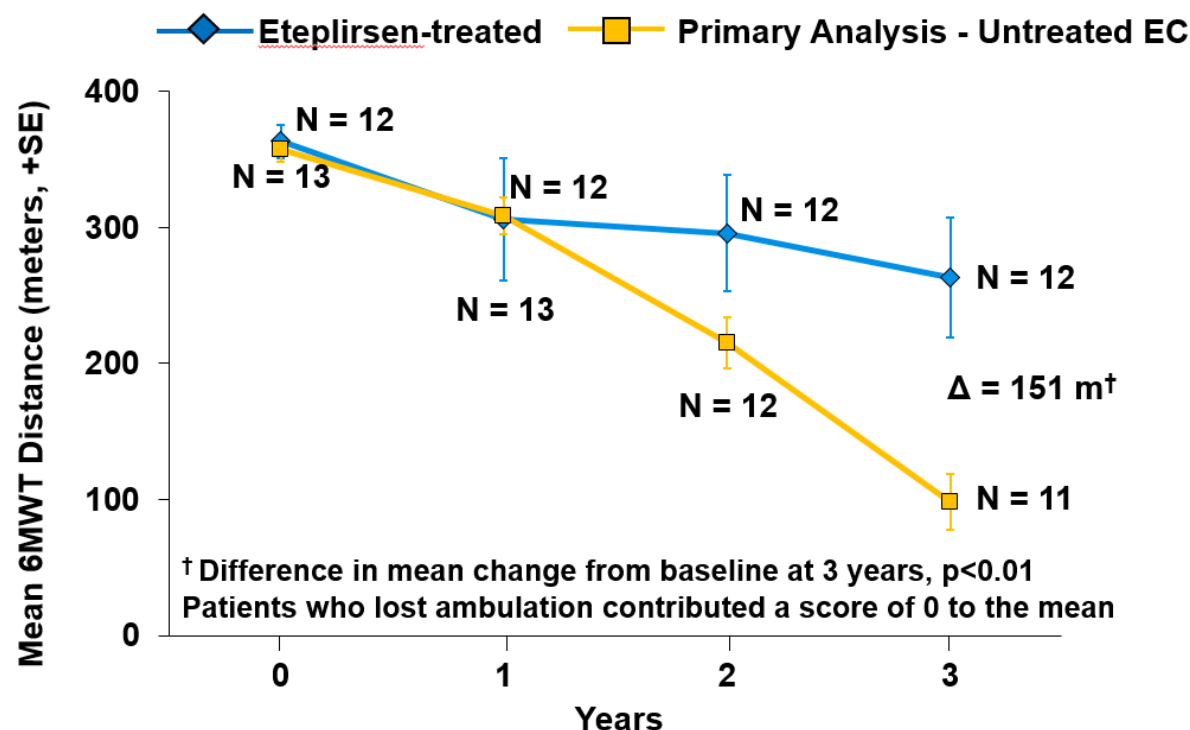
	Baseline	Year 1	Year 2	Year 3
Eteplirsen, n	12	12	12	12
Mean (SD)	363.2 (42.19)	305.8 (155.32)	295.9 (148.98)	263.1 (151.74)
Controls, n	13	13	12	11
Mean (SD)	357.6 (66.75)	309.0 (118.78)	215.4 (148.85)	98.5 (136.28)

In the analysis of individual 6MWT values over time, 2 of the eteplirsen-treated patients (blue lines) lost ambulation relatively early in the study (<48 weeks) compared to 1 of the external control patients (yellow lines). The eteplirsen patients (twin brothers, identified as Patients 009 and 010) had the lowest 6MWT scores at Baseline, hence they may have been at greater risk for loss of ambulation prior to initiation of treatment. The external control patient (identified as Patient (b)(6)) had a Baseline 6MWT of 327 meters, which was also relatively low.

All boys from either the eteplirsen Study 201/202 or the external control cohort who lost ambulation over the 3 year course were included in the analysis contributing a 6MWT value of "0" meters to the mean (Figure 16).

Figure 16:   **Individual 6MWT Values over Time in Eteplirsen-Treated Patients (Studies 201/202, N = 12) vs. Primary External Controls**
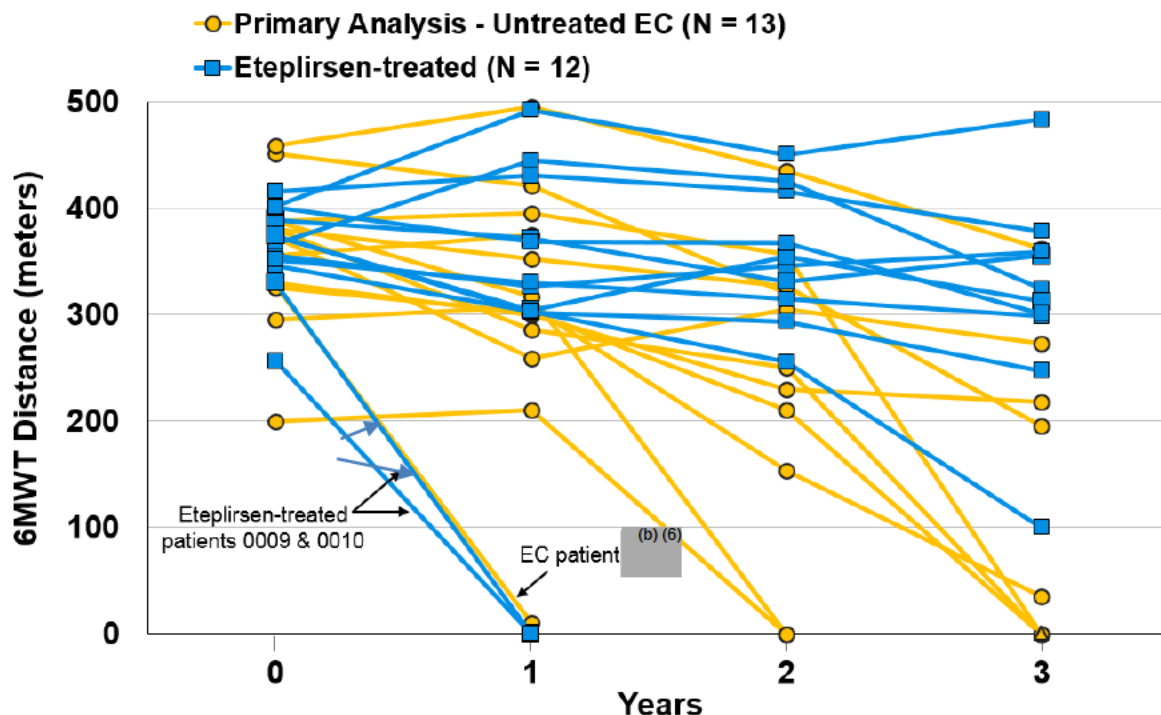
6MWT of Eteplirsen (N=12) vs External controls amenable to any exon skipping 51 (N = 50)

In the analysis between eteplirsen (N = 12) and the external controls amenable to any exon skipping (N = 50), a substantive difference of 79.9 meters on the 6MWT was shown. Although the secondary control was not as well matched as it included a broader population amenable to any exon skipping, this group was larger and provides a more conservative comparison. As shown in Figure 17, the 2 patient groups walked comparable mean distances at Baseline and demonstrated similar disease progression trajectories through Year 1 (12 months). After Year 1, however, they began to diverge, with the eteplirsen-treated patients showing a less severe disease progression, resulting in a clinically relevant difference between the 2 groups of 43.7 and 79.9 meters in 6MWT decline at Years 2 and 3, respectively.

A list of individual 6MWT results for both Study 201/201 patients (N = 12) as well as the external control cohort amenable to exon 51 skipping (N = 13) is provided in Appendix 4.

Figure 17:   **Mean 6MWT Values over Time in Eteplirsen-Treated Patients (Studies 201/202) vs. Secondary External Controls (Any Exon Skipping)**
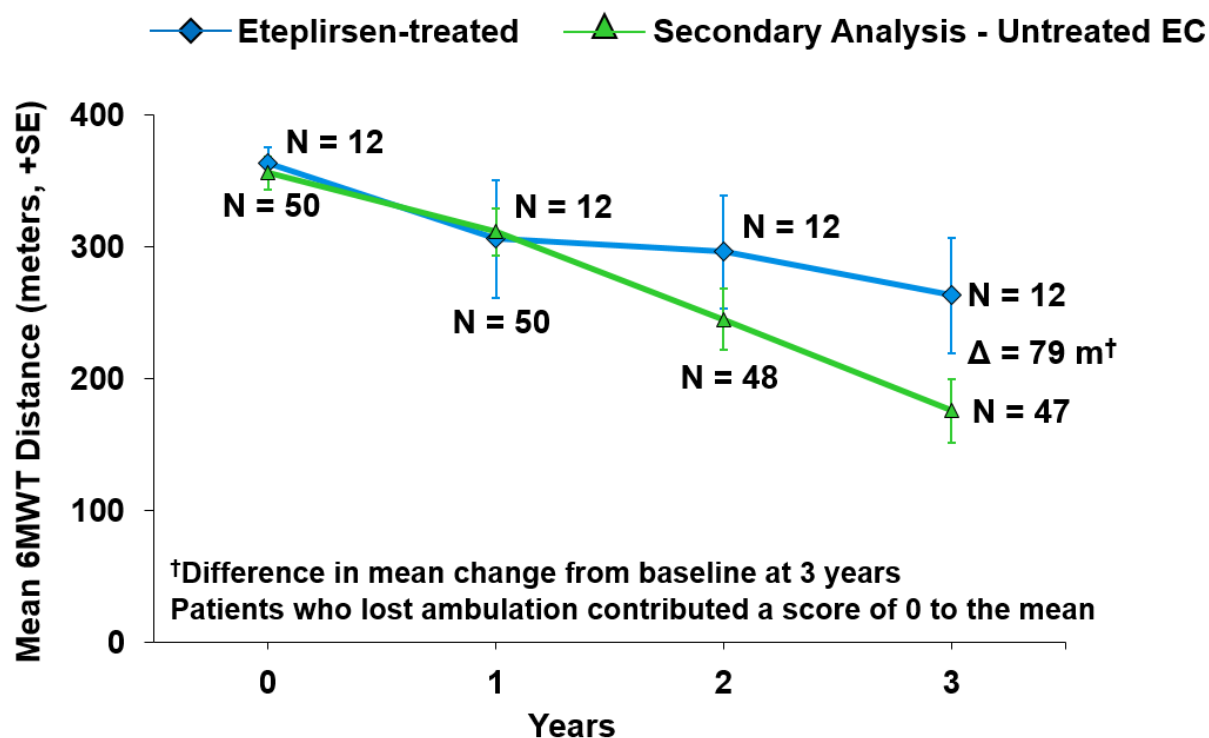
	Baseline	Year 1	Year 2	Year 3
Eteplirsen, n	12	12	12	12
Mean (SD)	363.2 (42.19)	305.8 (155.32)	295.9 (148.98)	263.1 (151.74)
Controls, n	50	50	48	47
Mean (SD)	355.7 (87.28)	311.3 (125.22)	244.8 (159.50)	175.7 (165.06)

6.5.1.1.   Sensitivity Analyses of the Primary Functional Efficacy Endpoint: 6MWT

A series of sensitivity analyses were performed on the 3-year 6MWT data to control for potential confounding factors and evaluate the robustness and validity of the primary efficacy analysis. These sensitivity analyses controlled for potential group imbalances in Baseline 6MWT and age. Additional sensitivity analyses accounted for potential violations of the data's normality assumption and missing data on the 6MWT. As described below and summarized in Table 9, for every analysis performed, the difference between the eteplirsen-treated and untreated external controls in the change from Baseline on the 6MWT remained clinically meaningful and statistically significant. Details for the methodologies of these analyses are provided in Appendix 5.

Table 9:   **Sensitivity Analysis for 6MWT in Eteplirsen-Treated (N = 12) vs. External Controls Amenable to Exon 51 Skipping (N = 13)**

Potential Issue Addressed	Row	Comparison: Change from Baseline in 6MWT in Eteplirsen-Treated (N = 12) vs. Untreated External Controls (N = 13)	LS Mean Difference (meters)	P-Value
Bias Caused by Imbalance in Important Baseline Prognostic Factors	1	ANCOVA with Baseline 6MWT and age as covariates	141	0.0115
Bias Caused by Violation of Normality Assumption	2	ANCOVA with Baseline as a covariate, rank transformation as the outcome for 6MWT	NA[a]	0.0055
	3	ANCOVA with Baseline and age as covariates, rank transformation as the outcome for 6MWT	NA[a]	0.0072
Bias Caused By Missing Data	4	MMRM analysis with Baseline and age as covariates	143	0.0026
	5	MMRM analysis with Baseline and age as covariates and rank transformation as the outcome for 6MWT	NA[a]	0.0091
	6	ANCOVA with Baseline as a covariate and LOCF for missing data	123	0.0221
	7	ANCOVA with Baseline and age as covariates and LOCF for missing data	123	0.0254
	8	ANCOVA with Baseline and age as covariates, LOCF for missing data, rank transformation as outcome for 6MWT	NA[a]	0.0228

Abbreviations: 6MWT = 6 Minute Walk Test; ANCOVA = analysis of covariance; LS = least squares; LOCF = last observation carried forward; MMRM = Mixed Model Repeated Measures; NA = not applicable.

[a] Not applicable as the data being analysed are rank-transformed.

An identical series of sensitivity analyses was applied to the comparison of eteplirsen-treated patients (N = 12) to external controls amenable to any exon skipping (N = 50). Although the difference in the change from Baseline on the 6MWT did not reach significance with the pre-specified ANCOVA analysis, the difference between the 2 groups did reach significance (p <0.05) in 2 analyses:

- Sensitivity analysis for the potential for violation of the Normality Assumption using an ANCOVA of rank transformed data with Baseline 6MWT as a covariate.

- Sensitivity analysis for potential for bias due to missing data was addressed using the MMRM analysis with Baseline 6MWT and age as covariates (see Appendix 6).

6.5.1.2.   Decline of 6MWT for External Controls Compared to Published Literature

To evaluate the possibility that the external control had worse outcomes than would have been expected in a cohort of patients from a randomized clinical trial, comparison of the 6MWT for the external control groups to published data from a drisapersen trial was conducted (placebo group N = 32). As shown in Figure 18, both external control groups (exon 51 skipping N = 13 and any exon skipping N = 50) declined at a slower rate than the drisapersen placebo cohort over the course of one year. This supports the conclusion that the 6MWT 150.8 meter difference observed for eteplirsen patients (N = 12) vs the untreated external cohort (N = 13) is attributable to eteplirsen treatment, rather than the chance occurrence of an atypically rapid decline of the external control group.

Figure 18:   Eteplirsen-Treated Patients Compared to External Controls and Drisapersen Placebo Cohort
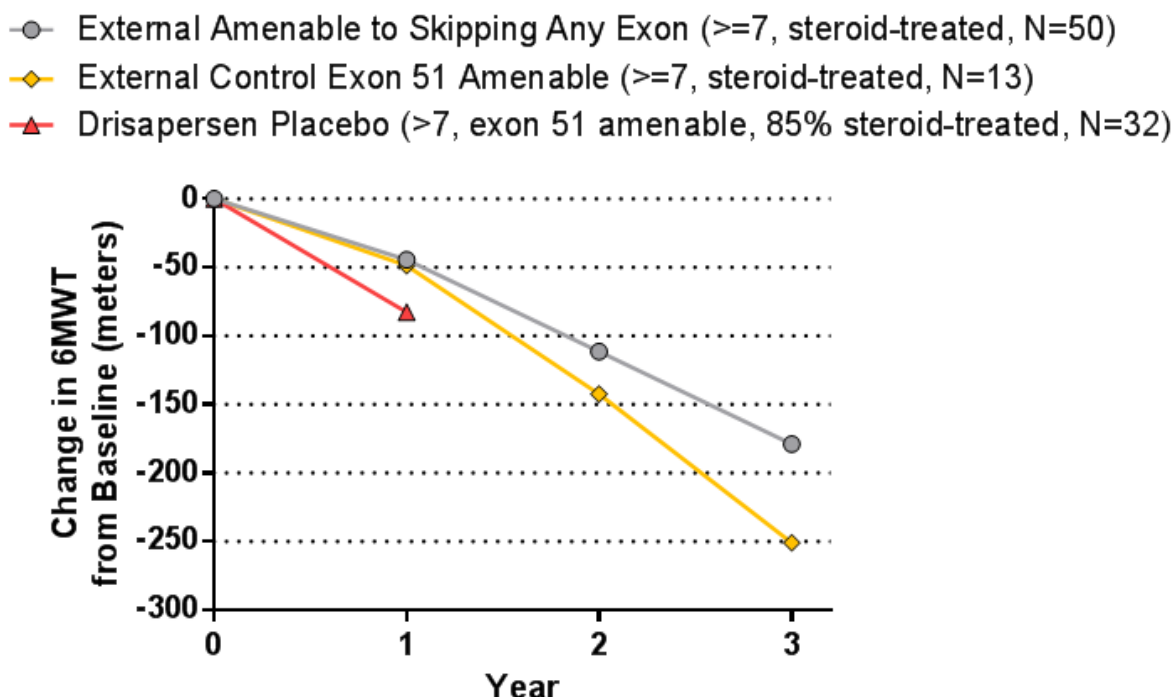
Source: Goemans, et al. Drisapersen Efficacy and Safety in Duchenne Muscular Dystrophy: Results of a Phase III, Randomized, Double-Blind, Placebo-Controlled Trial (Study DMD114044). Late Breaking Oral Presentation, WMS 2013, Asilomar, CA.

6.5.2.   Loss of Ambulation

Ambulatory compromise and loss of ambulation are hallmarks of the progressive muscle degeneration characteristic of DMD (Bushby 2010a; Ciafaloni 2009; van Ruiten 2014). Once confined to a wheelchair, other symptoms tend to follow in rapid succession including loss of upper limb function, such that self-care, unsupported sitting, and eating become impaired, severely affecting patient quality of life, as well as that of caregiver and families (Bendixen 2012; Bendixen 2014; Magliano 2014; Uzark 2012). Moreover loss of ambulation is associated with an earlier need for ventilation and premature death (Bushby 2010a; Humbertclaude 2012; Kinali 2007; Van Essen 2004).

Fewer eteplirsen treated boys lost ambulation (defined here as a score of 0 meters on the 6MWT) over the 3-year time period evaluated compared to untreated external control boys (Figure 19). After 3 years, only 2 of 12 (16.7%) eteplirsen-treated patients (i.e., Patients 009 and 010) lost ambulation. In comparison, 6 of 13 (46.2%) external controls amenable to exon 51 skipping lost ambulation, and 18 of 50 (36%) external controls amenable to any exon skipping, lost ambulation over 3 years. A listing of baseline characteristics of the 8 boys who lost ambulation (eteplirsen N = 2 and EC exon 51 N = 6) may be found in Appendix 7.

Figure 19: **Cumulative Loss of Ambulation Over 3 Years in Eteplirsen-Treated Patients (Studies 201/202) vs. External Controls**
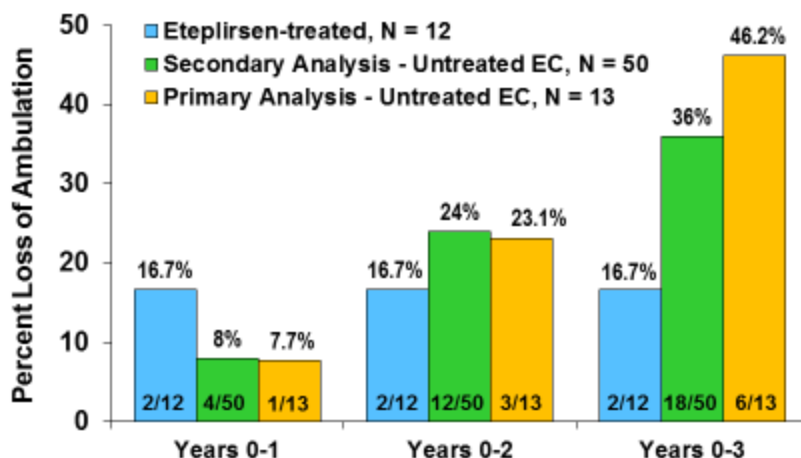
Abbreviations: EC=untreated external control patients.

Loss of ambulation of external controls compared to published literature

To evaluate the possibility that the observed difference in loss of ambulation between eteplirsen and the external controls could be due to an atypical rate of loss of ambulation for external control, a comparison to published data regarding the median age for loss of ambulation (11.2-13.9 years) was conducted (Ricotti 2015; Bello 2015). Based on the literature it would be expected that ~50% of DMD boys would have lost ambulation by the age of ~12.5 years. Since the median age of the external control group was approximately 12.5 years, after 3 years of follow-up, the loss of ambulation rates of 46.2% and 36% which were observed for the external cohorts of exon 51 skipping (N = 13) and the external cohort of any exon skipping (N = 50) appear to be aligned and no worse than published literature. This supports the conclusion that the lower rate for loss of ambulation observed for eteplirsen patients (16.7%) vs the external cohorts is attributable to eteplirsen treatment rather than an atypically rapid loss of ambulation for boys in the external control group.

6.5.3. North Star Ambulatory Assessment

The NSAA is a clinician-reported outcome instrument specifically designed to measure ambulatory function in patients with DMD (Scott 2012). In contrast to the 6MWT, which is a continuous measure, the NSAA is a multiple-item rating scale (17 items) that includes 3 ordered response categories (2, 1, or 0). Items are scored either 2 ('normal' with no obvious modification of activity), 1 (modified method but achieves goal), or 0 (unable to achieve independently). A total 'ambulatory function' composite score is generated by summing items. The 17 items

assessed include a 10-meter walk/run, rising from a sit to stand, standing on 1 leg, climbing and descending a step, rising from lying to sitting, rising from the floor, lifting the head, standing on heels, and jumping. The NSAA has undergone detailed psychometric evaluations based on traditional (reliability and validity) and modern (Rasch) methods, and has been included in international DMD clinical trials and natural history studies (Mazzone 2009; Mazzone 2010; Scott 2012; Mayhew 2011). A listing of the 17 items in the NSAA is provided in Appendix 8.

NSAA data (total scores) from eteplirsen-treated patients in Studies 201/202 were compared with longitudinal data from an external cohort. In both the eteplirsen-treated and external control patients, the NSAA was performed according to published methods (Mazzone 2009). For eteplirsen-treated patients (N = 12), data for the 30 and 50 mg/kg dose groups were pooled with those for the placebo-to-eteplirsen cohort after correcting for the 24-week placebo-treatment period (i.e., by counting Week 24, the last week before receiving eteplirsen, as Baseline = year 0.

NSAA data from eteplirsen-treated patients in Studies 201/202 were compared with longitudinal data from an external control cohort that included DMD patients amenable to exon 51 skipping (N = 10). As shown in Figure 20, at baseline, eteplirsen-treated patients had a total score of 24.9 compared to a score of 22.0 for the external control group, representing a difference of 2.9. Over the first year, both the eteplirsen treated boys and the EC group declined in function. However, as with the 6MWT, following Year 1, the decline in function for the eteplirsen group became slower. At the end of Year 3, there was a total mean score of 14.0 for the eteplirsen boys compared to a mean score of 8.1 for the external control boys, representing a 5.9 difference in score. Overall the untreated external control had experienced a 3-point decrease relative to eteplirsen treated boys. This is of relevance given that a 3-point decrease in NSAA may represent impairment in 3 activities, or the loss of an entire activity with impairment in another. Moreover, in patients ≥7 years of age, NSAA scores below 10 have been associated with a high risk of loss of ambulation within 1 year (Ricotti 2015).

Below is a figure of the baseline distribution of NSAA between eteplirsen treated boys vs EC boys both at baseline and the end of 3 years. A plot of individual NSAA patient data is provided in Appendix 4.

Figure 20:   **Mean NSAA Total Scores over Time in Eteplirsen-Treated Patients (Studies 201/202) vs. External Controls Amenable to Exon 51 Skipping**
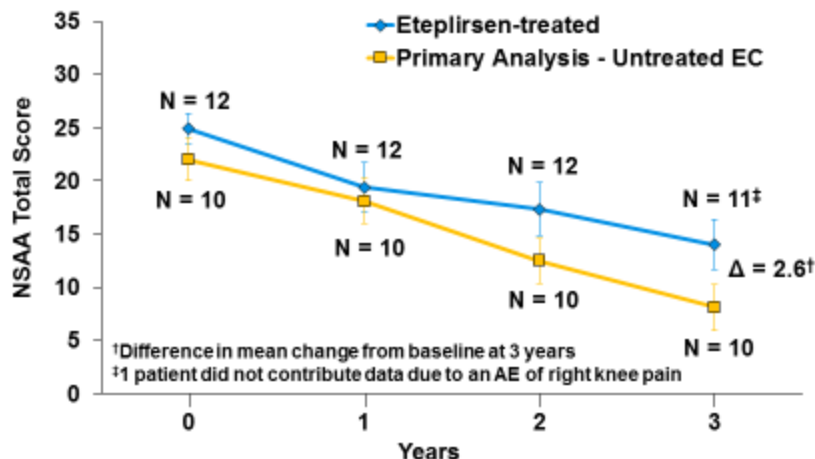
	Baseline	**Year 1**	**Year 2**	**Year 3**
Eteplirsen, n	12	12	12	11
Mean (SD)	24.9 (4.93)	19.4 (7.99)	17.3 (8.55)	14.0 (7.80)
Controls, n	10	10	10	10
Mean (SD)	22.0 (6.27)	18.1 (6.74)	12.5 (6.69)	8.1 (6.92)

6.5.4.   Comparison of Rise Time: Eteplirsen Treated (N = 12) vs Italian Telethon (N = 10)

The ability to rise from supine is a critical activity for DMD patients and it is one of the early abilities to be lost in DMD and is predictive of loss of ambulation. Information regarding the ability to rise without external support, was obtained from the Italian Telethon registry.

The proportion of eteplirsen vs Italian Telethon patients who were able to rise at Baseline (Year 0) and Year 1, 2 and 3 was calculated. At Baseline, 92% of eteplirsen treated patients vs 88% of the Italian Telethon patients had the ability to rise independently. However by the end of Year 3, 55% of eteplirsen treated boys had maintained the ability to rise, compared to only 14% of the Italian Telethon patients as illustrated in Figure 21.

Figure 21:   **Study 201/202 Eteplirsen Treated Patients (N = 12) Vs. Italian Telethon (N = 10) Ability to Rise Without External Support**
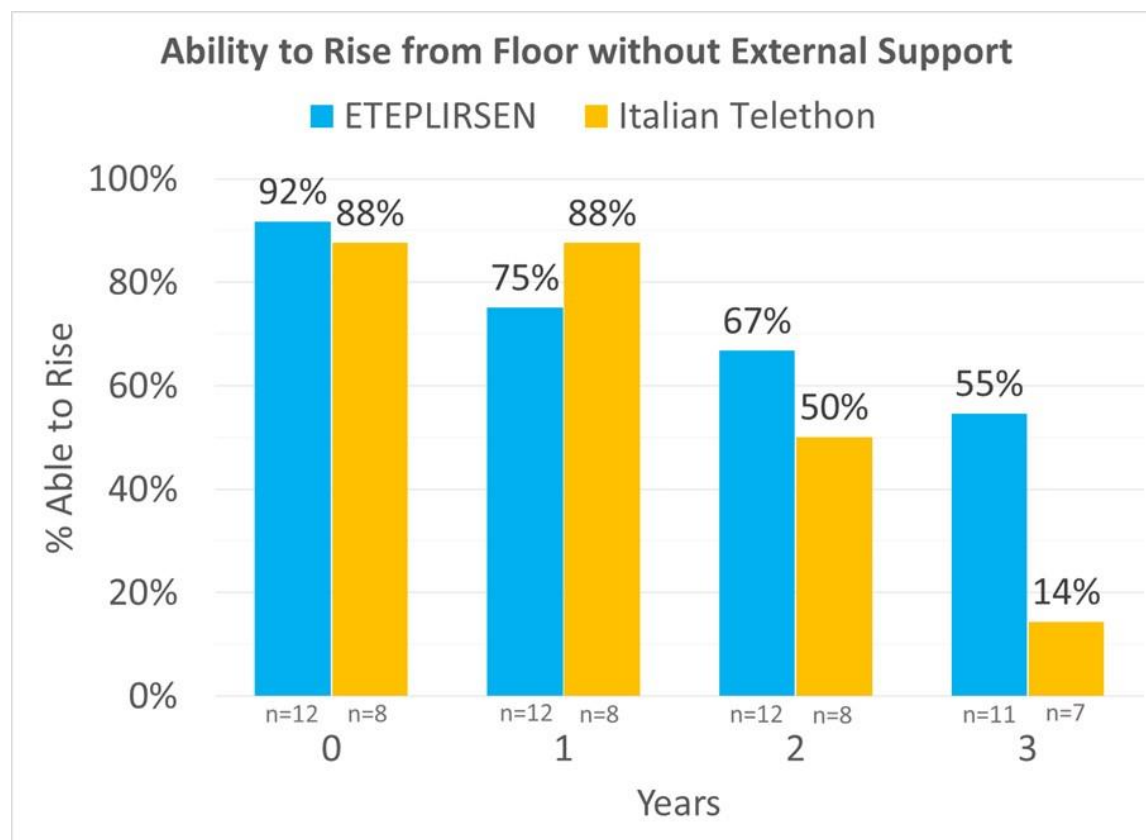
6.5.5.   Pulmonary Function Tests

Respiratory function in DMD is progressively impaired over time as the dystrophic process affects respiratory muscles, including the diaphragm, and leads to significant morbidity and mortality. Respiratory function is measured by pulmonary function testing (PFTs), which includes measurement of lung volume (forced vital capacity, FVC) and the ability to generate pressure during inspiration (maximum inspiratory pressure, MIP) and expiration (maximum expiratory pressure, MEP).

FVC measures integrity of both inspiratory and expiratory muscles, is an excellent measure of respiratory function reserve, and is widely used in DMD to assess respiratory muscle function. Most studies that define the natural history of PFTs in patients with DMD include measurement of FVC, and therefore FVC provides the best available comparator for patients treated with eteplirsen (Mayer 2015; Buyse 2015; Khirani 2014; Henricson 2013; Hahn 1997; McDonald 1995; Miller 2005). MEP and MIP are also used as measures of expiratory and inspiratory muscle function in neuromuscular diseases including DMD (Khirani 2014; Henricson 2013; Hahn 1997). MEP and MIP are indicators of decreased respiratory muscle strength, but are subject to variability given that patients with muscle weakness and especially very young patients may have a reduced ability to perform the test.

Measurements of PFTs such as volume (FVC) or pressure (MEP and MIP) are converted to values relative to normal (% predicted). PFT values in adults decline with age. In children, in

contrast, there is a dramatic increase in PFT parameters over time, which parallels increases in growth and age. Hence, a correction factor accounting for growth/age needs to be incorporated for calculation of predicted values in the pediatric population. The methodology for calculation of predicted FVC values was described by Polgar (Polgar 1971) and corrects based on height. The most widely used correction for predicting MEP and MIP, which adjusts by age (MEP) or weight (MIP), was established by Wilson (Wilson 1984).

A comparison of FVC% predicted for eteplirsen treated patients (N=12) to patient level data from a contemporary, external natural history DMD patient cohort has been conducted. Dr. Mayer et al kindly provided FVC and FVC% predicted data from a subset of the patients that form the basis for their recently published work (Mayer 2015). A dataset of 44 patients with valid data described in the publication was received, of whom 24 were treated with corticosteroids and 14 were non-ambulant. These baseline characteristics were shown by Mayer et al not to impact the rate of decline in FVC% predicted, and therefore, all data received was used in the analyses.

