

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**206488Orig1s000**

**MEDICAL REVIEW(S)**



**Food and Drug Administration**  
**CENTER FOR DRUG EVALUATION AND RESEARCH**  
**Division of Neurology Products**  
10903 New Hampshire Ave.  
Silver Spring, MD 20993-0002

### **Errata of Clinical Review**

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**NDA** 206488

**Drug Name (Generic):** Exondys 51 (eteplirsen)

**Sponsor:** Sarepta

**Indication:** Treatment of Duchenne Muscular Dystrophy

**Date of Original Review:** 5/9/16

**Reviewer:** Christopher D. Breder, MD PhD

**Project Manager:** Yuet (Fanny) Choi

#### **I) Background**

The original primary clinical review of NDA was submitted on 5/9/2016. The Adverse Event Table (Table 35, pp. 119-120/159) was generated from the analysis dataset (ADAE) from the placebo-controlled study, 201. However, the variable in the dataset that defined the placebo-controlled portion did not accurately reflect the division between the placebo and active controlled portions of the 201 and 202 study.

The table below was generated by the clinical reviewer by using the start dates of adverse events and selecting only those starting between 0 and 24 Weeks. From that set, a subset was identified that occurred with greater incidence than placebo. Similar to conventional incidence calculations, the numbers in Table 1 reflect the number of subjects who have experienced each Preferred Term. Subjects experiencing an AE more than once were counted as having had one event.

**Table 1 Absolute counts of Unique Treatment Emergent Adverse Events Occurring at a Rate Greater than Placebo from Study 201 (Safety Population)**

<b>AEBODSYS</b>	<b>AEDECOD</b>	<b>N (Eteplirsen 30 mg/kg)</b>	<b>N (Eteplirsen 50 mg/kg)</b>	<b>N(Placebo)</b>
<b>Ear and labyrinth disorders</b>	Motion sickness	0	1	0
<b>Gastrointestinal disorders</b>	Diarrhoea	0	1	0
	Vomiting	1	2	0
<b>General disorders and administration site conditions</b>	Injection site pain	0	1	0
	Malaise	0	1	0
	Non-cardiac chest pain	1	0	0
	Pain	0	1	0
<b>Injury, poisoning and procedural complications</b>	Arthropod bite	1	0	0
	Back injury	1	0	0
	Joint injury	1	0	0
<b>Musculoskeletal and connective tissue disorders</b>	Arthralgia	0	1	0
	Bone pain	1	0	0
	Muscle spasms	0	1	0
	Musculoskeletal pain	1	0	0
<b>Nervous system disorders</b>	Balance disorder	1	2	0
	Somnolence	1	0	0
<b>Renal and urinary disorders</b>	Polyuria	1	0	0
<b>Respiratory, thoracic and mediastinal disorders</b>	Sinus congestion	1	0	0
	Upper respiratory tract congestion	1	0	0
<b>Skin and subcutaneous tissue disorders</b>	Dermatitis contact	1	0	0
	Petechiae	1	0	0
	Urticaria thermal	1	0	0

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CHRISTOPHER D BREDER  
07/20/2016

RONALD H FARKAS  
07/20/2016

## Cross-Discipline Team Leader Review - **ADDENDUM**

<b>Date</b>	June 28, 2016
<b>From</b>	Ronald Farkas, MD, PhD
<b>Subject</b>	Cross-Discipline Team Leader Addendum
<b>NDA/BLA #</b>	206488
<b>Supplement#</b>	
<b>Applicant</b>	Sarepta Therapeutics
<b>Date of Submission</b>	June 6, 2015
<b>PDUFA Goal Date</b>	May 26, 2015
<b>Proprietary Name / Non-Proprietary Name</b>	EXONDYS 51/eteplirsen
<b>Dosage form(s) / Strength(s)</b>	For intravenous infusion / 50 mg/mL

### **1. Introduction**

This addendum to the Cross-Discipline Team Leader (CDTL) Review of May 17, 2016, is based on new efficacy data submitted by the applicant subsequent to the completion of the CDTL review.

To address FDA concerns about whether substantial evidence of efficacy had been presented that eteplirsen increases dystrophin protein in patients with Duchenne muscular dystrophy (DMD) caused by mutations amenable to exon 51 skipping, the applicant performed Western blot analysis of muscle tissue that had been obtained in the ongoing Study 4658-301 (PROMOVI). The PROMIVI study is an open-label, multi-center, 96-Week study of eteplirsen in patients with mutations amenable to exon 51 skipping compared with a concurrent untreated control arm composed of patients not amenable to exon 51 skipping. Western blot was performed on matched pre- and post-treatment biopsies from 13 patients treated with 30 mg/kg/week eteplirsen for 48 weeks.

Review of assay methods and the technical reliability of the Western blot results was conducted by Ashutosh Rao, Ph.D., from the Office of Biotechnology Products. Inspection of the applicant's laboratory testing site was conducted by Dr. Rao along with Young Moon Choi, Ph.D., from the Office of Study Integrity and Surveillance, and Mark Babbit, from the Office of Regulatory Affairs. Statistical review was conducted by Xiang Ling, Ph.D., from the Office of Biostatistics.

### **2. Results**

For 12 of the 13 patients the paired muscle biopsies were from biceps, whereas for 1 patient paired biopsies were from triceps muscle. Table 1 contains the demographic information provided by the applicant for the 13 patients from whom biopsies were obtained.

Table 1: Study 4658-301 Week 48 Patient Information

<b>Anatomical Location</b>	<b>Dystrophin Mutation</b>	<b>Gender</b>	<b>Age at Baseline Biopsy (yrs)</b>
biceps	deletion 45-50	Male	7.10
biceps	deletion 50	Male	7.16
biceps	deletion 50	Male	7.40
biceps	deletion 49-50	Male	7.51
triceps	deletion 49-50	Male	7.60
biceps	deletion 45-50	Male	7.86
biceps	deletion 48-50	Male	8.05
biceps	deletion 45-50	Male	8.99
biceps	deletion 52	Male	9.70
biceps	deletion 49-50	Male	9.93
biceps	deletion 50	Male	10.61
biceps	deletion 48-50	Male	10.88
biceps	deletion 49-50	Male	12.59

A newly obtained normal control muscle sample was used for these Western blot studies, obtained from biceps muscle of a 14 year old male with no pathologic diagnosis. Negative control tissue was obtained from untreated (baseline) biopsies from 3 exon 51 skippable patients enrolled in the PROMOVI study. The Western blot methods used for PROMOVI muscle samples were generally similar to those used for the Week 180 muscle samples from Study 202.

Tables 2 and 3 contain the summary Western blot results. Dr. Xiang Ling, Ph.D., from the Office of Biostatistics, verified the analytical results of the applicant's statistical calculations that are presented in these tables. Dystrophin levels that were below the level of quantification (BLOQ) of 0.25% of normal were imputed as 0.24 in Table 2, or presented as the observed value in Table 3.

Table 2: Western Blot Results, BLOQ Reported as 0.24%

	<b>Baseline</b>	<b>Week 48</b>	<b>Change From Baseline</b>
n	12	12	12
Mean (% Dystrophin)	0.260	0.478	0.218
SD (SE)	0.0469 (0.0135)	0.4066 (0.1174)	0.4173 (0.1205)
Median	0.240	0.330	0.018
Min, Max	0.24, 0.37	0.24, 1.57	-0.07, 1.33
			<b>P = 0.041</b>

Table 3: Western Blot Results, BLOQ Reported as Observed Value

	<b>Baseline</b>	<b>Week 48</b>	<b>Change From Baseline</b>
n	12	12	12
Mean (% dystrophin)	0.157	0.440	0.283
SD (SE)	0.1159 (0.0335)	0.4341 (0.1253)	0.4153 (0.1199)
Median	0.150	0.330	0.098
Min, Max	0.02, 0.37	0.09, 1.57	-0.07, 1.33
			<b>P = 0.008</b>

Individual patient-level Western blot results are presented in Table 5, with BLOQ values imputed as 0.25% of normal. Information was not submitted by the applicant to determine which Western blot results were obtained from which patient, such that FDA review of effects of the specific dystrophin mutation or other demographic factors on dystrophin production was not possible.

Table 4: Individual Patient Western Blot Results (Values BLOQ imputed as 0.24)

Patient Number*	Time Point	Pass/Fail	%Dystrophin
1	Baseline	Pass	0.24
	Baseline	Pass	0.24
	Week 48	Pass	0.24
	Week 48	Pass	0.29
2	Baseline	Pass	0.35
	Baseline	Fail	0.26
	Week 48	Pass	0.36
	Week 48	Fail	0.24
3	Baseline	Pass	0.24
	Baseline	Pass	0.24
	Week 48	Pass	0.5
	Week 48	Pass	0.24
4	Baseline	Pass	0.24
	Baseline	Fail	0.24
	Week 48	Pass	0.24
	Week 48	Fail	0.24
5	Baseline	Fail	0.24
	Baseline	Pass	0.24
	Week 48	Fail	0.92
	Week 48	Pass	1.02
6	Baseline	Pass	0.37
	Baseline	Fail	0.46
	Week 48	Pass	0.3
	Week 48	Fail	0.29
7	Baseline	Fail	0.24
	Baseline	Pass	0.24
	Week 48	Fail	0.24
	Week 48	Pass	0.42
8	Baseline	Fail	0.24
	Baseline	Fail	0.24
	Week 48	Fail	0.24
	Week 48	Fail	0.24
9	Baseline	Fail	0.24
	Baseline	Pass	0.24
	Week 48	Fail	1.17
	Week 48	Pass	1.57
10	Baseline	Pass	0.24
	Baseline	Fail	0.24
	Week 48	Pass	0.24
	Week 48	Fail	0.24
11	Baseline	Pass	0.24
	Baseline	Pass	0.24
	Week 48	Pass	0.31
	Week 48	Pass	0.63
12	Baseline	Pass	0.24
	Baseline	Fail	0.24
	Week 48	Pass	0.24
	Week 48	Fail	0.24
13	Baseline	Fail	0.34
	Baseline	Pass	0.24
	Week 48	Fail	0.34
	Week 48	Pass	0.24

\*Patient number in this table does not correspond to patient order in Table 1

Dr. Rao concluded that the Western blotting procedure and quantification appeared to have been conducted within the scope of the applicant's predetermined standard operating procedures.

### **3. Discussion and Conclusions**

The May 17, 2016 CDTL review concluded that there was some evidence that eteplirsen increases the expression of a Becker-type dystrophin protein in DMD patients, but that the amount of evidence was less than is usually considered to be "substantial evidence" in the context of FDA approval. In brief, shortcomings of dystrophin quantification for the Week 180 biopsy from 11 patients in Study 201/202 included controls that were not matched by muscle group or patient, and lack of independent confirmation of findings. In the April 15, 2014, letter to the applicant discussing data that would be filed with the NDA, FDA stated the expectation that additional biomarker data from newly exposed patients would become available at about the time of the NDA submission or shortly thereafter.

The CDTL review also concluded that Becker-type dystrophin protein could be considered under the accelerated approval provisions as a biomarker reasonably likely to predict benefit in DMD, but that the amount of the protein produced by eteplirsen would be a key consideration in such a determination. The level of dystrophin in the Week 180 biopsies,  $\approx 1\%$  of normal, was low enough that an FDA finding that such a level was reasonably likely to predict clinical benefit would have to be based on a low threshold for reasonably likely.

The new Western blot data described in this addendum was first examined in regard to whether substantial evidence has now been presented that eteplirsen increases the expression of at least some amount of Becker-type dystrophin protein. Patient- and muscle-group matched pre- and post-treatment muscle biopsies were available for each of the 13 patients studied, and FDA inspection found that the Western blots were conducted within the scope of the applicant's predetermined standard operating procedures. Important characteristics for concluding independence of the present findings from previous studies were present, including different DMD patients, muscle biopsy specimens, and laboratory facilities used to conduct the studies. At least one Western blot data point (pre- and post-treatment samples) was available for all but one patient, and statistical analysis using a number of different methods of addressing BLOQ results provided evidence against the null hypothesis. Thus, in combination with the Western blot data from the Week 180 biopsy from Study 201/202, I conclude that substantial evidence has now been presented that eteplirsen increases the expression of at least some amount of Becker-type dystrophin protein.

The new Western blot data was then examined regarding whether the amount of dystrophin produced by eteplirsen is enough to be considered reasonably likely to predict benefit. The mean dystrophin level in the new patients was roughly 0.5% of normal, an increase of about 2 to 3 fold over matched baseline, depending on the imputation for BLOQ values. This is less than levels estimated from the Week 180 biopsies from Study 201/202, but in the context of experimental uncertainties (e.g. biopsies from different muscle groups, different time points, different normal control sample used) it does not appear to be interpretable as a categorically different result. Therefore, the previous conclusion of the CDTL review that approval of

eteplirsen under accelerated approval provisions would have to be based on a low threshold for reasonably likely remains unchanged.

Importantly, the new Western blot results suggest that the response to eteplirsen may be dichotomous, with a majority of patients experiencing no or negligible increase in dystrophin. Resolving this question is clearly of utmost importance, with a wide range of implications from appropriate patient selection in clinical use to the design of confirmatory efficacy studies of eteplirsen and the development of similar therapies for non-exon 51 skippable patients.

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RONALD H FARKAS  
07/15/2016

## Division Director Summary Review for Regulatory Action

<b>Date</b>	(electronic stamp)
<b>From</b>	Eric Bastings, MD. Deputy Director, DNP
<b>Subject</b>	Division Director Summary Review
<b>NDA/BLA #</b>	206488
<b>applicant</b>	Sarepta Therapeutics, Inc.
<b>Date of Submission</b>	June 26, 2016
<b>PDUFA Goal Date</b>	May 26, 2016
<b>Proprietary Name / Non-Proprietary Name</b>	Exondys 51 Eteplirsen
<b>Dosage Form(s) / Strength(s)</b>	Solution/ 30 mg/kg intravenously once-weekly
<b>applicant Proposed Indication(s)/Population(s)</b>	Treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping
<b>Recommended Action:</b>	Complete Response
<b>Approved/Recommended Indication/Population(s)</b>	None

<b>Material Reviewed/Consulted</b>	<b>Names of discipline reviewers</b>
OND Action Package, including: Project Manager	Yuet (Fannie) Choy; Laurie Kelley
Medical Officer Clinical Review	Christopher Breder
Clinical Pharmacology Review	Ta-Chen Wu; Yuxin (Angela) Men, Venkatesh (Atul) Bhattaram; Kevin Krudys; Hobart Rogers; Christian Grimstein; Mehul Mehta
Statistical Review	Xiang Ling; Kun Jin; Hsien Ming (Jim) Hung
Pharmacology Toxicology	David Hawver; Lois Freed; Paul Brown
Office of Biotechnology Products (Bioassay)	Ashutosh Rao; Amy Rosenberg
OPQ/Chemistry Manufacturing and Controls	Joseph Leginus; Donna Christner; Mariappan Chelliah; Denise Miller; Neal Sweeney; Sung Kim; Edwin Jao; Zhong Li; Zhihao Peter Qiu; Dahlia Woody; Martha Heimann; Wendy Wilson-Lee
OPQ / Environmental Assessment	James Laurenson; M. Scott Furness
Method Validation	Michael Hadwiger; Michael Trehy
Statistical Review – Stability data	Zhuang Miao; Xiaoyu Dong, Meiyu Shen; Yi Tsong
Controlled Substance Staff	Katherine Bonson; Martin Rusinowitz; Michael Klein; Sandy Saltz
Office of Scientific Investigation	Antoine El Hage; Cara Alfaro; Susan Thompson; Kassa Ayalew; Ni Aye Khin
Division of Advisory Committee and Consultant Management	Diem Ngo; Moon Hee Choi

Office of Prescription Drug Promotion	Aline Moukhtara
OSE PMs	Ermias Zerislassie; Corwin Howard; Davis Mathew
Division of Medication Error Prevention and Analysis	Deborah Meyers; Justine Harris, Danielle Harris; Todd Bridges
Division of Risk Management	Robert Pratt; Jamie Wilkins Parker; Kellie Taylor; Cynthia LaCivita
Associate Director for Labeling, DNP	Tracy Peters
Cross-Discipline Team Leader	Ronald Farkas

# 1. Benefit-Risk Assessment

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Dimension	Evidence and Uncertainties	Conclusions and Reasons
<p><b>Analysis of Condition</b></p>	<ul style="list-style-type: none"> <li>• Duchenne Muscular Dystrophy (DMS) is a degenerative X-linked recessive genetic disorder associated with mutations in the dystrophin that result in the absence or near absence of functional dystrophin protein. Lack of dystrophin results in degeneration of muscle fibers, inflammation, and ultimately replacement of muscle by fibrotic and adipose tissue.</li> <li>• Exon 51 skip-amenable DMD, a subgroup of DMD, is defined by the presence of exon 51 in the dystrophin gene and the deletion of one or more exons contiguous with exon 51, resulting in an out-of-frame deletion in which the reading frame is potentially restorable by the skipping (removing) of exon-51. Mutations that are potentially treatable by skipping exon 51 are thought to comprise about 13% of the DMD population, resulting in a prevalence of about 2000 boys in the US.</li> <li>• Loss of muscle strength is progressive, typically beginning a waddling gait and inability to jump in young boys, progressing to a loss of ability to ambulate. The loss of ambulation is generally considered to occur between ages 8 to 16 years, but about 25% of patients may still be ambulatory at age 16. While pulmonary and cardiac function are generally normal during early childhood,</li> </ul>	<p>DMD is a serious and life-threatening disease. The loss of muscle strength in DMD is progressive, leading to loss of ambulation in the teens, followed by decline in respiratory and cardiac function, resulting in death typically in the third decade.</p>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<p>muscles of the heart and diaphragm progressively weaken during adolescence, and patients often die from cardiac or respiratory failure in their early 20s.</p>	
<p><b>Current Treatment Options</b></p>	<ul style="list-style-type: none"> <li>There is no FDA-approved treatment for DMD. The current standard of care is glucocorticoids, which are thought to provide a modest beneficial effect on function and survival. In addition, supportive care, such as assisted ventilation and physiotherapy, is modestly effective in prolonging function and survival.</li> </ul>	<p>There is a substantial unmet need for therapies in DMD.</p>
<p><b>Benefit</b></p>	<ul style="list-style-type: none"> <li>Clinical efficacy was evaluated in a 24 week placebo-controlled trial (Study 201), which was followed by open-label extension (Study 202, for which data up to Week 240 have been submitted to the application).</li> <li>Study 201 was negative.</li> <li>The applicant has requested accelerated approval based on an endpoint of 6-minute walk distance in Study 202, comparing open-label experience with two dose levels of eteplirsen (30 mg/kg and 50 mg/kg weekly) to an external historical control. The applicant proposes that 6-minute walk be considered an intermediate endpoint demonstrating delayed disease progression. The division considers an effect on walking distance to be a clinical benefit that, if demonstrated, would support full approval. Therefore, the division sees no justification for using 6-minute walk distance as an intermediate endpoint here, in particular as the period of observation is unusually long, around 4 years, which is more than sufficient to identify a possible clinical benefit. The clinical evidence provided by the applicant, which includes a number of clinically meaningful endpoints, is therefore to be examined in the context of “conventional” approval. The comparison to historical control made by the applicant in Study 202 failed to show a</li> </ul>	<p>The applicant has not provided substantial evidence of efficacy from adequate and well controlled trials to support “conventional” approval.</p> <p>The applicant has provided substantial evidence that eteplirsen induces production of dystrophin. This is unprecedented for Duchenne Muscular Dystrophy, establishes proof of concept, and gives hope that this therapeutic approach may address the fundamental pathology of DMD. However, the amount of dystrophin produced in response to eteplirsen treatment is very small. While it is somewhat possible that the amount of dystrophin produced may lead to a modest clinical benefit, such a benefit does not appear reasonably likely.</p>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<p>clear separation between the disease course in eteplirsen-treated patients and historical control patients. Instead, all patients in the eteplirsen treatment group appeared to experience the sequential worsening of functional abilities and muscle weakness expected in patients with Duchenne muscular dystrophy.</p> <ul style="list-style-type: none"> <li>• Biomarkers that reliably reflect the health and amount of skeletal muscle may, if supported by sufficient scientific evidence and acceptable analytical methods, be used as surrogate endpoints to support accelerated approval of a new drug for Duchenne muscular dystrophy. Such a biomarker would have to be “reasonably likely to predict clinical benefit” in order to be acceptable as a basis for accelerated approval. In Study 201/202, the applicant obtained 4 muscle biopsies, spaced between baseline (pre-treatment) and Week 180 of treatment. Pharmacodynamic effects of eteplirsen are potentially demonstrable at two levels: expression of an altered messenger RNA in muscle, and production of dystrophin protein in muscle. There is evidence of production of an altered messenger RNA in the muscle of all patients of Study 201/202. However, this biomarker provides little support of efficacy for eteplirsen. Demonstration of messenger RNA production is necessary to establishing proof of concept, but not sufficient. In Study 201/202, the mean dystrophin level in patients who have been treated with eteplirsen for three and a half years was 0.93% ± 0.84% of normal. As baseline dystrophin level was only available in two of these patients, and because of methodological issues, it was difficult to ascertain whether there was any increase from baseline in dystrophin in Study 201/202. Therefore, the applicant was asked to provide additional dystrophin data from an additional 13 patients participating in an ongoing study (PROMOVI study) and who had a muscle biopsy at baseline and at Week 48 (with data available in 12 of those patients). In those 12 patients, there was a small (mean = 0.3%)</li> </ul>	

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<p>but statistically significant increase from baseline in dystrophin level. Overall, the applicant has provided substantial evidence that eteplirsen produces an increase in dystrophin, but the mean increase is very small. Based on a comparison of Week 48 to baseline using reported dystrophin values, most patients (about 60%) from the PROMOVI study had no increase in dystrophin content, or an increase smaller than 0.25% of normal levels. A single patient in the PROMOVI study had a dystrophin increase greater than 1%, and no patient had a dystrophin increase greater than 2%. In comparison, about a third of patients from Study 201/202 had no increase in dystrophin content, or an increase smaller than 0.25% of normal levels, while about a third of patients had dystrophin increases greater than 1% of normal levels. A single patient had a dystrophin increase greater than 2%, and no patient had a dystrophin increase greater than 3%. The minimum level of dystrophin that might be reasonably likely to predict clinical benefit in patients with DMD remains unknown, and there are no data to support the concept that the small increase in dystrophin induced by eteplirsen at the doses that were studied is reasonably likely to predict clinical benefit. In Study 201/202, there was no correlation between dystrophin levels and clinical outcome, and no dose-response in the amount of dystrophin.</p>	
Risk	<ul style="list-style-type: none"> <li>The clinical safety database for eteplirsen is small: 114 total patients exposed, with only 36 exposed for <math>\geq 24</math> weeks and 12 exposed for <math>\geq 1</math> year. Most of these exposures were outside of placebo-controlled studies, limiting ability to determine if adverse events were the result of drug effect or chance. However, the serious and severe adverse events that occurred were generally consistent with events expected in DMD. The 12 patients in Study 202 were exposed for <math>&gt;3</math> years, which provides some reassurance against delayed toxicity.</li> </ul>	<p>The safety database for patients exposed at the intended dose is small, but sufficient to assess frequent adverse events, and acceptable for this serious disease with great unmet medical need.</p>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<ul style="list-style-type: none"> <li>In animal studies, the primary target organ was the kidney, with dose-dependent renal tubular cytoplasmic basophilia and/or vacuolation and, at the high dose, tubular degeneration/necrosis. In a mouse study, dilatation of the lateral ventricles of the brain was observed at the mid and high doses. The mechanism of this effect, and its relevance to humans, is unknown. Mean eteplirsen plasma exposures (AUC) at the NOAELs for monkey and juvenile rat were 20-fold and 6-fold, respectively, greater than that in patients dosed once weekly with 30 mg/kg IV eteplirsen.</li> </ul>	
<b>Risk Management</b>	<ul style="list-style-type: none"> <li>Safety risks have not been identified that would require risk management beyond standard pharmacovigilance. A patient registry may be useful to acquired additional safety information in the postmarketing setting.</li> </ul>	Safety risks have not been identified that would require risk management beyond standard pharmacovigilance.