Some proprietary data could not be accessed, however, Sarepta has received assurance that this should not affect the overall analyses. Patient-level data for eteplirsen-treated patients from study 201/202 up to Week 168 was used in the analysis, since all 12 patients had data up to this point. FVC% predicted vs age (in years) was plotted for eteplirsen-treated patients from study 201/202 and for the control patient data from Mayer et al. Simple regression models estimated the slope of the best-fit lines for each group. Given the exploratory nature of these analyses, and the difference in datasets between the more extensive longitudinal analysis in the eteplirsen cohort and the fewer data points per patient in the Mayer cohort, only descriptive statistics are presented.

When all patient level data from Mayer 2015 are plotted relative to age, the linear relationship between age and FVC% predicted in patients with DMD becomes apparent (Figure 22A). Based on regression analysis, the slope in the untreated cohort (N=44) is -6.8, which translates to an overall annual decline in FVC% predicted of 6.8%. This decline is slightly higher than the one published in Mayer et al (Mayer 2015). In order to exclude a potential difference between the overall slope and the slope in the age group of interest for comparison with eteplirsen, i.e. 7-15 years of age, a sensitivity analysis has been performed in a subset of patients in the Mayer population between the ages of 7-15 years old (Figure 22B). The sensitivity analysis showed an overall annual decline of 5.8%. In comparison, the slope in the eteplirsen treated group is -3.2, or approximately half of the annual decline in the similarly aged external control cohort (Figure 23). If only 10-15 year old patients were included, patients from Mayer et al had a slope of -4.7 and eteplirsen treated patients has a slope of -3.3 over 168 weeks. Using only data from 11-15 year old patients, those from Mayer et al had a slope of -3.6 and eteplirsen treated patients has a slope of -1.3 over 168 weeks. Thus the comparison with the external control data favors the eteplirsen treated group regardless of age cut-off.

Figure 22:   FVC% Predicted: Natural History per Mayer et al by Age Group
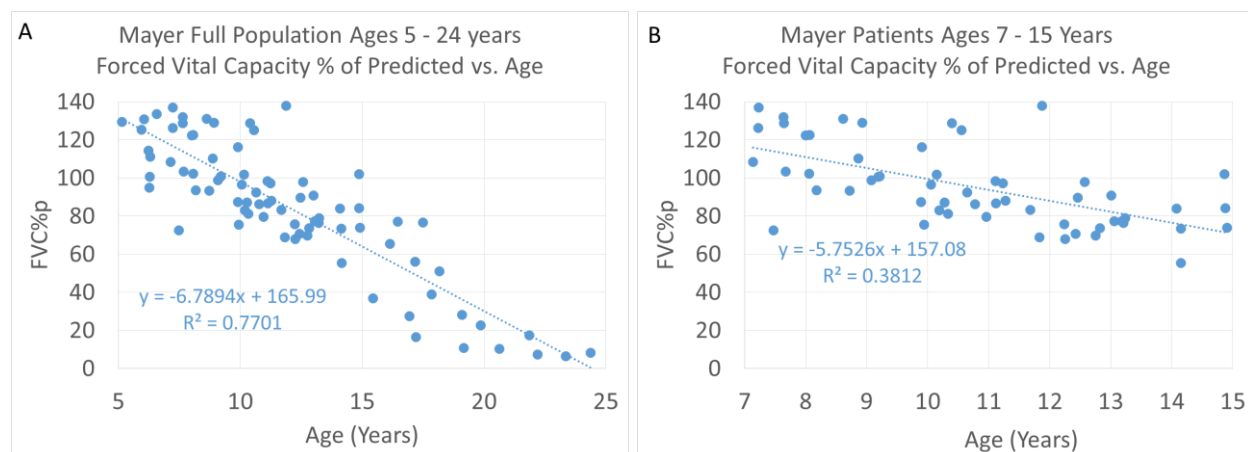
	Mayer Full Population (Age 5-24)	Mayer Subset (Age 7-15)
% Annual Decline	6.8%	5.8%

(A) FVC% predicted vs age based on full population (N=44, ages 5-24 years) patient level data from Mayer et al with an annual decrease of FVC% predicted of 6.8%. (B) If patients <7 years and >15 years are excluded (n=33), the resulting annual decrease is 5.8%.

Figure 23:   FVC% Predicted: Mayer et al Age Matched Subset Compared to Eteplirsen Treated Patients
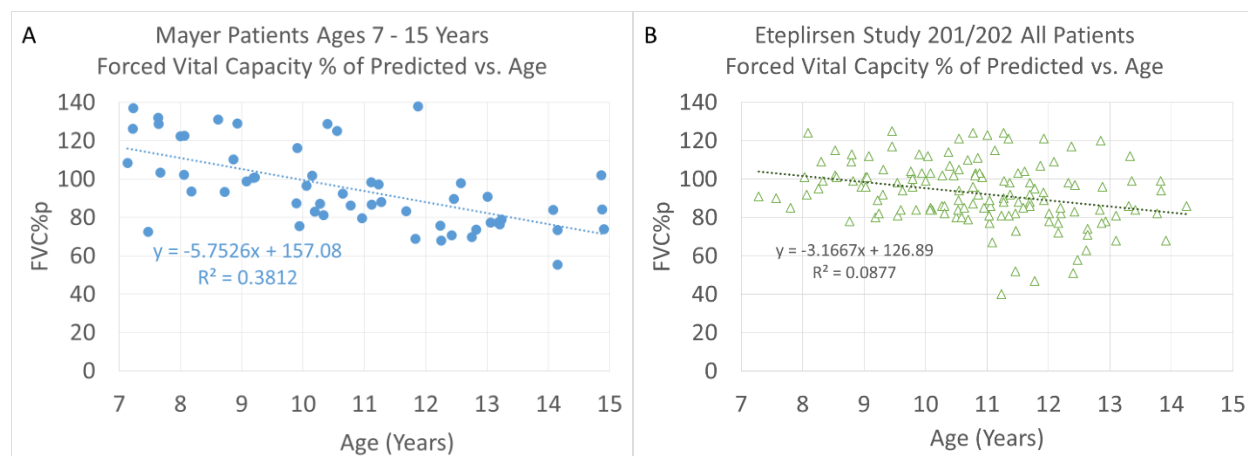
	Mayer Subset (Age 7-15)	Eteplirsen Full Population (Age 7-15)
% Annual Decline	5.8%	3.2%

(A) Mayer age matched (Ages 7-15 years) subset, with an annual decline of 5.8%. (B) Eteplirsen treated patients (30 or 50 mg/kg/week IV) from study 201/202: all available on-treatment data plotted vs. age, with an annual decline of 3.2%.

The most recently published dataset on annual decline of FVC% predicted in DMD patients (Khirani 2014) is similar to the data presented above (Mayer 2015), and therefore also supports a beneficial effect of eteplirsen. Findings in other publications have generally provided a higher estimated annual decrease in FVC% predicted than those found by Mayer and Khirani (Miller 2005, McDonald 1995, Hahn 1997, Buyse 2015), and are therefore also supportive.

Measurement of MIP and MEP has been done in patients with DMD, albeit to a lesser extent than FVC, and only 3 relevant scientific publications have been identified (Khirani 2014, Hahn 1997, Henricson 2013), Based on these data, an annual decline of MIP% predicted and MEP% predicted of approximately 3-4% would be expected in patients with DMD during their second decade. The change from baseline over 3 years with eteplirsen treatment (-3.6% for MIP% predicted and -8.4% for MEP% predicted, which translates into an average annual decline of 1% and 2.4%, respectively) appears to be within the same range or lower. However, interpretation of these comparisons is limited by the small number of scientific publications describing these specific assessments in DMD patients over time; the lack of availability of patient level comparator data; and the increased effort dependency compared to FVC for assessment of MIP and MEP, leading to higher variability especially in a pediatric population.

In summary, pulmonary function data from DMD patients who received eteplirsen for approximately 3 years were compared to data from the scientific literature. Analyses of FVC% predicted are strengthened by a comparison to patient level data (Mayer 2015). Using these comparator data, the deterioration of respiratory muscle function as measured by FVC% predicted appears to be slower than expected with eteplirsen treatment. Additionally, MEP% predicted and MIP% predicted may also decline more slowly, though interpretation of this comparison is limited by the aforementioned considerations.

7. PHARMACODYNAMIC RESULTS

7.1. Methods for Assessing Pharmacodynamic Endpoints

Primary Pharmacodynamic/Biological Efficacy Endpoint: Dystrophin Production

Several complementary methods were used to provide a detailed assessment of eteplirsen's effects on exon skipping and dystrophin protein expression, as no single measurement of dystrophin production can provide a complete evaluation of eteplirsen's pharmacodynamic and biological effects. In the eteplirsen clinical program, the evaluation of exon skipping was accomplished by RT-PCR and sequencing of the PCR product. Dystrophin protein production was evaluated by assessment of the percentage of dystrophin positive fibers and dystrophin intensity in histological specimens, and by Western blot.

Overall, in the 4 studies evaluating pharmacodynamics endpoints, there was a high ascertainment rate with all muscle biopsies resulting in evaluable samples. In Studies 201/202, analysis of muscle tissue biopsy samples by IHC was performed according to written procedures in a central laboratory by blinded personnel who were not otherwise involved in the study. Biopsy processing and analysis of samples for Studies 28 and 33 were performed at a separate laboratory. A high-level summary of the methods used to evaluate exon skipping and dystrophin production as well as study specific biopsy schedules are summarized in Table 10.

Table 10:   Methods for Evaluation of Pharmacodynamic/Biologic Endpoints by Studies

	Study 33	**Study 28**	**Study 201/202**
Anatomic Location of Muscle Biopsy	Extensor digitorum brevis muscle of treated and opposite placebo foot at baseline and Day 14-28	Biceps brachii Biopsy samples at Baseline and Week 14	Biceps brachii (Baseline and Week 24) and deltoid biopsy samples (Week 48 and Week 180)
Mechanism of Action Confirmation			
Detection of internally deleted dystrophin mRNA	RT-PCR assessment of mRNA exon 51 skipping	RT-PCR assessment of mRNA exon 51 skipping	RT-PCR assessment of mRNA exon 51 skipping
Sequencing of mRNA to confirm correct exon skipping	Direct sequencing of RT-PCR product	Direct sequencing of RT-PCR product	Direct sequencing of the RT-PCR product (Week 180 only)
Dystrophin Production and Localization			
Dystrophin protein levels	Western Blot assessment of Dystrophin protein levels with NCL-DYS1[a]	Western Blot assessment of Dystrophin protein levels with NCL-DYS1 [a]	Western Blot assessment of Dystrophin protein levels with MANDYS106 [a], NCL-DYS2 [a] and NCL-DYS1 [a] (Week 180 only)
Percent dystrophin-positive muscle fibers	Scoring of digital images for presence of dystrophin-positive muscle fibers following indirect immunofluorescence staining with MANDYS106 [a]	Scoring of digital images for presence of dystrophin-positive muscle fibers following indirect immunofluorescence staining with MANDYS106 [a]	Scoring of digital images for presence of dystrophin-positive muscle fibers following indirect immunofluorescence staining with MANDYS106 [a], NCL-DYS2 [a], NCL-DYS3 [a]
Dystrophin Intensity	Assessment of fluorescence signal intensity following indirect immunofluorescence staining with MANDYS106 [a] antibody against dystrophin	Assessment of fluorescence signal intensity following indirect immunofluorescence staining with MANDYS106 [a] antibody against dystrophin	Assessment of fluorescence signal intensity following indirect immunofluorescence staining with MANDYS106 [a], NCL-DYS2 [a] antibody against dystrophin

[a] Dys1: NCL-DYS 1 Clone Dy4/6D3 (Leica); Dys2: NCL-DYS 2 Clone DY8/6C5; MANDYS106: Nguyen 1992.

7.2.   Pharmacodynamic/Biological Endpoints

7.2.1.   RT-PCR Demonstrates Exon 51 Skipping in Studies 201/202, 28 and 33

All studies used qualitative nested end-point RT-PCR to detect the presence or absence of internally shortened dystrophin mRNA to confirm exon 51 skipping. PCR primers were specific for each patient's known dystrophin mutation, and RT-PCR products were visualized on agarose gels. In addition, the accurate skipping of exon 51 was confirmed by sequencing of the skipped RT-PCR product in Studies 28 and 33, and for the Week 180 biopsy in Studies 201/202. Exon 51 skipping was demonstrated using RT-PCR in all eteplirsen-treated patients evaluated to date (N = 36).

- **Proof of Concept Study 33**: (N = 7) patients were given a single dose of eteplirsen IM 0.9 (N = 5) or 0.09 (N = 2) mg directly into the EDB muscle and a single dose of placebo in the contralateral foot. RT-PCR demonstrated exon 51 skipping in the eteplirsen-treated foot of all patients, although only low-level exon skipping was observed in the 2 patients receiving the low dose of 0.09 mg.

- **Dose-Ranging Study 28**: (N = 17) patients were given 12 weekly IV doses of eteplirsen (0.5-20 mg/kg/week) with muscle biopsies at Baseline and post-treatment Week 14. In this study, exon skipping in post-treatment biopsies was most easily and reliably detected in those patients within the 2 highest dose groups (10 and 20 mg/kg) suggesting a dose-dependent effect of eteplirsen on exon skipping.

- **Pivotal Study 201/202**: (N = 12) tested higher doses of 30 or 50 mg/kg/week with muscle biopsies taken at Baseline and on-treatment Week 12, 24, 48 and 180. Exon skipping was observed for all 12 patients.

Skipping of exon 51 was confirmed with Sanger sequencing (Sanger 1975). Accurate sequences of the flanking DNA at the new exon junction formed by skipping exon 51 were confirmed in all assessed patients from Studies 33 (N = 7), Study 28 (N = 17) and Studies 201/202 (Week 180 biopsies only, N = 11). Observed sequences were consistent with those found in BMD patients with the corresponding in-frame mutation, supporting the hypothesis that eteplirsen results in the production of functional dystrophin protein capable of attenuating the DMD phenotype.

7.2.2.   Dystrophin Protein Expression – Percent Dystrophin Positive Fibers

Across studies, the production of dystrophin protein pre- and post-treatment was evaluated using immunohistochemical (IHC) methods. The percentage of dystrophin-positive muscle fibers was determined by a pathologist who counted both the number of dystrophin positive muscle fibers and the total number of muscle fibers allowing the calculation of a percentage of dystrophin-positive muscle fibers. In pivotal studies 201/202, treatment with eteplirsen produced reliable increases in the percentage of dystrophin-positive fibers.

Study 201/202

The primary endpoint in the 24-week placebo-controlled portion of Study 201 was change from baseline in percent dystrophin positive fibers. At Week 25, the placebo patients were rolled over onto open-label treatment and all patients continue to receive treatment in the on-going extension study today.

A key question that Study 201/202 sought to address, was whether dose or duration was more important in the induction of novel dystrophin protein. Accordingly, while all patients had muscle biopsies at baseline, Week 48 and Week 180, in order to minimize the number of required biopsies, the study design varied the biopsy schedule at Week 12 (only 50 mg/kg and 2 placebo patients) and Week 24 (only 30 mg/kg and 2 placebo patients). Figure 24 displays the biopsy schedule by treatment group in Studies 201/202.

Figure 24:   Biopsy Schedule in Study 201/202

Treatment Group	Baseline	Week 12	Week 24	Week 25	Week 48	Week 180
30 mg/kg	√		√	Placebo patients rolled onto treatment	√	√
Placebo	√		√			
50 mg/kg	√	√			√	√*
Placebo	√	√				

*1 patient declined the optional Wk 180 biopsy

Treatment with 50 mg/kg did not demonstrate a significant increase in the amount of mean percentage of dystrophin-positive fibers at Week 12. However, treatment with 30 mg/kg eteplirsen (N = 4) for 24 weeks significantly increased the mean percentage of dystrophin-positive fibers from a baseline of 18.19% to 41.14% resulting in a 22.95% change from baseline p ≤0.004). This change was also statistically significantly different than the change from baseline observed in the placebo-treated patients (p ≤0.002). Mean change from Baseline in PDPF for each individual patient is shown in Figure 25. Therefore, it was determined that a 12-week treatment duration may not be sufficient to observe significant dystrophin production, whereas significant dystrophin production was shown by Week 24.

Figure 25: **Individual Patient Data: Mean Change from Baseline in Percent Dystrophin Positive Fibers (MANDYS106) in Patients Treated with 50 or 30 mg/kg Eteplirsen vs. Placebo at Week 12 and 24, respectively (Study 201)**
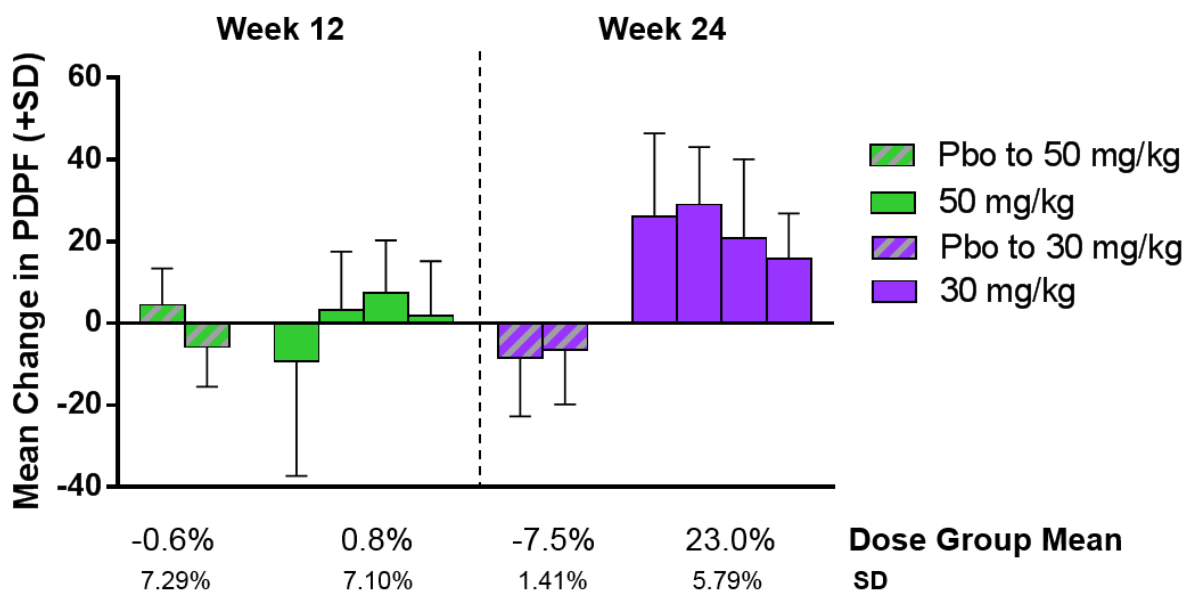
Dose selection of 30 mg/kg based on Week 48 analysis of Studies 201/202

Analysis of mean change in percent dystrophin positive fibers at Week 48 showed that both 30 and 50 mg/kg weekly doses significantly increased the mean percentage of dystrophin-positive fibers compared to Baseline (p ≤0.001, p ≤0.008, respectively), with no significant difference in the magnitude of this change between the 2 dose groups. Statistically significant changes from Baseline were also observed when the dystrophin results at Week 48 were combined for the 4 patients who received placebo for the first 24 weeks (p ≤0.009) and for the 8 patients who received eteplirsen continuously (p ≤0.001). Mean change from Baseline in PDPF for each individual patient is shown in Figure 26.

Given the equivalency of Week 48 mean change in PDPF between 30 or 50 mg/kg, and the need for chronic lifelong administration in a pediatric population, the lower dose of 30-mg/kg dose was selected.

Figure 26:   **Mean Change from Baseline in Percent Dystrophin Positive Fibers in Patients Treated with 30 vs 50 mg/kg/week Eteplirsen for Week 24 or 48 (Studies 201/202)**
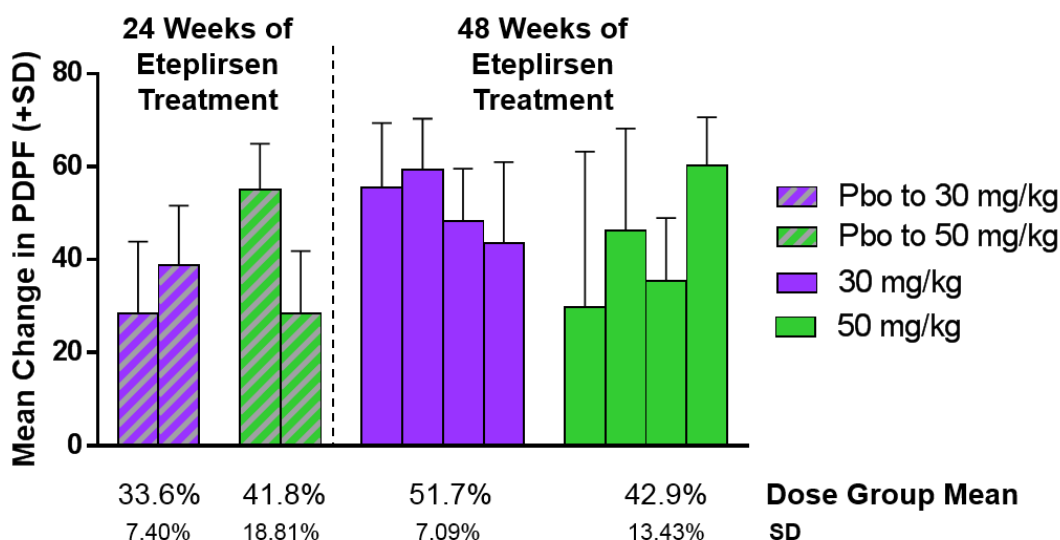
Independent Verification of Percent Dystrophin-Positive Fibers

In response to a request by the Agency, the original images used in scoring the percentage of dystrophin-positive fibers in biopsy samples from patients in Studies 201/202 and Study 28 were re-assessed by 3 independent and blinded raters. Per the Agency's request, the primary endpoint for the image reassessment was the percentage of dystrophin-positive fibers at Week 24 and changes from Baseline. Sarepta engaged 3 trained pathologists (through Flagship Biosciences) to independently identify dystrophin-positive muscle fibers utilizing the same archived digital images obtained from Study 201 and Study 28 used for the original study assessments.

Independent Verification of Study 201/202 Percent Positive Dystrophin Fibers

The mean ratings from the independent pathologists showed the mean percentage of dystrophin-positive fibers for the 4 patients in the 30 mg/kg eteplirsen group increased from 13.63 at Baseline to 27.33 at Week 24, representing a 1.37-fold, statistically significant ($p \leq 0.007$) increase, consistent with the results of the original analysis performed at Nationwide Children's Hospital in Columbus, OH. Evaluation of inter-rater and intra-rater reliability confirmed that consistency was achieved through training of the independent raters. The inter-rater reliability for the 3 blinded pathologists performing the reassessment was high (interclass correlation coefficient [ICC] = 0.793), as was the intra-rater reliability, with ICCs for each rater ranging from 0.932 to 0.955.

Study 28 Percent Positive Dystrophin Fibers

Once weekly IV infusions of eteplirsen (0.5 to 20 mg/kg) for 12 weeks in Study 28 resulted in a 3-fold increase in the mean percentage of dystrophin-positive fibers, which increased from a mean of 2.2% at Baseline to a mean change from Baseline of 6.5% at Week 14 (biopsies were

taken 2 weeks after the last dose), with the greatest mean increases observed for highest doses (i.e., 10 or 20 mg/kg), although variation across individual results was noted. The original analysis of the percent dystrophin-positive fibers, which was performed at the University College London, was also confirmed by a blinded reassessment of the data by 3 independent pathologists, who noted mean increases from 1.83% at Baseline to 8.19% at Week 14 for 4 patients who received 10 mg/kg/week and mean increases from 2.87% at Baseline to 15.87% at Week 14 for 4 patients who received 20 mg/kg/wk eteplirsen. Although increases in dystrophin were observed as early as 14 weeks in Study 28, this was not consistent across individuals.

Study 33 Percent Positive Dystrophin Fibers

Results from Study 33, in which 7 patients received a single IM dose of 0.09 (N = 2) or 0.9 mg (N = 5) eteplirsen into the EDB muscle of one foot and a single dose of placebo into the EDB of the opposite foot, further support eteplirsen's ability to induce dystrophin production in patients with DMD. In this study, dystrophin-positive fibers were observed in biopsies obtained from the eteplirsen treated feet of the 5 patients who received the higher dose of eteplirsen, 4 of whom were already non-ambulatory at the time of the study. The mean percentage of dystrophin-positive fibers in EDB muscle biopsy specimens from the eteplirsen-treated feet was 59.7% compared with 0% in EDB muscle biopsy specimens from the placebo-treated feet of the same 5 patients. The lower dose of eteplirsen (0.09) did not have a measurable effect on dystrophin production in this study

7.2.3.   Dystrophin Protein Expression – Dystrophin Fiber Intensity

Analysis of dystrophin fiber intensity as measured by an automated software system (Bioquant® or MetaMorph®) was used as a complementary method to verify the de novo production of dystrophin. In Studies 201/202, 28 and 33, eteplirsen's effect on dystrophin production was examined by measuring the fluorescence staining intensity of dystrophin following indirect immunostaining with primary anti-dystrophin antibodies. In both studies, the mean changes from Baseline in dystrophin fiber intensity were similar in magnitude and direction to the results of the percentage of dystrophin-positive fibers analyses, supporting eteplirsen's ability to increase dystrophin levels in patients with DMD.

Studies 201/202 Dystrophin Intensity

Once weekly treatment with eteplirsen (30 or 50 mg/kg) significantly increased (p ≤0.001) mean dystrophin fiber intensity from 10.57% of normal at Baseline to 25.98% of normal at Week 48 in the 8 patients in Studies 201/202 who received eteplirsen from the start of the study. Eteplirsen also significantly (p ≤0.006) increased mean dystrophin fiber intensity from 8.95% of normal at Baseline to 23.43% of normal at Week 48 in the 4 patients in Studies 201/202 who received placebo from Baseline to Week 24 and eteplirsen from Weeks 24 to 48 (and hence had only received eteplirsen for 24 weeks at the time of the Week 48 biopsy).

Study 28 Dystrophin Intensity

Similarly, in Study 28, once weekly treatment with eteplirsen increased mean dystrophin fiber intensity in the 17 patients with evaluable data from 7.9% of normal at Baseline to 11.5% of normal at Week 14. The results were variable within and across dose groups, but the largest and most consistent increases tended to occur in the 10 and 20 mg/kg/wk eteplirsen groups.

Study 33 Dystrophin Intensity

In Study 33, a single IM dose of 0.9 mg eteplirsen increased the mean dystrophin fiber intensity from 9.4 % in the contralateral saline-injected muscle to 26.4% in the eteplirsen-treated muscle. The lower dose of eteplirsen (0.09 mg) did not have a measurable effect on dystrophin production in this study.

7.2.4.   Dystrophin Quantity By Western Blot

Study 33, 28 Western BLOT

Dystrophin expression was also evaluated by Western Blot in Studies 33, 28 and 201/202. In Study 28, the most consistent increase in dystrophin expression from baseline tended to occur in the 10 and 20 mg/kg/wk eteplirsen group. Similarly, the higher dose group in Study 33 showed a more consistent expression above baseline than the lower dose group. Results for Study 201/202 are presented in the Week 180 section below.

7.2.5.   Studies 201/202: Week 180 Results For Dystrophin Production

Patients in Studies 201/202 underwent a fourth muscle biopsy after 180 weeks in the study. The primary purpose of this optional biopsy was to evaluate the ability of eteplirsen to sustain dystrophin production during chronic treatment. This biopsy also afforded an opportunity to examine dystrophin production using the optimized method previously described for evaluation of percent dystrophin-positive fibers. In addition, new Western blot methodology was developed in alignment with the NIH-FDA Dystrophin Methodology Workshop (March 2015) and in collaboration with the FDA.

Eleven of the 12 patients provided muscle biopsies at Week 180. As mentioned previously, it is important that biopsy results be compared to pre-treatment or untreated controls in order to evaluate the treatment effect on dystrophin expression. Frozen archived Baseline muscle biopsy tissue from Study 201 was available for re-analyses from only a limited number of patients, resulting in baseline values for only 3 patients for each of the 3 dystrophin parameters. Since baseline tissue was not available for all patients, these samples were supplemented with tissue from untreated control patients amenable to exon 51 skipping in order to provide a total of 9 untreated samples as a comparator group. The additional 6 untreated control samples for each assay were from confirmatory Study 301 (PROMOVI) and were simply the first baseline biopsies collected to have sufficient excess biopsy material available to repurpose for use in the Week 180 analysis. Characteristics for the untreated control patients are summarized in Appendix 9.

A series of methodologies was utilized including: scoring of digital images for the percentage of dystrophin-positive fibers following indirect immunofluorescence staining; BIOQUANT® assessment of dystrophin fiber intensity following indirect immunofluorescence staining; and Western blot assessment of dystrophin protein levels.

Study 201/202 Week 180 Percent Dystrophin-Positive Fibers (PDPF)

As shown in Figure 27, the mean percentage of dystrophin-positive fibers in the eteplirsen-treated patients at Week 180 (37.33%), as determined by a blinded analysis of digital images performed by the expert pathologist at NCH, was 7.4 times greater than that observed in untreated patients (5.04%), a difference that was statistically significant (p <0.001).

Confirmation of this finding (treated PDPF > untreated PDPF) was provided by an independent analysis of identical images conducted by 3 blinded independent pathologists, with mean differences between the eteplirsen-treated patients and the untreated controls ranging from 14.15% to 19.99% for the 3 raters (all p-values <0.001). Refer to Appendix 10 for individual patient PDPF data and Appendix 12 for representative images of dystrophin-positive fibers.

Figure 27:   Mean Percentage of Dystrophin-Positive Fibers in Eteplirsen-Treated Patients (Week 180, Studies 201/202) vs. Untreated DMD Controls
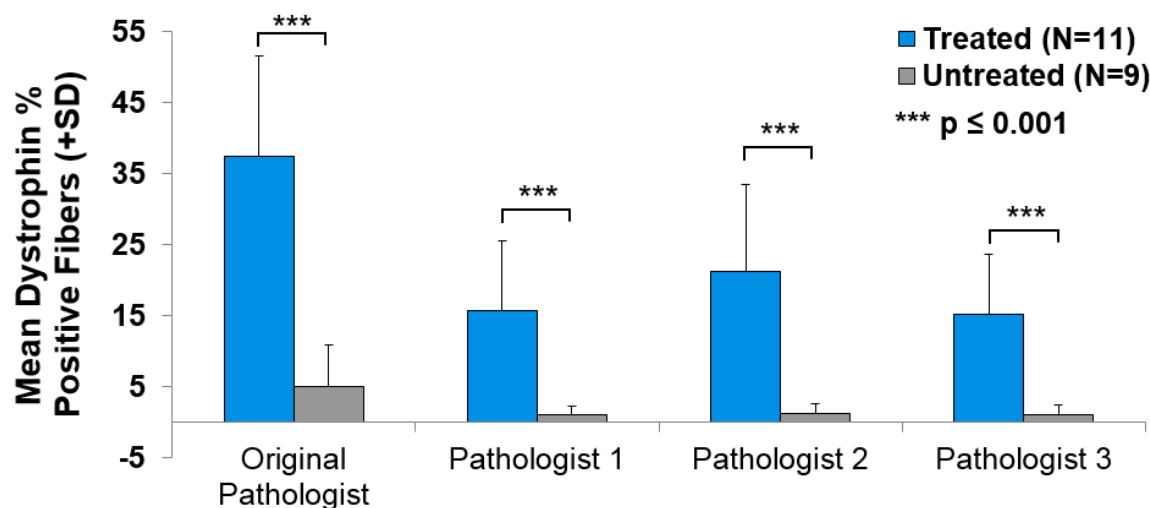
	Original Assessment	Reassessment		
	NCH Pathologist	Pathologist 1	Pathologist 2	Pathologist 3
Eteplirsen, mean (SD)	37.33 (14.267)	15.67 (9.846)	21.30 (12.219)	15.20 (8.442)
Untreated, mean (SD)	5.04 (5.85)	1.02 (1.293)	1.31 (1.294)	1.05 (1.371)
Mean Diff. (95% CI) p-value	32.29 (22.15, 42.43) p <0.001	14.66 (8.01, 21.30) p <0.001	19.99 (11.75, 28.22) p <0.001	14.15 (8.44, 19.87) p <0.001

Pathologist(s)	Absolute Difference of Mean PDPF (Treated vs. Untreated)	Relative Difference of Mean PDPF (Treated vs. Untreated)	p-value
Multi-rater (3)	16.27%	1,453%	<0.001

Study 201/202 Week 180 Dystrophin Intensity

The IHC images were also evaluated by an automated computer algorithm to assess for dystrophin staining intensity. Generally higher dystrophin staining was observed for individual eteplirsen-treated patients in comparison to untreated control patients, with the overall mean dystrophin fiber intensity in eteplirsen-treated patients at Week 180 (22.61) significantly (p <0.001) greater than that observed in untreated controls (9.41) as shown in Figure 28, Table 11. Results for dystrophin fiber intensity from individual patients are provided in Appendix 11.

Figure 28:   **Mean Dystrophin Intensity in Eteplirsen-Treated Patients (Week 180, Studies 201/202) vs. Untreated DMD Controls**
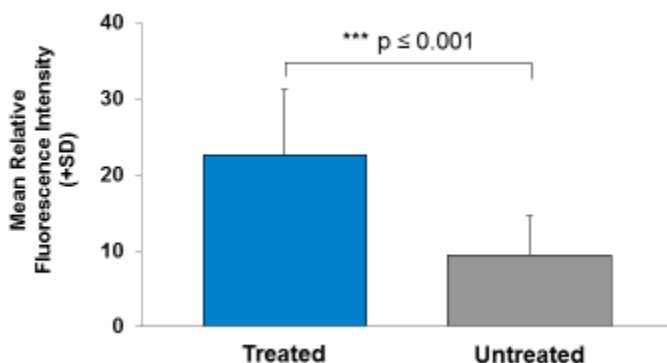
Table 11:   **Absolute and Relative Differences of Mean Dystrophin Intensity (Week 180, Studies 201/202)**

Absolute Difference of Mean Intensity (Treated vs. Untreated)	Relative Difference of Mean Intensity (Treated vs. Untreated)	p-value
13.2%	140%	<0.001

Study 201/202 Week 180 Western Blot

Dystrophin expression was also evaluated using a validated, optimized Western Blot method developed in collaboration with the FDA and in alignment with the FDA-NIH Dystrophin Methodology Workshop (March 2015). This validated method is capable of quantifying dystrophin as low as 0.25% of normal, enabling sensitive robust determination of % normal dystrophin levels.

In Western blot analysis, 9 of 11 biopsied eteplirsen-treated patients had an observable dystrophin band. Comparing these results to biopsies from 9 untreated exon 51 amenable DMD patients, eteplirsen-treated patients demonstrated statistically significant higher mean dystrophin expression levels than untreated patients. The mean dystrophin protein level as assessed by Western blot in eteplirsen-treated patients at Week 180 (0.93) was significantly (p = 0.007) greater than that observed in untreated controls (0.08) (Figure 29, Table 12). Results for Western blot analysis from individual patients are provided in Appendix 13.

Figure 29:   **Mean Percent Normal Dystrophin in Eteplirsen-Treated Patients (Week 180, Studies 201/202) vs. Untreated DMD Controls (Western Blot)**
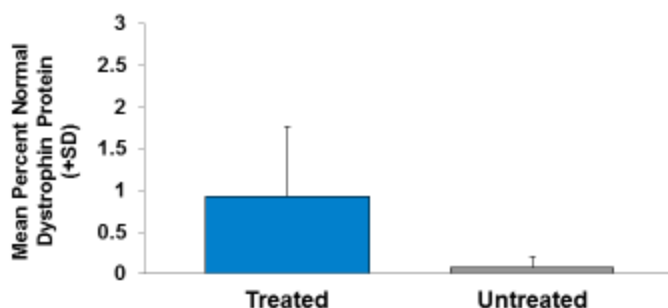
Table 12:   **Absolute and Relative Differences of Mean Dystrophin by Western Blot (Week 180, Studies 201/202)**

Absolute Difference of Means (Treated vs. Untreated)	Relative Difference of Means (Treated vs. Untreated)	p-value
0.85%	104%	<0.007

There is a wide body of literature reporting on dystrophin levels in DMD and BMD. The range of values for Western blot presented in literature sources cannot be directly compared to each other or to the values from eteplirsen studies. Western blot results are not directly comparable due to method variations and most critically, the absence of a universal dystrophin reference standard. This is illustrated by levels of dystrophin across different normal muscle biopsy tissue; samples can vary from as low as 50 % to over 100 % relative to a "normal" reference control (FDA-NIH Dystrophin Methodology Workshop (March 2015) and eteplirsen NDA 206,488). This variability is likely due to variation between individuals, muscle type and/or different regions of a muscle biopsy sample. A uniform, absolute dystrophin standard to enable direct comparison of % normal values between individual assays or laboratories has not been established.

In the Week 180 analysis muscle biopsy samples were measured relative to a single "normal" non BMD/DMD individual's deltoid muscle biopsy tissue sample in order to enable consistent and accurate quantification and comparison of dystrophin expression in the eteplirsen treated and untreated samples. Western Blot method validation and acceptance standards are detailed in Appendix 14 along with representative gel images.

In summary, the results from the entire set of patient samples from eteplirsen-treated (N = 11) and untreated (N = 9) patients with DMD mutations amenable to exon 51 skipping were concordant across all of the assays (Table 13), supporting both the robustness of each assay's underlying methodology as well as the conclusion that long-term treatment of Exon 51 amenable patients with eteplirsen continues to produce sustainable increases in dystrophin muscle tissue.

Table 13:   **Summary of Week 180, Studies 201/202 Dystrophin Data in Eteplirsen Treated Patients and Untreated DMD Controls**

Week 180	Treated (Mean % Dystrophin of Normal)	Untreated (Mean % Dystrophin of Normal)	Difference of Means (Treated vs. Untreated)	Relative Difference of Means	P-value
PDPF (Flagship)	17.39 %	1.12 %	+16.27 %	+1453%	<0.001
Intensity	22.61 %	9.41 %	+13.20 %	+140%	<0.001
Western Blot	0.93 %	0.08 %	+0.85 %	+1063%	<0.007

7.2.6.   Cellular Localization of Dystrophin, nNOS, and Sarcoglycan Complex Proteins

The functionality of the dystrophin produced by eteplirsen is supported by IHC analysis showing localization of dystrophin with other components of the DAPC, including nitric oxide synthase (nNOS), and α-, β-, and γ-sarcoglycan, at the sarcolemma membrane. The restoration of nNOS to the membrane is especially notable as it is consistently absent in DMD muscle tissue lacking dystrophin.

Figure 30 shows muscle tissue taken from an untreated DMD patient (Panel A), a single DMD patient (Patient 006) after 48 weeks of treatment with eteplirsen in Studies 201/202 (Panels B/D), and normal healthy muscle (Panel C) and stained for nNOS. While no evidence of nNOS binding is evident in muscle taken from the untreated DMD patient (Panel A), there is clear evidence of nNOS staining in both the healthy control tissue (Panel C) and in tissue obtained from the patient treated with eteplirsen for 48 weeks (B and D). Muscle tissue from the same eteplirsen-treated patient (006) positively stained for β-Sarcoglycan (Panel E) and γ-Sarcoglycan (Panel F) confirm restoration of the DAPC complex in this patient.

Similar results, were obtained in Studies 28 and 33 (as published in Cirak 2011 and Cirak 2012).

Figure 30:   **Positive Staining for nNOS and Sarcoglycan Complex Proteins in an Eteplirsen-Treated Patient in Studies 201/202**
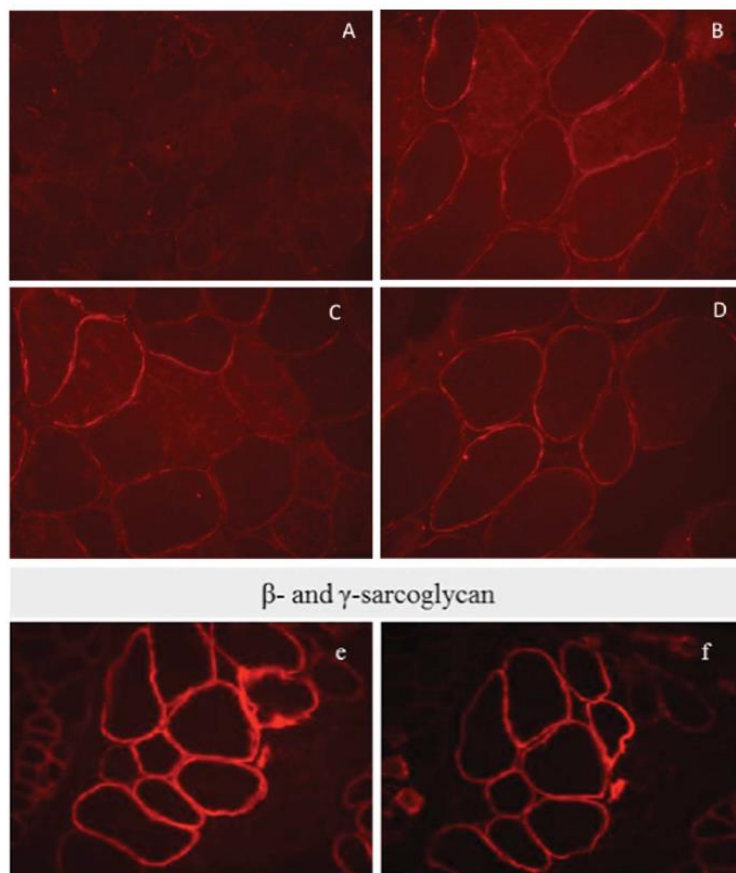
Source: Mendell 2013.

8. ONGOING AND PLANNED STUDIES

8.1. Ongoing Studies Supportive of Safety

Sarepta is also conducting 2 additional studies to further evaluate the safety and efficacy of eteplirsen in younger boys and boys with advanced DMD. Study 203 is an ongoing, 96-week, open-label study to evaluate the safety, efficacy, and tolerability of eteplirsen in DMD patients 4 to 6 years of age; this study includes an untreated control group of DMD patients not amenable to exon 51 skipping. Study 204 is an ongoing, 96-week, open label study of eteplirsen in non-ambulatory patients or unable to walk ≥300 meters on the 6MWT.

Efficacy data are not yet available for these studies; however, as of 14 August 2015 (cutoff date for the 120-Day Safety Update) safety data were available for 4 and 24 patients in Studies 203 and 204, respectively. Key aspects of these studies are summarized below.

Table 14: Ongoing Supportive Studies 203 and 204

	Study Number	
	Study 203	**Study 204**
Study Design	Multi-center, open-label study (US)	Multi-center, open-label study (US)
Dosing Regimen	Eteplirsen 30 mg/kg/week (IV) Includes untreated concurrent control group of DMD patients not amenable to exon 51 skipping	Eteplirsen 30 mg/kg/week (IV)
Endpoints	**Primary**=Safety and tolerability; **Secondary**=Change from BL to Wk 48 and Wk 96 in PDPF **Exploratory**=Dystrophin intensity; Dystrophin protein levels (Western blot); exon 51 skipping (RT PCR); T-cell infiltration; Change from BL to Wk 96 for NSAA, Time to walk 100 meters; PODCI; PK	**Primary**=Safety and tolerability; **Exploratory**=Change from BL to Wk 96 in PFTs, PUL Scale, Brooke Score for Arms and Shoulders, 9-hole peg test, ACTIVE, 10-Meter Walk/Run Test, and EK Scale
Required Age at Entry (yrs)	4-6	7-21
Study Status	Ongoing	Ongoing
No. Enrolled	4	24
No. Completed	NA	NA
Study Period	June 2014 –14 Aug 2015[a]	Nov 2014 –14 Aug 2015[a]
Planned Study Duration	96 weeks	96 weeks

Abbreviations: ACTIVE = Ability Captured Through Interactive Video Evaluation; BL = Baseline; EK = Egen Klassifikation; IV = intravenous; NSAA = North Star Ambulatory Assessment; PDPF = percentage of dystrophin positive fibers; PK = pharmacokinetics; PFT = pulmonary function testing; PODCI = Pediatric Outcomes Data Collection Instrument; PUL = Performance Upper Limb Scale; RT-PCR = reverse transcriptase-polymerase chain reaction; US = United States; Wk = week.

[a] Cut-off date for 120 Day Safety Update

8.2.   Confirmatory Studies to Support Accelerated Approval

Sarepta will conduct 2 confirmatory studies in accordance with the requirements for Accelerated Approval. Study 4658-301 (also referred to as PROMOVI) will confirm the efficacy of eteplirsen in a population of boys with DMD that is amenable to exon 51 skipping. The second study, 4045-301 (also referred to as ESSENCE) will confirm the efficacy of the PMO platform testing the efficacy of 2 other PMOs in a population of boys that is amenable to exon 45 or 53 skipping.

PROMOVI is an ongoing 96-week, open-label study evaluating the effects of eteplirsen in DMD patients amenable to exon 51 skipping in boys 7 to 16 years of age; this study includes an untreated control group of DMD patients with deletion mutations amenable to skipping exons other than 51 (Figure 31).

Figure 31:   Study Schematic for PROMOVI
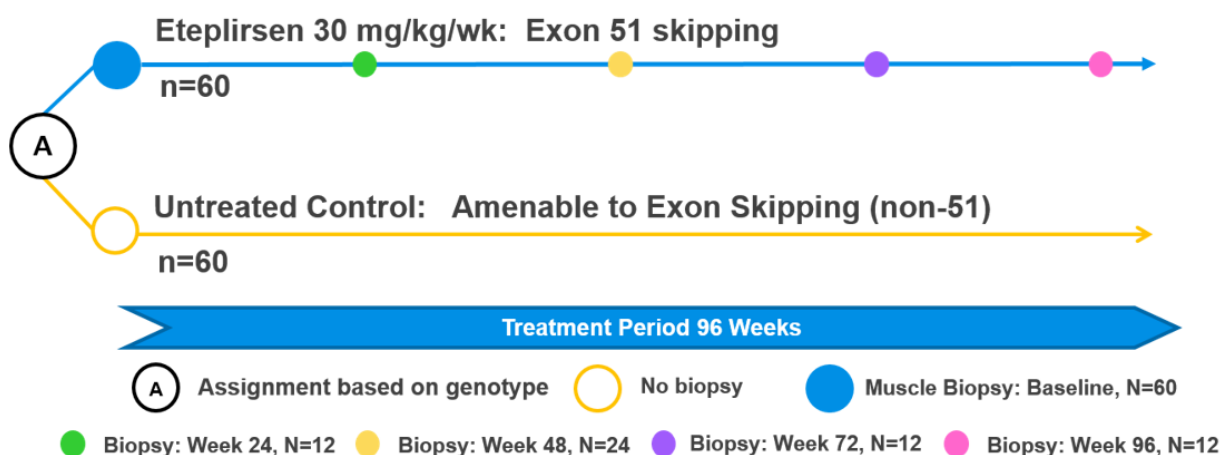
ESSENCE is a planned, double-blind, placebo-controlled study to evaluate the efficacy and safety of 2 other PMOs, SRP-4045 and SRP-4053, in DMD patients amenable to exon 45 and 53 skipping, respectively. This study is expected to begin dosing in Q1 2016 (Figure 32).

Figure 32:   Study Schematic for ESSENCE
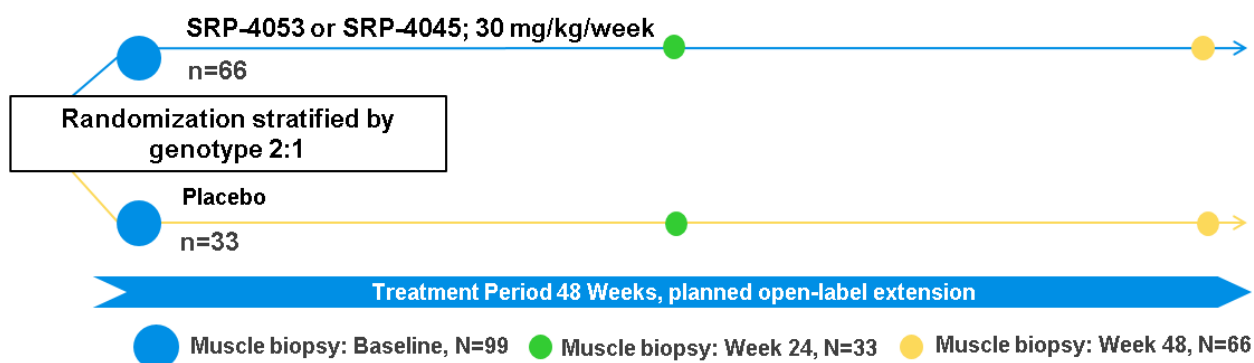
Efficacy data are not yet available for these studies; however, as of 14 August 2015 (cutoff date for the 120-Day Safety Update) safety data were available for 48 patients from Study 4658-301 (PROMOVI). Key aspects of the confirmatory studies are summarized below in Table 15.

Table 15:   Confirmatory Studies: 4658-301 (PROMOVI) and 4045-301 (ESSENCE)

	Study Number	
	4658-301 PROMOVI	**4045-301 ESSENCE**
Study Design	Multi-center, treatment assigned open-label study of eteplirsen in DMD patients amenable to skipping exon 51 (US) compared to an untreated control group of DMD not amenable to skipping exon 51	Multi-center, randomized double-blind, placebo-controlled study of PMOs for DMD patients amenable to skipping exon 45 or 53 (US) Ratio of 2:1
Dosing Regimen	Eteplirsen 30 mg/kg/week (IV) Includes untreated concurrent control group of DMD patients not amenable to exon 51 skipping	SRP-4045, SRP-4053 (according to genetic mutation) or placebo (IV)
Endpoints	**Primary Efficacy**=Change from BL to Week 48 on the 6MWT **Secondary Efficacy**=Change from BL to Wks 24 and 48 in PDPF (treated patients only); Change from BL to Wk 48 in PFT	**Primary Efficacy**=Change from BL to Wk 48 on the 6MWT **Secondary Efficacy**=Change from BL to Wk 24 or Wk 48 in PDPF (treated patients only); Change from BL to Wk 48 in PFT
Required Age at Entry (yrs)	7-16	7-16
Study Status	Ongoing	Planned
No. Planned Enrolled	60:60	60:30
No. Enrolled	48 treated/15 untreated	0

Abbreviations: 6MWT = 6 Minute Walk Test; BL = Baseline; IV = intravenous; PDPF = percentage of dystrophin positive fibers; PFT = pulmonary function testing; QMT = quantitative muscle testing; RT-PCR = reverse transcriptase-polymerase chain reaction. SAM = Step Activity Monitor; US = United States; Wk = week.

[a] Cut-off date for 120-Day Safety Update.

9. SAFETY EVALUATION

9.1. Methods for Assessing Safety

Across the 7 clinical studies providing safety data, the safety and tolerability of eteplirsen were evaluated using standard safety assessments including review of adverse events (AEs), serious adverse events (SAEs), study or treatment discontinuations; safety laboratory tests (serum chemistry, hematology and coagulation, and urinalysis); electrocardiograms (ECGs); vital signs; and physical examination findings. In addition, echocardiography (ECHO) was performed to further evaluate the clinical course of cardiomyopathy associated with the underlying disease (Spurney 2014). In Studies 201/202, 204, 203 and 301, renal function was closely monitored via serial testing of blood urea, blood creatinine, urine protein, as well as serum cystatin C, due to nonclinical renal findings in kidneys (Section 4.2.2).

9.2. Safety Population

The safety population includes all patients who were randomized / enrolled and received at least 1 dose of study drug (placebo or eteplirsen) or, for untreated control patients from Study 301, all patients who had completed the Week 1 visit. The eteplirsen-treated safety population is shown in Table 16. Eteplirsen is proposed for accelerated approval for the treatment of DMD patients with mutations that are amenable to exon 51 skipping at a dose of 30 mg/kg administered by weekly IV infusion. The safety database provides data from 114 patients from 4-19 years of age at study entry, including 88 patients treated with eteplirsen 30 mg/kg or higher by weekly IV infusion. Twelve (12) patients were treated for up to 208 weeks in Studies 201/202 and 76 patients were treated with eteplirsen 30 mg/kg/wk in Studies 203, 204, and 301 for up to 40 weeks. Although the safety database is not extensive, DMD is a rare disease and the intended patient population is a discrete subpopulation that represents approximately 13% of DMD patients (Aartsma-Rus 2009) which consists of a total of 1,300 to 1,900 patients in the US. As such, the eteplirsen safety database represents approximately 6-9% of the intended US patient population for eteplirsen.

Table 16: Studies Comprising the Eteplirsen Safety Database

Study and Description	Dose (mg/kg)	Route of Administration	Duration of Dosing (weeks)	N
Study 33 [a] (*proof of concept*)	0.09, 0.9	IM	Single dose	7
Study 28 (*dose ranging*)	0.5, 1, 2, 4, 10, 20	IV	12	19
Studies 201/202 (*placebo-controlled / open-label*)	30, 50	IV	184-208	12
Studies 301 (*confirmatory*), 204 (*advanced DMD*), and 203 (*younger patients*)	30	IV	1-40	76
All eteplirsen-treated patients				**114**

IM = single intramuscular dose; IV = once weekly intravenous infusion.

[a] A single intramuscular dose was administered in Study 33.

9.3.   Statistical Analysis

Descriptive statistics were used to summarize the safety data. For the purposes of the integrated safety analyses, treatment-emergent adverse events (TEAEs) were defined as any adverse event that began after the start of the first infusion (or injection) of study drug (eteplirsen or placebo) and within (≤) 28 days after the last dose of study medication. Events with missing start dates were considered treatment emergent.

9.4.   Exposure to Eteplirsen

A total of 88 patients have received once weekly eteplirsen IV at the proposed clinical dose of 30 mg/kg or higher. Of these, 61 have been treated for ≥12 weeks. Six patients each at 30 and 50 mg/kg eteplirsen have been treated over 4 years (Table 17).

Mean exposure for patients treated at 30 mg/kg/wk (N = 82) and 50 mg/kg/wk (N = 6) IV is 213.7 and 1394.8 days, respectively. Twelve (12) patients received eteplirsen 30 mg/kg or 50 mg/kg for approximately 4 years in Study 201/202.

Exposure to placebo for the 4 patients who received once weekly IV infusions of placebo for the first 24 weeks of Study 201 was a mean of 162.3 days. Any comparison of adverse event rates between study dose and placebo must take the variation of exposure into account.

Table 17:   Extent of Exposure to Study Drug: Integrated Analyses (Safety Population)

		Eteplirsen			
	Placebo (N = 4)	**30 mg/kg IV (N = 82)**	**50 mg/kg IV (N = 6)**	**All IV (N = 107)**	**All Eteplirsen (N = 114)**
Days on Study Drug					
N	4	82	6	107	114
Mean	162.3	213.7	1394.8	255.4	239.8
SD	1.26	342.29	87.03	412.93	404.62
Median	162.0	126.5	1449.5	97.0	89.5
Min, Max	161, 164	1, 1451	1282, 1453	1, 1453	1, 1453

Eteplirsen exposure	**Route**	**Dose**	**Patients Exposed (N)**
≥1 dose	IM or IV	any	114
≥1 dose	IV	any	107
≥1 dose	IV	≥ 30 mg/kg	88
≥3 months	IV	≥ 30 mg/kg	61
≥6 months	IV	≥ 30 mg/kg	36
≥4 years	IV	≥ 30 mg/kg	12

Abbreviations: IM = intramuscular; IV = intravenous; max = maximum; min = minimum; SD = standard deviation.

9.5.   Treatment-emergent Adverse Events

9.5.1.   General Overview of Adverse Events

The majority of patients in each treatment group, including placebo, experienced at least
1 TEAE. TEAEs were reported for 88 (82.2%) patients in the 'eteplirsen IV' group (i.e., all
patients receiving eteplirsen IV at any dose, N = 107), and 63 (76.8%) patients in the 30 mg/kg
IV group (N = 82).

No deaths or life-threatening events occurred during the eteplirsen clinical studies (see
Section 9.5.4). Two (2) patients experienced a treatment-emergent SAE; none of the SAEs were
considered related to treatment. One (1) patient discontinued treatment with eteplirsen due to a
TEAE (Section 9.5.5).

Five (5) patients (4.7%) in the 'eteplirsen IV' group (3 of whom received 30 mg/kg IV) and
1 patient in the untreated group experienced severe TEAEs (Section 9.5.6). TEAEs that were
considered related to study drug occurred in 35 (32.7%) patients in the 'eteplirsen IV' group,
18 (22.0%) patients in the 30 mg/kg group, and in 1 patient (25.0%) in the placebo group
(Section 9.5.7).

The limited numbers of adverse events that were severe, serious, or resulted in discontinuation
were observed across dose groups with no suggestion of a dose effect.

9.5.2.   Adverse Events in the Placebo-Controlled Period of Study 201

Safety data in patients who received 30 or 50 mg/kg/wk eteplirsen or placebo (N = 4 per group)
over a 24-week period are available from Study 201.

All patients experienced at least 1 TEAE during the 24-week placebo-controlled period of the
study. Review of all TEAEs by system organ class (SOC) did not show any increases in the
frequency of events within any SOC in eteplirsen-treated patients versus placebo-treated patients
or with increasing dose of eteplirsen.

There were 19 TEAEs that occurred in ≥2 patients as presented below in Table 18. A table of all
TEAEs occurring in the placebo-controlled period of Study 201 is provided in Appendix 15.

Table 18:   Treatment-emergent Adverse Events Occurring in ≥2 Patients during the 24-Week Placebo-controlled Period of Study 201

System Organ Classification Preferred Term	Placebo N = 4 n (%)	Eteplirsen		
		30 mg/kg IV N = 4 n (%)	50 mg/kg IV N = 4 n (%)	All Eteplirsen N = 8 n (%)
Number of Subjects With a TEAE	4	4	4	8
Injury, poisoning & procedural complications				
Procedural pain	3 (75.0%)	1 (25.0%)	3 (75.0%)	4 (50.0%)
Fall	1 (25.0%)	1 (25.0%)	0	1 (12.5%)
Incision site pain	1 (25.0%)	1 (25.0%)	0	1 (12.5%)
Respiratory, thoracic & mediastinal disorders				
Oropharyngeal pain	3 (75.0%)	3 (75.0%)	0	3 (37.5%)
Cough	2 (50.0%)	1 (25.0%)	1 (25.0%)	2 (25.0%)
Nasal congestion	2 (50.0%)	1 (25.0%)	0	1 (12.5%)
Musculoskeletal & connective tissue disorders				
Pain in extremity	3 (75.0%)	0	1 (25.0%)	1 (12.5%)
Back pain	2 (50.0%)	1 (25.0%)	0	1 (12.5%)
Nervous system disorders				
Balance disorder	0	1 (25.0%)	2 (50.0%)	3 (37.5%)
Headache	2 (50.0%)	1 (25.0%)	0	1 (12.5%)
General disorders & administration site conditions				
Pyrexia	2 (50%)	1 (25.0%)	0	1 (12.5%)
Metabolism & nutrition disorders				
Hypokalaemia	2 (50.0%)	2 (50.0%)	2 (50.0%)	4 (50.0%)
Gastrointestinal disorders				
Vomiting	0	1 (25.0%)	2 (50.0%)	3 (37.5%)
Abdominal pain	2 (50.0%)	0	0	0
Diarrhoea	1 (25.0%)	0	1 (25.0%)	1 (12.5%)
Nausea	1 (25.0%)	0	1 (25.0%)	1 (12.5%)
Infections & infestations				
Rhinitis	1 (25.0%)	0	1 (25.0%)	1 (12.5%)

Table 18:   **Treatment-emergent Adverse Events Occurring in ≥2 Patients during the 24-Week Placebo-controlled Period of Study 201**

		Eteplirsen		
System Organ Classification Preferred Term	**Placebo N = 4 n (%)**	**30 mg/kg IV N = 4 n (%)**	**50 mg/kg IV N = 4 n (%)**	**All Eteplirsen N = 8 n (%)**
Vascular disorders				
Haematoma	1 (25.0%)	1 (25.0%)	1 (25.0%)	2 (25.0%)
Skin & subcutaneous tissue disorders				
Dermatitis contact	0	2 (50.0%)	0	2 (25.0%)

Abbreviations: IV = intravenous; TEAE = treatment-emergent adverse event.
Note: Patients were counted once in each body system and preferred term.

9.5.3.   Adverse Events in the Integrated Safety Analysis

Common adverse events, defined as TEAEs reported in ≥10% of all eteplirsen-treated patients, are summarized in Table 19 and all adverse events that occurred in eteplirsen-treated patients are summarized in Appendix 16.

The most commonly experienced TEAEs were consistent with the underlying diagnosis of DMD, steroid treatment, and/or the pediatric nature of the patient population and included headache (27 patients; 23.7%); back pain and vomiting (24 patients each; 21.1%); cough (18 patients, 15.8%); pain in extremity (17 patients; 14.9%); procedural pain (16 patients; 14.0%); upper respiratory infection (15 patients; 13.2% each); arthralgia, contusion, excoriation, oropharyngeal pain, and nasopharyngitis (14 patients each, 12.3%); and nasal congestion (13 patients; 11.4%). Of these, the following occurred more frequently in patients who received 30 or 50 mg/kg eteplirsen IV than in patients who received placebo: headache, vomiting, cough, procedural pain, upper respiratory tract infection, arthralgia, contusion, excoriation, nasopharyngitis and nasal congestion, however this needs to be interpreted in the context of the shorter exposure period for placebo treated patients. The majority of these common TEAEs were mild in severity, considered not related to study drug, and resolved during continued treatment with study drug.