## 2. Background

The NDA under review is for eteplirsen, proposed for the treatment of patients with DMD who have a confirmed mutation of the dystrophin gene amenable to exon 51 skipping ( $\approx 13\%$  of patients with DMD).

Duchenne Muscular Dystrophy (DMS) is a fatal, degenerative, X-linked recessive genetic disorder associated with mutations in the gene encoding dystrophin, a sarcolemma protein critical to the structural stability of myofibers in skeletal and cardiac muscle. Dystrophin mutations induce a shift in the open reading frame of the dystrophin transcript, leading to a reduction or absence of functional dystrophin. In the absence of dystrophin, the stress of muscle contraction causes progressive muscle damage. Duchenne muscular dystrophy is usually first diagnosed before age 5. Progression in DMD occurs in a generally predictable stepwise fashion, starting with loss of ability to stand from the floor, followed by a loss of ability to walk independently, itself preceding a decline in pulmonary function.

There are no drugs approved for the treatment of DMD, and there is an enormous unmet medical need. Corticosteroids are standard of care for the condition, and appear to slow down progression, but they have many side effects.

Eteplirsen is a phosphorodiamidate morpholino oligomer (PMO) designed to target the pre-mRNA transcripts of the dystrophin gene so that exon 51 is excluded, or skipped, from the mature spliced mRNA, thereby restoring the mRNA reading frame. If successful, this shift may enable the production of a truncated dystrophin protein, which, if functional, may lead to clinical benefit.

Pharmacodynamic and clinical effects of eteplirsen are therefore potentially demonstrable at three levels: expression of an altered messenger RNA for dystrophin in muscle (assessed by nested polymerase chain reaction [PCR]), production of dystrophin protein in muscle, and improvement or preservation of muscle function.

The applicant undertook two exploratory studies (Study 28 and Study 33) to assess eteplirsen's potential to increase expression of an altered mRNA and dystrophin expression, and a 12-patient controlled clinical study (Study 201/202) to assess whether eteplirsen increased expression of dystrophin protein, and led to clinical benefit.

Study 201/202 began as a 24-week randomized placebo-controlled study (Study 201). After Study 201 did not meet its primary endpoint, and as FDA did not consider the post hoc analyses of Study 201/202 conducted by the applicant to be scientifically valid, FDA advised the applicant to conduct an adequately powered, randomized, placebo-controlled trial to assess the clinical benefit of eteplirsen. But in the context of an ongoing series of reports from the applicant and its academic associates describing marked effects on dystrophin production and

stabilization of disease progression, many in the DMD community had strong reservations regarding the ethics and practicality of conducting another placebo-controlled trial of eteplirsen. Given the apparent difficulty of doing such a trial, FDA expressed willingness to consider an externally controlled trial, although stating clearly that interpretation of the data could be difficult, and that the acceptability of the study would be a matter for NDA review. FDA advised the applicant to identify external control groups appropriately matched to Study 202 patients, including similar treatment modalities, and to provide patient-level data. The applicant identified two DMD patient registries as a source of external data, the “Italian DMD Registry” and the “Leuven Neuromuscular Reference Center” registry, and conducted a post hoc comparison of the patients in Study 201/202 with patients from the two external registries.

The applicant is proposing approval primarily based on a post hoc comparison of patients of all available open-label data from Study 202 (up to Week 144) to a natural history cohort of untreated patients. The applicant believes that the results of their external control comparison provide evidence of benefit on an “intermediate clinical endpoint” – 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, and that could suffice as a basis for accelerated approval.

### 3. Product Quality

From a product quality perspective, NDA 206488 is recommended for approval.

#### Drug substance

As discussed by the product quality reviewer, eteplirsen contains a sequence of 30 linked (b) (4) -phosphorodiamidate morpholino subunits. (b) (4)

The chemical name for eteplirsen is:

(b) (4)

#### Drug Product

Eteplirsen injection is a sterile solution containing 50 mg eteplirsen per mL. The applicant proposes two single dose vial configurations: 100 mg/2 mL and 500 mg/10 mL. All excipients are within the ranges used in previously approved intravenous drug products.

The product must be diluted with saline prior to infusion. The product does not contain an antimicrobial preservative and should be used within 4 hours after dilution if stored at room temperature, or 24 hours after dilution if refrigerated.

Based on evaluation of stability data from primary and supportive batches, an expiration dating period of 18 months is established for eteplirsen, when stored refrigerated (5°C).

The inspection of the drug substance and of the drug product manufacturing facilities is acceptable.

The applicant has agreed to the following CMC post-marketing commitments:

1. Investigate the root cause of the increasing assay trend observed in the drug product stability study.
2. Revalidate the accuracy of the in-process (b) (4) method used during drug product manufacture.
3. Revalidate the robustness of the in-process (b) (4) method in terms of (b) (4).
4. Investigate the consistent bias in the in-process (b) (4) results and the release (b) (4) results.

## 5. Nonclinical Pharmacology/Toxicology

From a nonclinical perspective, NDA 206488 is recommended for approval.

Dr. Hawver, nonclinical reviewer, notes that pharmacological studies have demonstrated that administration of eteplirsen can induce exon 51 skipping in dystrophin mRNA in human muscle cell cultures, muscle explant cultures, in transgenic hDMD mice, and in cynomolgus monkeys.

In cynomolgus monkeys, samples of quadriceps muscle, heart, and diaphragm tissues, collected from cynomolgus monkeys after 12 weekly doses of eteplirsen at 0, 5, 40, or 320 mg/kg IV, or 320 mg/kg SC. The samples were analyzed using PCR for exon 51 skipping of the dystrophin gene. Dr. Hawver discusses that all three target muscles showed increased skipping of exon 51 of the dystrophin gene after treatment with IV or SC eteplirsen. There is also a very clear dose-response in exon 51 skipping, as shown in Table 1, which is adapted from Dr. Hawver's review. Of note, a similar dose response was observed in DMD patients in exploratory Study 33 (see Clinical/Statistical-Efficacy), in which direct intramuscular injection of eteplirsen led to increased skipping of exon 51 in all five patients at a 0.9 mg dose, but not in patients injected with 0.09 mg eteplirsen or placebo. Similarly, dystrophin expression by western blot was noted in all patients treated with 0.9 mg of eteplirsen, but in no patient who received with 0.09 mg of eteplirsen. On immunofluorescence testing, there was also a high

percentage of dystrophin-positive fibers with eteplirsen 0.9 mg (ranging from 44 to 79%), versus no expression with eteplirsen 0.09 mg.

**Table 1: Dose-response on exon 51 skipping in the cynomolgus monkey with eteplirsen treatment**

<i>Tissue</i>	<i>Average % Exon 51 Splicing ± 1 SD</i>				
	0 mg/kg IV	5 mg/kg IV	40 mg/kg IV	320 mg/kg IV	320 mg/kg SC
Quadriceps muscle	0.0 ± 0.0	0.5 ± 0.5	0.6 ± 0.3	8.2 ± 7.4	1.3 ± 0.5
Heart	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.2	4.5 ± 2.9	1.4 ± 0.5
Diaphragm	0.0 ± 0.0	0.2 ± 0.2	0.9 ± 0.7	6.1 ± 3.5	2.2 ± 0.9

SD standard deviation

Dr. Hawver also discusses that published studies present evidence for exon skipping and induction of dystrophin protein expression in mouse and dog DMD models using species-specific exon skipping phosphorodiamidate morpholino oligomer (PMOs), and often correlated these changes with reductions in muscle pathology and/or improvements in muscle function. In reference to the eteplirsen NDA, Dr. Hawver notes that the most robust finding among the studies provided or referenced is the wide variability in the extent of PMO-induced dystrophin expression within a single muscle and among different muscles. Dr. Freed, Supervisory nonclinical reviewer, describes a clear dose-response in a study<sup>1</sup> in mdx and C57Bl that tested the effects of a mouse-specific PMO targeting exon 23. At the low dose, dystrophin-positive fibers were increased up to 5% of normal in skeletal muscle. The maximum amount of (truncated) dystrophin protein was 2.6% of normal, based on Western blot analysis. At the mid-dose, 10 to 50% fibers were dystrophin-positive were in skeletal muscle, and levels of dystrophin protein were up to 17.1% of normal (Western blot). The distribution of protein-positive fibers was reported to be highly variable among muscle groups in an individual animal and in the same muscle type among animals. Significant improvement in muscle function was observed. Further enhancement of exon skipping and muscle function was observed at the higher doses, e.g., with dystrophin-positive fibers close to 100%, and levels of dystrophin protein 25-50% of normal.

Another study discussed by Dr. Freed was conducted in mdx mice in order to address the issue of how much dystrophin is needed to protect muscles. In that study, higher acute doses of peptide-conjugated PMO were associated with dystrophin expression in the tibialis anterior at levels of 5-15% of wild type; none was detected at the lower acute doses. The authors concluded that 15% of wild type (“low level dystrophin restoration”) was sufficient to protect muscle (eccentric contraction-induced muscle damage) but not sufficient to “substantially” improve muscle function (maximum isometric force). The effects of repeated dosing (Q2W)

<sup>1</sup> Wu B et al. *Mole Therap* 19(3):576-583, 2011. The study was referenced by the applicant in the eteplirsen NDA.

on muscle pathology and function were also tested in tibialis anterior from mdx mouse. Western blot analysis indicated dystrophin expression around 50% of wild type, which positively correlated with maximal isometric force and reduced muscle pathology. Dr. Freed concludes that the applicant conducted only a minimal PD assessment of eteplirsen in animals, assessing exon skipping in muscles from a 12-week monkey study. Dr. Freed notes that the monkey study demonstrated dose-related increases in exon skipping. She also notes that published literature suggests that a minimum threshold for functional benefit or protection of muscle has not been identified, but that higher doses and/or longer duration may be associated with greater effects.

Dr. Hawver comments that pivotal toxicology studies of eteplirsen were conducted in male monkeys (39-week study) and juvenile male rats (10-week study), and that a 26-week study was conducted with a mouse-specific surrogate in male transgenic mdx mice. Dr. Hawver observes that the primary target organ of toxicity was the kidney in all three species, as evidenced by dose-dependent renal tubular cytoplasmic basophilia and/or vacuolation and, at the high dose, tubular degeneration/necrosis. Dr. Hawver also notes that in the mdx mouse study, dilatation of the lateral ventricles of the brain was observed at the mid and high doses. The mechanism of this effect and its relevance to humans is unknown. Dr. Freed believes that although toxicities were observed in mouse, juvenile rat, and monkey (kidney in all species; dilatation of lateral ventricles in mdx mouse; bone morphology in juvenile rat at all doses), the kidney toxicity was minimal and is monitorable and bone growth is monitorable in children. Dr. Freed notes that the dilatation of lateral ventricles is not monitorable and may be relevant to DMD patients, but was not thought to be of sufficient concern to halt clinical development. Dr. Freed notes that safety margins based on plasma exposures at the NOAELs are low (or non-existent in the case of bone) (<1 in mdx mouse, 3.4 in monkey), but observes that plasma exposures at the highest doses tested, which, with the exception of the moderate dilatation of lateral ventricles, were associated with minimal-to-slight toxicity were 17 and 20 times the anticipated human exposure. So, presuming that toxicities can be monitored in humans, Dr. Freed believes that nonclinical data would support doses >30 mg/kg in humans. Considering the seriousness of DMD, the unmet medical need, and the nature of the toxicities observed in animals, I believe that the nonclinical data would support, with proper monitoring, dosing in DMD patients at least up to 200 mg/kg, a dose expected to provide exposure similar to the most sensitive species NOAEL for the toxicities seen in animals. If the human safety experience at these doses is acceptable, further dose escalation is possible in DMD patients.

Dr. Hawver and Dr. Freed recommend that carcinogenicity studies in two species be conducted as a post-marketing requirement. I agree that for this serious indication with unmet need, carcinogenicity studies could be deferred to after marketing of the drug has started.

## **6. Clinical Pharmacology**

The Office of Clinical Pharmacology (OCP) concludes that a relationship between eteplirsen dose and changes in 6-minute walk distance (6MWD) cannot be characterized based on the results of Study 201/202, and that comparison of changes in 6MWD and NSAA score between eteplirsen-treated patients and historical controls does not provide clear evidence of efficacy. As I will discuss later in this memo, I am in full agreement with those conclusions.

The Office of Clinical Pharmacology (OCP) further concludes that due to lack of clear evidence of benefit from eteplirsen in Study 201/202, and considering the pharmacokinetics of eteplirsen (3 to 4 hours plasma half-life, urinary excretion of 60-70% of the dose within 24 h post-dose), the applicant should evaluate doses greater than 50 mg/kg (administered weekly), or alternate regimens that would include loading and maintenance doses. As I discussed above, nonclinical data do support testing higher doses of eteplirsen in DMD patients, and I find the OCP recommendation fully justified, based on all nonclinical and clinical data generated to date for eteplirsen.

In their review of the pharmacokinetics of eteplirsen, the Office of Clinical Pharmacology observes that approximate dose-proportionality and linearity in PK properties were observed following multiple doses of eteplirsen. There was insignificant drug accumulation following weekly dosing across the dose range of 0.5 to ~50 mg/kg. Following single or multiple IV infusion, the peak plasma concentrations of eteplirsen occurred near the end of infusion, and plasma concentration-time profiles of eteplirsen were generally similar and showed multi-phasic decline, with the majority of drug elimination occurring within 24 hours. Plasma protein binding of eteplirsen ranges between about 5 to 15%. Eteplirsen is metabolically stable in vitro, with no evidence of metabolism or metabolites. At 30 and 50 mg/kg weekly doses, urinary excretion accounts for about two thirds of the dose. Elimination half-life is about 3.5 hours. Inter-subject variability of eteplirsen PKs ranges between 20 and 55%.

The Office of Clinical Pharmacology expects eteplirsen to have a low potential for drug-drug interaction in human, based on results of in vitro investigation on microsomal metabolism, plasma protein binding, inhibition or induction of major CYP isozymes or major drug transporters at the concentration range studied for clinical dosing regimen.

## **7. Clinical Microbiology**

Not applicable.

## 8. Clinical/Statistical-Efficacy

From a clinical and statistical perspective, a complete response action is recommended for NDA 206488 by all members of the efficacy review team: Dr. Breder, clinical reviewer, Dr. Farkas, Clinical Team Leader, and Dr. Yin, statistical reviewer. In addition, Dr. Atul Bhattaram, from OCP, played a key role in the evaluation of the efficacy database, and produced many of the graphs presented below. As discussed above, OCP also concluded that there is no clear evidence of efficacy of eteplirsen.

### Clinical Development Program

As explained by the applicant, eteplirsen's intended mechanism of action is by removal of exon 51 of the pre-messenger ribonucleic acid (RNA), thereby restoring the messenger RNA "reading frame." This shift would enable the production of a truncated form of the dystrophin protein. By increasing the quantity of an abnormal, but potentially functional, dystrophin protein, the objective is to slow or prevent the progression of DMD.

To support the efficacy of eteplirsen, the applicant conducted two small exploratory studies (Study 28 and Study 33) to assess the potential for eteplirsen to increase expression of an altered mRNA and to increase dystrophin expression, and a single controlled clinical study (Study 201/202) in 12 patients to assess whether eteplirsen increased expression of dystrophin protein, leading to clinical benefit.

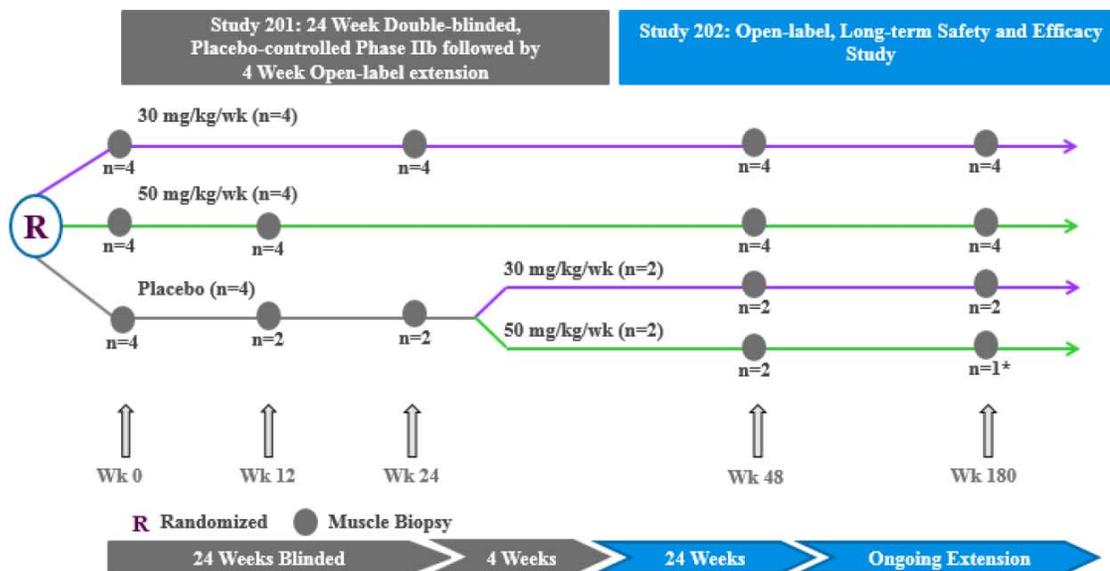
**Study 33** was an exploratory study in which small doses of eteplirsen (up to 0.9 mg) were injected directly into a foot muscle in seven patients with DMD. The study showed a clear dose-response in mRNA expression and dystrophin production, with no effect at the initial dose tested, strongly supporting the importance of appropriate dose-finding. Also, as the drug was administered intramuscularly, it is very difficult to extrapolate what intravenous doses would be necessary to achieve similar intramuscular exposures to those obtained by direct injection in Study 33. The clear conclusion, though, is that adequate dose-finding is critical. Also, in Study 33, there was a ten-fold difference between the tested dose that led to pharmacodynamic activity and the dose that did not. As will be discussed below, there is less than a two-fold difference between the two eteplirsen doses tested in Study 201/202, and there is no information as to whether higher doses of eteplirsen administered intravenously may lead to levels of dystrophin expression as high as those reported in Study 33.

**Study 28** was an exploratory study in which eteplirsen was administered intravenously once a week for 12 weeks at doses up to 20 mg/kg in 19 patients with DMD. As discussed by Dr. Breder, the applicant reported that across the 17 evaluable patients, the mean percentage of dystrophin-positive fibers increased from about 2% at baseline to up to 19% with the highest dose tested (20 mg/kg weekly). However, there was no clear dose-response, and the results appeared highly variable, with the 2 mg/kg weekly dose leading to a 12% absolute increase in

dystrophin positive fibers, while the 4 mg/kg weekly dose led to a decrease in the percentage of positive fibers. The study also had major methodological issues, similar to those discussed below for Study 201/202, and is overall inconclusive.

**Study 201/202** was the only concurrently controlled clinical trial conducted by the applicant intended to assess a clinical endpoint. Study 201/202 (Figure 1) began as a 24-week randomized placebo-controlled study (Study 201) comparing three groups of four patients each, treated weekly with intravenous eteplirsen 50 mg/kg, eteplirsen 30 mg/kg, or placebo (the 4 placebo patients were divided in two subgroups, 2 patients switched to eteplirsen 30 mg/kg at Week 24, and 2 switched to eteplirsen 50 mg/kg at Week 24).

**Figure 1: Design of Study 201/202 (copied from applicant’s Advisory Committee Briefing materials, page 51)**



The prospectively planned primary endpoint of Study 201 was an assessment of dystrophin in skeletal muscle. In Study 201, all twelve patients had a muscle biopsy at baseline (*first biopsy*) and Week 48 (*third biopsy*). In addition, patients had a *second biopsy* either at Week 12 (50 mg/kg group) or Week 24 (30 mg/kg group). The randomized controlled phase (Study 201) was followed by an open-label extension phase (Study 202) in which patients continued to receive eteplirsen at the same dose as they did after Week 24 of Study 201, i.e., six patients on eteplirsen 30 mg/kg weekly, and six patients on 50 mg/kg weekly. Study 202 had a 6-Minute Walk Test (6MWT) at Week 48 as prespecified primary endpoint, but continued beyond Week 48, and is still ongoing at the time of writing this memo. In Study 202, 11 of the 12 patients had a *fourth biopsy* at Week 180 (~3.5 years).

After the first 3 biopsies were analyzed, FDA conducted an inspection of the facility which completed the biomarker analyses, and identified significant methodological issues, which cast

serious doubts on the reliability of assessments from the first three biopsies. These issues are discussed in detail by Dr. Rao in his review. In light of these concerns, FDA worked collaboratively with the applicant on methods for a reassessment of the images of the first three biopsies, as well as collection of additional data that could be more reliable. The goal of this effort was to help the applicant apply suitable, consistent, and objective methods for measuring dystrophin protein that would be amenable to independent verification for any future biopsies for patients in Study 201/202 and other planned studies. These improved methods were applied to the following:

- Week 180 biopsy
- Re-read of immunofluorescence images from the first three biopsies
- Re-do of immunofluorescence and Western blot analysis of the baseline samples for the three eteplirsen-treated patients who had archived pre-treatment muscle tissue<sup>2</sup>.
- Immunofluorescence and Western blot analysis for six external untreated patients with DMD amenable to exon 51 skipping (i.e., patients who were not participants in Study 201/202). These external untreated patients and three baseline samples from eteplirsen-treated patients were compared with the treated week-180 samples from eleven treated patients together in the same experimental analyses.