Table 19:   **Treatment-emergent Adverse Events Observed in ≥10% of 'All Eteplirsen' Patients by System Organ Classification and Preferred Term: Integrated Analyses (Safety Population)**

System Organ Classification Preferred Term	Placebo (N = 4)	0.09 & 0.9 mg IM (N = 7)	Eteplirsen						
			≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Number of Patients With a TEAE Occurring in ≥10% of Patients While on Eteplirsen	4 (100%)	0	11 (100%)	3 (75.0%)	2 (50.0%)	47 (57.3%)	6 (100%)	69 (64.5%)	69 (60.5%)
Musculoskeletal and connective tissue disorders									
Back pain	2 (50.0%)	0	3 (27.3%)	1 (25.0%)	0	17 (20.7%)	3 (50.0%)	24 (22.4%)	24 (21.1%)
Pain in extremity	3 (75.0%)	0	2 (18.2%)	1 (25.0%)	0	10 (12.2%)	4 (66.7%)	17 (15.9%)	17 (14.9%)
Arthralgia	0	0	3 (27.3%)	0	0	8 (9.8%)	3 (50.0%)	14 (13.1%)	14 (12.3%)
Injury, poisoning and procedural complications									
Procedural pain	3 (75.0%)	0	2 (18.2%)	0	0	8 (9.8%)	6 (100%)	16 (15.0%)	16 (14.0%)
Contusion	0	0	1 (9.1%)	0	0	10 (12.2%)	3 (50.0%)	14 (13.1%)	14 (12.3%)
Excoriation	0	0	0	0	1 (25.0%)	11 (13.4%)	2 (33.3%)	14 (13.1%)	14 (12.3%)
Respiratory, thoracic and mediastinal disorders									
Cough	2 (50.0%)	0	2 (18.2%)	0	0	12 (14.6%)	4 (66.7%)	18 (16.8%)	18 (15.8%)
Oropharyngeal pain	3 (75.0%)	0	0	0	0	10 (12.2%)	4 (66.7%)	14 (13.1%)	14 (12.3%)
Nasal congestion	1 (25.0%)	0	0	0	0	11 (13.4%)	2 (33.3%)	13 (12.1%)	13 (11.4%)
Nervous system disorders									
Headache	2 (50.0%)	0	5 (45.5%)	2 (50.0%)	1 (25.0%)	14 (17.1%)	5 (83.3%)	27 (25.2%)	27 (23.7%)
Gastrointestinal disorders									
Vomiting	0	0	2 (18.2%)	1 (25.0%)	0	18 (22.0%)	3 (50.0%)	24 (22.4%)	24 (21.1%)

Table 19:   **Treatment-emergent Adverse Events Observed in ≥10% of 'All Eteplirsen' Patients by System Organ Classification and Preferred Term: Integrated Analyses (Safety Population)**

System Organ Classification Preferred Term	Placebo (N = 4)	0.09 & 0.9 mg IM (N = 7)	Eteplirsen						
			≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Infections and infestations									
Upper respiratory tract infection	0	0	3 (27.3%)	2 (50.0%)	1 (25.0%)	5 (6.1%)	4 (66.7%)	15 (14.0%)	15 (13.2%)
Nasopharyngitis	1 (25.0%)	0	0	0	0	10 (12.2%)	4 (66.7%)	14 (13.1%)	14 (12.3%)

Abbreviations: IM = imtramuscular; IV = intravenous; TEAE = treatment-emergent adverse event.

Note: AEs are coded using MedDRA V14.0. AEs were attributed to the treatment being received at start of AE. TEAEs are those starting during or after the first infusion of study drug (or week 1 for untreated patients) or within 28 days after the last infusion (or last visit for untreated patients). Patients who experience a coded event more than once are only counted once per treatment received

9.5.4.   Deaths and Other Serious Adverse Events

There have been no deaths and no life-threatening SAEs with an overall exposure of 72 patient years.

Two (2) treatment-emergent SAEs (one event each of moderate vomiting and severe femur fracture) have been reported in the eteplirsen clinical development program. These were considered as unrelated to study drug. A brief narrative summary for each of these is provided below.

In addition, 4 SAEs that did not occur on eteplirsen have also been reported: 1 'treatment emergent' event that occurred in an untreated patient in Study 301 (lymphadenitis viral); 2 events (ankle fracture, wound infection) that occurred more than 28 days after the patient had discontinued study medication; and 1 event (postoperative oxygen saturation decreased due to emesis) that occurred prior to treatment in a post-operative setting.

<u>Patient 201/202-01-009</u> (eteplirsen 30 mg/kg)

Femur Fracture (fracture of right distal femur)
   Severe, unrelated

Patient 201/202-01-009 was a 9-year-old boy with DMD enrolled in Studies 201/202 in the 30 mg/kg IV group. On ▓▓▓▓▓▓ (b) (6) (Study Day 608), he sustained a fracture of the right distal femur after falling out of his wheelchair when his mother made a sudden stop in their van. He had taken off his seatbelt. He was taken to the emergency room where an X-ray confirmed he had suffered a closed stable femoral fracture; a cast was applied. He received versed, opioids, fentanyl, and ibuprofen for pain relief. Approximately 2 months later, the patient recovered from this event.

<u>Patient 28-01-107</u> (eteplirsen 2 mg/kg)

Vomiting (post-operative nausea and vomiting)
   Moderate, Unrelated

Patient 28-01-107 was a 10-year-old boy diagnosed with DMD and enrolled in Study 28. He received 12 doses of once weekly eteplirsen 2.0 mg/kg IV beginning on ▓▓▓▓▓▓ (b) (6).

On ▓▓▓▓▓▓ (b) (6), 12 days after the last dose of study drug, he was admitted to the hospital for the protocol-specified muscle biopsy to be performed under general anesthesia. Per standard procedure prior to general anaesthesia, the patient fasted the night before surgery. Initially following the procedure, he made a good recovery and was given liquid and a light diet that evening; however, later that evening (at approximately 20:00 hours), he developed nausea and vomiting. On physical examination, his vital signs were normal and he looked well. Laboratory results from that day were consistent with his underlying DMD condition and were considered unremarkable. His sodium, chloride, and potassium levels were within the normal range. The following day (▓▓▓▓▓▓ (b) (6)), the event of vomiting was considered resolved and he was discharged. The Investigator attributed the event to the prolonged fasting (12 hours) prior to general anesthesia.

9.5.5.   Adverse Events Leading to Drug or Study Discontinuation

One (1) patient, a 10-year-old boy with DMD enrolled in Study 28, discontinued treatment due to a TEAE (cardiomyopathy).

Patient 28-02-202 (eteplirsen 4 mg/kg)

Cardiomyopathy (Cardiomyopathy [left ventricular dysfunction])
   Severe, Possibly related

Patient 28-02-202, a 10-year-old boy in the 4 mg/kg IV dose group, had 3 reported TEAEs of mild tachycardia and 1 TEAE of sinus tachycardia on Days 1, 8, 24 and 36, with heart rate up to 127 beats per minute. An echocardiogram was performed and revealed decreased fractional shortening of 22% (ejection fraction [EF] of 40% to 45%). The investigator reported this finding as an adverse event of cardiomyopathy (described as 'cardiomyopathy [left ventricular dysfunction]') that was possibly related to eteplirsen and led to study drug discontinuation on Day 47 (after 7 doses of eteplirsen 4 mg/kg IV). Retrospective review of echocardiograms obtained prior to study entry showed evidence for pre-existing cardiomyopathy per the investigator's report. Moreover, subsequent re-evaluation of all study echocardiograms of this patient by an independent cardiologist (without the cardiologist being provided clinical details of the adverse event) determined normal left ventricular ejection fraction (>55%) on all study echocardiograms.

Given the possibility of presence of left ventricular dysfunction prior to study treatment, and inconsistency with respect to independent interpretation of the echocardiographic data, a relationship between study drug and this event is difficult to establish. Further discussion of cardiac function, including the diagnosis and prevalence of cardiomyopathy in the DMD population, is provided in the section on adverse events of special interest (Section 9.5.8.1).

9.5.6.   Severe Adverse Events

The majority of TEAEs across all treatment groups were mild or moderate in intensity, as assessed by the Investigator. A total of 5 eteplirsen-treated patients (4.4%) experienced 8 severe TEAEs, including incision site haemorrhage, haemorrhoids, back pain, cardiomyopathy, nasal congestion, balance disorder, bone pain, and femur fracture. With the exception of cardiomyopathy (Section 9.5.5), which was considered by the investigator to be possibly related to treatment, all other severe events were considered unrelated to study drug. In addition, one untreated patient experienced one event of lymphadenitis viral that was considered severe in intensity and that met the criteria for seriousness.

9.5.7.   Treatment-Related Adverse Events

TEAEs assessed by the investigator to be possibly, probably, or definitely related to study treatment were considered treatment-related. Overall, treatment-related TEAEs were reported in 36 (31.6%) patients in the 'all eteplirsen' treatment group and in 1 (25%) patient in the placebo group. In the 'all eteplirsen' group, the most frequent treatment-related TEAEs were headache (8 patients, 7.0%), proteinuria (4 patients, 3.5%), and dizziness, fatigue, vomiting, and tachycardia (each in 3 patients, 2.6%). One (1) patient was reported to have a treatment-related TEAE of nausea while receiving placebo. Treatment-related TEAEs occurred across dose groups with no indication of a dose effect.

Treatment-related TEAEs were reported in 21 (23.9%) of the 88 patients who received eteplirsen at either 30 or 50 mg/kg IV weekly, the treatment groups that represent the greatest exposure to eteplirsen. Of these, proteinuria, protein urine present, thrombosis in device, vomiting, and flushing were reported in >1 patient; these TEAEs are discussed in more detail in Section 9.5.8, Adverse Events of Special Interest.

9.5.8.   Adverse Events of Special Interest

Adverse events of special interest (AESIs) for the eteplirsen program included potential safety related findings based on manifestations of the underlying DMD disease (cardiac function), nonclinical observations with eteplirsen (renal function, see Section 9.5.8.2), AEs associated with other RNA analogs (renal and hepatic function, coagulopathy and infusion site reactions), and general precautions with administration of a compound in clinical development (infusion related reactions, hypersensitivity, severe cutaneous reactions, leukopenia and neutropenia).

The inclusion of adverse events associated with other RNA analogs in Adverse Events of Special Interest for eteplirsen, a phosphorodiamidate morpholino oligomer, is a conservative approach, since the non-clinical toxicity data for eteplirsen did not show a signal except for renal findings at the highest dose administered (Section 4.2.2). Other RNA analogs, specifically phosphorothioate oligonucleotide therapeutics have been associated with renal toxicity, including increases in proteinuria, α1 microglobulin, and KIM 1 (McGowan 2012, Goemans 2011); elevated levels of transaminases and hepatic steatosis (McGowan 2012); thrombocytopenia and other coagulation related adverse events (Goemans 2014); and injection site reactions (Voit 2014, McGowan 2012).

To identify potential AESIs, search criteria for specific MedDRA preferred terms were developed. In addition, medical review of all TEAEs, as well as relevant laboratory, vital sign, ECG and echocardiogram results was performed.

9.5.8.1.   Cardiac Function

In addition to progressive muscle weakness and wasting, manifestations of DMD typically include cardiac symptoms. While cardiac function is generally normal during early childhood, they progressively worsen over time, and patients typically die from cardiac or respiratory failure (Brooke 1989, Eagle 2002).

Boys with DMD have a resting heart rate that is consistently higher than normal even when cardiac function remains normal. Although elevation in resting heart rate in this patient population is likely multifactorial, it is associated with increased risk of cardiomyopathy (Thomas 2012), which is usually diagnosed after the age of 10 years as dilated cardiomyopathy with reduced left ventricular ejection fraction in boys with DMD. While cardiomyopathy rarely manifests clinically in the early teens in DMD patients, the prevalence of cardiomyopathy as measured by a left ventricular ejection fraction of <55% has been estimated at 27% overall. Cardiomyopathy shows an increasing prevalence with age and disease progression, with 10% to 20% of patients affected between 6 and 13 years of age and over 60% of patients ≥18 years affected (Spurney 2014). A long latency between initial abnormal cardiac findings in laboratory assessments and clinically manifest cardiomyopathy exists, and the pathology includes myocyte atrophy, hypertrophy and fibrosis.

In the 'all eteplirsen' group, 6 patients (5.3%) had a total of 12 reported events potentially indicative of a cardiac disorder. None of the events was serious, and the majority of events were assessed by the investigator as mild in intensity (9/12) and as possibly related (7/12) to eteplirsen (Table 20).

The observed TEAEs included cardiomyopathy, congestive cardiomyopathy, pericardial fibrosis, tachycardia, and sinus tachycardia, and were distributed across dose groups with no suggestion of a dose effect. One (1) 10-year-old patient (28-02-202) with mild events of tachycardia and sinus tachycardia prematurely discontinued treatment in Study 28 due to an event of cardiomyopathy; this event was described above in Section 9.5.5. The other event of cardiomyopathy was reported in a 13-year-old patient 27 days after a single low dose of eteplirsen 0.09 mg IM. An event of pericardial fibrosis was identified by routine cardiac MRI as part of DMD natural history surveillance at the study site and was considered to be related to the underlying disease of DMD:

Patient 204-206-104 (eteplirsen 30 mg/kg)

Pericardial fibrosis
   Mild, not related

Patient 204-206-104 was a 14-year-old with advanced DMD who experienced mild, asymptomatic pericardial fibrosis on Day 120, after receiving 18 eteplirsen doses. The finding was identified by routine cardiac MRI as part of DMD natural history surveillance at the study site. The event is ongoing and the patient remains asymptomatic. The Investigator assessed causality of the event as unrelated to study drug or study procedures, and definitely related to the underlying disease of DMD. The patient's cardiologist started him on spironolactone. No action was taken with study drug administration and the patient remained in the study through the D120 data cutoff.

Table 20:   Cardiac Function TEAEs

Patient ID	Age (yr)	Dose (mg/kg)	Preferred Term	Treatment Related	Severity	Outcome
204-206-104	14	30	Pericardial fibrosis	No	Mild	Not recovered
201/202-01-006	10	30	Tachycardia	No	Moderate	Not recovered
			Tachycardia (worsening)	No	Moderate	Recovered
			Tachycardia	No	Mild	Recovered
28-02-205	10	20	Tachycardia	Possibly	Mild	Not recovered
28-02-207	9	20	Tachycardia	Possibly	Mild	Not recovered
28-02-202	10	4	Cardiomyopathy	Possibly	Severe	Not recovered
			Sinus tachycardia × 3	Possibly	Mild	Recovered
			Tachycardia	Possibly	Mild	Recovered
33-01-002	13	0.09 [a]	Congestive cardiomyopathy	No	Mild	Unknown

[a] Patient 33-01-002 received a single 0.09-mg intramuscular dose of eteplirsen.

In addition, 3 patients had 5 reported events of tachycardia and sinus tachycardia.

To further evaluate the cardiac clinical course of patients with DMD, predefined criteria were established for abnormal changes for QTcF; there were no patients who met predefined criteria for QTcF. Of the predefined criteria for abnormal ECG results, only the criterion for abnormal HR (HR >120 bpm) was met. A total of 7 patients (1 in ≤4 mg/kg, 2 in 20 mg/kg, and 4 in 30 mg/kg) met this criterion; 3 of these patients had reported TEAEs of tachycardia or sinus tachycardia (Table 20). The remaining 4 patients had a single occurrence of HR >120 bpm with no reported cardiac events associated with elevated heart rate. It should be noted that 4 additional patients also experienced heart rates above 120 bpm prior to treatment initiation.

In addition, serial echocardiograms were conducted in Studies 201/202.None of the patients in the safety population had left ventricular ejection fraction results that met the criteria for a predefined abnormal change. Longitudinal analysis of the left ventricular ejection fraction as assessed at the annual milestone visits in Studies 201/202 is provided in Table 21 below. In this table, the Week 24 assessment was used as baseline in patients originally randomized to placebo to ensure that only the period on eteplirsen treatment is represented. These data characterize the stability of LVEF in patients treated with eteplirsen over 4 years.

Table 21:   Left Ventricular Ejection Fraction over Time in Studies 201/202

	Patients Treated with Eteplirsen at 30 or 50 mg/kg IV (N = 12)		
Timepoint	**n**	**Median LVEF**	**Min, Max LVEF**
Baseline	12	61.5	50, 74
Year 1	11	66.0	52, 71
Year 2	12	62.5	54, 67
Year 3	12	65.0	53, 71
Year 4	8[1]	62.0	55, 76

Abbreviations: LVEF = left ventricular ejection fraction; max = maximum; min = minimum

[1] At the time of the data cut for the Day 120 Safety Update, the 4 patients initially on placebo had not been on eteplirsen treatment for 4 years.

The occurrence of tachycardia, cardiomyopathy and cardiac fibrosis observed during clinical trials with eteplirsen was not related to study dose or duration of administration, is not unexpected in the DMD population enrolled, and appears consistent with the underlying disease.

9.5.8.2.   Renal Function

The primary elimination pathway for eteplirsen is renal, and the kidney was identified as the primary target organ for toxicity in nonclinical toxicology studies. In addition, other RNA analogs, specifically phosphorothioate oligonucleotides, have been associated with renal toxicity, including increases in proteinuria, α1 microglobulin, and KIM 1 (McGowan 2012, Goemans 2011).

However, it should be noted that renal dysfunction is a common complication in advanced stages of DMD, and DMD patients have additional risk factors, including dehydration, for renal dysfunction (Bratt 2015). While serum creatinine levels are typically a fairly reliable indicator of

kidney function, this is not the case in patients with more advanced DMD whose basal creatinine levels tend to be low or low normal due to decreased muscle mass (Viollet 2009); therefore, elevations that would typically be seen in patients with renal dysfunction would not necessarily be observed in patients with DMD. Thus, serum cystatin C may provide a better measure of renal function than creatinine.

In the 'all eteplirsen' group, 16 patients (14.0%) had a total of 21 TEAEs potentially representative of renal toxicity. One (1) patient in the placebo group had an event of proteinuria and one patient in the 30 mg/kg treatment group had an event of proteinuria prior to treatment initiation. None of the events were serious, and all were assessed by the investigator as mild in intensity. The majority of events were transient and spontaneously resolved with ongoing study drug administration. Nine (9) patients (7.9%) had 9 TEAEs that were reported by the investigator as treatment related (Table 22).

Table 22:   Treatment-related TEAEs Potentially Indicative of Renal Toxicity

Patient ID	Age (yr)	Dose (mg/kg)	Preferred Term	Treatment Related	Severity	Outcome
201/202-01-004	8	50	Hypercalciuria	Possibly	Mild	Not recovered
201/202-01-015	9	50	Proteinuria	Possibly	Mild	Recovered
204-202-101	13	30	Protein urine present	Possibly	Mild	Recovered
204-202-104	11	30	Protein urine present	Possibly	Mild	Recovered
204-233-105	17	30	Blood urine present	Possibly	Mild	Not recovered
301-213-001	12	30	Urine analysis abnormal	Possibly	Mild	Recovered
301-218-004	9	30	Proteinuria	Possibly	Mild	Recovered
301-234-001	12	30	Proteinuria	Possibly	Mild	Recovered
201/202-01-006	10	30	Proteinuria	Possibly	Mild	Recovered

Proteinuria/urine protein present were the most common reported adverse events; these events were transient or sporadic, spontaneously resolved with ongoing treatment, and were not associated with increasing renal laboratory values, with the exception of 1 patient who had evidence of transient renal laboratory abnormalities in the setting of dehydration (Patient 201/202-01-003, 50 mg/kg IV). No other concurrent indicators of renal toxicity were reported. Only 1 event of proteinuria (Patient 204-202-101, 30 mg/kg IV) led to interruption of study drug. This patient resumed treatment after missing 1 dose without further TEAEs or abnormal laboratory findings.

Adverse Events of Proteinuria / Protein Urine Present

In the 'all eteplirsen' group, of the 16 patients with reported renal events, 11 patients (9.6%) had reported TEAEs of either proteinuria (10 TEAEs) or protein urine present (2 TEAEs). Of these 12 events, 6 (50.0%) were considered possibly related to treatment. In addition, 1 event of proteinuria was reported in 1 patient who was receiving placebo. All events were mild with no

consistent pattern of time to onset (onset ranged from Day 1 to Day 785), and all patients continued treatment uninterrupted with 1 exception (1 dose of study medication was withheld as a precautionary measure for Patient 204-202-101).

In 10 of the 11 patients, the events were isolated with no increases in serum BUN, serum cystatin C, or urine KIM 1, had no accompanying symptoms of renal disease, were generally mild, transient, and resolved in subsequent assessments. The case of proteinuria with associated changes in laboratory values is briefly summarized below.

Patient 201/202-01-003 (eteplirsen 50 mg/kg)

Blood creatinine increased, Blood urea increased, Dehydration, Proteinuria
  Mild, Not related

Patient 201/202-01-003 (50 mg/kg IV), who was 7 years of age at baseline, experienced blood creatinine increased, blood urea nitrogen increased and proteinuria at Week 60, with concurrent abnormal laboratory findings of creatinine 102.5 μmol/L, blood urea nitrogen (BUN) 14.6 mmol/L, and trace urine protein at the time of the observed laboratory abnormalities. Of note, the serum cystatin C and urine KIM 1 values at the time of the TEAEs were normal. Both BUN and creatinine abnormalities had resolved by the time of re-testing 11 days later and remained normal with continued eteplirsen treatment through data cutoff at Week 208. Subsequent urinalysis was sporadically positive for trace or 1+ protein. The investigator interpreted this event in the context of dehydration, noting that this patient had a history of dehydration on several occasions, and a TEAE of dehydration was recorded. The patient remained in the study and continued to receive study drug through data cutoff at Week 208.

In addition, a value of 2+ urine protein on dipstick, corresponding to ≥100 mg/dL and <300 mg/dL, was predefined as the criterion for a markedly abnormal value; 5 treated patients had a post-treatment 2+ urine protein value that was not recorded as an AE. In all cases, the finding was a single occurrence that spontaneously resolved with ongoing treatment. It should be noted that 2+ urine protein values were also recorded prior to treatment.

Additional renal TEAEs included dehydration, chromaturia, crystalluria, hypercalciuria, blood creatinine increased, blood urea increased, blood urine present, and urine analysis abnormal; with the exception of dehydration, which was reported in 2 patients, these events were reported in only 1 patient (per event).

Laboratory observations of protein in urine (Study 201/202)

To assess whether or not elevations in urine protein were increasing over time with eteplirsen, a longitudinal analysis of positive (≥1+ by dipstick, corresponding to ≥30 mg/dL and <100 mg/dL) urine protein findings over time in Studies 201/202 was performed. Over the 4-year period, a total of 721 urinalysis assessments were performed in the 12 patients. Overall, 702 (>97%) of the assessments were normal, and no increase in the occurrence of urine protein was observed over time, suggesting no cumulative effect (Table 23).

Table 23:   **Instances of Urine Protein ≥1+ Over Time in Studies 201/202 (based on urinalysis by dipstick assay)**

Timepoint	Instances of protein in urine ≥1+	Number of Assessments
Placebo and Prior to dosing	2	68
Week 0-48	3	183
Week 48-96	6	150
Week 96-144	5	152
Week 144-Week 208 (data cutoff)	3	168

Laboratory Observations of Serum Creatinine and Cystatin C

In patients with DMD, creatinine levels tend to be low or low normal due to decreased muscle mass. Serum cystatin C is less dependent on muscle mass and therefore, may provide a better measure of renal function. Thus, serum cystatin C levels were evaluated in the eteplirsen clinical program as an additional biomarker of kidney function.

One patient (201/202 01 003), described above, had a creatinine value that met predefined criteria for an increase over baseline of ≥35 μmol/L and a clinically noteworthy treatment-emergent value. No other patient treated with eteplirsen met the criterion for abnormal change, and no other patient had a creatinine value above the ULN while on eteplirsen treatment. Patient 201/202 01 003 was discussed above.

Two (2) patients (1 in the 30 mg/kg IV dose group and 1 untreated) had a shift from normal to high for serum cystatin C. Concurrent BUN and creatinine values were normal, urine protein was negative, and cystatin C levels returned to normal at the next assessment for both patients.

Adverse events of Myoglobinuria

There were 4 AEs of myoglobinuria reported in eteplirsen studies. All of the myoglobinuria events were reported in Study 33, after a single intramuscular (IM) dose, in the 0.9mg/kg IM arm. The myoglobinuria was reported on the day that study drug was administered for 3 of the 4 subjects, and was reported on the day of the post-treatment biopsy for the remaining subject (on Day 29). The myoglobinuria events were self-limited and resolved, without treatment. Due to the temporal relationship between the administration of general anesthesia and the onset of myoglobinuria, the general anaesthesia may have contributed to the onset of these events; however, it is more likely that direct injury to muscle following the IM injection or the muscle biopsy caused the observed myoglobinuria. Myoglobinuria events were not observed in subsequent eteplirsen studies.

In summary, protein in urine was observed not only during treatment with eteplirsen, but also in patients prior to dosing. In addition, there was a lack of concurrent elevation of other markers of renal function, including BUN, creatinine, and cystatin C, (with the exception of one event of increased BUN and creatinine as described above) and spontaneous resolution was observed with ongoing eteplirsen dosing. The data suggest that protein in urine may occur in the background population. Additional renal events were isolated, mild in intensity, and the majority resolved

with ongoing treatment. Thus, the data generated to date do not suggest an association between renal dysfunction and eteplirsen at this time.

9.5.8.3. Hepatic Function

There were no treatment emergent adverse events representative of a potential drug-induced hepatotoxicity.

Laboratory Observations of Liver Function Tests

Traditional criteria to assess liver function may have limited applicability in the DMD population, because high transaminase levels (alanine aminotransferase [ALT] and aspartate aminotransferase [AST] up to approximately $22 \times$ ULN) are generally observed in these patients due to leakage of the enzymes from degenerating muscle fibers (McMillan 2011). Therefore, abnormal change criteria in the eteplirsen clinical development program were defined as $\geq 2 \times$ baseline for ALT and $\geq 3 \times$ baseline for AST.

Three (3) patients in the 30 mg/kg IV dose group, met the predefined abnormal criterion of $\geq 2 \times$ baseline for ALT. No patients in the untreated or the 50 mg/kg IV dose group met this criterion. In all 3 instances, the patient had no increase in bilirubin or GGT and a pre-treatment ALT value that was higher than the on treatment value designated as meeting the predefined abnormal criterion. The abnormal ALT values on-treatment were, therefore, considered consistent with fluctuations in ALT that may be seen with the underlying DMD.

Two (2) patients in the 30 mg/kg IV dose group had elevated AST levels that met the predefined abnormal change criterion of $\geq 3 \times$ baseline. In one case, the patient also had recorded pre-treatment ALT and AST values that were higher than the on-treatment values that met the abnormal criteria. In both cases, AST decreased with ongoing study drug, and both patients were asymptomatic. No changes were made to study drug administration, and the patients continued in the study.

In addition, 1 patient in the 30 mg/kg IV group met the predefined criterion of $\geq 1.5 \times$ ULN for bilirubin. This patients had elevated bilirubin levels prior to study drug administration that were higher than on study values. No action was taken with study drug, and the patient continues to be followed.

Overall, there were no adverse events suggestive of hepatic effect of eteplirsen, and the observed transaminase levels appeared consistent with the underlying disease.

9.5.8.4. Coagulopathy

In the 'all eteplirsen' group, 21 patients (18.4%) had a total of 42 TEAEs which were reviewed to evaluate whether they were potentially indicative of a coagulation disorder. None of the events were serious, and the majority were reported by the investigator as mild in intensity and unrelated to eteplirsen. There were no discontinuations or changes to study treatment due to any of these events, and at the time of data cutoff, all events had resolved. Three (3) patients (2.6%) had 5 TEAEs that were reported by the investigator as treatment related and/or as moderate or severe in intensity (Table 24). Four (4) events involved the Port a Cath device; in each case, there were no abnormal platelet, prothrombin time (PT), international normalized ratio (INR), or activated partial thromboplastin time (aPTT) values. Therefore, the sponsor considers these events to be not related to study drug, but rather to the Port-a-Cath device. For the event of

platelet anisocytosis, there were no concurrent events indicative of a bleeding disorder, and platelet counts, aPTT, PT, and INR were normal around the time of the reported event.

Table 24:   **Treatment-related and/or Moderate or Severe TEAEs Potentially Indicative of Coagulopathy**

Patient ID	Age (yr)	Dose (mg/kg)	Preferred Term	Treatment Related	Severity	Outcome
201/202-01-009	9	30	Thrombosis in device	Possibly	Moderate	Recovered
201/202-01-010	9	30	Thrombosis in device	Possibly	Moderate	Recovered
			Thrombosis in device	Possibly	Moderate	Recovered
			Device occlusion	Possibly	Moderate	Recovered
28-01-108	10	4	Platelet anisocytosis	Possibly	Mild	Recovered

Other unrelated events included infusion and injection site haematoma, prolonged aPTT, ecchymosis, thrombosis in device, catheter site haematoma, device occlusion, and petechiae.

Ten (10) patients had more than 1 adverse event potentially indicative of coagulopathy. Most of the events for the 10 patients with multiple AEs in this category were consistent with catheter site hematomas or device thrombosis. Two (2) of the 10 patients experienced events of prolonged aPTT and ecchymosis. One (1) of the 2 patients, had an elevated aPTT measurement from a normal baseline test (baseline 25.7 seconds) ranging from 36.0-53.3 seconds (normal range 23.6-32.5 seconds) on Study Days 52-95, and concurrent ecchymosis on Study Days 67-97. This patient had another asymptomatic episode of aPTT elevation (35.2 seconds) on Study Day 162-177 without an associated AE. The second patient experienced bilateral lower limb ecchymosis of 3-day duration (Study Days 501-504) 11 months prior to the onset of aPTT elevation ranging from 43.0-48.0 seconds (baseline 30.0 seconds) on Study Days 918-936. All of the events of aPTT and ecchymosis experienced by the two patients above were of mild severity and resolved without treatment.

Overall review of the events potentially related to coagulopathy suggested no consistent pattern of eteplirsen drug effect.

9.5.8.5. Infusion Site Reactions

Patients receiving eteplirsen were monitored closely for events related to potential infusion site reactions. In the analysis of infusion site reactions focus was on the eteplirsen IV group, as the route of administration for eteplirsen is IV. The 7 patients who received a single low dose of intramuscular eteplirsen in Study 33 are not included in this analysis.

In the 'eteplirsen IV' group, 24 patients (22.4%) had a total of 55 TEAEs which were reviewed to evaluate whether they were potentially representative of infusion site reactions. None of the events were serious, and the majority were assessed by the investigator as mild in intensity and unrelated to eteplirsen. At the time of data cutoff, the majority of events had resolved without changes to treatment administration. Two (2) patients (1.9%) each had 1 TEAE that was reported by the investigator as treatment-related and/or as moderate or severe in intensity (Table 25). The event that was considered to be possibly related resolved the same day and the patient had no

other reported infusion site reactions; and the event of moderate intensity was described as pain post-operative to port placement.

Table 25:   Treatment-related and/or Moderate or Severe TEAEs Potentially Indicative of an Infusion Site Reaction

Patient ID	Age (yr)	Dose (mg/kg)	Preferred Term	Treatment Related	Severity	Outcome
204-233-102	8	30	Catheter site pain	Possibly	Mild	Recovered
201/202-01-010	9	30	Catheter site pain	No	Moderate	Recovered

Additional mild and unrelated events included catheter or infusion site haematoma (21 events in 9 patients); catheter, infusion or injection site pain (15 events in 11 patients); pyrexia (5 events in 4 patients); infusion site extravasation (4 events in 4 patients); application or infusion site erythema or rash (4 events in 3 patients); catheter site hemorrhage, inflammation, and related reaction (1 event each in 1 patient each); and infusion site swelling (1 event in 1 patient). Four (4) patients (3.7%) had their infusion interrupted as the result of a mild, unrelated TEAE of either extravasation (n = 3) or infusion site pain (n = 1).

Overall, the majority of events were mild (98.2%) and considered unrelated to eteplirsen (98.2%) and were generally reflective of the types of events due to catheter placement rather than due to a direct effect of eteplirsen.