It is important to note that Week 180 biopsies in eteplirsen-treated patients came from the deltoid, while biopsies for the external controls and preserved baseline muscle samples came from the biceps in all but one patients. As dystrophin expression is known to vary between muscles, this difference creates an additional source of variability in the study results.

#### Expression of the dystrophin messenger RNA in DMD patients muscle

The applicant evaluated the effect of eteplirsen on production of dystrophin messenger RNA in Study 33, Study 28, and Study 201/202. Skipping of the mRNA exon was assessed using reverse transcriptase polymerase chain reaction (RT-PCR), a standard technique commonly used in molecular biology laboratories to detect RNA expression. The PCR results of Study 33 showed an apparent dose-response in exon 51 skipping. As discussed by Dr. Rao, some baseline samples of Study 201 also showed a skipped mRNA band, likely due to revertant or trace dystrophin mRNA. Dr. Rao also observes that after eteplirsen-treatment, an appreciably pronounced band for the skipped mRNA was apparent in each of the 11 post-treatment samples of patients from Study 201. Dr. Rao also notes that the applicant's RT-PCR technique is not quantitative due to a lack of a reference gene. In addition, the presence of an exon skipped band does not indicate that the mRNA was translated into a functional protein.

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<sup>2</sup> An important limitation of the re-do of immunofluorescence and Western blot s that tissue (and protein) for the 3 patients who had preserved (frozen) baseline samples is that degradation of proteins is known to occur over time, and the effect that extended freezing of the sample samples had on dystrophin results is impossible to quantify.

Therefore, this biomarker provides little support of efficacy for eteplirsen; it does, however, provide evidence that eteplirsen causes at least some degree of exon 51 skipping, as intended.

### Production of Dystrophin Protein in Muscle

The applicant evaluated the effect of eteplirsen on dystrophin expression primarily in Study 201/202, but also in Study 28 and Study 33. Production of dystrophin was assessed by two different methods: immunofluorescence (IF) and Western blot. In considering these two measures, it is important to note that Western blot is considered to be a quantitative method, whereas immunofluorescence is generally considered to be less quantitative, and is more often relied upon to show the localization of protein in tissue sections. The applicant used Western blot to quantify dystrophin protein. Immunofluorescence methods were used to distinguish “positive” muscle fibers, i.e., those with at least some degree of positivity, from “negative” muscle fibers in tissue biopsy sections, and the data were also analyzed based on the staining intensity of identified areas of tissue sections. I discussed above the dystrophin expression results of Study 28 and 33. I will now review the dystrophin expression results for Study 201/202.

### **Immunofluorescence (IF)**

The immunofluorescence technique can be used to look at the percentage of dystrophin-positive fibers, and at the levels of dystrophin intensity per fiber. As discussed by Dr. Farkas, the applicant’s definition of a positive fiber was not based on a threshold amount of dystrophin or staining brightness, but rather only on “a majority of the fiber perimeter stain at an intensity judged by eye to be above background of the image.” Consequently, “17% positive fibers” does not correspond to 17% of normal dystrophin levels, or to 17% of fibers being as bright as in BMD. The percent positive fiber result is, instead, mainly useful for localization of dystrophin, not quantification.

#### *Percentage of dystrophin positive fibers*

The percentage of dystrophin-positive fibers in tissue obtained from muscle biopsies was the prospectively planned primary endpoint of Study 201. Substantial increases in dystrophin in Study 201 were initially reported in a publication,<sup>3</sup> which stated the “...percentage of dystrophin-positive fibers was increased to 23% of normal; no increases were detected in placebo-treated patients ( $p \leq 0.002$ ). Even greater increases occurred at week 48 (52% and 43% in the 30 and 50 mg/kg cohorts, respectively....)”. However, as discussed above, there were technical problems with the initial analyses of the first three biopsies, and the biopsies were reanalyzed by three blinded readers. It is important to note that this reanalysis, as

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<sup>3</sup> *Ann Neurol* 2013;74:637

discussed by Dr. Rao and Dr. Farkas, does not address all of the methodological issues that were identified, and still has significant interpretability concerns.<sup>4</sup>

With these limitations in mind, on re-analysis of the first three biopsies by the three blinded readers, the changes in percent of positive fibers were considerably lower than those initially reported in the Nationwide Children's Hospital analysis, and also were inconsistent between the treatment groups, as illustrated in Table 2. For example, for the patients who were started on eteplirsen 50 mg/kg weekly from the beginning of Study 201, the mean percent dystrophin-positive fibers had an apparent modest increase, from 15% at baseline to 17% at Week 12, and to 25% at Week 48. However, for patients initially on placebo and switched to eteplirsen 50 mg/kg weekly at Week 24, there was no increase noted in the percent dystrophin-positive fibers between baseline and Week 48. As these patients, by Week 48, had received 24 weeks of treatment with eteplirsen, the results can directly be compared with the first 24 weeks of treatment in patients who immediately received eteplirsen treatment in Study 201. The discrepancy is obvious, and adds to the multiple concerns noted about the robustness and interpretability of the dystrophin data in Study 201/202. Of note, the change from baseline in percent of dystrophin positive fibers as measured in the muscle biopsy tissue using immunohistochemistry was the primary endpoint of Study 201. As noted by Dr. Ling, statistical reviewer, there was no statistically significant difference between the 50 mg/kg eteplirsen group and placebo at Week 12 ( $p=0.958$ ). At Week 24, the mean percentage of dystrophin positive muscle fibers was higher in the eteplirsen 30 mg/kg group than the placebo. However, the nominal p-value (0.002) for the comparison between eteplirsen 30 mg/kg group and the placebo group can only be considered exploratory, as there was no plan to control the type-1 error due to multiple comparisons, and because the other primary endpoint comparison between the 50 mg/kg group and placebo was negative.

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<sup>4</sup> For example, Week 48 samples were processed separately for dystrophin immunofluorescence from earlier samples, and had higher background staining. As a consequence, valid comparison is not possible with earlier time points for percent positive fibers or total immunofluorescence because the higher background staining, and not necessarily an effect of drug, could be responsible for any differences observed.

**Table 2: Study 201 immunofluorescence results for first three muscle biopsies (% positive fibers)**

	Nationwide Children’s Hospital analysis				Re-analysis by 3 blinded readers				
	Baseline	Week 12	Week 24	Week 48	Baseline	Week 12	Week 24	Week 48	Week 180
30 mg/kg (n=4)	18		41	70	14		27	23	17
50 mg/kg (n=4)	11	12		54	15	17		25	
Placebo to 30 mg/kg (n=2)	24		24	58	10		10	9	
Placebo to 50 mg/kg (n=2)	7	7		49	11	9		10	

For the eleven eteplirsen-treated patients who had a biopsy at Week 180, the three blinded veterinary pathologists reported a mean of 17% of dystrophin-positive fibers for the eteplirsen-treated patients, a level considerably lower than reported by Nationwide Children’s Hospital for the first three biopsies.<sup>3</sup> Week 180 biopsies were also compared with untreated controls (i.e., preserved baseline tissues of three eteplirsen-treated patients and the six external controls). The untreated control patients were reported as having about 1% dystrophin-positive fibers. For the three eteplirsen-treated patients who had retained baseline samples, the proportion of dystrophin-positive fibers upon reanalysis respectively was 1.1%, 2.6%, and 0.2% of normal. This contrasts with original baselines values, respectively, of 11.7%, 17%, and 18.9%. As discussed by Dr. Breder, the basis for the differences in the percent positive fibers from the time they were originally stained and the time of the 4th biopsy is not known; however, because they were stained with the same antibody and nearly the same procedure, one would expect the levels to be similar. One factor which is concerning to Dr. Breder, and to me, is that the tissue for the fiber staining as well as the other biomarker assays had been in the freezer for about 3 years. Without a method to control for or evaluate the potential loss of immunoreactivity, the protein may have undergone changes which would result in a lesser level in the biomarker assays. For the two patients with retained baseline muscle samples who also had a biopsy at Week 180 (Patient 013 and Patient 015), the proportion of dystrophin-positive fibers at Week 180 respectively was 19.1%, and 18.5%. This number contrasts with

baseline values in eteplirsen-treated patients (as reanalyzed by the three blinded readers), ranging between 10 and 15% of fibers; it is unclear what role differences between the analytical methods, or other factors, such as a difference in muscle sampled, or protein degradation over time, played in the discrepant results. Also, the data were analyzed in a single laboratory, fraught with methodological issues during the development program and have not been independently substantiated.

#### *Levels of dystrophin intensity per fiber (“Bioquant”)*

As discussed by Dr. Breder and by Dr. Rao, after breaking the blinding code, the applicant discarded their original analysis, as according to the applicant, this magnification did not “allow for optimal differentiation of the muscle fibers for quantitation”. It is important to note that this original analysis was negative, while the post hoc analysis conducted by the applicant shows some numerical increases in the average fiber intensity in the eteplirsen treatment group, compared with placebo. As noted by Dr. Rao, dismissing the original analysis is not good scientific practice.

For the fourth biopsy, the applicant reported that the muscle biopsy from Week 180 displayed a statistically significant increase in the relative associated fluorescence intensity. The mean relative fluorescence value for treated patients was reported as 22.6 versus 9.4 for the untreated control samples, which came from a population of six untreated DMD boys, and the baseline biopsy from three of the original eteplirsen treated patients. An important limitation of the Week 180 Bioquant analysis is that there were no matched controls from the same patients and same muscle groups for all treated samples. As discussed by Dr. Breder, it is not clear how similar the external controls were to the treated patients, and it is not clear that the applicant selected the external controls completely at random, i.e., bias may have been introduced.

Overall, the immunofluorescence data do not provide consistent evidence that the percent of dystrophin positive fibers may have increased as a result of eteplirsen treatment. The issues described above deeply affect the interpretability of the findings, and make any quantification of the changes unreliable. In addition, as analyses based on immunofluorescence overestimate the amount of dystrophin in tissue sections because a muscle fiber can be considered “positive” if it exhibits any staining at all, the percent dystrophin-positive fibers by immunofluorescence is not the most meaningful way to estimate dystrophin content. The Western blot analyses are informative for that purpose.

#### **Western Blot**

The applicant provided a second line of evidence, Western blot analysis, to support the concept that eteplirsen increases dystrophin production in skeletal muscle. As discussed by Dr. Rao, the Western blots from the first 3 biopsies had oversaturated bands, did not have appropriate

controls or quality control metrics and were essentially uninterpretable. Therefore, the results of Western blot analyses for the first three biopsies do not merit discussion in this memo.

As discussed by Dr. Rao, the methodologies used by the applicant were relatively improved for the 4th biopsy. The applicant, however, used a different antibody (Dys 1) for the fourth biopsy Western blots, potentially confounding comparisons to the patients' original pre-treatment baseline values (which were assessed with Mandys106 antibody in all but one patients). As the Western blot assessments prior to Week 180 were essentially uninterpretable, and used a different antibody, FDA suggested that the applicant attempt reassessing baseline dystrophin levels, i.e., pre-treatment, for patients who had available baseline muscle samples, together with the Week 180 samples. Of the three patients who had retained baseline samples, only two also had a biopsy at Week 180: Patient 13, and Patient 15 (presented in Table 4). In that reassessment on the retained sample which had been frozen for about 3 years, both of these patients had baseline dystrophin levels below the level of quantification, i.e., below 0.25%. As for immunofluorescence analyses, data from external controls were used to supplement the limited baseline samples that were available for re-analysis. When all of the untreated and baseline samples are considered, the applicant reports a value of dystrophin level of 0.08% of normal in controls.

There are, however, important limitations with respect to interpretation of the results of these controls. We already discussed that Week 180 biopsies in eteplirsen-treated patients were obtained from the deltoid, while control biopsies came from the biceps in all but one patient, for whom the biopsy also came from the deltoid. As discussed by Dr. Farkas, the deltoid is one of the few muscle groups that, along with the calf muscle, can be hypertrophied in DMD. It is not clear to what extent differences in dystrophin expression between muscle groups may have contributed to the change in dystrophin reported for the 4th biopsy. Also, as discussed by Dr. Breder, the untreated DMD controls used in the fourth biopsy analyses were not necessarily selected at random from a representative patient population, as they came from patients from the ongoing eteplirsen Phase 3 confirmatory study 4658-301. Finally, the tissue was not of comparable quality (i.e., fresh versus frozen for about 3 years) for Week 180 biopsies vs. those of controls. Because of these issues, Dr. Rao concluded that it is not clear exactly how much dystrophin, if any, was made based on a drug effect at the time of the fourth biopsy.

Notwithstanding these critical limitations, by Western blot, the most accurate quantitative method used by the applicant, the mean dystrophin level after about 3.5 years of eteplirsen treatment (at Week 180) was 0.93%. Table 3, adapted from the applicant's submission, shows the results for dystrophin quantification from the fourth biopsy for the eleven patients who consented to muscle biopsies at Week 180. Most patients had two separate Western Blot estimates, and the values were averaged to provide the final results. It is also noteworthy that three of the patients had a variability of 0.7% or greater between their measurements. Also,

there was a poor correlation between immunofluorescence and Western blot data.

**Table 3: Applicant’s Quantification of Dystrophin by Western Blot at Week 180 (% of normal)**

Subject	Test 1 (%)	Test 2 (%)	Mean	Intra-Patient variability
002	0	0.28	0.14	0.28
003	0 0	0	0	0
004	1.22	0.69	0.955	0.53
006	2.83	2.11	2.47	0.72
007	0	0	0	0
008	0.93	1.02	0.975	0.09
009	0.58	0.46	0.52	0.12
010	1.45	1.78	1.615	0.33
012	0.75	0	0.375	0.75
013	1.15		1.15	-
015	2.43	1.67	2.05	0.76

Because of the limitations in controls used to interpret Week 180 dystrophin findings, it was not clear exactly how much dystrophin, or even if any dystrophin at all, was made in response to the drug. As additional muscle biopsies at baseline and after 48 weeks of eteplirsen treatment were available in an ongoing eteplirsen study (“PROMOVI Study<sup>5</sup>”), the applicant was asked to analyze these samples and submit the results in order to provide substantiation of the dystrophin findings of Study 201/202. Western blots were conducted on samples from 13 patients treated with eteplirsen 30 mg/kg/week for 48 weeks. The Western blot methods used for these additional analyses were generally similar to those used for the Week 180 muscle samples from Study 201/202. Twelve of the 13 patients had paired biceps biopsies, and a

<sup>5</sup> The PROMOVI study is an open-label, multi-center, 96-Week study of eteplirsen in patients with mutations amenable to exon 51 skipping compared with a concurrent untreated control arm composed of patients not amenable to exon 51 skipping

single patient had paired triceps biopsies. Results are available for 12 out of the 13 patients, as both gels for one patient failed acceptance criteria. Table 4, Table 5, and Table 6 summarize the Western blot results. Dystrophin levels that were below the level of quantification (0.25% of normal) were imputed as 0.24% in Table 4, imputed as zero in Table 5, or presented as the observed value in Table 6. Of note, actual values under 0.25% may represent less accurate estimates, because of the validation cutoffs (0.25% to 4%) for the assay, but still represent actual values that can be used to estimate the treatment effect, while keeping in mind the lower accuracy of these values. On the other hand, considering all values under 0.25% as zero introduces a greater imprecision, and magnifies changes from baseline if the actual value is greater than zero percent. Regardless of the method of imputation of baseline dystrophin data, there was a statistically significant difference in dystrophin levels between baseline and Week 48. The magnitude of the effect, however, is very small, in the order of 0.3% of normal values, on average.

**Table 4: Western Blot results in boys from the Promovi Study (levels below level of quantification imputed as 0.24%)**

	Baseline	Week 48	Change From Baseline	Fold Change From Baseline
n	12	12	12	12
Mean (% Dystrophin)	0.260	0.478	0.218	1.915
SD (SE)	0.0469 (0.0135)	0.4066 (0.1174)	0.4173 (0.1205)	1.7331 (0.5003)
Median	0.240	0.330	0.018	1.066
Min, Max	0.24, 0.37	0.24, 1.57	-0.07, 1.33	0.81, 6.54
			<b>P = 0.041</b>	

**Table 5: Western Blot results in boys from the Promovi Study (levels below level of quantification imputed as 0%)**

	Baseline	Week 48	Change From Baseline	Fold Change From Baseline
n	12	12	12	12
Mean (% Dystrophin)	0.060	0.378	0.318	3229.320
SD (SE)	0.1402 (0.0405)	0.4760 (0.1374)	0.5026 (0.1451)	4986.0753 (1439.3560)
Median	0.000	0.275	0.078	725.514
Min, Max	0.00, 0.37	0.00, 1.57	-0.07, 1.57	0.00, 15700.00
			<b>P = 0.023</b>	

**Table 6: Western Blot results in boys from the Promovi Study (actual values)**

	Baseline	Week 48	Change From Baseline	Fold Change From Baseline
n	12	12	12	12
Mean (% dystrophin)	0.157	0.440	0.283	3.723
SD (SE)	0.1159 (0.0335)	0.4341 (0.1253)	0.4153 (0.1199)	3.0189 (0.8715)
Median	0.150	0.330	0.098	2.485
Min, Max	0.02, 0.37	0.09, 1.57	-0.07, 1.33	0.81, 10.44
			<b>P = 0.008</b>	

Individual dystrophin results for the “PROMOVI” patients are presented in Table 7.

**Table 7: Individual Western Blot results in boys from the Promovi Study**

SR-CR-16-003 Patient WB Analysis  
4658-301 Week 48 Interim Analysis

June 27, 2016

Western Blot Analysis

Listing 1.1  
Western Blot Results  
Values < 0.25 Treated as Reported

Dummy ID	Time Point	Image Filename	Gel #	Blinded Sample ID	Pass/Fail	Calculated % Dystrophin	Average Value	Change from Baseline	Fold Change
301-01	Baseline	SR-CR-16-003_GEL#1_DYS1_30MIN.TIF	1	FORD-22559	PASS	0.15	0.13		
	Week 48	SR-CR-16-003_GEL#2_DYS1_30MIN.TIF	2	FORD-22559	PASS	0.11	0.26	0.13	1.96
301-02	Baseline	SR-CR-16-003_GEL#3_DYS1_20MIN.TIF	3	CHEVY-27336	PASS	0.35	0.35		
	Week 48	SR-CR-16-003_GEL#4_DYS1_30MIN.TIF	4	CHEVY-27336	FAIL	0.26	0.36	0.01	1.03
301-03	Baseline	SR-CR-16-003_GEL#5_DYS1_30MIN.TIF	5	FORD-24422	PASS	0.06	0.06		
	Week 48	SR-CR-16-003_GEL#6_DYS1_30MIN.TIF	6	FORD-24422	PASS	0.06	0.37	0.31	6.17
301-04	Baseline	SR-CR-16-003_GEL#7_DYS1_30MIN.TIF	7	FORD-27138	PASS	0.04	0.04		
	Week 48	SR-CR-16-003_GEL#8_DYS1_30MIN.TIF	8	FORD-27138	FAIL	0.06	0.10	0.06	2.50
301-05	Baseline	SR-CR-16-003_GEL#9_DYS1_30MIN.TIF	9	FORD-28500	FAIL	0.10	0.17		
	Week 48	SR-CR-16-003_GEL#10_DYS1_30MIN.TIF	10	FORD-28500	PASS	0.17	1.02	0.85	6.00
301-06	Baseline	SR-CR-16-003_GEL#11_DYS1_20MIN.TIF	11	CHEVY-24986	PASS	0.37	0.37		
	Week 48	SR-CR-16-003_GEL#12_DYS1_30MIN.TIF	12	CHEVY-24986	FAIL	0.46	0.30	-0.07	0.81
301-07	Baseline	SR-CR-16-003_GEL#13_DYS1_15MIN.TIF	13	FORD-20841	FAIL	0.04	0.17		
	Week 48	SR-CR-16-003_GEL#14_DYS1_15MIN.TIF	14	FORD-20841	PASS	0.17			

Note: For calculation of Fold Change, baseline values of 0 were imputed as 0.0001.

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Page 17 of 2  
Sarepta Therapeutics, Inc.  
4658-301  
Western Blot Analysis

Listing 1.1  
Western Blot Results  
Values < 0.25 Treated as Reported

Dummy ID	Time Point	Image Filename	Gel #	Blinded Sample ID	Pass/Fail	Calculated % Dystrophin	Average Value	Change from Baseline	Fold Change
301-07	Week 48	SR-CR-16-003_GEL#13_DYS1_15MIN.TIF	13	CHEVY-20841	FAIL	0.22	0.42	0.25	2.47
		SR-CR-16-003_GEL#14_DYS1_15MIN.TIF	14	CHEVY-20841	PASS	0.42			
301-08	Baseline	SR-CR-16-003_GEL#15_DYS1_15MIN.TIF	15	FORD-22355	FAIL	0.08	0.11		
		SR-CR-16-003_GEL#16_DYS1_15MIN.TIF	16	FORD-22355	FAIL	0.14			
	Week 48	SR-CR-16-003_GEL#15_DYS1_15MIN.TIF	15	CHEVY-22355	FAIL	0.08			
		SR-CR-16-003_GEL#16_DYS1_15MIN.TIF	16	CHEVY-22355	FAIL	0.05			
301-09	Baseline	SR-CR-16-003_GEL#17_DYS1_15MIN.TIF	17	CHEVY-28907	FAIL	0.14	0.24		
		SR-CR-16-003_GEL#18_DYS1_15MIN.TIF	18	CHEVY-28907	PASS	0.24			
	Week 48	SR-CR-16-003_GEL#17_DYS1_15MIN.TIF	17	FORD-28907	FAIL	1.17			
		SR-CR-16-003_GEL#18_DYS1_15MIN.TIF	18	FORD-28907	PASS	1.57			
301-10	Baseline	SR-CR-16-003_GEL#19_DYS1_15MIN.TIF	19	FORD-29648	PASS	0.11	0.11		
		SR-CR-16-003_GEL#20_DYS1_15MIN.TIF	20	FORD-29648	FAIL	0.05			
	Week 48	SR-CR-16-003_GEL#19_DYS1_15MIN.TIF	19	CHEVY-29648	PASS	0.12			
		SR-CR-16-003_GEL#20_DYS1_15MIN.TIF	20	CHEVY-29648	FAIL	0.11			
301-11	Baseline	SR-CR-16-003_GEL#21_DYS1_15MIN.TIF	21	CHEVY-29727	PASS	0.01	0.05		
		SR-CR-16-003_GEL#22_DYS1_15MIN.TIF	22	CHEVY-29727	PASS	0.08			
	Week 48	SR-CR-16-003_GEL#21_DYS1_15MIN.TIF	21	FORD-29727	PASS	0.31			
		SR-CR-16-003_GEL#22_DYS1_15MIN.TIF	22	FORD-29727	PASS	0.63			
301-12	Baseline	SR-CR-16-003_GEL#23_DYS1_15MIN.TIF	23	CHEVY-29751	PASS	0.02	0.02		
		SR-CR-16-003_GEL#24_DYS1_15MIN.TIF	24	CHEVY-29751	FAIL	0.00			
	Week 48	SR-CR-16-003_GEL#23_DYS1_15MIN.TIF	23	FORD-29751	PASS	0.09			
		SR-CR-16-003_GEL#24_DYS1_15MIN.TIF	24	FORD-29751	FAIL	0.01			
301-13	Baseline	SR-CR-16-003_GEL#25_DYS1_15MIN.TIF	25	FORD-25715	FAIL	0.34	0.18		
		SR-CR-16-003_GEL#26_DYS1_15MIN.TIF	26	FORD-25715	PASS	0.18			
	Week 48	SR-CR-16-003_GEL#25_DYS1_15MIN.TIF	25	CHEVY-25715	FAIL	0.34			
		SR-CR-16-003_GEL#26_DYS1_15MIN.TIF	26	CHEVY-25715	PASS	0.21			

Note: For calculation of Fold Change, baseline values of 0 were imputed as 0.0001.  
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The categorical changes from baseline in dystrophin muscle content across the PROMOVI study and Study 201/202 are summarized in Table 8. Importantly, the table must be read with an understanding that the percent changes are not directly comparable between the studies, as the Western blots were not run concurrently and methodological differences may have affected the results. The results, for example, cannot be reliably be used to assess whether longer duration of treatment leads to greater dystrophin production, unless the differences are large.

Based on a comparison of Week 48 to baseline using reported dystrophin values, most patients (about 60%) from the PROMOVI study had no increase in dystrophin content, or an increase smaller than 0.25% of normal levels. A single patient had a dystrophin increase greater than 1%, and no patient had a dystrophin increase greater than 2% (see Table 8).

In comparison, about a third of patients from Study 201/202 had no increase in dystrophin content, or an increase smaller than 0.25% of normal levels, while about a third of patients had dystrophin increases greater than 1% of normal levels. A single patient had a dystrophin increase greater than 2%, and no patient had a dystrophin increase greater than 3% (see Table 8).

Across both studies, about 20% of patients had a dystrophin increase of 1% of normal values or greater, while an increase greater than 2% was seen in a single patient (which represents 4% of the sample). Of note, there is some variability in normal values of dystrophin in healthy subjects, and using as a normal reference a lower dystrophin level would obviously lead to higher estimates of increases in dystrophin levels (e.g., using as “normal” reference a level 50%

lower than used as a reference by the applicant would have led to conclude that about 20% of patients had a dystrophin increase of 2% or more).

**Table 8: Categorical changes from baseline in Study 201/202 and in the PROMOVI study**

	PROMOVI (n=12) using actual values	Study 201/202 (n=11) using a baseline of 0.08% <sup>‡</sup>	Study 201/202 (n=11) using a baseline of 0.16%*
0% to 0.24%	7 (58%)	3 (27%)	4 (36%)
0.25% to 0.49%	3 (25%)	2 (18%)	1 (9%)
0.5% to 0.99%	1 (8%)	2 (18%)	3 (27%)
1% to 1.49%	1 (8%)	2 (18%)	1 (9%)
1.50% to 1.99%	0	1 (9%)	1 (9%)
2% to 2.5%	0	1 (9%)	1 (9%)

<sup>‡</sup>Based on dystrophin levels in controls of Study 201/202 (primarily external)

\*Based on actual baseline value of 0.157% in the PROMOVI sample

### **Clinical Effects Reflecting Muscle Function**

Study 201/202 is the only efficacy study submitted by the applicant (Figure 1).

Study 201/202 began as a 24-week randomized controlled study comparing three groups of patients treated weekly with intravenous eteplirsen 50 mg/kg, eteplirsen 30 mg/kg, or placebo (Study 201). After the randomized placebo-controlled phase, patients entered an open-label extension phase, i.e., Study 202. Study 201 and Study 202, however, assessed the same patients, and de facto constitute two phases of the same study.

The prospectively planned primary endpoint in Study 201 was the change from baseline in percent of dystrophin positive fibers in muscle tissue. The study had two pre-specified secondary endpoints: 1) change from baseline in CD3, CD4, and CD8 lymphocyte counts in muscle biopsy tissue at Week 12 or Week 24; and 2) change from baseline to Week 24 in 6-Minute Walk Test (6MWT).

The primary functional endpoint of Study 202 was comparison of Week 48 6MWT results for boys originally randomized to eteplirsen versus those originally randomized to placebo. A co-primary endpoint was dystrophin production at Week 48.

For the prospectively planned analysis in Study 201, there was no statistically significant difference on the change from baseline to Week 24 in 6MWT distance between eteplirsen 50 mg/kg, eteplirsen 30 mg/kg, and placebo.

Similarly, for the prospectively planned 6MWT analysis in Study 202, there was no significant difference between eteplirsen treated and placebo patients.

Two patients in the 30 mg/kg group became unable to ambulate soon after the study started. The applicant then pooled the six remaining eteplirsen patients and compared them with the four placebo patients, an unplanned post hoc analysis. No nominally significant difference between eteplirsen and placebo was identified in that post hoc analysis.

The applicant conducted a number of additional post hoc analyses, comparing the six patients who received eteplirsen in the 24-week double-blind phase of Study 201 and could still ambulate at the end of Study 201 (and continued on open-label eteplirsen in Study 202) to those originally treated with placebo in the double-blind phase of Study 201, and later switched to open-label eteplirsen. Based on these analyses, the applicant stated<sup>6</sup> that “48 weeks of treatment with eteplirsen resulted in an unprecedented and clinically meaningful 67.3-meter clinical benefit on the 6MWT compared to placebo for 24 weeks followed by eteplirsen for 24 weeks.” Considering the post hoc nature of the analyses, the post-randomization exclusion of two patients who lost ambulation in Study 201, and the limitations of the open-label design for protecting against expectation bias on effort-dependent endpoints such as the 6MWT, FDA indicated to the applicant that data from Study 202, as presented, did not provide interpretable evidence of benefit.

The applicant continued open-label administration of eteplirsen in Study 202, and is proposing approval primarily based on a post hoc comparison of patients of all available open-label data from Study 202 (up to Week 144) with a natural history cohort of untreated patients from the “Italian DMD Registry” and the “Leuven Neuromuscular Reference Center” registry. The applicant attempted to match patients in Study 202 with patients from these two external registries based on five factors: 1) corticosteroid use at baseline (use/non-use); 2) sufficient longitudinal data for 6MWT available (Y/N); 3) age  $\geq 7$  years (Y/N); 4) genotype amenable to any exon skipping therapy (Y/N); and 5) genotype amenable to exon 51 skipping therapy (Y/N). Patients did not have to match for baseline 6MWT distance. Based on these factors, the applicant matched 13 historical control patients to the 12 eteplirsen-treated patients.

Under the proper circumstances, FDA regulations (21 CFR 314.126) recognize that historical control studies can be considered adequate and well-controlled studies, but there are many concerns with the interpretability of such studies. These are discussed in detail in international guidelines (International Conference on Harmonization Guideline, “Choice of Control Group

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<sup>6</sup> End-of-Phase 2 meeting of March 13, 2013.

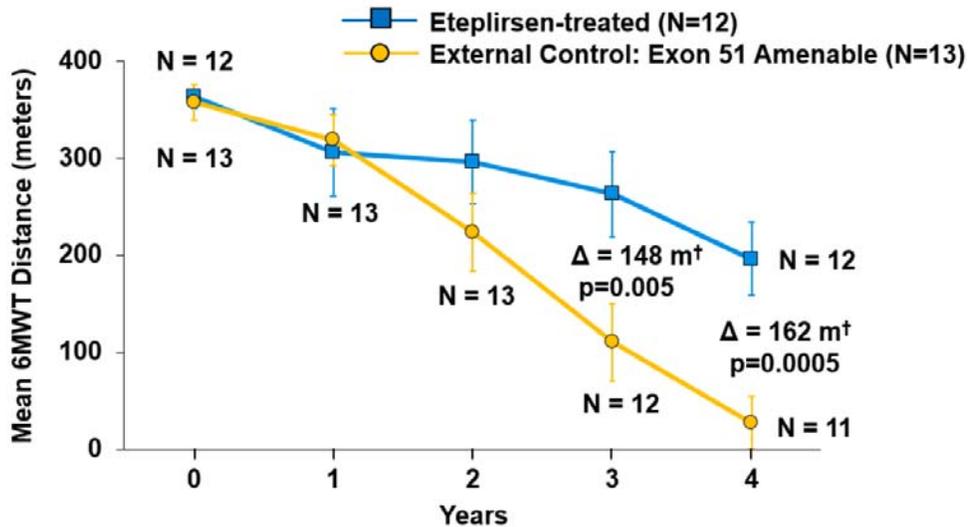
and Related Issues in Clinical Trials” – ICH E10 [2000]). FDA identified several issues related to the use of an external natural history for the applicant to address in the NDA. FDA asked the applicant to establish that treatment modalities, including the physical therapy programs and steroid regimens used, were similar between patients from Study 201/202 and the externally-controlled population. FDA also noted that for most of its duration, Study 201/202 was open-label, with all patients receiving eteplirsen, and that performance on the 6-minute walk test could be influenced by expectation bias, motivation, and coaching. The patients in the external control group may not have been subject to these factors because they were not in a study and were not receiving an investigational therapy. Another issue is that the registries that served as the external control were identified and patient selection criteria were developed in February 2015, at a time when data on the 6-minute walk test were available in Study 201/202 for more than three years, and much of the data had already been generated in the external control group. A limited amount of the longitudinal data for the external control group was generated after selection of the patients, from February to December, 2015. The impact of these factors on the interpretability of the between-group comparisons cannot be determined.

With these issues in mind, I will now review the results of the comparison to the external historical control.

The baseline characteristics between eteplirsen-treated patients and external controls were reasonably well matched by age, height, and weight, but had some important differences. The main one probably is that the mean age of initiation of steroid treatment was over one year later in the control group than in eteplirsen-treated patients (age 6.4 years vs. 5.2 years). As described by Dr. Breder, there were also differences in steroid regimens used (e.g., in the proportion of patients using a continuous steroid treatment). In addition, mean NSAA scores at baseline were lower in historical control patients, indicating greater disease severity in those patients. The impact of these differences is impossible to estimate in the context of a non-randomized study.

The applicant describes highly statistically significant results in the comparison between boys treated with eteplirsen in Study 201/202 and external controls, presenting a difference of 162 meters between the groups ( $p=0.0005$ ). The applicant also describes that, in a comparison of eteplirsen to external control over 4 years, only two of the eteplirsen-treated boys lost ambulation, compared with 10 of the 13 untreated external controls (Figure 2).

**Figure 2: Mean 6MWT Distance over Time in Eteplirsen-Treated Patients vs. External Controls (copied from applicant's Advisory Committee Briefing materials, page 64)**



† Difference in mean change from baseline  
 Patients who lost ambulation contributed a score of 0 to the mean  
 1 EC Subject was missing data at Year 3 & 4, 1 EC Subject was missing data at Year 4 only

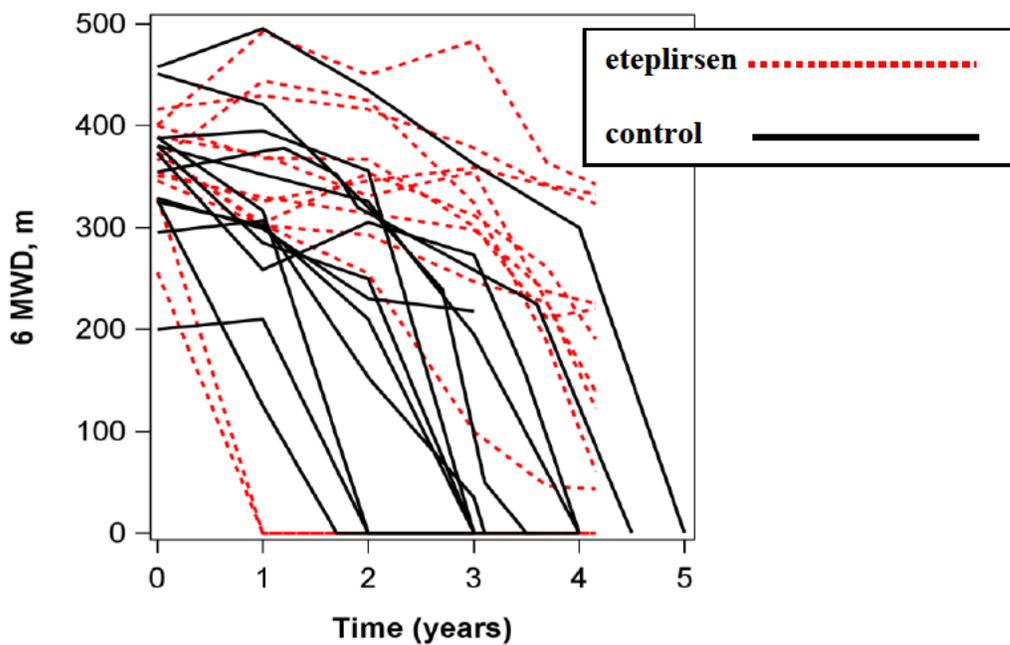
The natural history in patients with DMD amenable to exon 51 skipping indicates a wide age range at the time of loss of ambulation, from 8 to 18 years of age for most patients. As the applicant is proposing a comparison to a historical control, it is critical that convincing evidence be provided that the clinical course of the 12 patients participating in Study 201/202 differs appreciably from the expected natural history of DMD, and, in light of the nature of the control group, whether a difference, if present, is interpretable.

I agree that a 160-meter difference in 6-minute walk distance, if demonstrated in an adequate and well controlled study, would provide evidence of effectiveness. Several lines of evidence, however, raise considerable concerns that the differences in ambulation between eteplirsen-treated boys and external controls are not related to a treatment effect, and may be due to other factors:

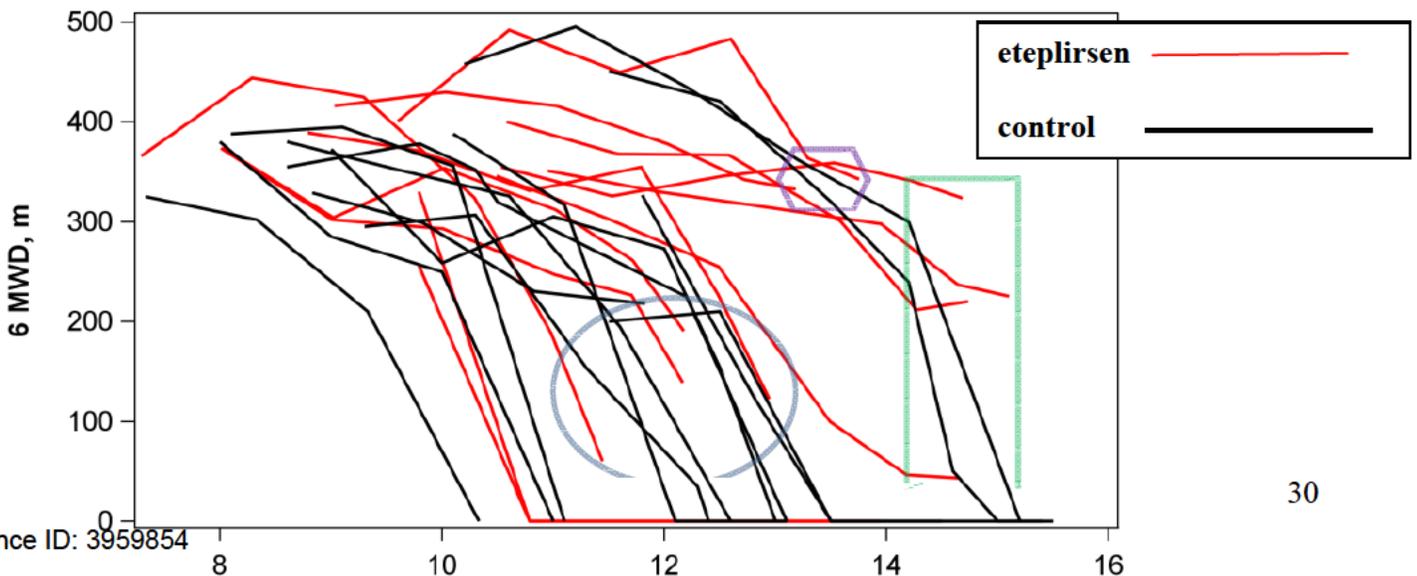
- a. As discussed above, there were differences between important baseline characteristics that could affect outcomes in boys enrolled in the eteplirsen study compared to those of the registries. Also, as described by Dr. Farkas, recent observational studies in DMD have been enrolling patients simultaneously with interventional trials of new drugs. Thus, patients in an observational cohort who were motivated to participate in an interventional drug study and who could qualify for enrollment might have dropped out of the observational study. With preferential loss of such subjects, patients who remained in the observational study may have been less motivated or less able to participate in interventional studies of new drugs, and in this sense, their prognosis could be worse.
- b. There is considerable overlap between 6MWT results for eteplirsen-treated patients and historical controls. Figure 3 and Figure 4 respectively show the evolution of 6MWT as a

function of time and as a function of age. It is important to note that both the analyses have limitations, and that there is no ideal way to present these data. However, as age has a major impact on ambulation in DMD patients, the analysis and display by age appear to be the most appropriate approach, acknowledging that all patients of a given age may have had a different duration of eteplirsen treatment, which also has a possible impact on test results. With these limitations in mind, Figure 4 and Figure 4 show that the patterns of progression are generally similar between study patients and controls.

**Figure 3: 6MWT distance vs. duration of observation in eteplirsen-treated patients in Study 201/202 and external control from the "Italian DMD Registry" and the "Leuven Neuromuscular Reference Center" registry (copied from Dr. Farkas' review)**



**Figure 4: 6MWT distance vs. age in eteplirsen-treated patients in Study 201/202 and external control from the "Italian DMD Registry" and the "Leuven Neuromuscular Reference Center" registry (adapted from Dr. Farkas' memo)**



It is noteworthy that, although only two eteplirsen-treated patients have lost ambulation by the time of data cutoff for NDA submission, four patients younger than age 14 at the time of their last observations (identified by a blue oval shape on Figure 4) appear to have a disease course extremely close to that of controls of similar age, and appear very likely to be on a path to loss of ambulation before or by age 14 (in fact, one of them recently did, as reported in a data update submitted by the applicant after the April 25 Advisory Committee meeting, and another patient has a 6MWT distance of 31 meters, which, as discussed below, would be considered as loss of ambulation in the registry studies). Two eteplirsen-treated patients (identified in the purple hexagon of Figure 4), still ambulatory after age 13, but having not yet reached age 14 at the time of their last observations, appear to have a course no different than the two control patients still ambulatory at age 14.

An interesting observation made by Dr. Farkas is that the patients who started eteplirsen treatment at younger ages appear to be declining more rapidly than patients who started at older ages. For example, the youngest patient, Patient 3, has essentially lost the ability to ambulate prior to age 12 years, and the second and third youngest patients, who are 12.2 years old, are now walking about 100 meters (98 m and 125 m). Each of these patients had baseline 6MW distances >350 meters, such that a decline in 6MWT distance seemingly could not be attributed to initiating treatment beyond a level of muscle loss that would have prevented the potential for benefit on ambulation. Age of loss of ambulation for these patients is thus similar to the mean age of loss of ambulation predicted by natural history (e.g., the applicant indicates a mean age of loss of ambulation of about 13 years for the Italian and Belgian external controls). Dr. Farkas notes that there are not enough observations for any reliable conclusions, but the limited available data do not appear to support the hypothesis that initiating eteplirsen at younger ages would lead to an increased potential for benefit. I agree.

Dr. Farkas believes that the observation that the 14 and 15 year old eteplirsen-treated patients are generally performing better on 6MWT than the 12 year old patients may be consistent with selection bias, as preserved function at younger ages in DMD is known to predict preserved function at older ages. Dr. Farkas believes that the fact that such patients continue to perform better than average is expected. I agree.

I have further comments and observations about the subgroup of the four eteplirsen-treated patients who were still ambulatory after age 14 years at the time of the Week 216 assessment<sup>7</sup>. If the natural history of DMD was for patients to almost never be ambulatory at age 14 years, the fact that four eteplirsen-treated patients were still walking at age 14 would, even in the context of a historical control trial, be supportive of efficacy. As discussed by Dr. Farkas,

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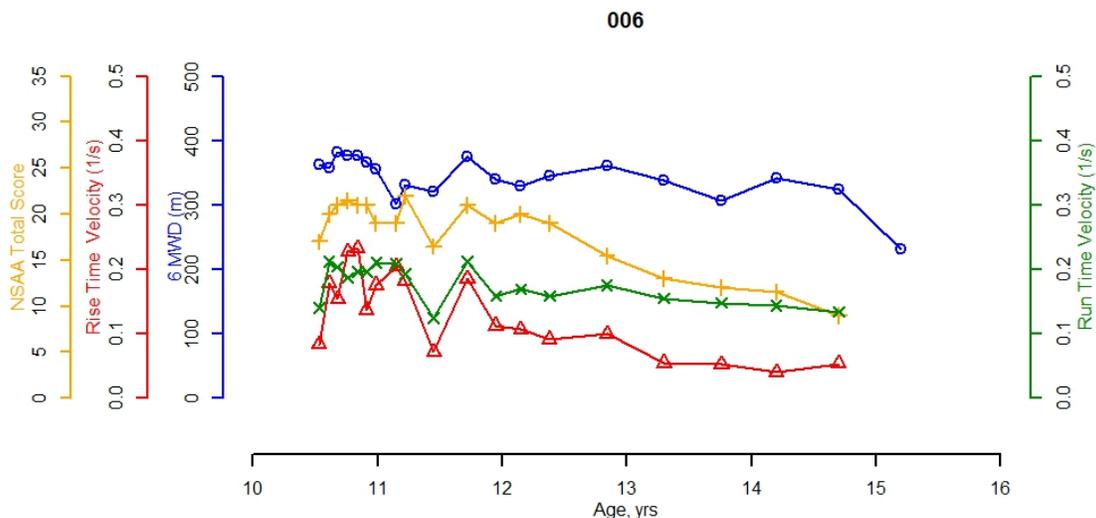
<sup>7</sup> Week 214 assessment, submitted to the NDA in December 2015, is the last one for which we have a complete set of clinical outcome measures. The applicant sent in May 2016 data from the Week 240 assessment limited to the 6MWT. These are presented in individual patient profiles, and have not been incorporated in graphs plotting mean values, because of time constraints.

natural history data from the CINRG<sup>8</sup> database supports that 25% of DMD boys may still be ambulatory at age 16, which is in line with the proportion of eteplirsen-treated patients observed to be ambulatory at age 14.