9.5.8.6.   Hypersensitivity

In the 'all eteplirsen' group, 27 patients (23.7%) had a total of 43 TEAEs which were reviewed to evaluate whether they were potentially representative of hypersensitivity, and 1 untreated patient (6.7%) had 1 event (mild rash). All of the events were reported as non-serious and recovered/resolved, and the majority of events in the 'all eteplirsen' group were mild (41/43, 95.3%) and considered by the investigator to be unrelated to study treatment (38/43, 88.4%). Study drug administration was interrupted for 2 events.

Six (6) patients (5.3%) had 7 TEAEs that were reported by the investigator as treatment related and/or as moderate or severe in intensity (Table 26). All of the events that were considered by the investigator as treatment related were mild in intensity and resolved with ongoing eteplirsen treatment; and the investigator provided an alternate etiology ('possible reaction to Ametop plastic') for 1 event (drug eruption) despite having recorded it as possibly related to treatment.

Table 26: **Treatment-related and/or Moderate or Severe TEAEs Potentially Indicative of Hypersensitivity**

Patient ID	Age (yr)	Dose (mg/kg)	Preferred Term	Treatment Related	Severity	Outcome
201/202-01-004	9	50	Erythema	Possibly	Mild	Recovered
			Erythema	Possibly	Mild	Recovered
201/202-01-005	8	50	Alopecia	No	Moderate	Recovered
301-218-001	10	30	Flushing	Definitely	Mild	Recovered
301-239-001	13	30	Flushing	Possibly	Mild	Recovered
201/202-01-002	9	30	Dermatitis contact	No	Moderate	Recovered
28-02-207	9	20	Drug eruption	Possibly	Mild	Recovered

Both events of flushing occurred on Day 1 (first infusion), resolved the same day, and did not recur despite continued dosing with eteplirsen. One (1) patient experienced 2 events of erythema (Days 974 and 988) that each occurred within 1 hour of drug infusion, resolved the same day, and did not recur despite continued dosing with eteplirsen.

There were also mild and unrelated events of rash, rash papular, rash pruritic, pruritus, erythema, urticaria, urticaria thermal, flushing, feeling hot, dermatitis contact, papule, seasonal allergy, hypersensitivity ('worsening of seasonal allergies'), lip swelling, and swelling.

Overall, no trends or patterns in these events were observed. The time to onset from last dose ranged from 44 minutes to 7 days; the number of doses prior to event onset ranged from 1 to 199; and event duration ranged from 15 minutes (dermatitis contact) to 50 days (feeling hot). Given the resolution and lack of recurrence for most events despite continued treatment, these events appear to be reflective of the background population rather than due to study drug treatment. The 2 events, mild erythema and flushing, that occurred on the day of study drug infusion may represent potential adverse drug reactions with eteplirsen.

9.5.8.7. Infusion-related Reactions

The 7 patients who received a single low dose of intramuscular eteplirsen in Study 33 were excluded from this analysis, because IV infusion is the proposed route of administration.

In the 'eteplirsen IV' group, 30 patients (28.0%) had a total of 55 TEAEs which were reviewed to evaluate whether they were potentially representative of an infusion-related reaction, and 3 events occurred in 2 patients while receiving placebo. The majority of events in the 'eteplirsen IV' group were non-serious (54/55, 98.2%), mild in intensity (51/55, 92.7%), and unrelated to study treatment (49/55, 89.1%). None of the events required a change in treatment administration, and as of the data cutoff date, 54 of the 55 events had resolved.

Nausea and/or Vomiting

Although nausea and vomiting are relatively non-specific events and may occur in a pediatric population, these events were medically reviewed to assess whether they potentially represented a type of infusion related reaction. The overall frequency of nausea and vomiting were comparable across dose groups and not suggestive of a dose effect, ranging from 4.9 to 50.0% in

the active groups and 0 to 25.0% in the placebo group. Seven (7) patients in the 'eteplirsen IV' group experienced events that were moderate in intensity and/or related (possibly or definitely) to eteplirsen according to the investigator. The time to onset for these 7 events was variable with only 1 patient experiencing intermittent nausea on a day of study drug infusion. All 7 patients continued to receive study drug, and nausea or vomiting did not recur except in 2 patients. Overall, events of nausea and vomiting were not considered to represent infusion related reactions.

Pyrexia

A total 5 events of pyrexia occurred in 4 patients in the 'eteplirsen IV' group and 2 events occurred in 2 patients in the placebo group. All of the events were assessed by the investigator as mild in intensity and unrelated to study drug. The time to onset from last dose for the events of pyrexia ranged from 10 hours to 5 days; the number of doses prior to event onset ranged from 4 to 194; and event duration ranged from 30 minutes to 6 days with 4 of the 5 events resolving within 1 day of onset. Only 1 patient, a 9-year-old boy, experienced a recurrence of pyrexia (after dose 13 and dose 124). No trends or patterns were observed. Given the resolution and lack of recurrence for most patients despite continued treatment, and the observation of pyrexia in 2 placebo patients, it may be concluded that these events are reflective of the background population.

However, there was one case of "mild temperature elevation" coincident with study drug infusion which the investigator considered related to study drug, and this event is therefore considered a potential adverse drug reaction. " (Patient 28-01-110 [10 mg/kg IV]) was described as a mild temperature elevation to 37.9°C after infusion of eteplirsen; this event resolved the same day, and the investigator did consider this event to be possibly related to study drug and this event is considered a potential adverse drug reaction.

Pruritus

Two (2) events of pruritus occurred in 2 patients in the 'eteplirsen IV' group. Both events were assessed by the investigator as mild in intensity and unrelated to study drug, and no changes were made to study treatment administration. Patient 204-201-103 (30 mg/kg IV) had a reported TEAE of pruritus from Day 106 to Day 112, and Patient 201/202-01-003 (50 mg/kg IV) had a reported TEAE of pruritus on Day 275 that resolved the same day.

Overall, non-specific symptoms of potential infusion-related reactions such as nausea and vomiting occurred in the eteplirsen-treated population at a relatively low rate, and also occurred prior to treatment or in the placebo group. Although some events have been noted on days of infusion, there was no consistent pattern of recurrence with subsequent infusions. These events were typically mild in intensity and similar across treatment groups. On review of events of pyrexia, there were no trends or patterns observed to suggest an association with study drug, as this event was also seen in the placebo group and all of the events resolved despite continued treatment. There was one case of "mild temperature elevation" coincident with infusion of eteplirsen, which the investigator considered related to study drug. This event is, therefore, considered a potential adverse drug reaction.

9.5.8.8.   Severe Cutaneous Reactions

In the 'all eteplirsen' group, 2 patients (1.8%) had a total of 2 TEAEs (one event of mild skin erosion resulting from an accident and one event of mild dermatitis bullous that resolved without intervention within 7 days). Neither event was serious; both resolved with no change to study treatment, both were reported by the investigator as mild in intensity and unrelated to study drug.

These events were mild and self-limited without sequelae. Both had alternative etiologies, including traumatic injury and post-biopsy complication. The events were not consistent with severe cutaneous reaction.

9.5.8.9.   Leukopenia/Neutropenia

In the 'all eteplirsen' group, 1 patient (0.9%) had 3 TEAEs that were potentially representative of leukopenia and/or neutropenia. The patient had 2 events of lymphocyte count decreased, both of which were considered by the investigator to be unrelated to treatment and mild in intensity, and 1 event of white blood cell count decreased that was mild and possibly related to treatment. All 3 events resolved with no action taken.

Given the clinical characteristics and spontaneous resolution of these events with ongoing eteplirsen treatment, there is no indication of leukocyte or neutrophil toxicity associated with eteplirsen.

9.6.   Clinical Laboratory Evaluations

Laboratory parameters including hepatic tests (i.e., ALT, AST, bilirubin, alkaline phosphatase, GGT), renal function tests (i.e., BUN, creatinine), along with hematologic parameters (i.e., hemoglobin, platelet counts, leukocytes, and leukocyte differential count) and parameters related to coagulation (aPTT and PT) were reviewed. These results were discussed above in relationship to AESIs.

Overall, review of serum chemistry data did not identify safety concerns or any consistent patterns of effect that were indicative of hepatic or renal toxicity. Likewise, review of coagulation and hematologic parameters did not identify any consistent effects suggestive of a coagulation disorder or hematologic toxicity. Markedly elevated transaminase levels that decrease over time were observed and are consistent with results expected in patients with DMD.

In addition, other chemistry laboratory parameters (glucose, albumin, potassium, and creatine phosphokinase) and immunogenicity assessments were also reviewed. Increases in glucose and decreases in potassium values were observed; however, these are considered reflective of the use of corticosteroids in the study population.

Creatine kinase (CK) and immunogenicity results are presented below.

9.6.1.   Creatine Kinase

Patients with DMD have grossly elevated CK values due to leakage of the enzyme from degenerating muscle fibers (Zatz 1991). Early in the disease, CK levels are usually 50 to $300 \times ULN$ (normal range 37 to 430 U/L) as muscles degenerate, and over time, the levels tend to decrease as muscle is lost.

Overall, CK values were elevated at baseline and last observation. A total of 20 patients who received eteplirsen at 30 mg/kg or higher met the predefined criterion of ≥2 × baseline for an abnormal change in CK value. However, in 10 of the 20 patients (30 mg/kg or higher), there was a recorded pre-treatment CK value that was higher than the reported on-treatment abnormality, and among the 20 patients, medical review determined that only 1 patient (301-210-004) had concurrent mild muscle related event (back pain lasting 5 hours), with no other reported myalgia or musculoskeletal pain. In addition, 1 untreated patient and 2 patients receiving placebo also had an increase in CK that was ≥2 × baseline. Therefore, these abnormal CK values are considered representative of fluctuations in CK laboratory values that occur during the clinical course of DMD.

9.6.2.   Immunogenicity

The potential for eteplirsen to cause immunotoxicity by complement activation was assessed in repeat-dose studies in juvenile rats, which included assays for T-cell dependent antibody response and blood immunophenotyping, and in non-human primates in complement activation assays. No biologically meaningful effects of eteplirsen on the immune system were detected in these studies.

Consistent with these findings, mean CD3, CD4 and CD8 lymphocyte counts (detected by IHC) decreased or remained stable from baseline to Week 48 in eteplirsen-treated patients in Studies 201/202, indicating a lack of immunogenicity of the newly formed dystrophin. Furthermore, there were no meaningful differences among the treatment groups in the number of interferon-gamma-induced spot-forming colonies on enzyme-linked immunosorbent spot assay (ELISPOT) from baseline through Week 48, indicating the newly expressed dystrophin in eteplirsen-treated patients did not elicit a T-cell response.

Similarly, in supportive Study 28, none of the patients had detectable levels of anti-dystrophin antibody following treatment and most of the patients in the 10 mg/kg and 20 mg/kg dose groups showed decreases in CD3, CD4 and CD8 counts. Finally, there were no clinically significant changes in immunoglobulins (IgA, IgG, or IgM) or in CD4 or CD8 counts following a single IM injection of eteplirsen in Study 33.

9.7.   Therapeutic Class Effects

Even though eteplirsen being a PMO is an RNA analog, it has significant, distinct chemical and biological properties that are not seen in other RNA analogues such as phosphorothioates. The difference in the nonclinical toxicity profile between phosphorothioate-based oligonucleotides, which are negatively charged, and eteplirsen is thought to be attributed to the uncharged nature of eteplirsen's phosphorodiamidate linkages that minimize protein binding and thus off-target effects.

Other RNA analogs, specifically those with phosphorothioate linkages, which are negatively charged, have been associated with renal toxicity, including increases in proteinuria (McGowan 2012; Goemans 2011). Elevated levels of transaminases, as well as an SAE of hepatic steatosis, have been observed in the context of treatment with a 2'-O-methoxyethyl (2'OME) phosphorothioate antisense oligonucleotide, mipomersen (McGowan 2012). SAEs of thrombocytopenia, as well as TEAEs related to coagulation, were observed in clinical trials of another phosphorothioate antisense oligonucleotide, drisapersen (Goemans 2014). Injection site

reactions comprised the most common TEAEs observed in clinical trials with both of these oligonucleotides, which share the common structural element of negatively-charged phosphorothioate linkages (Voit 2014, McGowan 2012).

These toxicities are dose limiting for phosphorothioate-based oligonucleotides in the clinical setting and are consistent with the nonclinical toxicity profile of phosphorothioate-based oligonucleotides (Levin 1998; Monteith 1999; Levin 2001; Henry 2008; Frazier 2014).

The safety data for eteplirsen in the clinical setting, including in 88 patients for an overall exposure of 72 patient years at the clinical dose of 30 mg/kg or higher for up 4 years, did not suggest a signal for the above-mentioned toxicities. These clinical data for eteplirsen are consistent with the nonclinical toxicity data, which showed only non-adverse renal findings at the highest doses administered to mice and NHPs and adverse renal findings, but no other toxicities, at the highest dose level in juvenile rats (Section 4.2.2).

Unlike phosphorothioates, PMOs thus may be less likely to be associated with off-target and serum protein binding, and immune activation. Eteplirsen thus has a chemical and biological profile that is distinct from phosphorothioates.

9.8.   Safety in Special Populations

9.8.1.   Intrinsic Factors

The safety profile of eteplirsen was evaluated in subgroups of patients in terms of specific age groupings, BMI, race, duration since DMD diagnosis, and ambulatory status. Due to the overall small number of patients (N = 107 in the 'eteplirsen IV' group) in safety dataset, interpretation of findings is limited when the dataset is split across subgroups; in addition, interpretation is further confounded by the low number of serious, severe, and 'uncommon' TEAEs (i.e., those occurring in <10% of patients).

The overall incidence for TEAEs was 82.2% for the 'eteplirsen IV' group and was comparable across patient subgroups of age, BMI, duration since DMD diagnosis and ambulatory status. The majority of patients were between ≥6 and <12 years of age, and all of the very few severe or serious adverse events occurred in ambulatory children aged ≥6 to <12 years.

Common adverse events observed in at least 10% of patients were also evaluated by patient subgroups. The frequency of these events was generally comparable across subgroups for age, ambulatory status, and duration since diagnosis except for lower frequency rates observed in the older age groups and children with non-ambulatory status.

9.8.2.   Pregnancy, Lactation, Geriatric Use

DMD is an X-linked genetic disease occurring in boys. Female carriers are, apart from extremely rare exceptions, asymptomatic. Therefore, eteplirsen has not been studied in pregnant and/or lactating women. In nonclinical testing, no evidence of eteplirsen-associated mutations, chromosomal aberrations, or clastogenic potential was observed in the ICH standard battery of genotoxicity tests. Geriatric patients have not been studied, because DMD is universally fatal during early adulthood.

10.   SUMMARY OF RESULTS

10.1.   Summary of Efficacy Results

Eteplirsen's ability to reliably induce the production of functional dystrophin in patients with DMD significantly slows the progression of this devastating disease as demonstrated by the following findings in eteplirsen-treated patients:

Biological Endpoints

- Confirmation of exon 51 skipping in all eteplirsen-treated patients with post-treatment biopsies (n = 36)

- De novo dystrophin production was demonstrated by Week 24 in Study 201 based on significant increases in the percent dystrophin positive fibers and intensity; these results were confirmed by independent, blinded pathologists

- Sustained dystrophin production was demonstrated by comparison of Study 201/202 Week 180 biopsy results to untreated controls. Utilizing methods agreed upon by FDA, significant increases in 3 complementary parameters (percent dystrophin positive fibers, dystrophin intensity, and Western Blot) were demonstrated.

- Correct localization of dystrophin at the sarcolemma, as well as localization of nNOS, and components of the DAPC at the sarcolemma, supporting the functionality of the newly expressed dystrophin protein

Primary Clinical Endpoint of 6MWT

- A significant reduction in the rate of decline for eteplirsen treated boys (N = 12) of 150.8 meters when compared to the external control group of exon 51 skippable (N = 13). Reduction in the rate of decline in 6MWT reflects amelioration of disease in terms of ambulation, endurance, and muscle function

  - Large magnitude of effect is clinically relevant (treatment effect of 150.8 meters); p<0.01

  - Substantive reduction in the rate of decline (78.7 meters) even when compared to the larger, but less well matched group of any exon skipping (N = 50)

- Temporal pattern for 6MWTin both analyses is divergence of trajectories after Year 1

  - Consistent with significant dystrophin production shown at Week 24

  - Sufficient time is required for decline of comparator in order to demonstrate eteplirsen stabilization of 6MWT

Supportive Endpoints Consistently Favor Eteplirsen vs External Controls

Analyses of supportive endpoints including the percent change from baseline in NSAA total score (Figure 33 Panel A) and preservation of function including the ability to walk and the ability to rise from supine (Figure 33 Panel B) are all directionally consistent with the results of the primary 6MWT. The analysis of mean adjusted treatment differences are directionally consistent and favor eteplirsen treated patients.

Figure 33:   Treatment Difference on Multiple Endpoints at Year 3: Study 201/201 Eteplirsen Treated Patients vs External Controls Amenable to Exon 51 Skipping
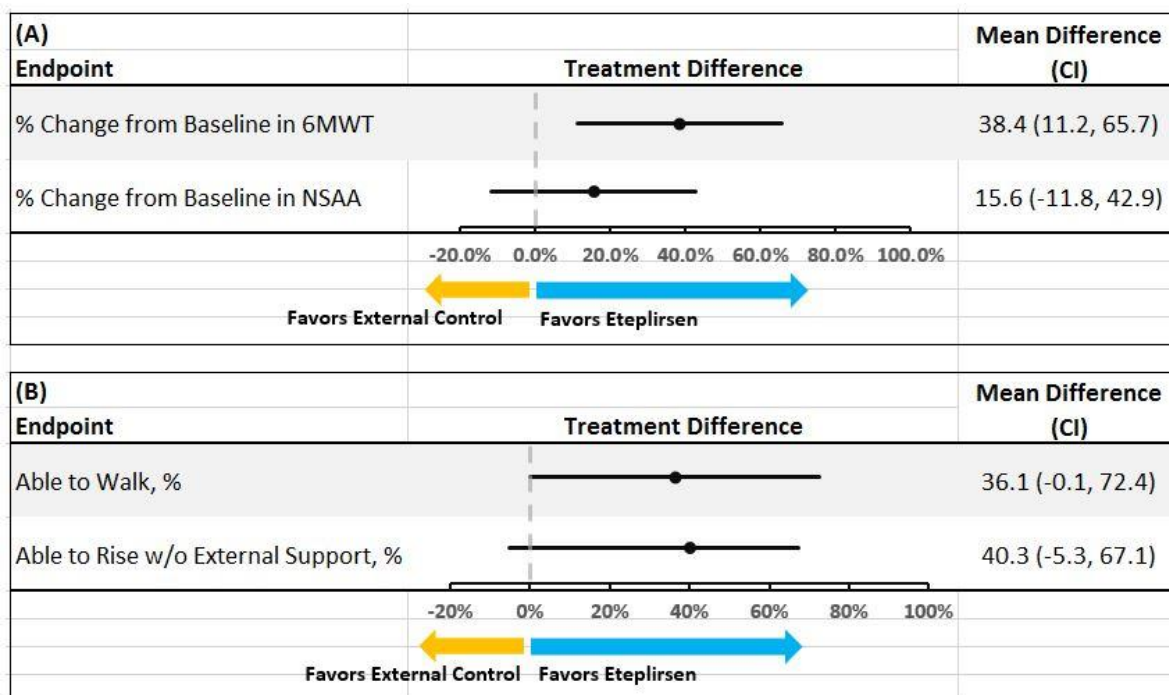
- **Loss of Ambulation**: Fewer eteplirsen treated boys lost ambulation over the course of 3 years (2/12; (16.7%) compared to 6 of 13 (46.2%) for external controls amenable to exon 51 skipping (N=13). The mean difference of 36.1% represents the larger proportion of eteplirsen treated boys maintaining the ability to walk.

- **NSAA Score:** A smaller decline in NSAA total scores over 3 years for eteplirsen boys (N = 12) compared to untreated external controls (N = 10) of 3 points representing loss or impairment of fewer abilities. In analysis of the difference in percent change from baseline the eteplirsen treated patients declined 15.6% less than the external control patients.

- **Ability to Rise from Supine without External Support:** More eteplirsen treated boys were able to rise from supine without external support (6/11; 55%) compared to the external control boys from the Italian Telethon cohort (1/7; 14%). The mean difference of effect is 40.3%.

Additionally comparisons of eteplirsen FVC% predicted to external patient level data provided by Mayer et al, confirmed the slower decline for eteplirsen treated patients compared to untreated DMD patients in a similar age group.

- **Pulmonary Function Tests:** Eteplirsen treated boys had slower deterioration of respiratory muscle function as measured by FVC% predicted (annual decrease of 3.2%) when compared to a cohort of patients in the 7-15 year age group (annual decrease of 5.8%). Additionally, based on review of published literature MEP% predicted and

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MIP% predicted may also decline more slowly with eteplirsen treatment, though the comparison is limited.

In summary, eteplirsen has been shown to slow the progression of DMD as measured by the 6MWT in DMD patients amenable to dystrophin exon 51 skipping over 3 years. This is supported by additional clinical measures, which are directionally consistent, including loss of ambulation NSAA and pulmonary function. The consistency of results across these endpoints supports the conclusion that eteplirsen is an effective treatment for DMD patients with genetic mutations amenable to exon 51 skipping therapy.

10.2.   Summary of Safety Results

Exposure and Demography

The overall safety analysis dataset includes a total of 114 eteplirsen-treated patients; 107 patients received once weekly IV infusions of eteplirsen at doses ranging from 0.5 to 50 mg/kg and 7 received a single IM dose of 0.09 mg or 0.9 mg eteplirsen. 88 patients received eteplirsen at either the proposed dose (30 mg/kg, N = 82) or higher (50 mg/kg, N = 6), including 61 patients who received the proposed dose or higher for at least 3 months. Collectively, these data represent over 72 patient-years of safety experience at the proposed once weekly dose of 30 mg/kg or higher. A safety database of this size is not unprecedented in the rare disease setting and the accelerated approval pathway which is reserved for serious and rare diseases with a high unmet medical need.

Treatment-emergent Adverse Events

The most common ($\geq$10% of patients) TEAEs occurring more frequently in patients treated with eteplirsen at either 30 or 50 mg/kg IV than in patients who received placebo were: headache, arthralgia, vomiting, upper respiratory tract infection, nasopharyngitis, cough, nasal congestion, contusion, excoriation and procedural pain. The majority of these common TEAEs were mild in severity, considered unrelated to study drug, and resolved during continued treatment with study drug.

No deaths or life-threatening events occurred during the eteplirsen clinical studies, and only 2 patients (1.8%) experienced a treatment-emergent SAE, both of which were unrelated to eteplirsen. Five (5) patients (4.4%) on eteplirsen and 1 patient in the untreated group experienced severe TEAEs, and 1 patient (0.9%) discontinued treatment prematurely due to a TEAE.

Adverse Events of Special Interest

TEAEs of special interest for the eteplirsen clinical program included medical topics that were selected based on: potential safety-related findings observed in nonclinical toxicity studies of eteplirsen (renal function), AEs associated with other RNA analogs (renal and hepatic function, coagulopathy and infusion site reactions), and general precautions with administration of a compound in clinical development (infusion-related reactions, hypersensitivity, severe cutaneous reactions, leukopenia and neutropenia). Inclusion of adverse events associated with other RNA analogs in Adverse Events of Special Interest for eteplirsen, is a conservative approach, since eteplirsen is structurally dissimilar and the nonclinical toxicity data for eteplirsen did not show a signal except for renal findings at high doses.

Renal function

Twenty-one (21) TEAEs potentially representative of renal toxicity were reported in 16 patients (14.0%) in the 'all eteplirsen' group, and an event of proteinuria was reported in both a placebo patient and a 30 mg/kg patient prior to treatment initiation. All of the events were mild; the majority were transient and spontaneously resolved with ongoing study drug administration. Proteinuria/urine protein present were the most common events observed; these events were transient or sporadic, spontaneously resolved with ongoing treatment, and were not associated with increasing renal laboratory values, with the exception of 1 patient who had adverse events of increased BUN and increased creatinine in the setting of dehydration.

Review of renal adverse events and laboratory parameters identified no pattern of drug effect.

Cardiac function:

Twelve (12) TEAEs potentially indicative of a cardiac disorder were reported in 6 patients in the 'all eteplirsen' group. These events included tachycardia and cardiomyopathy which are known to occur in the background population. None of the events were serious, and the majority were assessed by the investigator as mild in intensity and as possibly related to eteplirsen with the exception of a severe case of cardiomyopathy, which resulted in study drug discontinuation.

Echocardiogram data in the ongoing Study 201/202 did not suggest any pattern of decline in left ventricular ejection fraction for the 12 patients on eteplirsen at 30 or 50 mg/kg/wk for 4 years.

Based on the known prevalence (27%) of cardiomyopathy in patients with DMD, it is difficult to establish a causal association with drug therapy.

Hepatic function:

There have been no reported adverse events suggestive of drug-induced hepatotoxicity.

Coagulopathy: Forty-two (42) TEAEs potentially indicative of a coagulation disorder were reported in 21 patients (18.4%) in the 'all eteplirsen' group. None of the events were serious, and the majority were reported by the investigator as mild in intensity and not related to eteplirsen. Overall review of the events potentially related to coagulopathy suggested no consistent pattern of eteplirsen drug effect.

Infusion site reactions: Fifty-five (55) infusion site reactions were reported in 24 patients (22.4%) in the 'eteplirsen IV' group with over 3900 infusion (event rate <1.5%). Events of catheter-related pain, hematoma, or infusion site extravasation occurred during clinical studies of eteplirsen, but were generally reflective of the types of events due to catheter placement rather than due to a direct effect of eteplirsen. These events were all transient, mostly mild in severity, and consistent with catheter-related complications, which does not suggest an association with eteplirsen.

Infusion related reactions: Fifty-five (55) TEAEs were reported in 30 patients (28.0%) in the 'all eteplirsen IV' dose group and 3 events were reported in 2 placebo patients (50.0%). Non-specific symptoms of potential infusion-related reactions such as nausea and vomiting occurred in the eteplirsen-treated population at a relatively low rate, and also occurred prior to treatment or in the placebo group. Although some events have been noted on days of infusion, there was no consistent pattern of recurrence with subsequent infusions. There was one case of "mild temperature elevation" (coded to the Preferred Term "Infusion related reaction") coincident with

infusion of eteplirsen, which the investigator considered possibly related to study drug. This event is, therefore, being considered a potential adverse drug reaction.

Hypersensitivity: A total of 43 TEAEs potentially representative of hypersensitivity were reported in 27 patients (23.7%) in the 'all eteplirsen' group. None of the events were serious, and the majority were reported by the investigator as mild in intensity and not related to eteplirsen. There have been reports of mild and unrelated rash, contact dermatitis, papule, urticaria and pruritus coincident with eteplirsen treatment. There were no trends or patterns in time to onset from last dose, the number of doses prior to event, or event. Given the resolution and lack of recurrence for most events with continued treatment it may be concluded that these events are reflective of the background population rather than due to study drug treatment. There have been mild events of erythema and flushing occurring on days of study drug infusion, which may represent potential adverse drug reactions with eteplirsen.

Severe cutaneous reactions

Two (2) TEAEs potentially indicative of a severe cutaneous reaction were reported in 2 patients (1.8%) in the 'all eteplirsen' group. These events were mild and self-limited without sequelae. Both had alternative etiologies, including traumatic injury and post-biopsy complication. The events were not consistent with severe cutaneous reaction.

Leukopenia and neutropenia

The potential for leukopenia/ neutropenia was evaluated by review of TEAE data as well as pertinent laboratory parameters. TEAEs of mild leukopenia and lymphopenia were reported for a single patient. Both the leukocyte and lymphocyte counts subsequently normalized with ongoing eteplirsen treatment. There were no reported TAEs of neutropenia. Across all patients, evaluation of leukocytes, neutrophils, and lymphocytes identified no consistent pattern suggestive of drug effect.

Safety will continuously be evaluated in the post-marketed setting including spontaneous adverse event reports, reports from ongoing clinical studies and other sources. In addition a planned longitudinal observational safety registry in DMD patients will collect safety assessments including adverse events of special interest.

In summary, eteplirsen has been shown to be well tolerated, with low rates of serious or severe adverse effects, and the most common events are likely characteristic of the background population. The following common events occurred more frequently in patients who received 30 or 50 mg/kg eteplirsen IV than in patients who received placebo: headache, vomiting, cough, procedural pain, upper respiratory tract infection, arthralgia, contusion, excoriation, nasopharyngitis and nasal congestion. Due to their temporal occurrence relative to eteplirsen administration, the following events will be categorized as ADRs: erythema, flushing, and mild temperature elevation.

11. BENEFITS AND RISKS CONCLUSIONS

11.1. Medical Need

Duchenne muscular dystrophy is a rare, degenerative neuromuscular disease caused by mutations in the *DMD* gene leading to progressive muscle degeneration and ultimately death by early adulthood (Brooke 1989; Eagle 2002; Kohler 2009).

There are no approved therapies for DMD in the US. Although glucocorticoids may be used, their modest effects on delaying disease progression are accompanied by significant side effects. (Beenakker 2005; Biggar 2006; Pradhan 2006; Manzur 2009; Schram 2013; Henricson 2013a). Therefore, there remains a high unmet medical need for an effective therapy for these patients.

11.2. Benefits of Eteplirsen

Eteplirsen is a disease-modifying PMO therapeutic for DMD patients with mutations that are amenable to skipping exon 51. Clinical trials have demonstrated that, in this specific DMD population, eteplirsen treatment induced dystrophin expression resulting in the following sustained clinical benefits:

- Eteplirsen treated patients demonstrated significantly better performance on the 6MWT versus an untreated external control cohort bearing exon 51 skippable mutations, with a clinically meaningful 151-meter advantage after 3 years of therapy

- Fewer eteplirsen-treated patients lost ambulation over the course of 3 years compared to untreated external controls; 2 of 12 (16.7%) eteplirsen-treated patients lost ambulation, compared with 6 of 13 (46.2%) external controls with *DMD* mutations amenable to exon 51 skipping.

- Treatment with eteplirsen resulted in a slower rate of decline on the NSAA total score compared to untreated external control patients over 3 years; this was consistent with results for the 6MWT

- Eteplirsen treated patients experienced relative pulmonary function stability (annual decline of 3.2% on FVC% predicted) compared to an external cohort of patient level data provided by Mayer et al (annual decline of 5.8% on FVC% predicted)

11.3. Risks of Eteplirsen

Clinical trials have evaluated safety in a total of 114 patients with DMD, 88 of whom received a dose of $\geq$30 mg/kg.

- The favorable tolerability of eteplirsen is demonstrated by low rates of treatment emergent SAEs (N = 2, 1.8%), severe AEs (N = 5, 4.4%), and AEs resulting in study drug discontinuation (N = 1, 0.9%).

- The most common ($\geq$10% of patients) TEAEs occurring more frequently in patients treated with eteplirsen at the clinical dose of 30 mg/kg or higher than in patients treated with placebo were: headache, vomiting, cough, procedural pain, upper respiratory infection, arthralgia, contusion, excoriation, nasopharyngitis, and nasal congestion.

- In addition, due to their temporal relationship to eteplirsen administration, the following events are also categorized as ADRs: erythema, flushing, and mild temperature elevation.