Moreover, a look at the individual profile of these four patients, plotting on a single graph all key clinical outcome measures of Study 201/202 up to Week 216, indicate a clear functional decline in all patients, which may not be immediately obvious by only looking at the 6MWT data. Note that rise time and run time are expressed on these patient profiles as velocity, so that score increases or decreases, respectively, indicate clinical improvement or worsening.

Patient 006 (Figure 5), who was on eteplirsen 30 mg/kg, had highest 6MWT distance after age 14. Patient 006 is showing a marked decline in the North Star Ambulatory index, starting about age 12 and a half. Also, rise time velocity is slowly but steadily decreasing in this patient (rise time was greater than 20 seconds at the last visit, which indicates that the patient is nearing the loss of ability to rise). Week 240 clinical data<sup>9</sup> (final timepoint plotted for 6MWT), provided by the applicant after the advisory committee meeting, show that 6MWT distance has declined to 236 meters in Patient 006, which represents a decline of about 80 meters from Week 216. Dystrophin by Western blot at Week 180 in Patient 006 was 2.47% of normal, the highest value of any patient. No baseline muscle tissue sample was retained, so it cannot be determined if this represents an increase from baseline.

**Figure 5: Clinical profile of Patient 006**

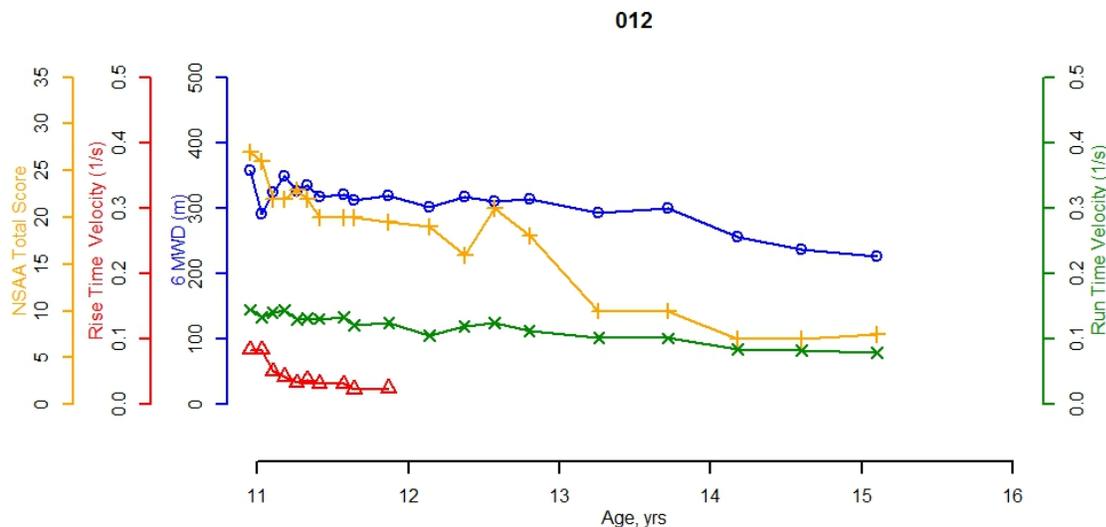


<sup>8</sup> CINRG: Cooperative International Neuromuscular Research Group <http://www.cinrgresearch.org/>

<sup>9</sup> The applicant provided a Week 240 update for 6MWT only. This update came after the Advisory Committee meeting.

Patient 012 (Figure 6), who was on eteplirsen 50 mg/kg, had the second highest 6MWT after age 14. Patient 012 is showing a marked decline on the NSAA, starting around age 12.5 years. Importantly, Patient 012 experienced a loss of ability to rise after age 12, an important milestone of disease progression. Week 240 6MWT distance is unknown in this patient, as he sustained a left femur fracture after the Week 216 visit. Dystrophin by Western blot at Week 180 in Patient 006 was 0.375% of normal. The low level of dystrophin in this patient assessed at Week 180 does not suggest that eteplirsen could have produced any significant amount dystrophin for this patient (who was on the highest dose of eteplirsen tested), and that the maintenance of relatively high 6MWT distance values at age 15 is not related to a drug effect, and instead illustrates the variability in the natural history of DMD.

**Figure 6: Clinical Profile of Patient 012**



For Patient 006 and Patient 012, the similarity in 6MWT distance, NSAA, and Run Time between age 11 years and age 15 years is striking (Figure 7). While Patient 006 had one of the highest dystrophin levels observed in eteplirsen-treated patients, Patient 012 had one of the lowest, in fact barely above the limit of quantification. These two patients illustrate that the temptation to assign the relative stability of Patient 006 to his dystrophin level must be restrained by the very similar progression of Patient 012 who, in fact, had extremely low dystrophin. That concern is reinforced by similar observations in other patients, as will be described below. In addition, a comparison with matched patients from the historical cohort (Patient PV12 and KB) shows that the course of Patient 006 and 012 is not exceptional for a DMD patient, and is compatible with the natural history of the disease (Figure 7). Specifically, the comparison of eteplirsen-treated Patient 006 to historical control Patient PV12, who both entered the study or registry around age 10 years and a half, shows the following:

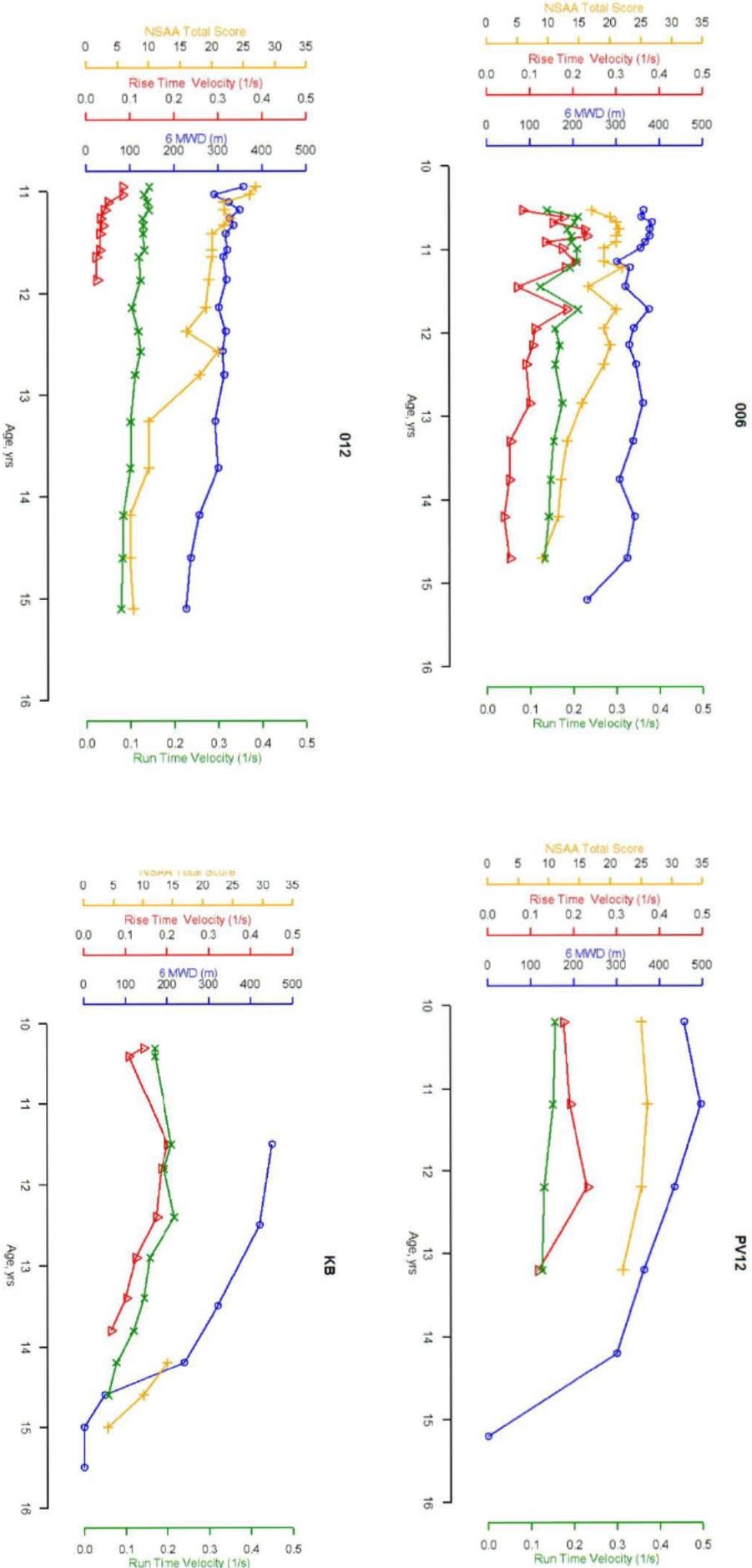
- At age 11 years, both patients had similar 6MWT distance, NSAA, rise time velocity and run time velocity.

- Between age 11 and age 12 years, both patients were fairly stable on all scales, with minor declines in some scores.
- Between age 12 and age 13 years, while 6MWT distance was more stable in Patient 006 than in Patient PV12, the NSAA score, a more comprehensive measure of ambulatory function, declined more sharply in Patient 006. The decline in rise time velocity was similar in both patients. Unfortunately, only 6MWT data are available for patient PV12 after age 13.
- Between age 13 and 14 years, patient PV 12 has a mild decline in 6MWT distance, remaining above 300 meters. By age 14 years, Patient 006 and Patient PV 12 have a similar 6MWT distance (300-350 meters), while NSAA and rise time velocity continue to decline in Patient 006.
- Between age 14 and age 15 years, Patient PV 12 was reported by the applicant as having a sharp drop in 6MWT distance, from over 300 meters to zero meters, and was considered as having lost ambulation. However, Patient PV12, in fact, fell just before age 15, and broke a leg. He was therefore unable to walk at testing time. On the other hand, between age 14 and 15 years, Patient 006 had a sharp (80 meters) decline in 6MWT distance. He has maintained ambulation at age 15. Unfortunately, NSAA, rise time and run time are not available for Patient PV12 for the last part of his observation period.

This detailed comparison of Patient 006 (the best performing patient of Study 006 up to age 14 years and a half) with Patient PV12 illustrates that the overall course of the disease is very similar in both patients, and that the course of Patient 006 is clearly within the boundaries of DMD natural history. This alone, in my opinion, is nearly sufficient to reject that a historical control design is capable of establishing the efficacy of eteplirsen, as the best performing eteplirsen-treated patient, in Study 201/202, does not have a course clearly different from natural history.

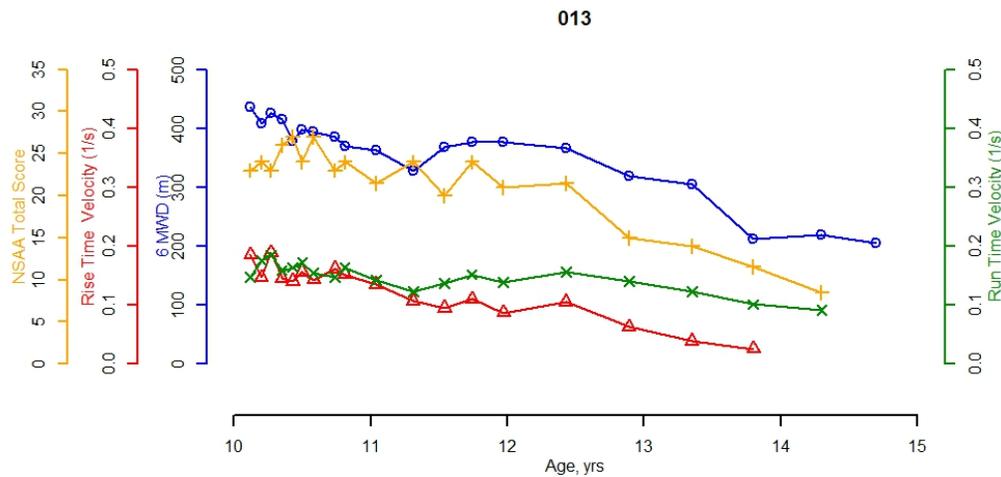
A similar observation can be made in a comparison between Patient 006 and Patient KB (Figure 7). Both patients had similar run time, rise time, and 6MWT around age 11. By age 14, they had similar 6MWT, NSAA, rise time and run time, indicating a similar disease course over a 3-year period of time. At age 14 and a half, patient KB had a sharp drop in ambulation, from ~ 300 meters to ~100 meters. At about the same time, Patient 006 has a sharp (80 meters) decline in 6MWT. Ambulation is reported as lost by age 15 in patient KB, so he has a zero 6MWT distance. Patient 006 still maintains ambulation at the same age. As discussed below, differences in the conduct of the 6MWT between patients of Study 201/202 and those of the historical control studies may account for some of the differences in reported 6MWT distances, in particular at the low end of the 6MWT distances, where encouragements, and decisions to record 6MWT even if ambulation has not lasted for a full 6 minutes can heavily bias the results. Notwithstanding the observed differences in 6MWT at the very end of the period of observation, the overall course of both patients is very similar, again indicating that Patient 006 has a progression compatible with the natural history of the disease.

Figure 7 : Comparison of Patient 012 and Patient 006 (from Study 201/202) with each other, and with Patient PV12 and KB from the historical patient registries



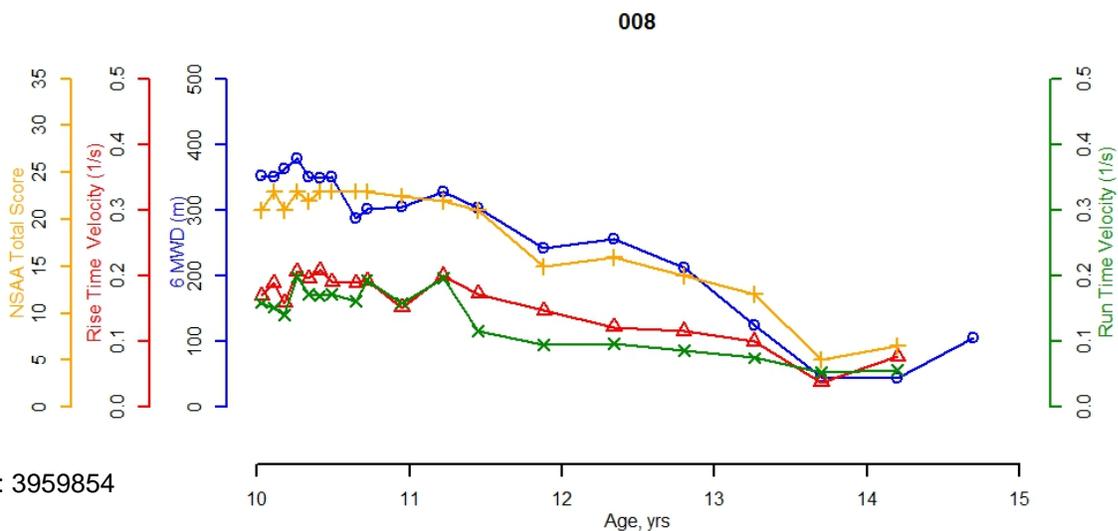
Patient 013 (Figure 8), who received placebo during Study 201, and was later switched to eteplirsen 50mg/kg, had the third highest 6MWT distance after age 14 years. Patient 013 is also showing a marked decline on most outcome measures, including rise time velocity from age 10.5, and a decline in NSAA scores, which started around age 12.5 (NSAA score is ~ 10 at the last visit). Patient 013 lost the ability to rise after age 12 (his last rise time was greater than 40 seconds). Dystrophin level by Western blot at Week 180 in this patient is 1.15%. Dystrophin level at baseline was below the level of quantification in (i.e., below 0.25%).

**Figure 8: Clinical Profile of Patient 013**



Patient 008 (Figure 9), who was on placebo during Study 201, and was later switched to eteplirsen 30 mg/kg, is the fourth patient still ambulating after age 14. At the final visit, Patient 008 has a very low 6MWT distance, less than 100 meters, and has experienced a sharp decline in NSAA score, rise time velocity, and 4-step velocity, declines which all started around age 11 years. Based on these results, it is likely that this patient is nearing loss of ambulation. At Week 240, this patient had a 6MWT distance of 103 meters. Dystrophin by Western blot at Week 180 in Patient 008 was 0.975% of normal. No baseline muscle tissue sample was retained, so it cannot be determined if this represents an increase from baseline.

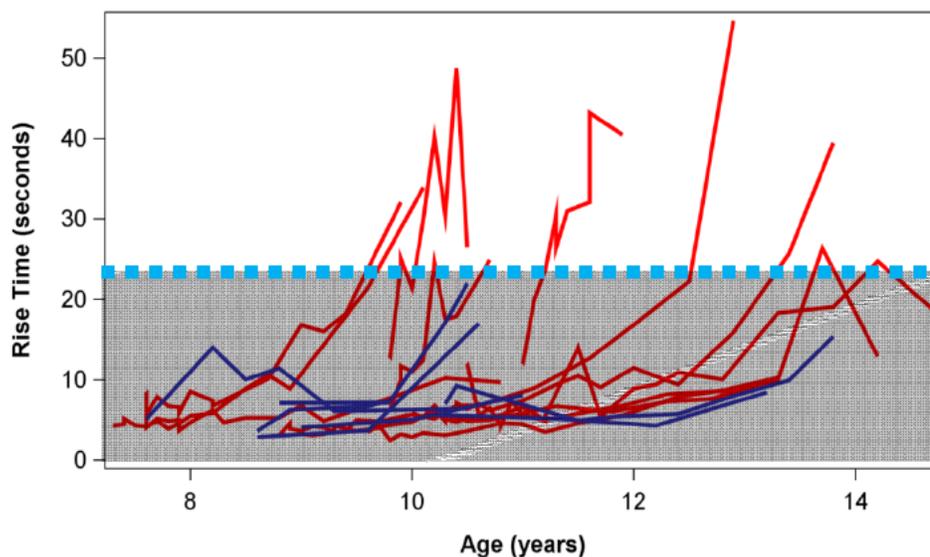
**Figure 9: Clinical profile of Patient 008**



A discussion of the patient profiles for the eight other eteplirsen-treated patients is provided in Appendix 1: Patient profiles. The clinical profile of these eight patients also show the expected worsening of clinical outcome measures related to ambulation over time, consistent with their stage of the disease.

- c. There were apparent differences in the administration and/or the performance of functional tests between eteplirsen-treated boys and those from the registries. It is striking that no boy in the Belgian or Italian registry had a recorded rise time greater than 22 seconds, whereas two-thirds of eteplirsen-treated boys did (Figure 10). Some rise times were extremely long, in some cases, even greater than 40 seconds. In addition, as discussed by Dr. Farkas, some boys in the Belgian or Italian registry had recorded 10-meter run/walk results and at the same time were declared unable to ambulate, which appears to be contradictory.

**Figure 10: Apparent differences in administration and/or performance of rise time.**



The advisory committee meeting did shed some light on this issue, as the applicant indicated at the meeting that boys in the eteplirsen study, upon reaching certain rise times, were allowed to receive external support for the test, which was not known to the review team up to the advisory committee meeting, and was not specified in the protocol. I looked further into the issue, and requested the applicant provide the “Study Operations Manual” for Study 202. The Manual, which is 24 pages long, includes no mention that external support was allowed during the performance of the rise time test, or any description of the point at which external support could be used. Regarding performance of the 6MWT, the Manual stated that “When the participant starts walking, walk along directly behind him at a distance of approximately 2 meters, giving positive verbal encouragement at approximately 15-second intervals. Encouragement should be similar to any of the following phrases: “You’re doing great (participant name)! Keep it up!,” “Remember, walk as fast as you can,!”, “Fantastic job (participant name)! Keep Going,!” or “Keep up the

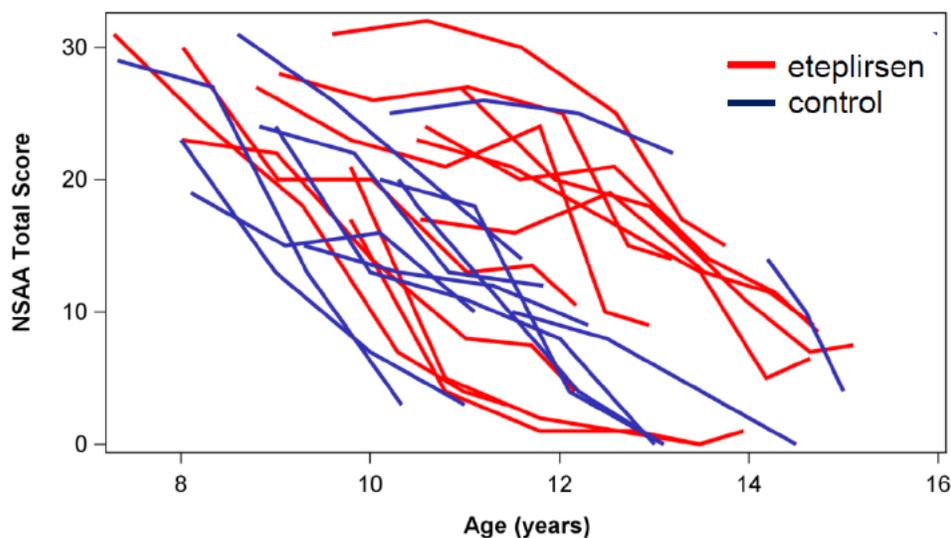
good work!,” The Manual for Study 202 also stated that if the patient fell or could not rise from the floor, the test was over and time and distance should be recorded. On the other hand, the protocols for the historical control studies were very scant (see Appendix 2: Protocol of the Leuven Neuromuscular Reference Center Registry and Appendix 3: Protocol of the Italian DMD Registry), and included no details on how the rise time test was to be performed, no mention with respect to encouragement during performance of the 6MWT, and no discussion about the situations under which boys should be declared unable to perform the test, without even attempting it.