- Adverse events of special interest in the following medical categories were not considered related to eteplirsen treatment (i.e. renal toxicity, hepatotoxicity, cardiac-related events, coagulopathy, severe cutaneous reactions, and leukopenia)

11.4.   Benefit:Risk Conclusions

The favorable benefit:risk profile of eteplirsen is demonstrated by the totality of evidence showing that weekly administration of eteplirsen is well-tolerated, and is an effective treatment in patients with DMD who are amenable to exon 51 skipping therapy. Specifically, eteplirsen slows the rate of decline in ambulation, endurance, and muscle function as measured by the 6MWT over a 3-year treatment period compared to external control data. Sarepta is committed to the completion of confirmatory trials that will not only aim to verify the clinical benefit of eteplirsen using the 6MWT (intermediate endpoint for accelerated approval), but will also provide an evolving understanding of the safety profile.

The benefits of eteplirsen are demonstrated by a significant difference in the 6MWT of 151 meters compared to external control and a reduction in the number of boys with a loss of ambulation (17% for eteplirsen compared to 46% for the external control cohort of exon 51 skippable patients). Given the highly comparable nature of the eteplirsen patients to the external control including Baseline age, 6MWT distance and longitudinal use of steroids, this difference can only be reasonably attributed to the beneficial intervention of eteplirsen. Moreover, additional clinical assessments using the NSAA and PFTs are supportive of the beneficial clinical effect of eteplirsen as well. In addition, to the demonstrated clinical benefit, the biologic endpoints confirm the predicted mechanism of action and that de novo dystrophin production occurs when boys are treated with eteplirsen.

Significantly, this clinical benefit is accompanied by a safety profile that indicates that eteplirsen is well tolerated with no apparent signal of safety risks. Although the safety dataset of 114 patients may not detect rare events and therefore carries the potential risk of uncertainty in characterization of such events, this needs to be weighed against the certainty of relentless disease progression and premature death for boys with DMD without treatment.

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APPENDIX 1. KEY FDA REGULATORY INTERACTIONS REGARDING ETEPLIRSEN

Date	Summary of Key Regulatory Activity
02 Aug 2007	AVI BioPharma (AVI) submits an initial IND for eteplirsen.
23 Oct2007	FDA grants orphan drug designation to eteplirsen for the treatment of DMD
27 Nov 2007	FDA designates the investigation of eteplirsen for DMD a Fast Track development program
25 Apr 2011	AVI submits a new proposed clinical protocol for study 4658-us-201, a randomized, double-blind, placebo-controlled, 24-week study of eteplirsen with two arms: • 50 mg/kg eteplirsen IV and matched placebo with a 12-week on-treatment biopsy time point • 30 mg/kg eteplirsen IV and matched placebo with a 24-week on-treatment biopsy time point
14 Jun 2011	A Type B End-of-Phase 1 meeting is held between the FDA and AVI. Key issues discussed at this meeting are: • Surrogate endpoints: FDA states that a statistically significant finding on a clinically meaningful functional outcome would be needed to support an efficacy claim for eteplirsen, and that findings on biomarkers and exploratory functional endpoints could only be supportive • Extension study: FDA agrees that an open-label rollover study, 4658-us-202, may initiate at the end of study 201 • Juvenile toxicology study: FDA makes various recommendations for the design of a 10-week repeat-dose toxicology study of eteplirsen in juvenile rats, including an assessment of immune function • FDA agrees that analysis of complement activation in the 9-month repeat dose cynomolgus monkey study will suffice to assess complement activation in NHPs
12 Jul 2012	AVI BioPharma changes its name to Sarepta Therapeutics

Date	Summary of Key Regulatory Activity

13 Mar 2013	A Type B End-of-Phase 2 meeting held between FDA and Sarepta. Key issues discussed at this meeting are:
	
	• Accelerated approval: FDA considers the study 201/202 dataset through Week 48 of the combined studies to be inadequate to support accelerated approval for the following reasons:
	
	   ○ No difference observed in the 6MWT in study 201 based on the ITT analysis
	
	   ○ 6MWT results in study 202 are uninterpretable due to the uncontrolled, open-label study design, and the effort-dependent nature of the 6MWT
	
	   ○ Inadequate characterization of the quantity of dystrophin in treated patients, due to the lack of western blot data
	
	   ○ Dystrophin as assessed by IHC appears of lesser quantity than in BMD
	
	   ○ "No good correlation" observed between the dystrophin and 6MWT results in 201/202
	
	FDA concludes that Sarepta should submit further information to support the use of dystrophin as a surrogate, as well as a discussion of all clinical functional outcomes assessed in eteplirsen studies , in order to determine whether it will consider filing an NDA for accelerated approval
	
	• Confirmatory study design: Sarepta proposes study 4658-301 (named PROMOVI), an open-label study of eteplirsen in exon 51 skipping amenable DMD patients, versus a concurrent untreated cohort of DMD patients with exon deletions not amenable to skipping exon 51, as a confirmatory study to support accelerated approval of eteplirsen. FDA indicates that placebo control is seemingly necessary to provide interpretable data of an effect on the 6MWT beyond the known variability range of DMD.
	
	• Safety population: FDA requires that additional exposed patients beyond the existing 38 in order to conclude that the drug has an acceptable risk/benefit profile.

23 Jul 2013	A Type C guidance meeting is held between the FDA and Sarepta as a follow-up to the March 2013 EOP2 meeting. • **FDA states that based on additional information submitted on dystrophin and clinical outcomes; *"We are now open to considering an NDA based on these data for filing"*.** FDA also makes the following general recommendations: • Creation of a proposed charter for dystrophin quantification methods to be used in future biopsies • Independent confirmation of the dystrophin-positive fiber results from study 201/202 • Collaborative development of a protocol for either a western blot or dot blot method to quantify total protein • Obtaining and analyzing a fourth biopsy from patients in study 201/202 • Obtaining additional safety data

Date	Summary of Key Regulatory Activity
08 Nov 2013	A Type C guidance meeting is held between the FDA and Sarepta to discuss the design of PROMOVI, and determine whether it will be placebo-controlled or open label. • **In preliminary comments received November 6th, FDA states that the negative reports of the large Phase 3 drisapersen study and Phase 2 PTC124 study, two drugs also thought to act by increasing dystrophin,** *"raises considerable doubt about the biomarker* **(dystrophin)***, and consequently, its ability to reasonably likely predict clinical benefit".* **Taken in combination with perceived difficulties in interpretation of the 6MWT results from studies 201/202,** *FDA "currently consider an NDA filing for eteplirsen as premature"* • **FDA also stated that** *"further biopsies should be delayed until a "validated assay to quantify dystrophin becomes available."* • Sarepta's presentation for this meeting focuses on the chemical differences eteplirsen and drisapersen (i.e. the backbone and sequence), the lack of publicly available evidence that drisapersen adequately induces either exon skipping or de novo dystrophin expression, and the superiority of eteplirsen over drisapersen reported in vivo (Heemskerk 2009).
15 Nov 2013	A teleconference is held between the FDA and Sarepta to continue the discussion from the November 8th Type C meeting regarding the design of PROMOVI. • Sarepta discusses the projected difficulty of enrolling a 120-patient placebo-controlled study of eteplirsen in the United States. • FDA concludes that it may be open to the open-label design of PROMOVI if analysis of DMD natural history data were to reveal subgroups with high degrees of predictability of decline on the 6MWT.
13 Dec 2013	FDA requests the methodology and protocols used for the dystrophin-positive fiber, dystrophin intensity, western blot, and RT-PCR assays in study 201/202
19 Dec 2013	A Type A guidance meeting is held between the FDA and Sarepta to continue discussion on the design of PROMOVI, including presentation of the study 201/202 Week 96 6MWT data. • **FDA requests that Sarepta contact the sponsors of the Italian Telethon and Belgian DMD natural history databases and request that their raw be provided to the FDA for analysis.** • FDA also recommends that Sarepta develop a plan to assess the immunogenicity of eteplirsen. • FDA concludes that it is not prepared to take a position on the open label design of the proposed confirmatory trial, nor resume a position on the feasibility of filing an NDA for eteplirsen based on the current dataset.

Date	Summary of Key Regulatory Activity
07 Feb 2014	An ad hoc teleconference is held between the FDA and Sarepta. The FDA requests all of the biomarker images and data listings from study 201/202 for review: • Dystrophin-positive fibers by IHC • Fluorescent intensity of dystrophin by BIOQUANT • Total protein by western blot • Exon skipping by RT-PCR
20 Feb 2014	Sarepta completes submission of all of the requested biomarker data
19 Mar 2014	A guidance meeting is held between the FDA and Sarepta. • **FDA states that it is open to filing an NDA for eteplirsen for consideration under accelerated approval** • **FDA proposed a potential approach of two confirmatory studies, an open-label study of eteplirsen and a randomized, double-blind, placebo-controlled trial of another exon skipping PMO** • In order for FDA reviewers to better understand the dystrophin-positive fiber methodology, FDA reviewers will visit the laboratory where the dystrophin assessments in study 201/202 were conducted. FDA adds the following remaining reservations regarding the 201/202 dataset, which it states will be NDA review issues: • The 6MWT analysis is based on a modified ITT population, excluding the two patients who became non-ambulant during study 201 • The supportive care given to 201/202 patients versus patients in a historically-controlled population • Potential bias in administration of the 6MWT during the open-label study 202
21 Mar 2014	In accordance with verbal agreement at the March 19th meeting, Sarepta sends correspondence to FDA outlining a new proposed clinical development plan for eteplirsen, including: • the open-label confirmatory study PROMOVI, • a safety study of eteplirsen in DMD patients with advanced disease (4658-204), • a safety study of eteplirsen in 4- to 6-year-olds (4658-203), • a randomized, placebo-controlled confirmatory study of SRP-4045 and SRP-4053 in a pooled population of DMD patients amenable to skipping exons 45 and 53 (protocol 4045-301, named ESSENCE) • Sarepta also commits to collaborating with FDA in development of bioassay methods for analysis of dystrophin in future biopsies.

Date	Summary of Key Regulatory Activity
15 Apr 2014	**FDA sends an advice letter verifying that an NDA for eteplirsen should be fileable based on the available dataset, and identifies additional data needed to support the efficacy and safety of eteplirsen. FDA proposes two potential pathways to accelerated approval:** • **Considering the 6MWT data from 201/202 as a finding on an intermediate clinical endpoint** • **Considering quantification of dystrophin in muscle biopsies via a number of modalities as a surrogate endpoint** FDA identifies two confirmatory trials to verify clinical benefit and urges Sarepta to initiate both studies as soon as possible: • A historically-controlled study of eteplirsen (PROMOVI) • A randomized, placebo-controlled study of another PMO with a similar mechanism of action, directed at a different exon (ESSENCE) FDA makes the following requirements for NDA filing: • **Obtain and submit patient-level historical control data, establishing that treatment modalities were similar to the 201/202 patients** • **Submitting additional patient exposure data beyond the existing 38 patients** FDA remains "skeptical" of the existing biomarker data and provides the following recommendations: • A collaborative effort between the FDA and Sarepta to develop a better understanding of the methods and analyses used for generation of the existing biomarker data and aid the development of suitable, consistent, and objective methods for collection and analysis of additional biomarker data • **A fourth biopsy of patients in study 202, with the samples compared in a blinded fashion to samples obtained from treatment-naïve patients with exon 51 skipping amenable DMD** • **Extending the duration of PROMOVI open-label confirmatory trial beyond 48 weeks**
09 May 2014	Protocol 4658-301, entitled "*An Open-Label Multi-center, 48-Week Study with a Concurrent Untreated Control Arm to Evaluate the Efficacy and Safety of Eteplirsen in DMD*" (PROMOVI) is submitted to the IND to initiate the first confirmatory trial.
03 Jul 2014	Protocol 4658-204, entitled "*An Open-Label Multi-center Study to evaluate the Safety and Tolerability of Eteplirsen in patients with Advanced Stage DMD*" is submitted to the IND to initiate the study.
29 Jul 2014	**FDA sends an advice letter requesting that Sarepta arrange for reassessment of the raw IHC images for determination of dystrophin-positive fibers from studies 201/202 and 28 by three independent experts, including assessment of the inter- and intra-operator reliability**

Date	Summary of Key Regulatory Activity
18 Sep 2014	**A Type B Pre-NDA meeting is held between FDA and Sarepta. FDA states that in addition to the available data, the following supplementary data are required to be included in the initial NDA submission in order to accept (file) the application for review:** • **3-month safety data from at least 12 to 24 newly exposed patients** • **Results of the University of Florida MRI natural history study** • **Patient-level historical control data on clinical endpoints, including timed tests, baseline factors, and ancillary care** • **Blinded reassessment of dystrophin-positive fiber data from studies 201/202 and 28 by 3 independent pathologists** • **Week 168 efficacy data from study 201/202** • **Presentation and analysis of the historical data available regarding dystrophin expression in BMD, including correlation between protein level and phenotype**
15 Oct 2014	FDA and Sarepta hold an ad hoc teleconference to discuss a design for the blinded reassessment of IHC images from study 201/202
28 Oct 2014	FDA sends correspondence regarding on Sarepta's proposed protocol for reassessment of IHC images from study 201/202, including comments that: • Quantification of protein level, which is not provided by the dystrophin-positive fiber assay, will be a "key" NDA review consideration • The primary statistical endpoint of the reassessment should be the baseline samples versus Weeks 12 and 24, as the Week 48 biopsy was taken from a different muscle type (deltoid vs. biceps) and processed in a separate batch, either of which could introduce confounding factors
14 Nov 2014	**FDA agrees to Sarepta's revised protocol for reassessment of IHC images from study 201/202**

Date	Summary of Key Regulatory Activity
18 Nov 2014	**A Type A guidance meeting is held between the FDA and Sarepta to discuss and agree on the design of the second proposed confirmatory study to support accelerated approval of eteplirsen. FDA and Sarepta agree to the following design aspects:** • **Randomized, double-blind, placebo control** • **A pooled study of SRP-4045 and SRP-4053 at a 30 mg/kg/week dose each** • **A patient population aged 7 to 16 years with a baseline 6MWT distance 300 to 450 meters and receiving a stable dose of oral corticosteroids** • **A primary endpoint of the 6MWT** • **Secondary endpoints of PFTs, dystrophin-positive fibers, protein level by western blot, the NSAA, and timed function tests** • **A 48-week duration** • **A total sample size of 99 patients, allocated to placebo or treatment in a 2:1 ratio**
23 Jan 2015	Protocol 4658-203, entitled "*An Open-Label Multi-center Study to evaluate the Safety, Efficacy and Tolerability of Eteplirsen in Early-Stage DMD*" is submitted to the IND to initiate the study.
30 Mar 2015	**FDA agrees that analysis of the Week 180 fourth biopsy tissue samples from study 202 may proceed with the assay protocols for western blot, dystrophin-positive fibers, dystrophin intensity, and RT-PCR submitted by Sarepta**
19 May 2015	**A Type C Pre-NDA meeting is held between FDA and Sarepta, as a follow-up to the September 18th Pre-NDA meeting.** **FDA states that Sarepta's proposed outline of the NDA is "*generally acceptable*" and requests submission of the following data to the NDA as soon as possible:** • Week 192 efficacy data from study 201/202 • Week 180 fourth biopsy data • FDA accepts that Sarepta was unable to obtain patient-level PFT natural history data from the Cooperative International Neuromuscular Research Group (CINRG), but requests that Sarepta continue efforts to obtain these data. • Sarepta states that they will submit the NDA as a rolling submission.
20 May 2015	Sarepta initiates the rolling NDA submission by providing the chemistry, manufacturing and control (CMC) and nonclinical portions of the NDA
26 Jun 2015	Sarepta submits the clinical portion of the NDA, completing the rolling NDA submission

Date	Summary of Key Regulatory Activity
25 Aug 2015	FDA accepts (files) the NDA for review and grants priority review designation, setting the user fee goal date of 26 February 2016

APPENDIX 2. INCLUSION AND EXCLUSION CRITERIA STUDY STUDY 201/202

Inclusion Criteria Study 201

Patients had to meet all of the following criteria to be eligible for this study:

1. Be a male with DMD and have an out-of-frame deletion(s) that may be corrected by skipping exon 51 [e.g., deletions of exons 45-50, 47-50, 48-50, 49-50, 50, 52, 52-63], as confirmed in a Clinical Laboratory Improvement Act (CLIA)-accredited laboratory by any peer-reviewed and published methodology that evaluates all exons (including, but not limited to, multiplex ligation-dependent probe, comparative genomic hybridization, and single condition amplification/internal primer analysis).
2. Be between the ages of 7 and 13 years, inclusive.
3. Have stable cardiac function and stable pulmonary function (forced vital capacity [FVC] ≥50% of predicted and not require supplemental oxygen) that, in the Investigator's opinion, is unlikely to decompensate over the duration of the study.
4. Be receiving treatment with oral corticosteroids and have been on a stable dose for at least 24 weeks before study entry. Patients may be allowed to take other (except RNA antisense or gene therapy) medication, including angiotensin-converting enzyme [ACE] inhibitors, β-blockers, losartan potassium, and coenzyme Q, as long as they have been on a stable dose of the medication for 24 weeks before the screening visit (Visit 1) and the dose will remain constant throughout the study.
5. Have intact right and left biceps muscles or an alternative upper arm muscle group.
6. Achieve an average distance within 200 and 400 meters ±10% (i.e. within 180 and 440 meters) while walking independently over 6 minutes.
7. Have a left ventricular ejection fraction (LVEF) of >40% based on the ECHO that is obtained at the screening visit (Visit 1). A patient who has abnormal ECHO findings but who has an LVEF of >40% may be enrolled in the study at the Investigator's discretion; however, the patient must have been receiving stable doses of ACE inhibitors or β-blockers for at least 24 weeks before study entry.
8. Have a parent(s) or legal guardian(s) who is able to understand and comply with the all of the study procedure requirements.
9. Be willing to provide informed assent and have a parent(s) or legal guardian(s) who is willing to provide written informed consent for the patient to participate in the study.

Exclusion Criteria Study 201

Patients who met any of the following criteria were excluded from this study:

1. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength or function within 12 weeks before study entry (e.g., growth hormone, anabolic steroids).
2. Previous treatment with the experimental agents eteplirsen, BMN-195, or PRO051.
3. Previous treatment with any other experimental agents or participation in any other DMD interventional clinical study within 12 weeks before entry into this study; including use of the shock training system or "STS," or planned use during this study.
4. Surgery within 3 months before study entry or planned surgery at any time during this study.
5. Presence of other clinically significant illness at the time of study entry, including significant renal dysfunction (as measured by urinary cystatin C, KIM-1, or urinary total protein), or average heart rate during screening Holter monitoring in excess of 110 bpm (unless subsequently treated and confirmed controlled and stable on a β-blocker) or QTc >450 ms.
6. Use of any aminoglycoside antibiotic within 12 weeks before the screening visit (Visit 1) or need for use of an aminoglycoside antibiotic during the study (unless discussed and agreed with the Principal Investigator and Medical Monitor).
7. Prior or ongoing medical condition that, in the Investigator's opinion, could adversely affect the safety of the patient or that makes it unlikely that the course of treatment or follow-up would be completed or could impair the assessment of study results.

Inclusion Criteria Study 202

In order to be considered eligible, all of the following criteria must have been met:

1. The patient and/or their parent/legal guardian are willing and able to provide signed informed consent.
2. The patient has successfully completed 28 weeks of treatment in Study 4658-us-201.
3. The patient has a parent(s) or legal guardian(s) who is able to understand and comply with all of the study procedure requirements.

Exclusion Criteria Study 202

Patients who met any one of the following criteria were ineligible for participation in the study:

1. The patient has a prior or ongoing medical condition that, in the Investigator's opinion, could adversely affect the safety of the patient or make it unlikely that the course of treatment or follow-up would be completed or impair the assessment of study results.

APPENDIX 3. BASELINE CHARACTERISTICS OF STUDY 201/202 ETEPLIRSEN TREATED (N = 12) VS. EXTERNAL CONTROL COHORT (N = 13)

SOURCE	SUBJ.	Deleted Exon(s)	AGE (YRS)	HEIGHT (CM)	WEIGHT (KG)	6MWT (M)	NSAA (TOTAL SCORE)	STEROID, DOSE, & REGIMEN (Dose: mg or mg/kg†)		
Eteplirsen Study 201/202	2	48-50	9.03	117	24.8	416	28	DFZ	18	Continuous
	3	49-50	7.29	118	23.7	366	31	DFZ	18	Continuous
	4	45-50	8.79	117	27.4	389	27	DFZ	24	Continuous
	5	50	8.01	117	23.5	374	23	DFZ	21	Continuous
	6	52	10.53	131	35.1	355	17	DFZ	21	Continuous
	7	49-50	8.02	120	40.9	374	30	PRD	20	Continuous
	8	49-50	10.49	117	35	346	23	DFZ	22.5	Continuous
	9	45-50	9.79	138	39.8	330	21	PRD	25	Continuous
	10	45-50	9.79	136	39.7	256	17	PRD	25	Continuous
	12	49-50	10.95	133	38.3	351	27	PRD	75	Intermittent
	13	49-50	10.58	126	34.2	400	24	DFZ	22.5	Continuous
	15	52	9.6	117	26.8	401	31	DFZ	15	Continuous
LNMRC	(b) (6)	50	11.8	*UNK*	*UNK*	327	*UNK*	DFZ or Equiv.	*UNK*	Continuous
		52	11.5	*UNK*	*UNK*	451	*UNK*	DFZ or Equiv.	*UNK*	Continuous
		45-50	8.6	*UNK*	*UNK*	355	*UNK*	DFZ or Equiv.	*UNK*	Continuous
Italian Telethon		48-50	11.5	133	47	200	10	DFZ	0.8	Continuous
		Ex 50	8.6	135	48	380	31	DFZ	0.9	Intermittent
		52	9	130	28	373	24	PRD	0.75	Intermittent
		45-50	8.83	136	42	329	24	DFZ	0.9	Continuous
		49-50	9.3	131	44	295	15	DFZ	0.9	Intermittent
		49-50	8	131	33	380	23	DFZ	0.8	Continuous
		45-50	7.33	131	26	325	29	DLT	17.5	Continuous
		52	10.2	126	27.1	458	25	DFZ	0.9	Continuous
		48-50	10.1	133	31	388	20	PRD	0.75	Intermittent
		49-50	8.1	127	39	388	19	PRD	0.75	Intermittent

Abbreviations: DFZ = Deflazacort; PRD = Prednisone; DLT = Deltacortene; UNK = Unknown

Note in the table of baseline characteristics, rows for patients with loss of ambulation during 3 years of follow-up are shaded.

APPENDIX 4.   LONGITUDINAL RESULTS FOR 6MWT, NSAA AND RISE TIME FOR ETEPLIRSEN TREATED (N = 12) VS. EXTERNAL CONTROL COHORT (N = 13)

		6MWT (m)				NSAA Total Score				Rise Time (sec)			
	Year	0	1	2	3	0	1	2	3	0	1	2	3
Source	Subject												
Eteplirsen Study 201/202	2	416	430	416	378	28	26	27	25	3.2	4.9	5.6	6.2
	3	366	444	425	324	31	24	18	7	4.3	5.9	12.6	53.5
	4	389	371	331	355	27	23	21	24	3.1	4.9	6.8	13.1
	5	374	302	293	247	23	22	14	8	8.2	16.8	27.7	unable
	6	355	326	346	359	17	16	19	13	12	13.4	10.9	17.9
	7	374	304	354	312	30	20	20	13	4.6	6.8	8.8	10.1
	8	346	303	255	100	23	21	17	13	4.9	5.8	6.6	8.9
	9	330	0	0	0	21	5	2	np	6.3	24.9	unable	unable
	10	256	0	0	0	17	4	1	1	12.7	unable	unable	unable
	12	351	330	314	298	27	20	18	11	12	34	unable	unable
	13	400	368	367	301	24	20	21	14	6	10.5	9.3	24.1
	15	401	492	450	483	31	32	30	25	3.5	3.6	4.6	8.2
Italian Telethon	(b) (6)	200.0	210.0	0.0	0.0	10.0	8.0	4.0	0.0	unable	unable	unable	unable
		380.0	352.0	326.0	195.0	31.0	26.0	20.0	14.0	2.9	3.7	17.0	unable
		373.0	259.0	305.0	273.0	24.0	13.0	11.0	8.0	4.09	5.04	8.09	np
		329.0	298.0	230.0	218.0	24.0	22.0	13.0	12.0	7.1	7.2	ES	no data
		295.0	307.0	153.0	35.0	15.0	13.0	12.0	9.0	6.2	no data	unable	unable
		380.0	285.0	250.0	0.0	23.0	13.0	7.0	3.0	no data	no data	no data	no data
		325.0	301.0	210.0	0.0	29.0	27.0	13.0	3.0	2.37	10.03	np	unable
		458.0	495.0	435.0	362.0	25.0	26.0	25.0	22.0	5.66	5.21	4.31	8.47
		388.0	317.0	0.0	0.0	20.0	18.0	4.0	0.0	no data	8.0	unable	unable
		388.0	395.0	356.0	0.0	19.0	15.0	16.0	10.0	9.0	10.0	16.0	unable
LNMRC		327	0	0	0	No Data							
		451	421	320	no data								
		355	375	no data	no data								

np=not performed; ES=external support required

APPENDIX 5.　SENSITIVITY ANALYSIS FOR 6MWT ETEPLIRSEN-TREATED VS. EXTERNAL CONTROLS

Sensitivity Analyses to Control for Potential Group Imbalances in Important Baseline Prognostic Factors

The pre-specified primary ANCOVA analysis included Baseline 6MWT as a covariate to control for potential imbalances between the groups (treated vs. untreated) in Baseline 6MWT distance, an important prognostic factor for loss of ambulation. When age, another important predictor of 6MWT was added as a covariate to the same analysis, the difference between the eteplirsen-treated patients and untreated external controls remained clinically and statistically significant (Row 1).

Sensitivity Analyses to Account for Potential Violations of the Data's Normality Assumption

To address potential violations of Normality Assumption, the changes from Baseline in 6MWT distance for all patients (N = 12 + 13 = 25) were ranked 1 25. Then the rank scores, which are not affected by large changes in 6MWT scores, were analyzed using ANCOVA. Two ANCOVA models were performed, the first included Baseline 6MWT as a covariate (Row 2) and the second included both Baseline 6MWT and age as covariates (Row 3). For both analyses, the difference between the eteplirsen-treated patients and untreated external controls remained statistically significant.

Sensitivity Analyses for Missing Data

Two patients in the external control cohort (N = 13) entered interventional clinical trials and therefore did not contribute data through Year 3. To account for any potential bias caused by this approach, a series of Mixed Model Repeated Measures (MMRM) analyses were performed to control for potential bias caused by missing data at later time points. The MMRM analysis uses all available data (i.e., Years 1, 2, and 3) to estimate the data's correlation structure between time points, thereby reducing the impact of missing data without explicit imputation. Two MMRMs were performed. The first included both Baseline 6MWT and age as covariates (Row 4), and the second was an MMRM analysis of the rank-transformed data, with both Baseline 6MWT and age as covariates (Row 5). For both of these analyses, the difference between the eteplirsen treated patients and untreated external controls remained statistically significant.

Additionally, a series of sensitivity analyses were conducted in which the last observed value for the first patient at 12 months (375 meters) and for the second patient at 29 months (252 meters) was imputed as a 36 month result (last observation carried forward or LOCF). This is a highly conservative approach as neither patient would be expected to remain stable over this period of time given their respective ages at Baseline (8.6 years and 11.5 years respectively). Three different ANCOVAs were performed using LOCF for missing data. The first included Baseline 6MWT as a covariate (Row 6), the second included both Baseline 6MWT and age as covariates (Row 7), and the third was an ANCOVA of the rank transformed data, with both Baseline 6MWT and age as covariates (Row 8). Even with these most conservative analyses, the difference between the eteplirsen-treated and untreated external control patients remained statistically significant.

APPENDIX 6. SENSITIVITY ANALYSIS FOR 6MWT IN ETEPLIRSEN TREATED (N = 12) VS. EXTERNAL CONTROLS AMENABLE TO ANY EXON SKIPPING (N = 50)

Potential Issue Addressed	Row	Comparison: Change from Baseline in 6MWT in Eteplirsen-Treated (N = 12) vs. Untreated External Controls (N = 50)	LS Mean Difference (meters)	P-Value
Bias Caused by Imbalance in Important Baseline Prognostic Factors	1	ANCOVA with Baseline 6MWT and age as covariates	74	0.0845
Bias Caused by Violation of Normality Assumption	2	ANCOVA with Baseline as a covariate, rank transformation as the outcome for 6MWT	NA [a]	0.0464
	3	ANCOVA with Baseline and age as covariates, rank transformation as the outcome for 6MWT	NA [a]	0.0544
Bias Caused By Missing Data	4	MMRM analysis with Baseline and age as covariates	73	0.0448
	5	MMRM analysis with Baseline and age as covariates and rank transformation as the outcome for 6MWT	NA [a]	0.0595
	6	ANCOVA with Baseline as a covariate and LOCF for missing data	71	0.0954
	7	ANCOVA with Baseline and age as covariates and LOCF for missing data	68	0.1128
	8	ANCOVA with Baseline and age as covariates, LOCF for missing data, rank transformation as outcome for 6MWT	NA [a]	0.0811

Abbreviations: 6MWT=6 Minute Walk Test; ANCOVA=analysis of covariance; LOCF=last observation carried forward; LS=least squares; MMRM=Mixed Model Repeated Measures; NA=not applicable

[a] Not applicable as the data being analysed are rank-transformed.

APPENDIX 7.   BASELINE CHARACTERISTICS OF STUDY 201/202 ETEPLIRSEN TREATED VS EXTERNAL CONTROL WITH LOSS OF AMBULATION

SOURCE	SUBJ.	Deleted Exon(s)	AGE (YRS)	HEIGHT (CM)	WEIGHT (KG)	6MWT (M)	NSAA (Total Score)	Steroid, Dose[†], Regimen		
Eteplirsen Study 201/202	9	45–50	9.79	138	39.8	330	21	PRD	25	Continuous
	10	45–50	9.79	136	39.7	256	17	PRD	25	Continuous
LNMRC	(b) (6)	50	11.8	UNK	UNK	327	*UNK*	DFZ or Eq.	UNK	Continuous
Italian Telethon		48–50	11.5	133	47	200	10	DFZ	0.8	Continuous
		49–50	8	131	33	380	23	DFZ	0.8	Continuous
		45–50	7.33	131	26	325	29	DLT	17.5	Continuous
		48–50	10.1	133	31	388	20	PRD	0.75	Intermittent
		49–50	8.1	127	39	388	19	PRD	0.75	Intermittent

Abbreviations: DFZ = Deflazacort; PRD = Prednisone; DLT = Deltacortene.