Two patients in the historical control group who were reported to have lost ambulation nevertheless had 10-meter walk test values reported at the same points in time, providing evidence that ambulation was, in fact, not lost in these patients. The FDA review team learned that a standard approach in the registries consisted in categorizing as “non-ambulatory” boys who did not complete the full 6MWT, which is very different from the procedure followed in Study 202. A clear illustration is that for the recently submitted Week 240 6MWT data for eteplirsen-treated boys, the applicant indicates that the 6MWT is “unknown” for Patient 12 because the patient recently experienced a femur fracture and the Week 240 assessment had not been performed at this time. In natural history studies, such a patient may have been deemed to be unable to perform 6MWT. Moreover, as discussed by Dr. Farkas, Patient 4 walked 7 meters on Day 1 and 22 meters on Day 2 of Week 240’s assessment, and is considered by the applicant in some analyses to have lost ambulation. Patient 3, on the other hand, walked 12 meters on Day 1 and 31 meters on Day 2, and is considered by the applicant to have maintained ambulation. In natural history studies, both patients may have been deemed unable to perform the 6MWT. These clear differences confound comparisons between patients in Study 201/202 and those from the registries. And these differences, obvious for the rise time testing, also clearly affected the performance of the 6MWT, the primary efficacy outcome, and the determination of loss of ambulation. The observed differences indicate that the functional tests had subjective elements, and that their performance may have been influenced by decisions made by boys, the caregivers, or the study investigators. These types of differences may have a large impact on test results, and there is no way to correct for them with statistics.

- d. Eteplirsen-treated patients experienced the expected sequential worsening of functional abilities and muscle weakness, as demonstrated by the North Star Ambulatory Assessment (NSAA) scores. The NSAA is particularly important to the interpretation of the study results of Study 201/202. The NSAA has been specifically designed to measure functional ability in ambulatory patients with DMD, and can be used across a range of patient functional abilities. Among other functions, the NSAA measures activities of standing, walking, standing up from a chair, standing on one leg, climbing onto and descending from a box step, getting from lying to sitting, rising from the floor, jumping, hopping, and

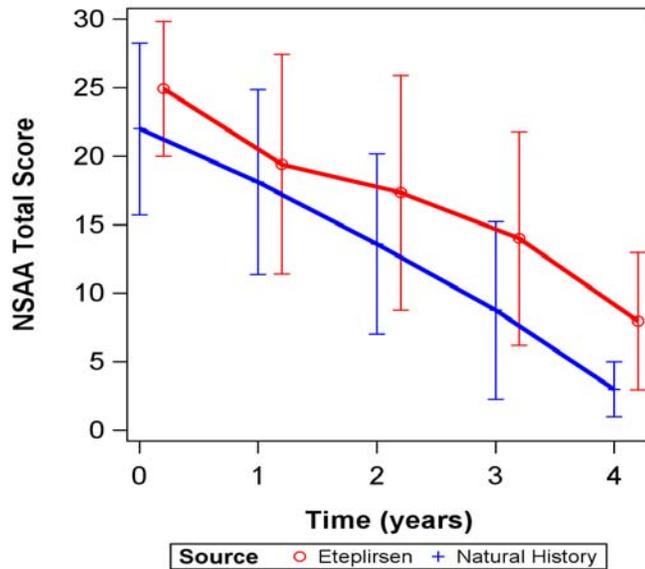
running. The NSAA is a comprehensive outcome measure, and arguably more fully reflects the functional abilities of DMD patients than the 6MWT. The NSAA remains, however, dependent on subject effort, and is not immune to possible bias. All eteplirsen-treated patients showed progressive declines in NSAA scores (with a single patient initially stable before declining), with no clear difference of pattern of decline between eteplirsen-treated boys and controls (Figure 11). In fact, all eteplirsen-treated patients were contained within NSAA decline boundaries set by control patients (shown as blue lines in Figure 11). This pattern, for the most comprehensive outcome measure used in Study 201/202, unequivocally shows no eteplirsen-treated patient had a clinical course clearly different from the natural history of the disease. It also shows that, despite the small sample size of the trial, there was assay sensitivity in Study 201/202 to determine whether eteplirsen meaningfully altered the expected course of the inexorable decline of function expected in DMD, as most patients experienced a large decline in functional abilities.

**Figure 11: North Star Ambulatory Assessment (NSAA) scores vs. duration of observation in eteplirsen-treated patients in Study 201/202.**



It is also remarkable that mean NSAA values over time show a very similar decline in eteplirsen-treated boys and external controls. As illustrated in Figure 12, patients in the external control group had a worse mean NSAA score at baseline, suggesting a worse prognosis in these patients. The curves are then similar over time, with large overlaps in confidence intervals.

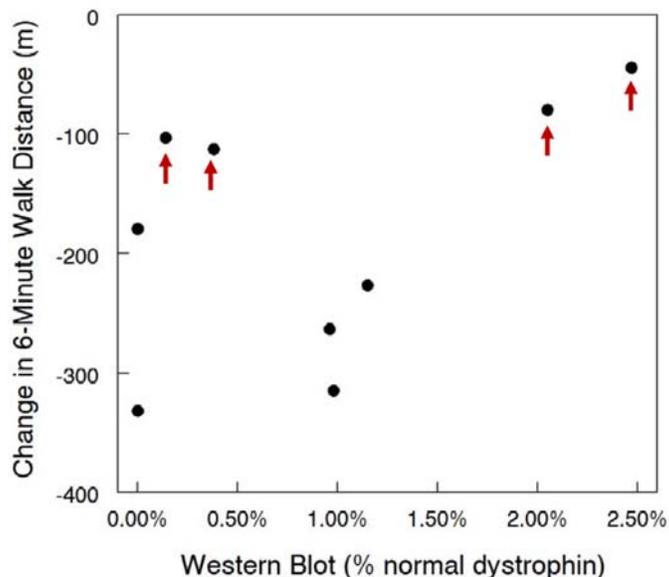
Figure 12: Mean NSAA scores over time



### Correlation between dystrophin levels and clinical outcome in Study 201/202

If production of dystrophin protein is reasonably likely to predict clinical benefit, one would expect a correlation between the level of dystrophin and ambulation in eteplirsen-treated patients. In Study 201/202, there were too few patients to perform a rigorous analysis. But for the nine patients who were able to ambulate and had a biopsy at Week 180, it is apparent that for the four patients whose 6MWT distances were best preserved, two had very low levels of dystrophin, and two had the highest levels. Thus, there is no apparent correlation between 6MWT and dystrophin levels in eteplirsen-treated patients (Figure 13).

Figure 13: Change in 6-minute walk distance (Week 180 minus Baseline) versus dystrophin level as determined by Western blot Study 201/202. (Two patients who lost ambulation are omitted.)



## **Conclusions about efficacy data**

Sponsors of marketing applications are required to establish a drug's effectiveness by providing "substantial evidence" of effectiveness from "adequate and well-controlled investigations." Positive findings on clinically meaningful endpoints in two adequate and well-controlled trials are typically required, but a single highly persuasive positive trial or a positive trial combined with independent findings that substantiate efficacy (confirmatory evidence) can also support approval in some cases. The intent of the statutory requirements is to reduce the chance of an incorrect conclusion that a drug is effective when, in fact, it is not effective.

The applicant is proposing approval based primarily on a post hoc comparison of 12 patients with Duchenne muscular dystrophy amenable to exon 51 skipping from the open-label portion of a single study (Study 201/202) to 13 patients from an external untreated control group. The applicant believes that the results of their external control comparison provide evidence of benefit on an intermediate clinical endpoint that could be the basis for accelerated approval. Accelerated approval can be based on an "intermediate clinical endpoint," i.e., a clinical endpoint that can be measured earlier than irreversible morbidity or mortality (IMM) that is reasonably likely to predict an effect on IMM or other clinical benefit. Accelerated approval can also be based on a biomarker surrogate endpoint that is reasonably likely to predict an effect on IMM or other clinical benefit. For eteplirsen, a possible path to accelerated approval could be based on substantial evidence from adequate and well-controlled studies that eteplirsen induces production of an amount of dystrophin that is reasonably likely to predict clinical benefit.

It must be noted that consideration for accelerated approval is based on the type of endpoints selected. Thus, the evidence of an effect on an intermediate endpoint, or of a surrogate biomarker, if it is to serve as the basis for accelerated approval, must meet the evidentiary standard for substantial evidence from adequate and well-controlled studies. The Agency's decision on whether to grant accelerated approval is based both on the appropriateness of the endpoints selected (surrogate marker or intermediate clinical endpoint), and on whether there is substantial evidence of an effect on these endpoints. Accelerated approval cannot be used to compensate for weak or inconsistent clinical findings (i.e., approval based on marginal data, to be buttressed with better data post-approval). When accelerated approval is used, post-approval studies to verify the expected clinical benefit are generally required.

### **Do the clinical results of Study 201/202 provide substantial evidence that eteplirsen is effective for the treatment of DMD, i.e., support "full approval"?**

The applicant proposed using clinical data from Study 201/202 on 6-minute walk distance as an intermediate clinical endpoint that could have the potential to support accelerated approval. Under that approach, the basis for accelerated approval would be a conclusion that eteplirsen reduced the rate of decline of walking performance to an extent that is reasonably likely to

predict a long-term beneficial effect on irreversible morbidity or mortality. It should be noted, however, that FDA would consider an effect on walking distance to be a clinical benefit that, if demonstrated, would support full approval. Therefore, there is no scientific justification for using 6-minute walk distance as an intermediate endpoint here, in particular as the period of observation is unusually long, around 4 years, which is more than sufficient to identify a possible clinical benefit. In the same sense, it is not clear what future clinical benefit would be prevented. The applicant proposed the following language for the indication section of labeling: *“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.”* The applicant’s statement that the intermediate endpoint demonstrate delayed disease progression clearly goes against the purpose of an intermediate endpoint, which, as discussed above, is to be a clinical endpoint that can be measured earlier than irreversible morbidity or mortality (IMM), and that is reasonably likely to predict an effect on IMM or other clinical benefit. Therefore, the clinical evidence provided by the applicant, which include a number of clinically meaningful endpoints, is to be examined in the context of “full approval.”

As discussed above, externally controlled trials can be considered well-controlled studies, and can contribute to the establishment of substantial evidence of effectiveness.

I agree with the review team that Study 201/202 does not provide substantial evidence that eteplirsen is effective for the treatment of DMD.

Before discussing the reasons for my conclusion, I want to point out that the size of the study, by itself, is not a reason for not approving eteplirsen, even in the context of a historical control study. Even though much larger studies have been conducted by other sponsors for the same indication, a drug that has a very clear effect on disease progression, e.g., preventing further worsening of the NSAA over a sufficient period of time (and not necessarily as long as 4 years, as in this case), may potentially be approved based on studies even smaller than Study 201/202. This being said, effects of that magnitude are very rare, and it would be prudent to have a larger sample size and an appropriate concurrent control in any future study.

Our review of Study 201/202 indicates that substantial evidence of effectiveness of eteplirsen was not provided by the applicant, for the following reasons:

- a. Study 201, the only randomized controlled study conducted by the applicant, did not meet its primary clinical endpoint, 6MWT at 24 weeks ( $p=0.026$ , in favor of placebo, for the 30 mg/kg group;  $p=0.563$  for the 50 mg/kg group).
- b. Study 202, the long-term extension of Study 201, did not meet its primary clinical endpoint, 6MWT at 48 weeks.

- c. The various post hoc analyses comparing the six patients who received eteplirsen in the 24-week double-blind phase of Study 201 and could still ambulate at the end of Study 201 (and continued on open-label eteplirsen in Study 202) with those originally treated with placebo in the double-blind phase of Study 201, and later switched to open-label eteplirsen, are not scientifically valid and not useful to support efficacy.
- d. The alternative analysis of Study 202 proposed by the applicant, using an external historical control, failed to show a clear separation between the disease course in eteplirsen-treated patients and historical control patients:
  - i. There were important differences in baseline characteristics of patients, e.g., age of onset of steroid treatment earlier in the eteplirsen group, and NSAA score at baseline lower in historical control patients
  - ii. There was considerable overlap of 6MWT results between eteplirsen-treated patients and historical controls. Detailed review of the clinical test results (6MWT, NSAA, rise time, run time) for the eteplirsen-treated patients who are still ambulating at age 14 show that these patients have, in fact, a disease course similar to natural history, and not clearly different from that of the historical cohort patients still ambulating at age 14. Similarly, all other eteplirsen-treated patients have a disease course compatible with the natural history of DMD.
  - iii. There were clear differences in the way clinical outcomes were evaluated and scored, or in the way patients were categorized as having lost ambulation, between Study 201/202 and the external patient registries. These differences created a bias favoring eteplirsen-treated patients, and affect the interpretability of the study results.
  - iv. All eteplirsen-treated patients experienced a worsening in rise time, and several patients lost the ability to rise.
  - v. All patients in the eteplirsen treatment group experienced the expected sequential worsening of functional abilities and muscle weakness, as demonstrated by their NSAA scores over time. The worsening of NSAA scores was similar between eteplirsen-treated patients and historical controls. In fact, the highest (i.e., better) NSAA individual scores between age 12.5 and 15 years were mostly held by historical control patients.
  - vi. Based on the CINRG<sup>10</sup> data, about 25% of exon-51 skippable patients maintain ambulation to age 16, and about 15% of patients to age 18.
- e. There is no independent substantiation of the findings, and Study 201/202 clearly does not have the potential to serve as a single study to establish efficacy.

Although the above issues strongly support that no large difference does exist between eteplirsen-treated patients and historical controls, additional non-identified differences may

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<sup>10</sup> Cooperative International Neuromuscular Research Group <http://www.cinrgresearch.org/>

have had an impact on study outcomes, as is often the case for historical control studies. Study 202, however, clearly had the potential to allow a demonstration of clinical stabilization, as all eteplirsen-treated patients experienced clear declines in all ambulatory outcome measures.

As discussed below, the members of the advisory committee largely agreed that the clinical results of Study 201/202 do not provide substantial evidence that eteplirsen is effective for the treatment of DMD, with 7 negative votes, 3 positive votes, and 3 abstentions.

The patient testimonies were very moving, and uniformly supportive of eteplirsen, indicating in multiple cases improvement of the patients' condition. Although many of the members of the advisory committee were as moved by the testimonies as I was, several members noted the disconnect between the testimonies and clinical outcome results, including the invited member who had Duchenne Muscular Dystrophy.<sup>11</sup> I myself have great difficulties reconciling the testimonies with the study results. I note that no eteplirsen-treated patient experienced a sustained functional improvement in the outcomes measures that were assessed in Study 202, and in particular in the NSAA, which is a rather comprehensive measure of mobility and transfers.

It is quite clear that eteplirsen does not have a dramatic effect, or even a moderate to large effect on disease progression in Duchenne muscular dystrophy. In fact, there is no clinical evidence of efficacy from Study 201/202. It is not impossible that lower magnitude differences could be identified on some outcome measures in future trials, but I have very serious doubts, given the results of Study 202, that a historical control study may be capable to identify such differences.

**Is there substantial evidence from adequate and well controlled studies that eteplirsen induces production of dystrophin to a level that is reasonably likely to predict clinical benefit?**

FDA indicated in the draft DMD guidance that biomarkers that reliably reflect the health and amount of skeletal muscle may, if supported by sufficient scientific evidence and acceptable analytical methods, be used as surrogate endpoints to support accelerated approval of a new DMD drug. Such a biomarker would have to be “reasonably likely to predict clinical benefit” in order to be acceptable as a basis for accelerated approval.

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<sup>11</sup> This member, Benjamin Dupree, stated at the end of the meeting that “the testimony that was given suggesting that boys are recovering abilities. I don't -- living with Duchenne I don't understand how that's even possible. But at the same time this study doesn't prove from a scientific -- like -- it doesn't provide what I think, is adequate evidence to support all this testimony that I'm seeing in here.”

Two questions must be sequentially addressed before considering accelerated approval:

1. Is there substantial evidence from adequate and well controlled studies that eteplirsen induces production of dystrophin?
2. If substantial evidence from adequate and well controlled studies that eteplirsen induces production of dystrophin is established, was the production to a level that is reasonably likely to predict clinical benefit?

### ***Production of dystrophin***

Pharmacodynamic effects of eteplirsen are potentially demonstrable at two levels: expression of an altered messenger RNA in muscle (assessed using reverse transcriptase polymerase chain reaction – RT-PCR), and production of dystrophin protein in muscle (assessed by immunofluorescence or Western blot). Western blot is considered to be a quantitative method, whereas immunofluorescence is generally considered to be less quantitative, and is more often relied upon to show the localization of protein in tissue sections.

The applicant obtained four biopsies in eteplirsen-treated patients in Study 201/202, spaced between baseline (pre-treatment) and Week 180 of treatment. In addition, the applicant obtained muscle biopsies in two exploratory studies (Study 33 and Study 28), and provided dystrophin data at baseline and after 48 weeks of treatment in 13 patients participating in the PROMOVI study.

#### *Dystrophin mRNA production*

Exon 51 skipping and production of an altered messenger RNA was clearly seen in the muscle of all patients of Study 201/202. As PCR is a highly sensitive technique that can detect even a few copies of messenger RNA, even a minimal PCR signal is interpreted as “positive.” Therefore, this biomarker provides little support of efficacy for eteplirsen; it does, however, provide evidence that eteplirsen causes at least some degree of exon 51 skipping, as intended.

#### *Immunofluorescence*

Overall, the immunofluorescence data provide do not provide consistent evidence that the percent of dystrophin positive fibers may have increased as a result of eteplirsen treatment. The issues described deeply affect the interpretability of the findings, and make any quantification of the changes unreliable. In addition, as analyses based on immunofluorescence overestimate the amount of dystrophin in tissue sections because a muscle fiber can be considered “positive” if it exhibits any staining at all, the percent dystrophin-positive fibers by immunofluorescence is not the most meaningful way to estimate dystrophin content; the Western blot analyses are informative for that purpose.

#### *Western Blot*

There is substantial evidence of production of dystrophin in response to eteplirsen treatment, by interim results from 13 patients participating in the PROMOVI study, showing a

statistically significant increase in dystrophin level after 48 weeks of eteplirsen treatment. Study 201/202 provides independent substantiation of the results of PROMOVI. In my opinion, these data establish clear proof of concept that eteplirsen is capable of increasing dystrophin in DMD patients. To the best of my knowledge, this is the first time a drug is documented to have that effect.

**Was the production of dystrophin to a level that is reasonably likely to predict clinical benefit?**

As substantial evidence of production of dystrophin in response to eteplirsen treatment has been provided, the next question to address in consideration of potential accelerated approval is whether the level induced is reasonably likely to predict clinical benefit.

The applicant's data support that dystrophin levels in DMD patients, in the absence of treatment, range between 0% and about 0.4% of dystrophin levels in healthy subjects. DMD experts, including those directly involved in the development of eteplirsen, have stated that levels less than 3% of that of normal healthy muscle are generally associated with the typical DMD phenotype, and the range observed by the applicant at baseline in DMD participants to eteplirsen studies is compatible with that figure. Baseline values greater than 0.4% have however not been observed by the applicant. It is unclear whether different methods of assessment of dystrophin content may explain that difference, or whether dystrophin levels greater than 0.4% can be present in some "outliers", and were not seen in this small database. The applicant's data suggest that dystrophin levels greater than 0.4% of normal are not common in DMD patients.

Based on a comparison of Week 48 results to baseline using reported dystrophin values, most patients (about 60%) from the PROMOVI study had no increase in dystrophin content, or an increase smaller than 0.25% of normal levels. A single patient had a dystrophin increase greater than 1%, and no patient had a dystrophin increase greater than 2%. In comparison, about a third of patients from Study 201/202 had no increase in dystrophin content, or an increase smaller than 0.25% of normal levels, while about a third of patients had dystrophin increases greater than 1% of normal levels. A single patient had a dystrophin increase greater than 2% of normal, and no patient had a dystrophin increase greater than 3% of normal. It is unclear whether the somewhat greater increases observed in Study 201/202 are related to duration of treatment or to methodological differences.

Based on a review of information that was presented to me by the review team or discussed at the advisory committee meeting, the minimum level of dystrophin that might be reasonably likely to predict clinical benefit in patients with DMD remains unknown. Unfortunately, the applicant's NDA does not provide any information suggesting that the dystrophin increases observed after eteplirsen treatment are reasonably likely to lead to clinical benefit, as there was no evidence of such benefit after about 4 years of treatment in Study 201/202. In fact, if

clinical data from Study 201/202 are used to inform whether the level of dystrophin increase hinted in eteplirsen-treated patients is reasonably likely to predict clinical benefit, the conclusion, based on the fact that not a single eteplirsen-treated patient clearly deviated from natural history, would have to be that the clinical data weaken, and clearly do not strengthen, the “reasonably likely” argument. Moreover, there was no correlation between the increases in dystrophin level reported in Study 201/202 and clinical outcome.

In addition, as discussed by the review team, DMD experts have proposed that “induction of approximately 10% of normal dystrophin levels sets a minimum level to confer measurable clinical benefit.” In fact, Dr. Chamberlain, who stated at the open public session at the advisory committee meeting that very low levels of dystrophin may be beneficial, discussed in a published paper<sup>12</sup> that “a majority of fibers must accumulate approximately 20% of wild-type levels of dystrophin for a significant correction of the muscle pathology,” which seems entirely contradictory to the comments he made at the advisory committee meeting.

Another consideration is that dystrophin levels in exon-51 model Becker’s Muscular Dystrophy patients have been observed to be roughly 80% of normal on average. This observation is not meant to say that levels that high would be needed to be likely to predict clinical benefit, but they provide an anchor point.

As discussed by Dr. Farkas, the only argument presented by the applicant about the relationship of dystrophin to DMD severity is that patients amenable to exon 44 skipping have been shown to express higher, albeit trace levels of dystrophin than are typically seen in DMD patients, and have a milder disease course compared with other types of DMD. The applicant also stated that “in a recent large prospective DMD natural history study (CINRG), an approximate 2-year delay of median loss of ambulation was observed in 20 participants who had mutations amenable to exon 44 skipping.” Dr. Farkas notes that it is not clear how much dystrophin is expressed in these patients, and that possible differences in functionality of the truncated dystrophin species produced in patients with different mutations can also confound interpretation of possible effects on clinical course of differences in dystrophin levels. Dr. Farkas conducted a detailed review of a publication of Anthony et al<sup>13</sup> describing a comparative immunohistochemical analysis of dystrophin expression in patients with in-frame (IF) or out-of-frame (OOF) deletions around exons 44 and 45 that was used in support of the applicant’s argument. Dr. Farkas notes that the two patients who had the highest dystrophin expression also had the mildest course of disease progression. However, the dystrophin levels in those two patients appeared to be similar to dystrophin levels in the in-frame Becker

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<sup>12</sup> Chamberlain JS. Dystrophin Levels Required for Genetic Correction of Duchenne Muscular Dystrophy. *Basic Appl Myol.* 7 (3&4): 251-255, 1997

<sup>13</sup> Anthony K, et al (2014) Biochemical characterization of patients with in-frame or out-of-frame DMD deletions pertinent to exon 44 or 45 skipping. *JAMA Neurol.* 71:32-40.

muscular dystrophy patients, and so their mild disease course is hardly surprising. I agree with Dr. Farkas that Western blot data from additional exon 44 skippable patients with various rates of disease progression would be highly desirable to increase understanding of dystrophin levels that might be reasonably likely to predict clinical benefit, and I believe that the publication referenced by the applicant does not address whether increases in dystrophin in the order of 1 to 2% of levels seen in healthy subjects are likely to confer any clinical benefit.

The advisory committee had mixed opinions about the “reasonably likely” question. A majority of members (n=7) voted that the production of dystrophin was not to a level reasonably likely to predict clinical benefit, while 6 members voted that it was. In explaining their “No” votes, 5 committee members opined that the studies were not adequate and well controlled; they questioned the techniques used to measure dystrophin as well as the appropriateness of the controls (see “Advisory Committee Meeting” section below). Four committee members expressed concern about the lack of correlation between the dystrophin levels and clinical measures. They agreed that even if some dystrophin was produced, there was no evidence that dystrophin production was to a level that would be reasonably likely to predict clinical benefit. The 6 members who voted “Yes” included the consumer representative and both patient representatives. A member who voted “Yes” stated that he was very troubled by not understanding what constitutes a clinically significant amount, but was impressed by the patients’ observations. Two members who voted “No” stated that their vote was justified by the way the question was phrased, but that the patient testimonies suggested the drug works.

In summary, DMD is characterized by the absence or near absence of functional dystrophin protein, leading to degeneration of muscle fibers. The finding of an increase (regardless of its size) in dystrophin in response to a drug treatment is unprecedented and provides great hope that therapies will be capable to address the fundamental defect that causes muscle damage in patients with DMD. There is no clear answer, however, to the question whether the small increases in dystrophin demonstrated in some DMD patients treated with eteplirsen are reasonably likely to predict clinical benefit. The clinical efficacy data are sufficient to conclude that a benefit, if any, would be very limited, and that eteplirsen would not fundamentally change the course of the disease. It is possible, however, that more modest benefits may be derived, but those benefits do not appear very likely. It is very unfortunate that the applicant did not conduct a reasonable development program that included appropriate exploration of dose response-response, as it is very possible that higher doses of eteplirsen may produce a greater pharmacodynamic effect that would be reasonably likely to predict clinical benefit. That information is not available to us, and we are left in a situation under which unequivocal proof of concept has been established, but the potential clinical significance of the effect has no clear answer.

Great flexibility must be applied in the FDA decision-making on possible accelerated approval for a precedent-setting new drug for the treatment of DMD, and is tempting to be applied for eteplirsen. While it is somewhat possible that the amount of dystrophin produced may lead to a modest clinical benefit, such a benefit does not appear likely. Considering the extent of the doubt about the potential clinical benefit of the pharmacodynamic effect of eteplirsen, FDA flexibility must be balanced with the risk of approving a drug at a subtherapeutic dose, before proper dose finding has been conducted, and its implications both for patients who would be prescribed the drug, and for future development programs of other drugs for the treatment of DMD, and other rare diseases.

If a decision is made to give a complete response to this application, which is my recommendation, I strongly support providing access to this drug for DMD patients through expanded access programs, with cost recovery, while an adequate dose-finding study is conducted. If a decision is made to give accelerated approval, labeling must make it very clear that no clinical benefit has been shown for eteplirsen. Also, no promotion of clinical benefit by the applicant should be allowed.

## 9. Safety

### Safety database

The safety population included data on a total of 114 patients who were exposed to eteplirsen. This number includes the 12 patients from Study 201/202, who have been treated with 30 mg/kg or 50 mg/kg/week for approximately 4 years, and 76 patients treated with 30 mg/kg in Study 20314, 20415, or 30116 (ongoing studies which contributed safety data only to the application). Overall, 12 patients have received eteplirsen for one year or longer (in fact, exposure of these patients is almost 4 years), 36 patients have received eteplirsen for 24 weeks or longer, and 61 have received eteplirsen for 13 weeks or longer.

### Deaths

No patients have died during the eteplirsen clinical development program.

### Nonfatal serious adverse events

Nonfatal SAEs were reported in six patients in the safety population. The SAEs included wound infection, vomiting, ankle fracture, femur fracture, oxygen saturation decreased, and viral lymphadenitis. These events were considered by Dr. Breder as unrelated to treatment. I agree.

### Adverse dropouts

A single patient (10 year old) discontinued treatment because of an adverse event, reported as cardiomyopathy, which was pre-existing. The boy discontinued treatment due to a decrease in left ventricular ejection fraction after having received seven once-weekly doses of eteplirsen 4 mg/kg. The event was judged by the investigator as possibly related to eteplirsen.

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<sup>14</sup> Study 203 is an open-label study designed to evaluate the safety, efficacy and tolerability of eteplirsen in patients with early stage DMD. Approximately 40 male ambulatory patients between the ages of 4 and 6 years, inclusive, who have a confirmed diagnosis of DMD amenable to exon 51 skipping will be enrolled. Patients will receive eteplirsen 30 mg/kg IV weekly for 96 weeks.

<sup>15</sup> Study 204 is an open-label study designed to evaluate the safety and tolerability of eteplirsen in patients with advanced stage DMD. Approximately 20 male ambulatory impaired or non-ambulatory patients between the ages of 7 and 21 years, inclusive, who have a confirmed diagnosis of DMD amenable to exon 51 skipping are being enrolled. Patients will receive eteplirsen 30 mg/kg IV weekly for 96 weeks.

<sup>16</sup> Study 301 is an open-label study of eteplirsen safety and efficacy in patients with DMD. Approximately 80 male ambulatory patients (able to walk >300 meters on 6MWT) between the ages of 7 to 16 years who have a confirmed diagnosis of DMD amenable to exon 51 skipping are being enrolled. Patients assigned to eteplirsen treatment will receive eteplirsen 30 mg/kg IV weekly for 48 weeks and will be compared with an untreated control group (i.e., patients who are non-amenable to exon 51 skipping).

### **Severe adverse events, or adverse events of concern**

Nine adverse events occurring in six patients were assessed as severe. The events included incision site hemorrhage, hemorrhoids, back pain, nasal congestion, bone pain, loss of balance, viral lymphadenitis, femur fracture, and cardiomyopathy with left ventricular dysfunction). All events, except for the case of cardiomyopathy, which is discussed above under “adverse dropouts”, were considered unrelated to treatment. I agree that no pattern of severe adverse events is present in the database.

### **Common adverse reactions**

As the placebo-controlled experience is extremely limited for eteplirsen (i.e., 8 patients on drug vs. 4 patients on placebo treated for 24 weeks), most of the safety experience comes from open-label studies, which greatly limits the interpretability of data, in particular considering the various events and complications that are expected as Duchenne Muscular Dystrophy progresses.

In Study 201/202, which has been ongoing for nearly 4 years, with most of the experience without a concurrent control, Dr. Breder describes that infections were noted, including an increase in respiratory infections, which is expected in that population. Dr. Breder also notes some adverse events related to neuromuscular symptoms and hypersensitivity-related events in the later part Study 201/202.

In the other open-label trials, adverse events expected in the DMD population were observed, and the lack of concurrent control makes it impossible to determine whether their incidence was increased by eteplirsen treatment.

### **Laboratory findings**

Dr. Breder describes various laboratory tests changes of unclear clinical significance in eteplirsen-treated patients.

### **Vital signs and ECGs**

There were no changes of clinical relevance in vital signs or ECGs.

## 10. Advisory Committee Meeting

An advisory committee was held on April 25, 2016. I integrated in my discussion above salient points from the advisory committee discussion and votes. The following is a copy of the “Quick Minutes” of the meeting.

### *Questions to the Committee:*

The applicant is proposing approval based primarily on a post hoc comparison of 12 patients with Duchenne Muscular Dystrophy (DMD) amenable to exon 51 skipping from the open-label portion of a single study (Study 201/202) to 13 patients from an external untreated control group. The Advisory Committee will be asked to discuss and vote on whether the application has met the statutory requirements for substantial evidence of effectiveness, based on that comparison. The Advisory Committee will also be asked to discuss the evidence provided by the applicant on dystrophin expression with eteplirsen treatment, and vote on whether the applicant has provided substantial evidence from adequate and well-controlled studies that eteplirsen induces production of an amount of dystrophin that is reasonably likely to predict clinical benefit.

### Statutory standards for approval

Although drug approval ultimately reflects a benefit-risk assessment, the statutory standards for approval are applied stepwise, with the law first requiring substantial evidence that the drug is effective. If the standard for substantial evidence of effectiveness is met, a determination must be made that the drug is safe for its intended use, i.e., that its benefits outweigh the risks, given the nature of the disease and available treatment options.

### **Standard Approval**

Sponsors of marketing applications are required to establish a drug’s effectiveness by providing “substantial evidence” of effectiveness from “adequate and well-controlled investigations.” Positive findings on clinically meaningful endpoints in two adequate and well-controlled trials are typically required, but a single highly persuasive positive trial or a positive trial combined with independent findings that substantiate efficacy (confirmatory evidence) can also support approval in some cases. The intent of the statutory requirements is to reduce the chance of an incorrect conclusion that a drug is effective when, in fact, it is not effective. In making its determination on whether the statutory standards for approval have been met, the Agency considers all the available data.

### *Accelerated Approval*

Under the Accelerated Approval provisions, an effect on a surrogate marker that is determined by FDA to be reasonably likely to predict clinical benefit can support approval, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments. An effect on an intermediate clinical endpoint - a clinical endpoint that can be measured earlier than irreversible morbidity or mortality (IMM) and that is reasonably likely to predict an effect on IMM or other clinical benefit - can also serve as a basis for accelerated approval.

Importantly, accelerated approval does not change the statutory requirement for substantial evidence; rather, it allows FDA to utilize a demonstrated effect on an endpoint other than clinical benefit as the basis for showing effectiveness if the sponsor provides substantial evidence from adequate and well controlled trials that the drug has an effect on a surrogate or intermediate clinical endpoint. The Agency's decision on whether to grant accelerated approval is based both on the appropriateness of the endpoints selected (surrogate marker or intermediate clinical endpoint), and on whether there is substantial evidence of an effect on these endpoints. Accelerated approval cannot be used to compensate for weak or inconsistent clinical findings (i.e., approval based on marginal data, to be buttressed with better data post-approval). When accelerated approval is used, post-approval studies to verify the expected clinical benefit are generally required.

### Biomarker Evidence

For DMD, there is obvious interest in dystrophin expression as a potential surrogate marker to support accelerated approval. Whether an effect on a biomarker such as dystrophin is reasonably likely to predict clinical benefit in DMD depends on a number of factors including, but not limited to, the reliability of the data, the magnitude of the effect on the biomarker, and confidence that the dystrophin produced is functional.

Eteplirsen's putative mechanism of action is to increase production of a truncated form of dystrophin. By Western blot, the most accurate quantitative method used by the applicant, mean dystrophin levels after 180 weeks of eteplirsen treatment are  $0.93\% \pm 0.84\%$  of normal (mean  $\pm$  standard deviation). The applicant reported a control (untreated) value of 0.08% dystrophin based on retained samples from the pre-treatment biopsy in 3 patients from Study 201/201, combined with data from six patients with DMD who were not enrolled in any study. FDA identified, however, some important limitations with respect to interpretation of the results of the untreated controls (e.g., limits of assay detection, different muscles sampled).

1. **DISCUSSION:** Discuss the evidence presented about dystrophin production, including the following:
  - a. The strength of evidence that eteplirsen increased the amount of dystrophin in muscles of treated patients, relative to their baseline.
  - b. Clinical meaning of the amount of dystrophin observed in the muscles of eteplirsen-treated patients, taking into consideration the range of amounts of dystrophin known to be typically present in patients with DMD and in patients with Becker muscular dystrophy.

***Committee Discussion:** The committee members did not reach a consensus on either the strength of evidence that eteplirsen increased the amount of dystrophin in muscles of treated patients relative to baseline, or the clinical meaning of the amount of dystrophin observed in the muscles of eteplirsen-treated patients.*

- a. *Production of dystrophin: About half of the committee members thought that there was evidence that eteplirsen increased the amount of dystrophin produced in the muscles of the treated patients. Among those who were not convinced, two members cited issues with the controls (lack of pre- and post-treatment biopsies in the same patients; differences in muscle groups biopsied), two had concerns about inconsistencies between dystrophin levels and clinical response, and one cited concerns about the lack of a dose-response. The Chair found it surprising that there wasn't more scientific consensus.*
- b. *Clinical meaning: Only four Committee members had explicit comments with respect to the clinical meaningfulness of the amount of dystrophin observed in treated patients, and their opinions were split. One opined that the amount of dystrophin needed to impart clinical benefit is unknown, but could be very low, or very low in a subset of patients. One of the Patient representatives felt strongly that dystrophin was produced, and that the amount was sufficient to produce clinical benefit. One committee member, having opined that some dystrophin was produced, stated that we have no idea how much dystrophin would be clinically significant, or whether the dystrophin is functionally active. Another committee member, one who had not opined on whether dystrophin was produced, noted that whatever the amount of dystrophin produced, it was not clinically meaningful, based on a lack of correlation between dystrophin results and clinical results. Please see the transcript for details of the committee discussion.*

2. **VOTE:** Has the applicant provided substantial evidence from adequate and well controlled studies that eteplirsen induces production of dystrophin to a level that is reasonably likely to predict clinical benefit?

**Vote Result:** YES: 5 NO: 8 ABSTAIN: 0

***Committee Discussion:** One panel member stated that he had pressed the wrong voting button and stated that his vote should be changed to "Yes" for the record, which would*

*make the vote 6 “Yes” and 7 “No.” Thus, 7 committee members voted “No” that the applicant did not provide substantial evidence from adequate and well controlled studies that eteplirsen induces production of dystrophin to a level that is reasonably likely to predict clinical benefit. In explaining their “No” votes, 5 committee members opined that the studies were not adequate and well controlled; they questioned the techniques used to measure dystrophin as well as the appropriateness of the controls. Four committee members expressed concern about the lack of correlation between the dystrophin levels and clinical measures. They agreed that even if some dystrophin was produced, there was no evidence that dystrophin production was at a level that would be reasonably likely to predict clinical benefit. The 6 members who voted “Yes” included the consumer representative and both patient representatives. They believed that there was some difference in dystrophin production and some evidence of improvement in endpoints. One of the members who voted “Yes” stated that he was very troubled by not understanding what constitutes a clinically significant amount, but was impressed by the patients’ observations. Please see the transcript for details of the committee discussion.*

### Clinical evidence

Study 201/202 began as a 24-week randomized controlled study comparing three groups of 4 patients each, treated weekly with eteplirsen 50 mg/kg, eteplirsen 30 mg/kg, or placebo (Study 201). Study 201, when analyzed according to the pre-specified intent-to-treat (ITT) methods, did not show an advantage of eteplirsen over placebo on the 6-minute walk test (6MWT) after 24 weeks of treatment.

After the randomized placebo-control phase, all patients entered an open-label extension phase beginning at Week 28, i.e., Study 202. The primary clinical endpoint of Study 202 was a comparison of Week 48 6MWT results for patients originally randomized to eteplirsen vs placebo. When analyzed according to the pre-specified ITT methods, Study 202 did not demonstrate an advantage of eteplirsen over placebo on the 6-minute walk test.

The applicant then continued open-label treatment with eteplirsen in Study 202, which is still ongoing, and is seeking approval primarily based on a post hoc comparison of 12 patients from Study 201 to 13 patients from an untreated external control group amenable to exon 51 skipping (from two DMD patient registries, the “Italian Telethon DMD Registry” database and the “Leuven Neuromuscular Reference Center” database).

Because of difficulty of controlling bias in historical control studies, important issues to consider include: 1) whether there are identified or possible differences between the treatment and control groups, at baseline or during treatment, that may have had an impact on clinical course; 2) whether the endpoint(s) used to assess benefit was (were) objective and assessed in a sufficiently similar way in the treatment and control groups to allow a valid comparison; and 3) whether the reported effect size is large enough to conclude that the course of patients in Study 201/202 is clearly different from the usual course of patients with DMD.

3. **DISCUSSION:** Discuss the strengths and weaknesses of the clinical evidence of efficacy provided by Study 201/202, with particular consideration of the design of the study, sample size, statistical methods, general concerns regarding a comparison to a historical control group, specific concerns with respect to the comparability of these two groups (in particular, how motivational factors and differences in assessment of physical performance outcomes may have affected the 6-minute walk endpoint and other endpoints), and any other issues that you think may be important.

***Committee Discussion:** Overall, the majority of the committee agreed that there were weaknesses to Study 201/202. One committee member noted that although placebo controlled trials can have flaws, studies with historical controls can have even more flaws and was uncomfortable with the study design of Study 201/202. Another committee member added that, considering the testimonies provided by the public, Study 201/202 might have been successful if the patient-reported results had been included. Other committee members noted that they would have liked to see a measurement of upper limb strength, which was reported to be improved in the testimonies from the public but was not captured in the North Star Ambulatory Assessment, 10-meter run/walk and 6-minute walk tests. Please see the transcript for details of the committee discussion.*

4. **VOTE:** Were decisions to administer the 6-minute walk test (vs. conclusions that the patient could no longer walk) sufficiently objective and free of bias and subjective decision-making by patients, their caregivers, and/or health care professionals to allow for a valid comparison between patients in Study 201/202 and an external control group?

**Vote Result:**      YES: 5              NO: 7              ABSTAIN: 1

***Committee Discussion:** A slight majority of the committee voted “No” i.e., that decisions to administer the 6-minute walk test (vs. conclusions that the patient could no longer walk) were not sufficiently objective and free of bias and subjective decision-making by patients, their caregivers, and/or health care professionals to allow for a valid comparison between patients in Study 201/202 and an external control group. These members explained that there were difficulties in assessing historical controls, that there were problems with the primary endpoints, which measured only lower body strength, and they questioned the objectivity of the conclusion that the people in the external control group were actually unable to perform the 6-minute walk test. The members who voted “Yes” agreed that the 6-minute walk test was sufficiently objective to be meaningful, and that there was no evidence of real bias. One committee member chose to abstain, explaining that the 6-minute walk, although subjective, could be a valid endpoint, but had trouble with the context in which it was used and therefore had difficulty interpreting the question to make a firm decision. Please see the transcript for details of the committee discussion.*

5. **VOTE:** What is the impact of the North Star Ambulatory Assessment results on the persuasiveness of the findings in Study 201/202?
  - a. Strengthen
  - b. Weaken

c. No effect

**Vote Result:** Strengthen: 2 Weaken: 5 No Effect: 6

***Committee Discussion:** Six members of the committee voted that the results of the North Star Ambulatory Assessment (NSAA) had no effect on the persuasiveness of the findings in Study 201/202. One panel member stated for the record that he wanted to change his vote from “Strengthen” to “No Effect.” These members agreed that, overall, there was no evidence of difference between the two groups on either measure. The members who voted that the impact of the NSAA results weakened the persuasiveness of the findings in Study 201/202 noted that NSAA is a more comprehensive measure of functional assessment and explained that the persuasiveness was weakened because there were no statistically significant differences between the treated vs. the control groups. Please see the transcript for details of the committee discussion.*

6. **VOTE:** What is the impact of the other tests of physical performance (e.g., rise time, 10-meter run/walk) on the persuasiveness of findings in Study 201/202?
- a. Strengthen
  - b. Weaken
  - c. No effect

**Vote Result:** Strengthen: 1 Weaken: 2 No Effect: 10

***Committee Discussion:** The majority of the committee voted that the impact of the other tests of physical performance (e.g., rise time, 10-meter run/walk) had no effect on the persuasiveness of findings in Study 201/202. These members noted that the FDA and applicant are in disagreement in assessing rise time. They agreed that overall, physical performance measures in the other tests were secondary outcomes and that there was no evidence of difference between the two groups, probably because of the small sample size of the studies.*

7. **VOTE:** Do the clinical results of the single historically-controlled study (Study 201/202) provide substantial evidence (i.e., evidence from adequate and well-controlled studies or evidence from a single highly persuasive adequate and well-controlled study that is accompanied by independent findings that substantiate efficacy) that eteplirsen is effective for the treatment of DMD?

**Vote Result:** YES: 3 NO: 7 ABSTAIN: 3

***Committee Discussion:** The majority of the committee voted “No,” i.e., that the clinical results of the single historically-controlled study (Study 201/202) did not provide substantial evidence that eteplirsen is effective for the treatment of DMD. These members agreed that Study 201/202 was not a well-controlled study and based on statistical and scientific findings, substantial evidence regarding the efficacy of eteplirsen was not evident. Most who voted “No” cited problems with the controls. One noted that a*

*historically-controlled study could provide evidence of effectiveness, but that this trial did not. Two committee members noted that the original placebo-controlled portion of the study was negative. One member, noting the disconnect between the trial data and the patient testimonies, suggested that the patient community should be more willing to participate in controlled trials. One member who cited problems with the controls also noted that a single trial is insufficient. The members who voted that “Yes” said that substantial evidence did exist, adding that the study correlated with the testimonies presented by the public. With respect to the members who abstained, one member stated he was torn between the data presented by the FDA and the testimonies presented by the public. One felt uncomfortable with what he thought was a leading question. Another stated that the study was not adequate and well controlled, but that he was moved by the patients’ testimony. Please see the transcript for details of the committee discussion.*

## **11. Pediatrics**

Because Duchenne muscular dystrophy is an orphan indication, this application is not affected by the Pediatric Research Equity Act.

## **12. Other Relevant Regulatory Issues**

### **Office of Scientific Investigations (OSI) Audit**

As described by Dr. Breder, Study 201/202 was inspected at Dr. Mendell's site at Nationwide Children's Hospital. The review included an inspection of the IRB records, sponsor and monitor audit activities, financial disclosures, adverse events reporting, Informed Consent Documents for all subjects, the medical records/source data for 8 subjects enrolled, and observation of four subjects performing their individual subject level 6-Minute Walk Test (6MWT), individual subject level data for other functional assessments such as North Star Ambulatory Assessment (NSAA), Maximum Voluntary Isometric Contraction Test (MVICT), Rise Time, 10-Meter Run Time, Timed 4-Step Test, and pulmonary function tests. There was no evidence of inaccuracy of the data captured on the above metrics.

DNP consulted OSI for inspection of the sites in Belgium and Italy from which natural history data was derived. These inspections were ongoing at the time of writing of this review.

As I do not believe the clinical data support full approval, the results of this inspection are not indispensable for me to provide scientific conclusions about the efficacy data and make recommendations to the signatory authority.