†   Dose unit for eteplirsen-treated: mg. Dose unit for DMD Italian Telethon in mg/kg except PI3 who received doses of 17.5mg

APPENDIX 8. INDIVUDUAL ITEMS OF NSAA

1. Stand

2. Walk

3. Stand from chair

4. Stand R leg

5. Stand L leg

6. Climb R leg

7. Climb L leg

8. Descend R leg

9. Descend L leg

10. Gets to sitting

11. Rise from floor

12. Lifts head

13. Stands on heels

14. Jump

15. Hop R leg

16. Hop L leg

17. Run

Total score

APPENDIX 9. UNTREATED CONTROL MUSCLE BIOPSY SAMPLES USED IN WEEK 180 DYSTROPHIN ANALYSIS

Sample ID	Age (age at biopsy years)	Anatomical Location	Dystrophin Mutation	Baseline 6MWT	Source
01005	7	Biceps	Δ50	357 m	Study 201
01008	10	Biceps	Δ49-50	341 m	
01013	10	Biceps	Δ45-50	418 m	
01015	9	Biceps	Δ52	401 m	
DMD #1	7	Deltoid	Δ45-50	425 m	Study 301
DMD #2	15	Biceps	Δ45-50	421 m	
DMD #3	9	Biceps	Δ48-50	352 m	
DMD #4	7	Biceps	Δ45-50	402 m	
DMD #5	10	Biceps	Δ50	538 m	
DMD #6	7	Biceps	Δ49-50	383 m	
DMD #7	9	Biceps	Δ49-50	441 m	
DMD #8	9	Biceps	Δ48-50	306 m	
DMD #9	9	Biceps	Δ52	371 m	

APPENDIX 10.　INDIVIDUAL PATIENT RESULTS FOR PERCENT DYSTROPHIN POSITIVE FIBERS (PDPF)

Patient ID	Multi-rater PDPF %	
	Week 180	**Baseline[1]**
01002	4.54	--
01003	1.42	--
01004	28.2	--
01006	20.72	--
01007	7.08	--
01008	12.75	1.09
01009	21.48	--
01010	23.96	--
01012	33.5	--
01013	19.14	2.58
01015	18.48	0.19

[1] Baseline muscle biopsy tissue was not available for most patients. For patients where it was available, baseline analysis was performed on the archived tissue using updated methodology coincident with Week 180 analysis.

Patient ID	Multi-rater PDPF % Untreated
DMD1	0.15
DMD2	0.29
DMD3	3.95
DMD4	0.39
DMD5	0.36
DMD6	1.11

APPENDIX 11. INDIVIDUAL PATIENT RESULTS FOR DYSTROPHIN FIBER INTENSITY

Patient ID	Fiber Intensity %	
	Week 180	Baseline[1]
01003	7.0	--
01004	28.8	--
01006	28.7	--
01007	12.0	--
01008	26.7	11.6
01009	23.0	--
01010	21.4	--
01012	26.1	--
01013	32.5	16.3
01015	30.7	7.4

[1] Baseline muscle biopsy tissue was not available for most patients. For patients where it was available, baseline analysis was performed on the archived tissue using updated methodology coincident with Week 180 analysis.

Patient ID	Fiber Intensity % Untreated
DMD1	9.9
DMD2	6.1
DMD3	9.2
DMD4	3.5
DMD5	17.6
DMD6	3.1

APPENDIX 12. DIGITAL MICROSCOPY IMAGES FOR ASSESSMENT OF PERCENT POSITIVE DYSTROPHIN FIBERS

Muscle biopsy cryosections were immunostained with monoclonal antibody MANDYS106 by indirect immunofluorescence. Fluorescence microscope digital images were captured at 20X magnification. To more clearly display relative intensity of fibers, the contrast was inverted from original fluorescence images to display a pseudo-bright field image.

One image per patient is shown and for comparison, one image per DMD untreated control sample.
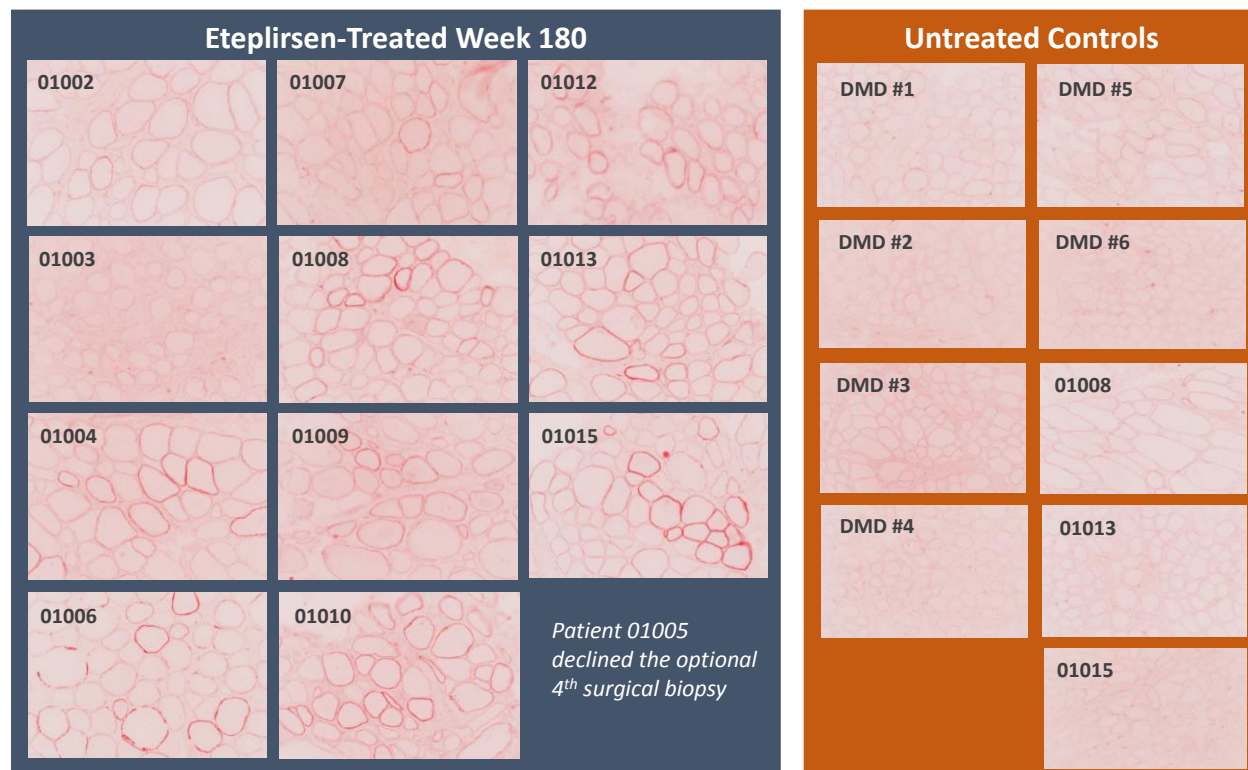
All 16 images are shown that were analyzed for patient 01015 for muscle biopsy sample at Week 180 and at Baseline. Systematic random sampling of image fields for each stained tissue section was used to capture four 20X images per tissue section. Tissue was sectioned at 2 levels from each of 2 distinct muscle biopsy samples, resulting in a total of 16 images per patient at Week 180 (4 images, 2 tissue levels, 2 biopsy samples) and 8 images at baseline (4 images, 2 tissue levels, 1 biopsy sample).
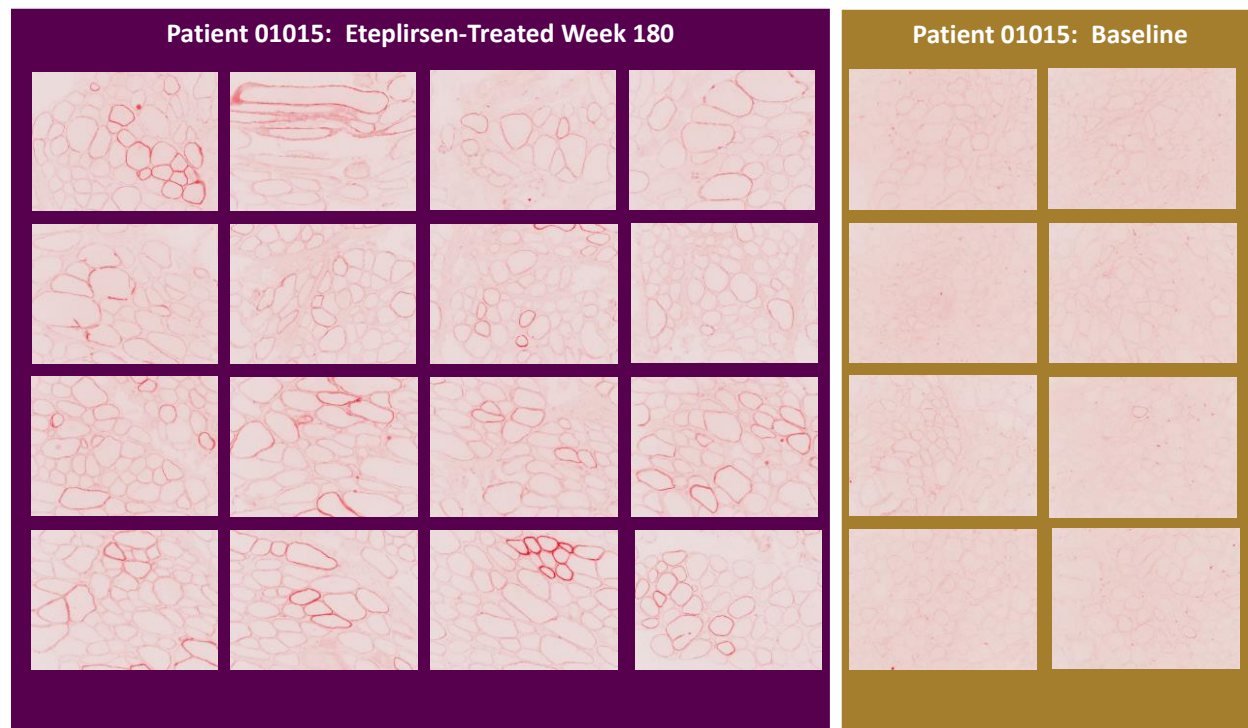
APPENDIX 13. INDIVIDUAL PATIENT RESULTS FOR WESTERN BLOT

Patient ID	Western blot %	
	Week 180	**Baseline[1]**
01002	0.14[4]	--
01003	0 (BLOQ)	--
01004	0.96	--
01005	n.a.[2]	0 (BLOQ)[3]
01006	2.47	--
01007	0 (BLOQ)	--
01008	0.98	--
01009	0.52	--
01010	1.62	--
01012	0.38	--
01013	1.15	0 (BLOQ)
01015	2.05	0 (BLOQ)

Abbreviations: BLOQ = Below Limit of Quantification

[1] Baseline muscle biopsy tissue was not available for most patients. For patients where it was available, baseline analysis was performed on the archived tissue using updated methodology coincident with Week 180 analysis.

[2] Patient 01005 did not consent for fourth biopsy.

[3] Baseline muscle biopsy tissue from patient 01005 was used in Western blot assays as there was not sufficient tissue remaining from patient 01008.

[4] One of two replicate gels was above BLOQ of 0.25% while the other was below and treated as zero. The average of two gels is reported.

Patient ID	Western blot % Untreated
DMD1	0 (BLOQ)
DMD2	0 (BLOQ)
DMD3	0.37
DMD7[1]	0.15[2]
DMD8[1]	0 (BLOQ)
DMD9[1]	0.20[2]

[1] Control DMD muscle biopsy tissue from DMD7, DMD8, DMD9 were used in Western blot assays as there was not sufficient tissue remaining from DMD4, DMD5, DMD6.

[2] One of two replicate gels was above BLOQ of 0.25% while the other gel was below and treated as zero. The average of two gels is reported.

APPENDIX 14. WESTERN BLOT ACCEPTANCE STANDARDS AND REPRESENTATIVE GEL IMAGES

All samples analyzed in the 4th biopsy by Western blot were run in duplicate on separate gels. NCL-DYS1 anti-dystrophin antibody was used to stain for dystrophin. A validated, sensitive method for Western blot was established with pass/fail criteria for each gel:

- 5-point standard curve (0.25 %-4 % of normal) included on every gel

  - Normal control muscle lysate spiked in DMD muscle lysate to control for equal muscle protein load in each lane

  - Lower limit of quantitation is 0.25 % of normal muscle

  - Pass criteria of $R^2 > 0.90$ to ensure standard curve linearity on each gel

- Negative control included on every gel

  - DMD muscle lysate used for standard curve (without normal control muscle lysate)

  - False positive reads prevented by setting gel pass criteria for negative lane density of < 0.25 % lane density to control

- Samples run blinded in duplicate on separate gels

- Alpha-actinin (muscle-specific protein expressed equally in DMD and non-DMD muscle tissue) used as control for equal protein load

  - Equal lane to lane protein load confirmed by pass criteria of RSD < 50 % of average actinin density for all lanes

Western Blot Images of 4 Separate Gels Illustrating Dystrophin Absence in Pretreatment Muscle of Patient 01015 (A Lane 7 + B Lane 8) and De Novo Dystrophin Protein Production After Eteplirsen Treatment at Week 180 in Tissue From the Same Patient 01015 (C Lane 8 + D Lane 7)
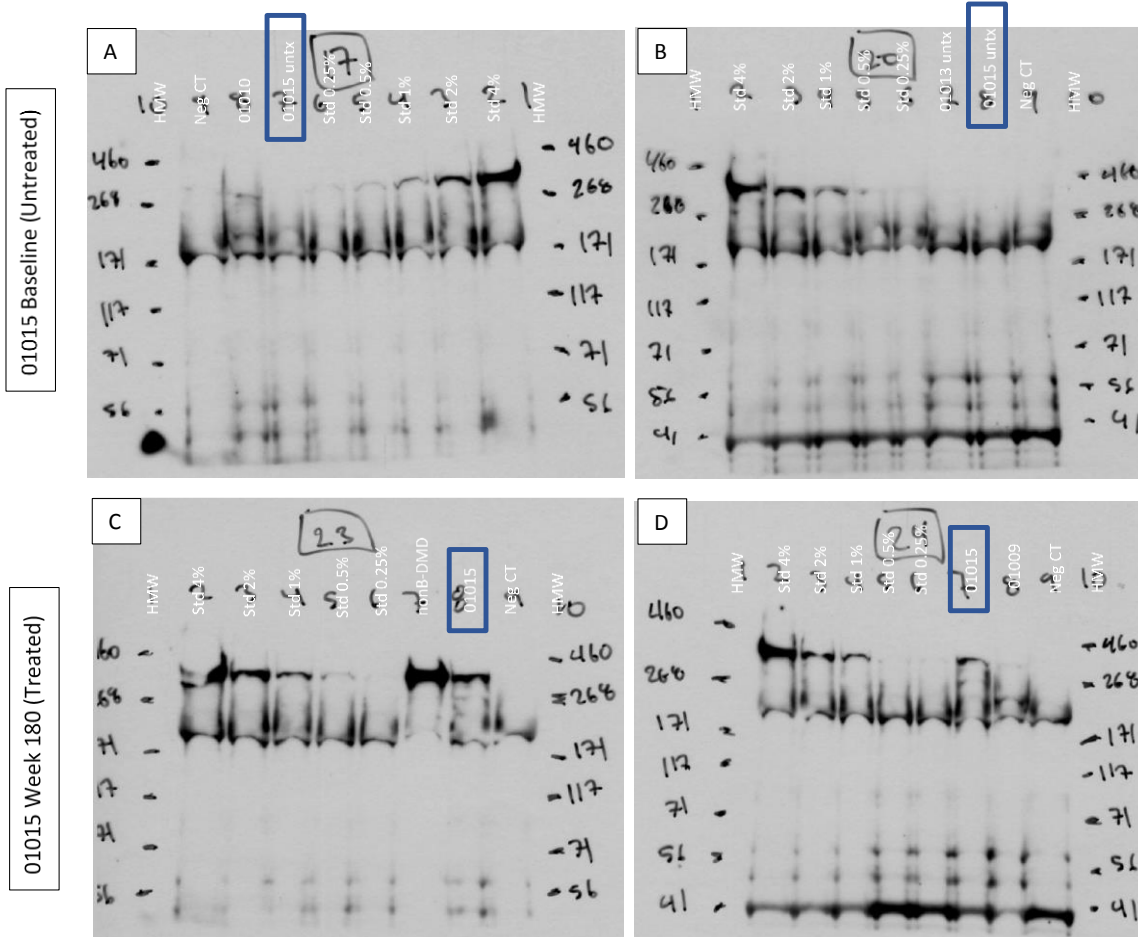
All gel images depicted here were obtained with a 30 minute exposure. All lanes (excluding high molecular weight lanes) were loaded with a consistent 50μg total protein load. Alpha-actinin, a muscle specific protein expressed equally in DMD and non-DMD muscle tissue, was used as a loading control to ensure equal protein load (not depicted here).

APPENDIX 15.   ALL TREATMENT-EMERGENT ADVERSE EVENTS DURING THE 24-WEEK PLACEBO CONTROL PERIOD OF STUDY 201

System Organ Classification Preferred Term		Eteplirsen		
	Placebo (N = 4) n	30 mg/kg/wk (N = 4) n	50 mg/kg/wk (N = 4) n	All Eteplirsen (N = 8) n
At Least One TEAE	**4**	**4**	**4**	**8**
Injury, poisoning & procedural complications	**4**	**3**	**4**	**7**
Procedural pain	3	1	3	4
Fall	1	1	0	1
Incision site pain	1	1	0	1
Arthropod bite	0	1	0	1
Back injury	0	1	0	1
Foot fracture	0	0	1	1
Joint injury	0	1	0	1
Wound dehiscence	1	0	0	0
Respiratory, thoracic & mediastinal disorders	**3**	**4**	**1**	**5**
Oropharyngeal pain	3	3	0	3
Cough	2	1	1	2
Nasal congestion	2	1	0	1
Sinus congestion	0	1	0	1
Upper respiratory tract congestion	0	1	0	1
Musculoskeletal & connective tissue disorders	**3**	**2**	**2**	**4**
Pain in extremity	3	0	1	1
Back pain	2	1	0	1
Arthralgia	0	0	1	1
Bone pain	0	1	0	1
Muscle spasms	0	0	1	1
Musculoskeletal pain	0	1	0	1
Nervous system disorders	**2**	**3**	**2**	**5**

System Organ Classification Preferred Term		Eteplirsen		
	Placebo (N = 4) n	30 mg/kg/wk (N = 4) n	50 mg/kg/wk (N = 4) n	All Eteplirsen (N = 8) n
At Least One TEAE	**4**	**4**	**4**	**8**
Balance disorder	0	1	2	3
Headache	2	1	0	1
Dizziness	1	0	0	0
Somnolence	0	1	0	1
General disorders and administration site conditions	**2**	**2**	**2**	**4**
Pyrexia	2	1	0	1
Injection site pain	0	0	1	1
Malaise	0	0	1	1
Non-cardiac chest pain	0	1	0	1
Pain	0	0	1	1
Metabolism & nutrition disorders	**2**	**2**	**2**	**4**
Hypokalaemia	2	2	2	4
Gastrointestinal disorders	**2**	**1**	**2**	**3**
Vomiting	0	1	2	3
Abdominal pain	2	0	0	0
Diarrhoea	1	0	1	1
Nausea	1	0	1	1
Infections & infestations	**3**	**0**	**1**	**1**
Rhinitis	1	0	1	1
Enterobiasis	1	0	0	0
Nasopharyngitis	1	0	0	0
Soft tissue infection	1	0	0	0
Vascular disorders	**1**	**1**	**1**	**2**
Haematoma	1	1	1	2
Renal & urinary disorders	**1**	**1**	**0**	**1**
Polyuria	0	1	0	1
Proteinuria	1	0	0	0

System Organ Classification Preferred Term	Placebo (N = 4) n	Eteplirsen		
		30 mg/kg/wk (N = 4) n	50 mg/kg/wk (N = 4) n	All Eteplirsen (N = 8) n
At Least One TEAE	**4**	**4**	**4**	**8**
Skin & subcutaneous tissue disorders	**0**	**2**	**0**	**2**
Dermatitis contact	0	2	0	2
Petechiae	0	1	0	1
Urticaria thermal	0	1	0	1
Cardiac disorders	**0**	**1**	**0**	**1**
Tachycardia	0	1	0	1
Ear & labyrinth disorders	**0**	**0**	**1**	**1**
Motion sickness	0	0	1	1

Note: AEs are coded using MedDRA v14.0. AEs were attributed to the treatment being received at start of AE. TEAEs are those starting during or after the first infusion of study drug. Patients who experience a coded event more than once are only counted once per treatment received.

APPENDIX 16. TREATMENT-EMERGENT ADVERSE EVENTS DURING THE ETEPLIRSEN CLINICAL DEVELOPMENT PROGRAM

| System Organ Classification Preferred Term | Placebo (N = 4) | Untreated (N = 15) | Eteplirsen | | | | | | | |
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Number of Subjects With a TEAE	**4 (100%)**	**9 (60.0%)**	**5 (71.4%)**	**11 (100%)**	**4 (100%)**	**4 (100%)**	**63 (76.8%)**	**6 (100%)**	**88 (82.2%)**	**93 (81.6%)**
Injury, poisoning and procedural complications	**4 (100%)**	**3 (20.0%)**	**0**	**9 (81.8%)**	**1 (25.0%)**	**1 (25.0%)**	**33 (40.2%)**	**6 (100%)**	**50 (46.7%)**	**50 (43.9%)**
Procedural pain	3 (75.0%)	0	0	2 (18.2%)	0	0	8 (9.8%)	6 (100%)	16 (15.0%)	16 (14.0%)
Contusion	0	0	0	1 (9.1%)	0	0	10 (12.2%)	3 (50.0%)	14 (13.1%)	14 (12.3%)
Excoriation	0	0	0	0	0	1 (25.0%)	11 (13.4%)	2 (33.3%)	14 (13.1%)	14 (12.3%)
Fall	1 (25.0%)	0	0	4 (36.4%)	0	0	7 (8.5%)	0	11 (10.3%)	11 (9.6%)
Arthropod bite	0	0	0	1 (9.1%)	0	0	5 (6.1%)	1 (16.7%)	7 (6.5%)	7 (6.1%)
Incision site haemorrhage	0	0	0	0	0	0	3 (3.7%)	1 (16.7%)	4 (3.7%)	4 (3.5%)
Joint injury	0	0	0	0	0	0	3 (3.7%)	1 (16.7%)	4 (3.7%)	4 (3.5%)
Joint sprain	0	1 (6.7%)	0	0	0	0	1 (1.2%)	3 (50.0%)	4 (3.7%)	4 (3.5%)
Foot fracture	0	0	0	0	0	0	2 (2.4%)	1 (16.7%)	3 (2.8%)	3 (2.6%)
Head injury	0	0	0	1 (9.1%)	0	0	1 (1.2%)	1 (16.7%)	3 (2.8%)	3 (2.6%)
Muscle strain	0	0	0	0	0	0	1 (1.2%)	2 (33.3%)	3 (2.8%)	3 (2.6%)
Post procedural haematoma	0	0	0	1 (9.1%)	0	0	1 (1.2%)	1 (16.7%)	3 (2.8%)	3 (2.6%)
Arthropod sting	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Limb injury	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Scratch	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)

System Organ Classification Preferred Term	Placebo (N = 4)	Untreated (N = 15)	Eteplirsen							
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Thermal burn	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Accident	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Ankle fracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Back injury	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Burns first degree	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Femur fracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Hand fracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Heat stroke	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Incision site erythema	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Incision site pain	1 (25.0%)	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Incision site pruritus	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Infusion related reaction	0	0	0	0	1 (25.0%)	0	0	0	1 (0.9%)	1 (0.9%)
Laceration	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Ligament sprain	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Lip injury	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Lower limb fracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Nail injury	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Radius fracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Skeletal injury	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Soft tissue injury	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Spinal compression fracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Sunburn	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Tooth fracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)

System Organ Classification Preferred Term	Placebo (N = 4)	Untreated (N = 15)	Eteplirsen							
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Wound dehiscence	1 (25.0%)	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Tooth avulsion	0	1 (6.7%)	0	0	0	0	0	0	0	0
Torus fracture	0	1 (6.7%)	0	0	0	0	0	0	0	0
Gastrointestinal disorders	**1 (25.0%)**	**2 (13.3%)**	**0**	**8 (72.7%)**	**1 (25.0%)**	**2 (50.0%)**	**27 (32.9%)**	**5 (83.3%)**	**43 (40.2%)**	**43 (37.7%)**
Vomiting	0	0	0	2 (18.2%)	1 (25.0%)	0	18 (22.0%)	3 (50.0%)	24 (22.4%)	24 (21.1%)
Nausea	1 (25.0%)	0	0	2 (18.2%)	0	1 (25.0%)	4 (4.9%)	2 (33.3%)	9 (8.4%)	9 (7.9%)
Abdominal pain upper	0	1 (6.7%)	0	2 (18.2%)	0	0	3 (3.7%)	3 (50.0%)	8 (7.5%)	8 (7.0%)
Abdominal pain	1 (25.0%)	0	0	2 (18.2%)	0	0	3 (3.7%)	2 (33.3%)	7 (6.5%)	7 (6.1%)
Diarrhoea	0	1 (6.7%)	0	1 (9.1%)	0	0	4 (4.9%)	2 (33.3%)	7 (6.5%)	7 (6.1%)
Dyspepsia	0	0	0	0	0	0	3 (3.7%)	1 (16.7%)	4 (3.7%)	4 (3.5%)
Constipation	0	0	0	1 (9.1%)	0	0	0	2 (33.3%)	3 (2.8%)	3 (2.6%)
Abdominal discomfort	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Haematochezia	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Abdominal distension	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Dental caries	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Dysphagia	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Flatulence	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Food poisoning	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Haemorrhoids	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Lip dry	0	0	0	0	0	1 (25.0%)	0	0	1 (0.9%)	1 (0.9%)
Lip swelling	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Oral pain	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)

			Eteplirsen							
System Organ Classification Preferred Term	**Placebo (N = 4)**	**Untreated (N = 15)**	**0.09 & 0.9 mg IM (N = 7)**	**≤4 mg/kg IV (N = 11)**	**10 mg/kg IV (N = 4)**	**20 mg/kg IV (N = 4)**	**30 mg/kg IV (N = 82)**	**50 mg/kg IV (N = 6)**	**All IV (N = 107)**	**All Eteplirsen (N = 114)**
Retained deciduous tooth	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Tooth impacted	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Toothache	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Musculoskeletal and connective tissue disorders	**3 (75.0%)**	**2 (13.3%)**	**0**	**8 (72.7%)**	**1 (25.0%)**	**0**	**28 (34.1%)**	**6 (100%)**	**43 (40.2%)**	**43 (37.7%)**
Back pain	2 (50.0%)	0	0	3 (27.3%)	1 (25.0%)	0	17 (20.7%)	3 (50.0%)	24 (22.4%)	24 (21.1%)
Pain in extremity	3 (75.0%)	0	0	2 (18.2%)	1 (25.0%)	0	10 (12.2%)	4 (66.7%)	17 (15.9%)	17 (14.9%)
Arthralgia	0	0	0	3 (27.3%)	0	0	8 (9.8%)	3 (50.0%)	14 (13.1%)	14 (12.3%)
Muscle spasms	0	0	0	0	0	0	3 (3.7%)	2 (33.3%)	5 (4.7%)	5 (4.4%)
Myalgia	0	0	0	1 (9.1%)	0	0	2 (2.4%)	2 (33.3%)	5 (4.7%)	5 (4.4%)
Musculoskeletal pain	0	0	0	0	0	0	3 (3.7%)	1 (16.7%)	4 (3.7%)	4 (3.5%)
Muscular weakness	0	2 (13.3%)	0	0	0	0	1 (1.2%)	2 (33.3%)	3 (2.8%)	3 (2.6%)
Coccydynia	0	0	0	1 (9.1%)	0	0	1 (1.2%)	0	2 (1.9%)	2 (1.8%)
Neck pain	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Tendonitis	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Bone pain	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Groin pain	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Joint swelling	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Osteopenia	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Scoliosis	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Tendinous contracture	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)

System Organ Classification Preferred Term	Placebo (N = 4)	Untreated (N = 15)	Eteplirsen							
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Tendon disorder	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
General Disorders and Administration Site Conditions	**2 (50.0%)**	**0**	**2 (28.6%)**	**3 (27.3%)**	**2 (50.0%)**	**2 (50.0%)**	**23 (28.0%)**	**5 (83.3%)**	**35 (32.7%)**	**37 (32.5%)**
Catheter site pain	0	0	0	0	1 (25.0%)	0	7 (8.5%)	2 (33.3%)	10 (9.3%)	10 (8.8%)
Infusion site haematoma	0	0	0	0	0	0	6 (7.3%)	1 (16.7%)	7 (6.5%)	7 (6.1%)
Fatigue	0	0	0	1 (9.1%)	1 (25.0%)	0	4 (4.9%)	0	6 (5.6%)	6 (5.3%)
Catheter site haematoma	0	0	0	2 (18.2%)	0	0	1 (1.2%)	1 (16.7%)	4 (3.7%)	4 (3.5%)
Infusion site extravasation	0	0	0	0	0	0	2 (2.4%)	2 (33.3%)	4 (3.7%)	4 (3.5%)
Pyrexia	2 (50.0%)	0	0	0	0	0	2 (2.4%)	2 (33.3%)	4 (3.7%)	4 (3.5%)
Influenza like illness	0	0	0	1 (9.1%)	1 (25.0%)	0	1 (1.2%)	0	3 (2.8%)	3 (2.6%)
Infusion site pain	0	0	0	0	0	0	2 (2.4%)	1 (16.7%)	3 (2.8%)	3 (2.6%)
Thrombosis in device	0	0	0	0	0	0	3 (3.7%)	0	3 (2.8%)	3 (2.6%)
Chest pain	0	0	0	1 (9.1%)	0	0	1 (1.2%)	0	2 (1.9%)	2 (1.8%)
Device occlusion	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Disease progression	0	0	0	0	1 (25.0%)	1 (25.0%)	0	0	2 (1.9%)	2 (1.8%)
Injection site pain	0	0	1 (14.3%)	0	0	0	0	1 (16.7%)	1 (0.9%)	2 (1.8%)
Non-cardiac chest pain	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Oedema peripheral	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Application site erythema	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Application site rash	0	0	0	0	0	1 (25.0%)	0	0	1 (0.9%)	1 (0.9%)
Catheter site haemorrhage	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Catheter site inflammation	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Catheter site related reaction	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)

System Organ Classification Preferred Term	Placebo (N = 4)	Untreated (N = 15)	Eteplirsen							
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Feeling hot	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Gait disturbance	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Infusion site rash	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Infusion site swelling	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Injection site haematoma	0	0	1 (14.3%)	0	0	0	0	0	0	1 (0.9%)
Irritability	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Malaise	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Pain	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Swelling	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Vaccination site pain	0	0	0	0	0	1 (25.0%)	0	0	1 (0.9%)	1 (0.9%)
Infections and infestations	**3 (75.0%)**	**2 (13.3%)**	**0**	**5 (45.5%)**	**4 (100%)**	**2 (50.0%)**	**19 (23.2%)**	**6 (100%)**	**36 (33.6%)**	**36 (31.6%)**
Upper respiratory tract infection	0	0	0	3 (27.3%)	2 (50.0%)	1 (25.0%)	5 (6.1%)	4 (66.7%)	15 (14.0%)	15 (13.2%)
Nasopharyngitis	1 (25.0%)	0	0	0	0	0	10 (12.2%)	4 (66.7%)	14 (13.1%)	14 (12.3%)
Rhinitis	1 (25.0%)	0	0	1 (9.1%)	3 (75.0%)	1 (25.0%)	0	1 (16.7%)	6 (5.6%)	6 (5.3%)
Ear infection	0	0	0	0	0	0	3 (3.7%)	0	3 (2.8%)	3 (2.6%)
Gastroenteritis viral	0	0	0	0	0	0	2 (2.4%)	1 (16.7%)	3 (2.8%)	3 (2.6%)
Hordeolum	0	0	0	1 (9.1%)	0	0	0	2 (33.3%)	3 (2.8%)	3 (2.6%)
Viral infection	0	0	0	1 (9.1%)	0	0	1 (1.2%)	1 (16.7%)	3 (2.8%)	3 (2.6%)
Gastroenteritis	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Influenza	0	0	0	0	0	0	0	2 (33.3%)	2 (1.9%)	2 (1.8%)
Pharyngitis streptococcal	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Post procedural cellulitis	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)

System Organ Classification Preferred Term	Placebo (N = 4)	Untreated (N = 15)	Eteplirsen							
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Body tinea	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Bronchitis	0	0	0	0	1 (25.0%)	0	0	0	1 (0.9%)	1 (0.9%)
Candidiasis	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Folliculitis	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Furuncle	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Incision site infection	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Sinusitis	0	1 (6.7%)	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Skin infection	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Tinea infection	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Tinea pedis	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Tooth abscess	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Tooth infection	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Viral upper respiratory tract infection	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Enterobiasis	1 (25.0%)	0	0	0	0	0	0	0	0	0
Lymphadenitis viral	0	1 (6.7%)	0	0	0	0	0	0	0	0
Otitis media	0	1 (6.7%)	0	0	0	0	0	0	0	0
Soft tissue infection	1 (25.0%)	0	0	0	0	0	0	0	0	0
Nervous system disorders	**2 (50.0%)**	**1 (6.7%)**	**0**	**6 (54.5%)**	**2 (50.0%)**	**2 (50.0%)**	**20 (24.4%)**	**6 (100%)**	**36 (33.6%)**	**36 (31.6%)**
Headache	2 (50.0%)	1 (6.7%)	0	5 (45.5%)	2 (50.0%)	1 (25.0%)	14 (17.1%)	5 (83.3%)	27 (25.2%)	27 (23.7%)
Dizziness	1 (25.0%)	0	0	2 (18.2%)	0	1 (25.0%)	3 (3.7%)	0	6 (5.6%)	6 (5.3%)
Balance disorder	0	0	0	0	0	0	2 (2.4%)	3 (50.0%)	5 (4.7%)	5 (4.4%)
Paraesthesia	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)

System Organ Classification Preferred Term	Placebo (N = 4)	Untreated (N = 15)	Eteplirsen							
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Psychomotor hyperactivity	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Somnolence	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Respiratory, thoracic and mediastinal disorders	**3 (75.0%)**	**1 (6.7%)**	**0**	**2 (18.2%)**	**0**	**0**	**28 (34.1%)**	**6 (100%)**	**36 (33.6%)**	**36 (31.6%)**
Cough	2 (50.0%)	0	0	2 (18.2%)	0	0	12 (14.6%)	4 (66.7%)	18 (16.8%)	18 (15.8%)
Oropharyngeal pain	3 (75.0%)	0	0	0	0	0	10 (12.2%)	4 (66.7%)	14 (13.1%)	14 (12.3%)
Nasal congestion	1 (25.0%)	0	0	0	0	0	11 (13.4%)	2 (33.3%)	13 (12.1%)	13 (11.4%)
Rhinorrhoea	0	0	0	0	0	0	5 (6.1%)	2 (33.3%)	7 (6.5%)	7 (6.1%)
Epistaxis	0	0	0	0	0	0	4 (4.9%)	1 (16.7%)	5 (4.7%)	5 (4.4%)
Pharyngeal erythema	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Upper respiratory tract congestion	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Productive cough	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Respiratory disorder	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Sinus congestion	0	1 (6.7%)	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Sneezing	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Upper-airway cough syndrome	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Skin and Subcutaneous Tissue Disorders	**0**	**1 (6.7%)**	**0**	**1 (9.1%)**	**0**	**1 (25.0%)**	**20 (24.4%)**	**5 (83.3%)**	**27 (25.2%)**	**27 (23.7%)**
Rash	0	1 (6.7%)	0	0	0	0	9 (11.0%)	1 (16.7%)	10 (9.3%)	10 (8.8%)
Dermatitis contact	0	0	0	0	0	0	3 (3.7%)	1 (16.7%)	4 (3.7%)	4 (3.5%)
Ecchymosis	0	0	0	0	0	0	3 (3.7%)	1 (16.7%)	4 (3.7%)	4 (3.5%)

| System Organ Classification Preferred Term | Placebo (N = 4) | Untreated (N = 15) | Eteplirsen | | | | | | | |
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
Erythema	0	0	0	0	0	0	2 (2.4%)	1 (16.7%)	3 (2.8%)	3 (2.6%)
Papule	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Pruritus	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Rash papular	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Acne	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Alopecia	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Dermatitis bullous	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Drug eruption	0	0	0	0	0	1 (25.0%)	0	0	1 (0.9%)	1 (0.9%)
Ingrowing nail	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Intertrigo	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Keloid scar	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Nail discolouration	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Nail dystrophy	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Petechiae	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Rash pruritic	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Skin erosion	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Skin hyperpigmentation	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Skin irritation	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Urticaria	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Urticaria thermal	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Investigations	**0**	**1 (6.7%)**	**0**	**0**	**0**	**0**	**12 (14.6%)**	**6 (100%)**	**18 (16.8%)**	**18 (15.8%)**
Activated partial thromboplastin time prolonged	0	0	0	0	0	0	2 (2.4%)	3 (50.0%)	5 (4.7%)	5 (4.4%)

| System Organ Classification Preferred Term | Placebo (N = 4) | Untreated (N = 15) | Eteplirsen | | | | | | | |
			0.09 & 0.9 mg IM (N = 7)	≤4 mg/kg IV (N = 11)	10 mg/kg IV (N = 4)	20 mg/kg IV (N = 4)	30 mg/kg IV (N = 82)	50 mg/kg IV (N = 6)	All IV (N = 107)	All Eteplirsen (N = 114)
C-reactive protein increased	0	0	0	0	0	0	3 (3.7%)	2 (33.3%)	5 (4.7%)	5 (4.4%)
Blood creatine phosphokinase increased	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Blood glucose increased	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Body height below normal	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Protein urine present	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Blood amylase increased	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Blood creatinine increased	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Blood urea increased	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Blood urine present	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Breath sounds abnormal	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Lymphocyte count decreased	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Neutrophil count increased	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Red blood cells urine positive	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Urine analysis abnormal	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Urine ketone body present	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
White blood cell count decreased	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Wound healing normal	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Influenza A virus test positive	0	1 (6.7%)	0	0	0	0	0	0	0	0
Renal and Urinary Disorders	**1 (25.0%)**	**0**	**3 (42.9%)**	**0**	**0**	**1 (25.0%)**	**7 (8.5%)**	**4 (66.7%)**	**12 (11.2%)**	**15 (13.2%)**

			Eteplirsen							
System Organ Classification Preferred Term	**Placebo (N = 4)**	**Untreated (N = 15)**	**0.09 & 0.9 mg IM (N = 7)**	**≤4 mg/kg IV (N = 11)**	**10 mg/kg IV (N = 4)**	**20 mg/kg IV (N = 4)**	**30 mg/kg IV (N = 82)**	**50 mg/kg IV (N = 6)**	**All IV (N = 107)**	**All Eteplirsen (N = 114)**
Proteinuria	1 (25.0%)	0	0	0	0	0	5 (6.1%)	4 (66.7%)	9 (8.4%)	9 (7.9%)
Myoglobinuria	0	0	3 (42.9%)	0	0	0	0	0	0	3 (2.6%)
Chromaturia	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Crystalluria	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Enuresis	0	0	0	0	0	1 (25.0%)	0	0	1 (0.9%)	1 (0.9%)
Glycosuria	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Hypercalciuria	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Polyuria	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Urine odour abnormal	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Metabolism and Nutrition Disorders	**2 (50.0%)**	**0**	**0**	**1 (9.1%)**	**0**	**0**	**7 (8.5%)**	**3 (50.0%)**	**11 (10.3%)**	**11 (9.6%)**
Decreased appetite	0	0	0	1 (9.1%)	0	0	3 (3.7%)	0	4 (3.7%)	4 (3.5%)
Hypokalaemia	2 (50.0%)	0	0	0	0	0	2 (2.4%)	2 (33.3%)	4 (3.7%)	4 (3.5%)
Dehydration	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Obesity	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Vitamin D deficiency	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Psychiatric Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**10 (12.2%)**	**1 (16.7%)**	**11 (10.3%)**	**11 (9.6%)**
Aggression	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Anxiety	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Insomnia	0	0	0	0	0	0	1 (1.2%)	1 (16.7%)	2 (1.9%)	2 (1.8%)
Antisocial behaviour	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Anxiety disorder	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Bruxism	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)

			Eteplirsen							
System Organ Classification Preferred Term	**Placebo (N = 4)**	**Untreated (N = 15)**	**0.09 & 0.9 mg IM (N = 7)**	**≤4 mg/kg IV (N = 11)**	**10 mg/kg IV (N = 4)**	**20 mg/kg IV (N = 4)**	**30 mg/kg IV (N = 82)**	**50 mg/kg IV (N = 6)**	**All IV (N = 107)**	**All Eteplirsen (N = 114)**
Euphoric mood	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Mood altered	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Sleep disorder	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Vascular Disorders	**1 (25.0%)**	**0**	**0**	**2 (18.2%)**	**0**	**1 (25.0%)**	**4 (4.9%)**	**1 (16.7%)**	**8 (7.5%)**	**8 (7.0%)**
Haematoma	1 (25.0%)	0	0	1 (9.1%)	0	1 (25.0%)	1 (1.2%)	1 (16.7%)	4 (3.7%)	4 (3.5%)
Flushing	0	0	0	0	0	0	3 (3.7%)	0	3 (2.8%)	3 (2.6%)
Pallor	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Cardiac Disorders	**0**	**0**	**1 (14.3%)**	**1 (9.1%)**	**0**	**2 (50.0%)**	**2 (2.4%)**	**0**	**5 (4.7%)**	**6 (5.3%)**
Tachycardia	0	0	0	1 (9.1%)	0	2 (50.0%)	1 (1.2%)	0	4 (3.7%)	4 (3.5%)
Cardiomyopathy	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Congestive cardiomyopathy	0	0	1 (14.3%)	0	0	0	0	0	0	1 (0.9%)
Pericardial fibrosis	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Sinus tachycardia	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Ear and Labyrinth Disorders	**0**	**0**	**0**	**1 (9.1%)**	**0**	**0**	**1 (1.2%)**	**2 (33.3%)**	**4 (3.7%)**	**4 (3.5%)**
Cerumen impaction	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Ear pain	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Motion sickness	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Tympanic membrane disorder	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Eye Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.2%)**	**2 (33.3%)**	**3 (2.8%)**	**3 (2.6%)**
Cataract	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Cataract subcapsular	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Conjunctivitis	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)

								Eteplirsen		
System Organ Classification Preferred Term	**Placebo (N = 4)**	**Untreated (N = 15)**	**0.09 & 0.9 mg IM (N = 7)**	**≤4 mg/kg IV (N = 11)**	**10 mg/kg IV (N = 4)**	**20 mg/kg IV (N = 4)**	**30 mg/kg IV (N = 82)**	**50 mg/kg IV (N = 6)**	**All IV (N = 107)**	**All Eteplirsen (N = 114)**
Erythema of eyelid	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Hypermetropia	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Immune System Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**2 (2.4%)**	**1 (16.7%)**	**3 (2.8%)**	**3 (2.6%)**
Seasonal allergy	0	0	0	0	0	0	2 (2.4%)	0	2 (1.9%)	2 (1.8%)
Hypersensitivity	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Endocrine Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2 (33.3%)**	**2 (1.9%)**	**2 (1.8%)**
Goitre	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Growth hormone deficiency	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)
Reproductive System and Breast Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**2 (2.4%)**	**0**	**2 (1.9%)**	**2 (1.8%)**
Pelvic pain	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Testicular pain	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Blood and Lymphatic System Disorders	**0**	**0**	**0**	**1 (9.1%)**	**0**	**0**	**0**	**0**	**1 (0.9%)**	**1 (0.9%)**
Platelet anisocytosis	0	0	0	1 (9.1%)	0	0	0	0	1 (0.9%)	1 (0.9%)
Congenital, Familial, and Genetic Disorders	**0**	**0**	**0**	**0**	**0**	**0**	**1 (1.2%)**	**0**	**1 (0.9%)**	**1 (0.9%)**
Cryptorchism	0	0	0	0	0	0	1 (1.2%)	0	1 (0.9%)	1 (0.9%)
Neoplasms Benign, Malignant, and Unspecified (Incl. Cysts and Polyps)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1 (16.7%)**	**1 (0.9%)**	**1 (0.9%)**
Skin papilloma	0	0	0	0	0	0	0	1 (16.7%)	1 (0.9%)	1 (0.9%)

APPENDIX 17. DMD, EXON SKIPPING AND ETEPLIRSEN MECHANISM OF ACTION

Central Dogma of Molecular Biology, Introns & Exons

Watson and Crick's proposed use of DNA by the cell, that is DNA is transcribed into RNA and then RNA is translated into protein, has been further elucidated to include the removal of introns from RNA prior to translation into protein. DNA and the pre-mRNA that is a direct copy of DNA contain both introns and exons. As shown in Figure A, the introns are removed from the pre-mRNA by protein complexes called spliceosomes to create the final, mature mRNA that is translated by the ribosome into protein.

Figure A:
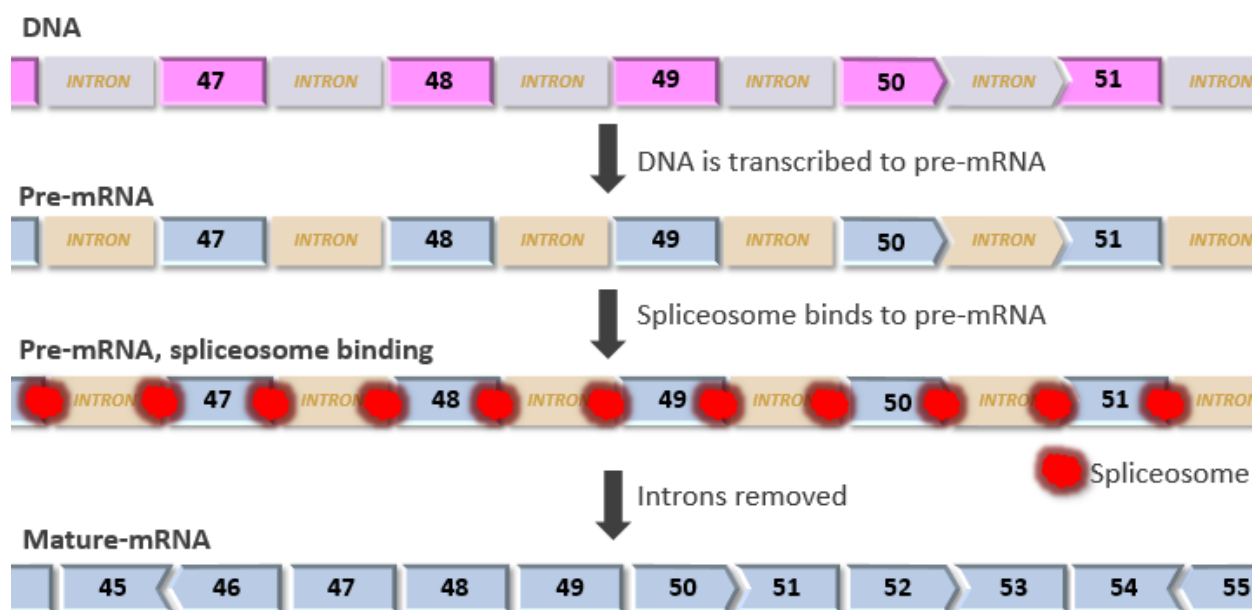
Translation

The ribosome translates mRNA into protein by reading the mRNA three nucleotides, or one codon, at a time. Each 3 nucleotide containing codon codes for a specific amino acid. Figure B, depicts a short mRNA sequence and its corresponding protein sequence.
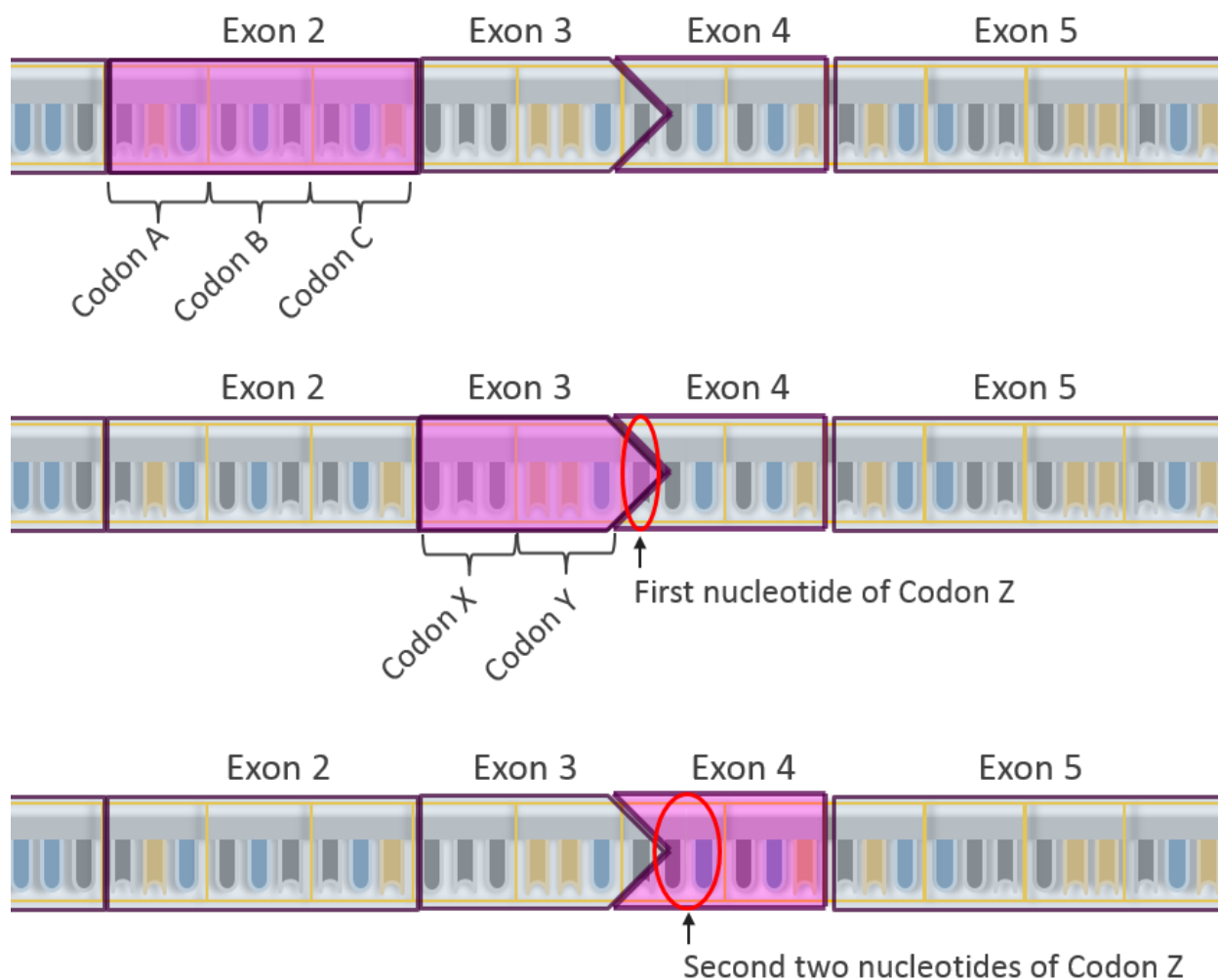
Figure B:
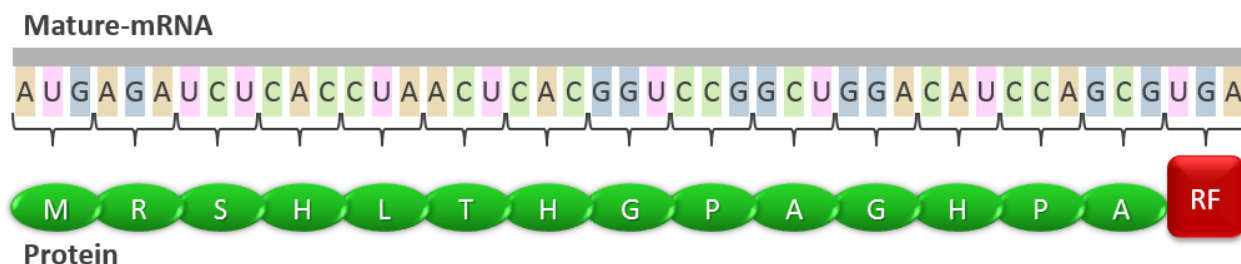
RF = release factor, binds to the stop codon to release the protein from the ribosome
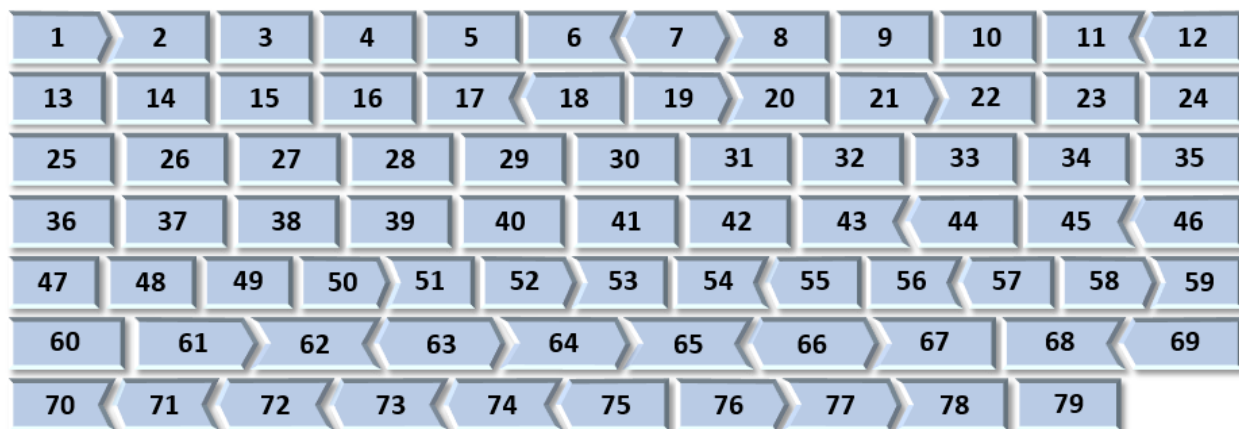
Codon Splitting by Exons

The codons of mRNA are not always evenly distributed between exons. The shape of the exon indicates the distribution of the codons. As shown in Figure C, the rectangular shaped exons contain whole codon units. In contrast, the arrow and chevron shaped exons split codons between them.
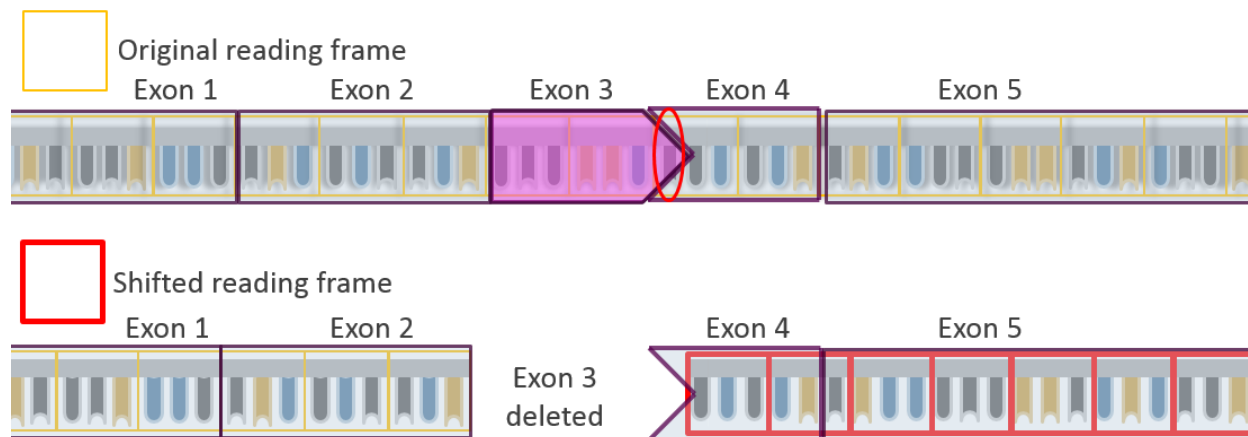
Figure C:

The dystrophin exon map is depicted in Figure D. Dystrophin is the longest known human gene, containing 2.4 million base pairs and 79 exons. A number of exons in the dystrophin gene split codons between them.

Figure D:

If all the exons are present, the splitting of codons between exons has no effect on the final protein. However, if as shown in Figure E, an exon that splits a codon is missing due to genetic mutation, the mRNA reading frame following the mutation is shifted and all subsequent amino acids will be incorrect. The resulting protein is non-functional and unstable.
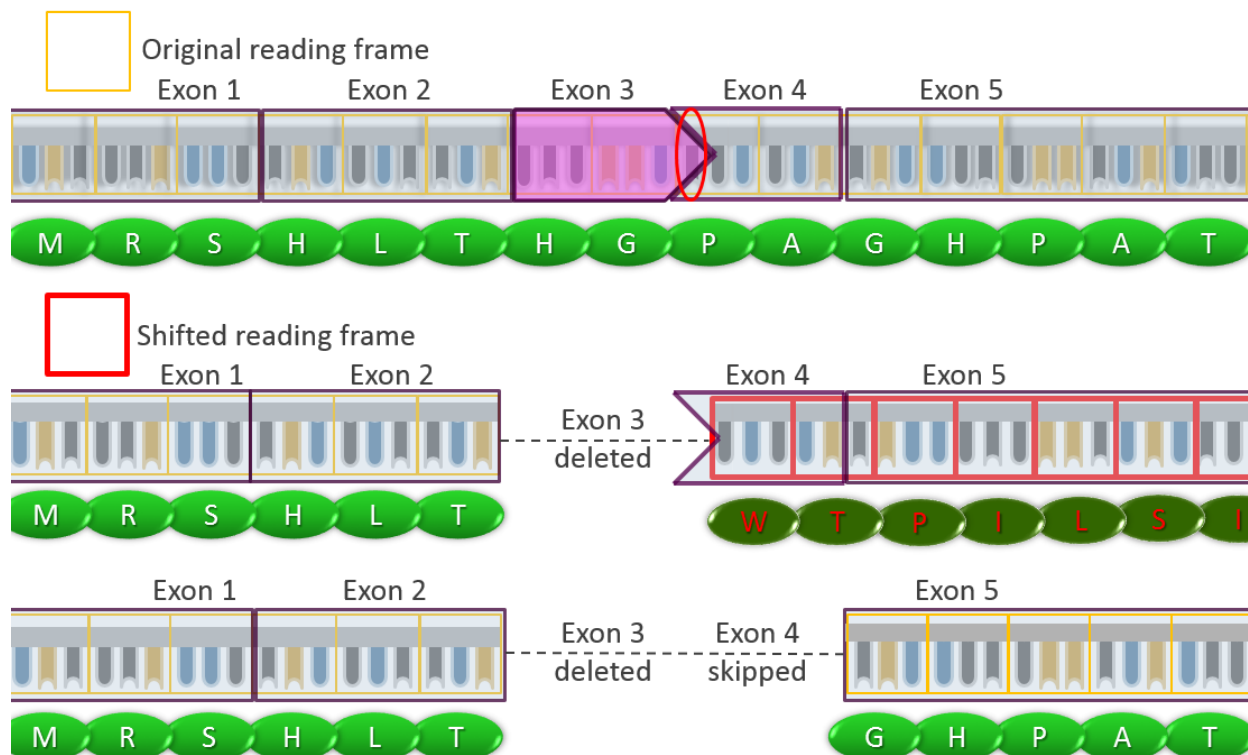
Figure E:

Restoration of the Reading Frame by Exon Skipping

Exon skipping aims to restore the mRNA reading frame by removing an additional exon from the final mRNA. As shown in Figure F, removal of an additional exon restores the reading frame following the mutation. The resulting protein will be missing the amino acids coded for by the missing exons, creating an internally deleted protein.
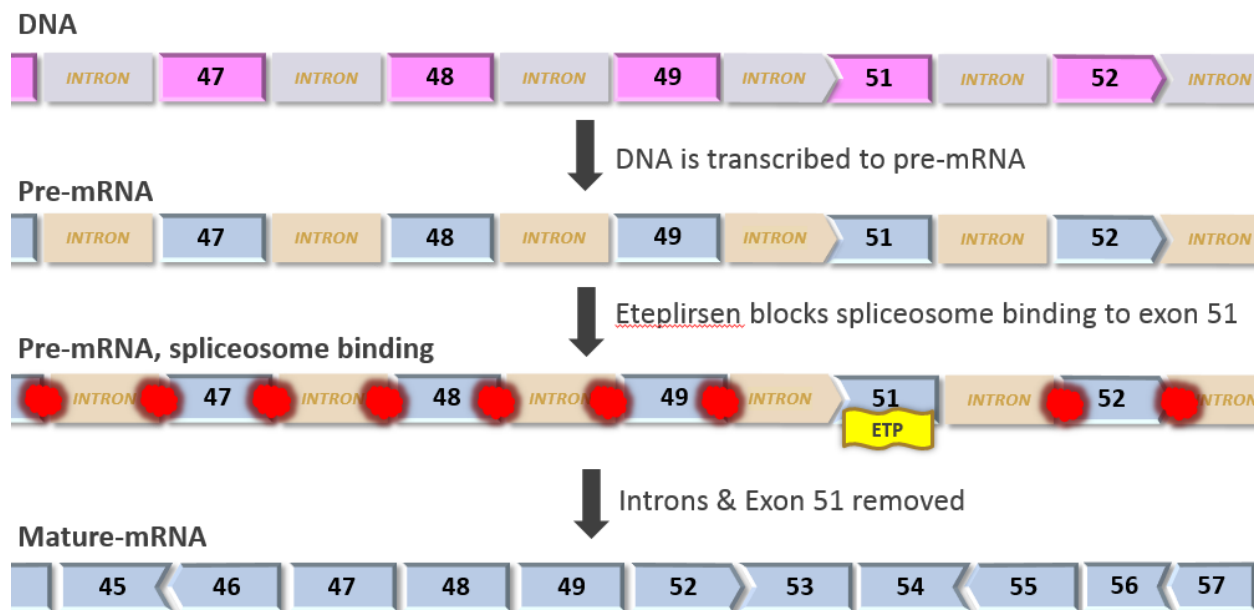
Figure F:

Eteplirsen Mechanism of Action

Eteplirsen enables exon skipping by binding to exon 51 of eteplirsen pre-mRNA and sterically hindering spliceosome binding. As shown in Figure G, if the spliceosome is unable to bind to exon 51, exon 51 will be removed along with the introns surrounding it and the reading frame will be restored.

Figure G:

Mutations Amenable to Exon 51 Skipping

A number of whole exon deletions are amenable to exon 51 skipping. Table A, provides examples that have been documented in the Leiden or UMD databases as well as the deletions tested in eteplirsen pivotal study 201/202.

Table A:

Population	Whole Exon Deletions Amenable to Exon 51 Skipping
Deletion documented in the Leiden or UMD databases[1]	13-50, 19-50, 29-50, 31-50, 35-50, 40-50, 42-50, 45-50, 47-50, 48-50, 49-50, 50, 52
Mutations tested in Eteplirsen study 201/202	45-50, 48-50, 49-50, 50, 52

[1] Leiden DMD Mutation Database [Internet]. Center for Human and Clinical Genetics – Leiden Medical Center. 2003 – [cited 2015 Dec 1]. Available from: http://www.dmd nl