### **Controlled Substance Staff review**

CSS concluded that eteplirsen does not have the profile of a drug with abuse potential and that an abuse potential assessment for eteplirsen is unnecessary.

### **Evaluation to determine if a REMS is necessary (DRISK)**

The Division of Risk Management (Office of Surveillance and Epidemiology) concluded that risk mitigation measures beyond the professional labeling are not warranted at this time to ensure that the benefits of eteplirsen outweigh the risks, based on the identified risks, the likely prescribing community of specialists, and the lethal nature of the disease.

### **Proprietary name review**

The Division of Medication Error Prevention and Analysis (Office of Surveillance and Epidemiology) finds the proposed proprietary name, Exondys 51, acceptable.

### **13. Labeling**

As I am recommending a complete response for this action, I do not have any recommendations regarding labeling at this time, besides noting that I am not aware of any safety issue that would warrant any contraindication, warning, or precaution. The indication section would need to reflect that the drug is for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping, and describe the basis for that indication if accelerated approval is considered by the signatory authority.

### **14. Postmarketing**

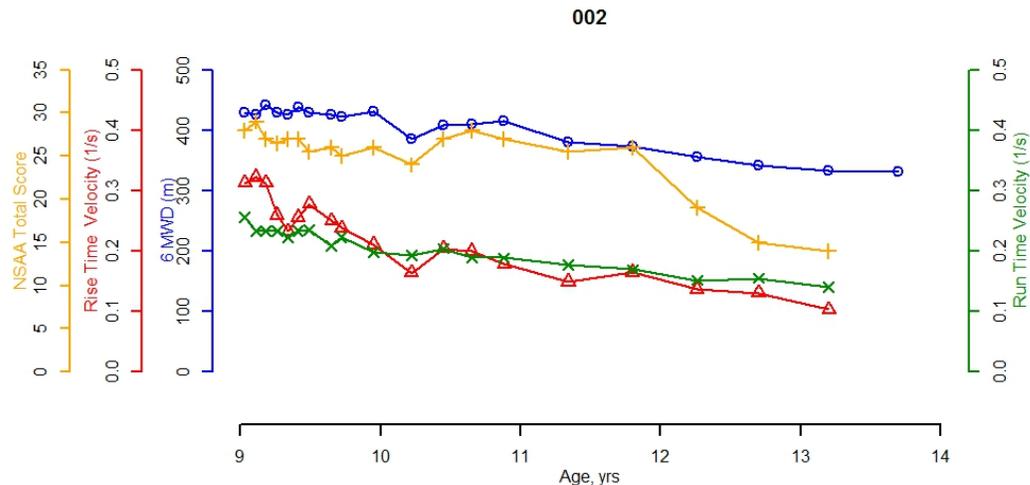
A Postmarketing Risk Evaluation and Mitigation Strategy is not needed for this product.

Other Postmarketing Requirements and Commitments should include those already agreed upon with the applicant by the OCP review team, and, if accelerated approval is considered by the signatory authority, postmarketing studies to confirm clinical benefit.

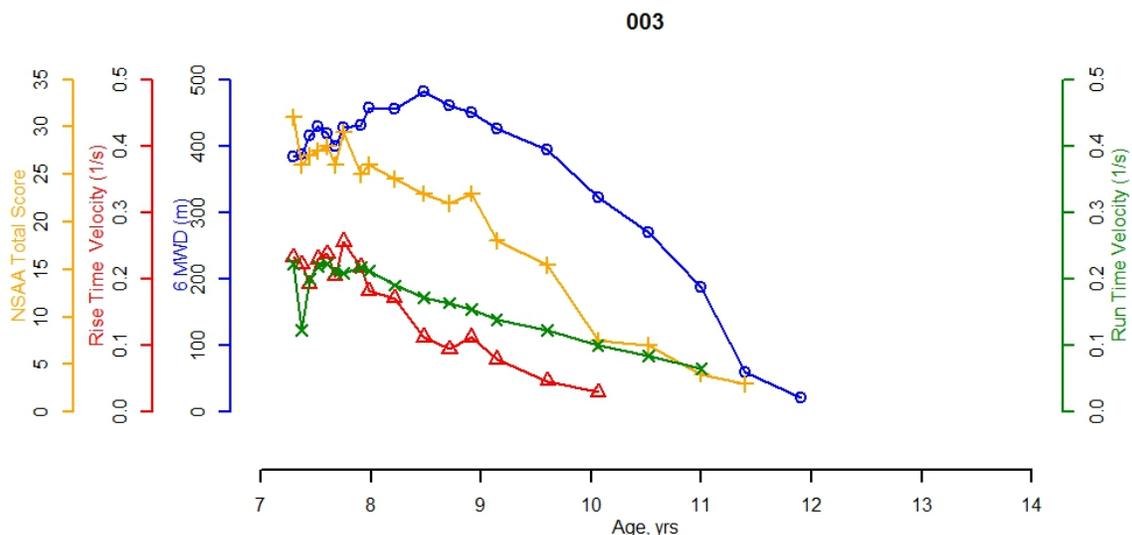
## Appendix 1: Patient profiles

The natural history in patients with DMD amenable to exon 51 skipping indicates a wide age range at the time of loss of ambulation, from 8 to 18 years of age for most patients. To obtain a full understanding of the disease progression in eteplirsen-treated boys, it is important to look at all individual patient profiles. We already reviewed earlier the profiles of the four patients who were still ambulating at age 14. Below are the profiles for the other 8 boys.

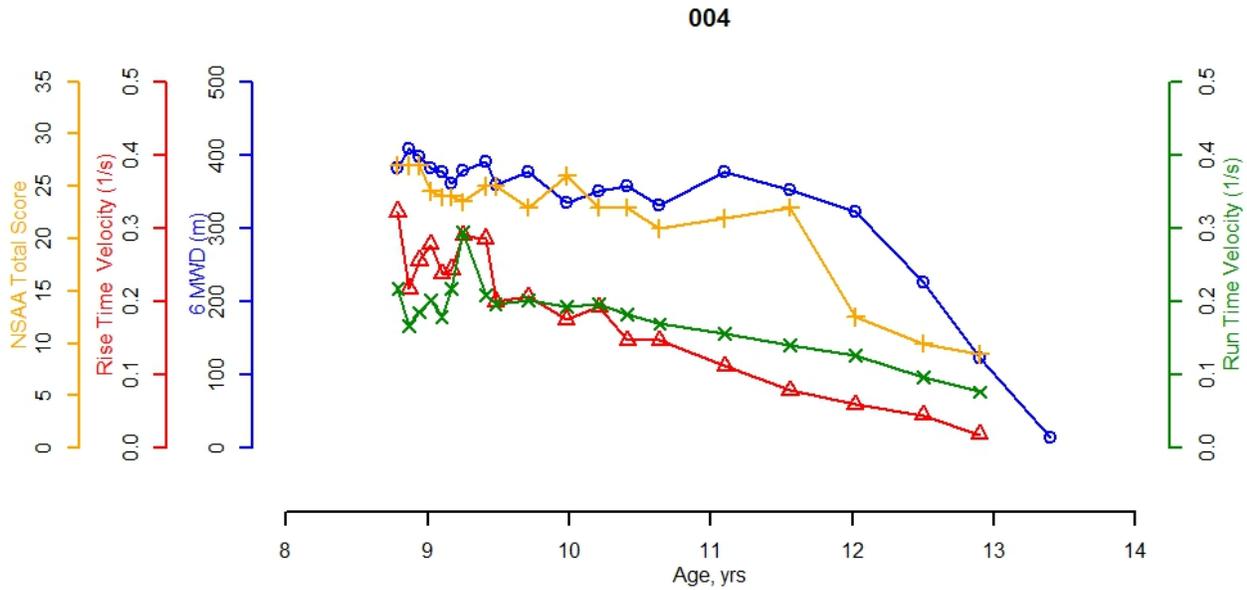
Patient 002 (eteplirsen 30 mg/kg) had a relatively mild course. Patient 002 has 0.14% dystrophin at Week 180, indication that eteplirsen is not likely to have contributed to the course of the disease in this patient.



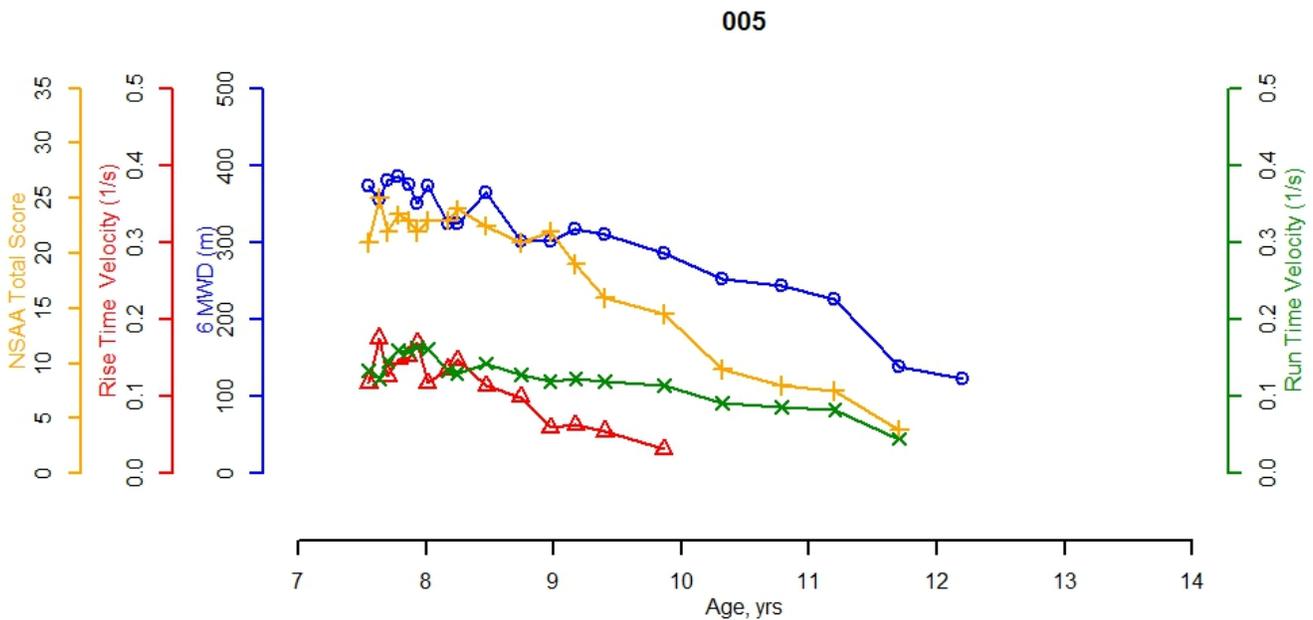
Patient 003 (eteplirsen 50 mg/kg) had a rapid decline in all outcome scales. Patient 003 had 0% dystrophin at Week 180.



Patient 004 (eteplirsen 50 mg/kg) had relative stability up to age 11 and a half, and then rapidly declined in all outcome scales. Patient 004 had 0.96% dystrophin at Week 180.

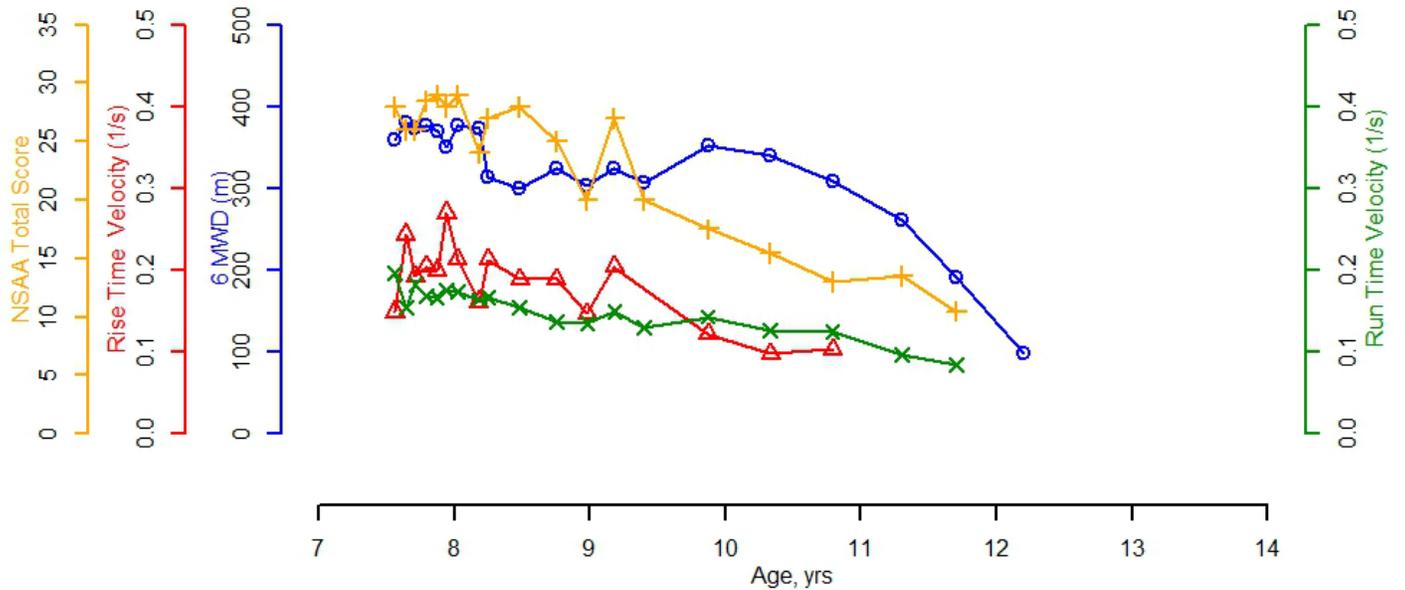


Patient 005 (eteplirsen 50 mg/kg preceded by placebo in Study 201) had a rapid decline in all outcome scales. Patient 005 lost the ability to rise at age 10 years. Patient 005 had no biopsy at Week 180.



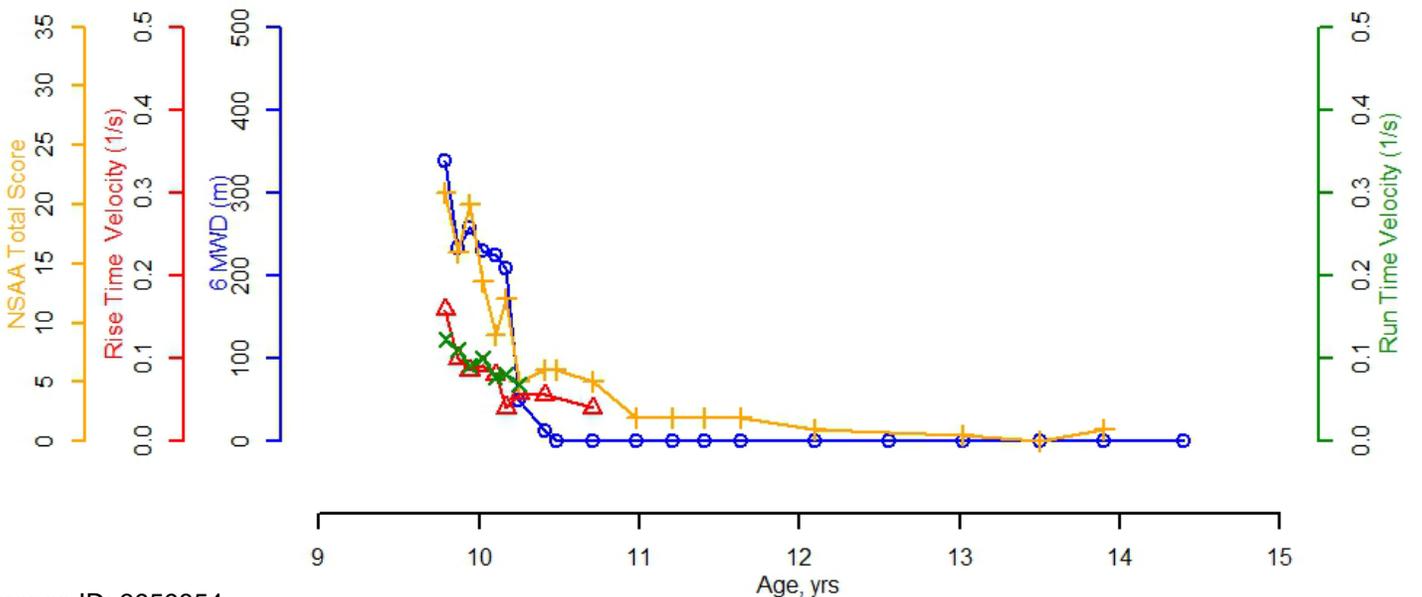
Patient 007 (eteplirsen 30 mg/kg preceded by placebo in Study 201) was relatively stable up to age 11 years, then had a steady decline in all outcome scales. Patient 007 had 0% dystrophin at Week 180.

**007**

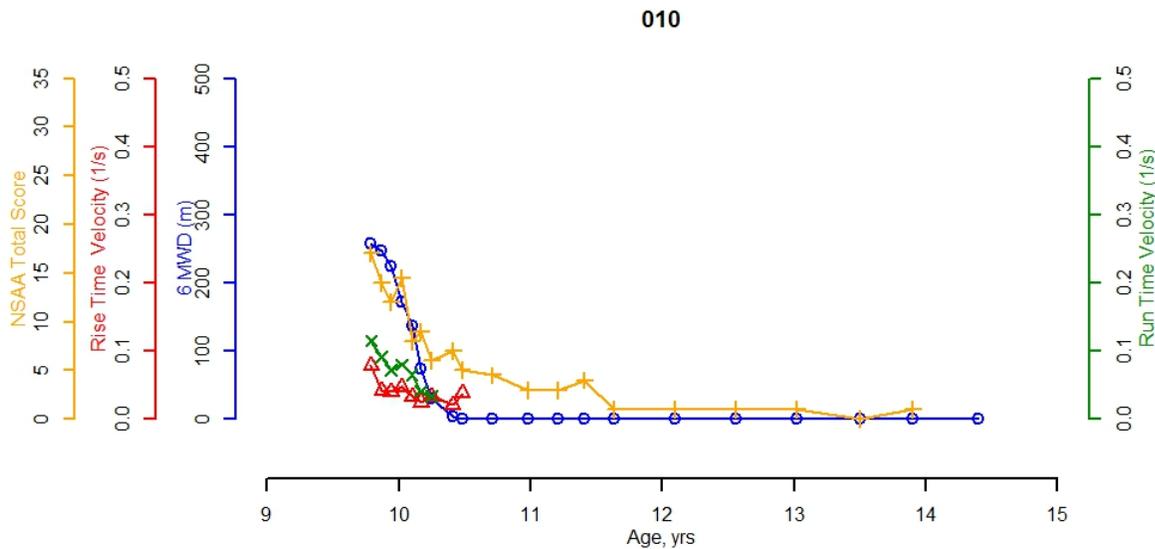


Patient 009 (eteplirsen 30 mg/kg) had a rapid decline in all scales from age 9.5 years. Patient 009 had 0.52% dystrophin at Week 180.

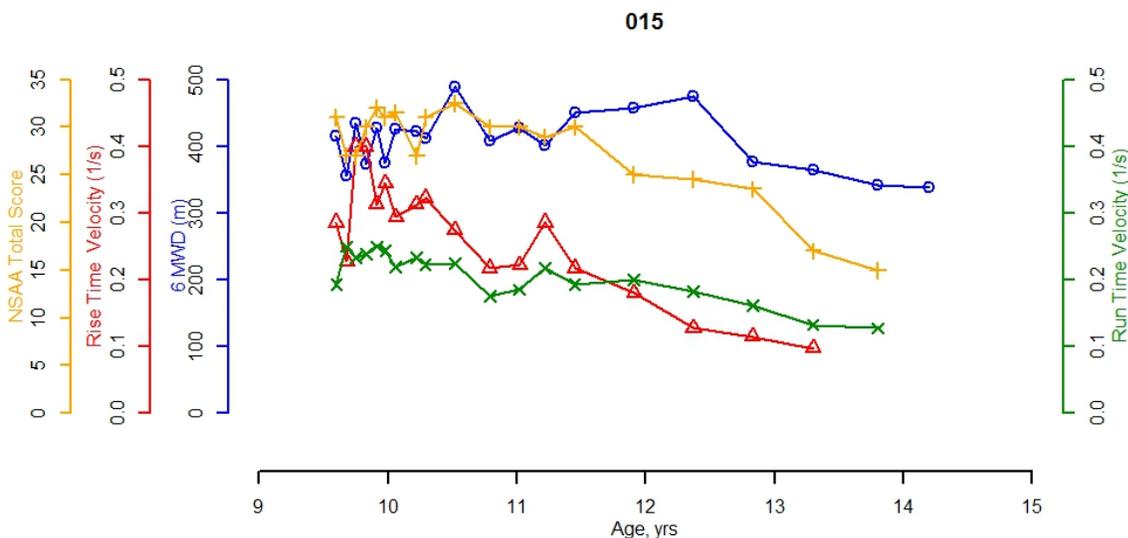
**009**



Patient 010 (eteplirsen 30 mg/kg) had a rapid decline in all outcome scales starting at age 9.5 years. Patient 010 had a dystrophin level of 1.615% at Week 180.



Patient 015 (eteplirsen 50 mg/kg), who had not reached age 14 years at the time of the Week 214 cutoff, had a 6MWT of 344 meters at Week 240, becoming the fifth patient ambulatory after age 14. Unfortunately, his NSAA, Rise time, and Run time are not available at the time of writing this memo. Patient 015 has showed a steady decline of NSAA score, starting at age 11 and a half, and steady worsening of rise time velocity and run time velocity, starting around age 10. Patient 15 had a dystrophin level of 2.05% at Week 180.



## Appendix 2: Protocol of the Leuven Neuromuscular Reference Center Registry

### DATABASE: FUNCTION TESTS, CLINICAL (Height, Weight, steroid use) AND GENETIC DATA FROM DMD PATIENTS ATTENDING THE NMRC UNIVERSITY HOSPITALS LEUVEN

#### Aim:

To create a database of all function test data (6 MWD, Timed function Tests, pulmonary function tests,..) in relation to age, weight, height, BMI , steroid use and gene mutation in patients with Duchenne Muscular Dystrophy attending the Neuromuscular Reference Centre for Children at the University Hospitals Leuven.

These data will improve the insights in the contemporary natural history of this disease in the context of new therapy developments.

#### Methods:

All data captured in clinical source documents on demographics, steroid use, genetic mutation and function tests from all DMD attending the NMRC Leuven were entered in the database. Start/stop date of steroid use and any participation in pharmacological trials are recorded. Physiotherapy assessments have been collected in great majority by Marleen vanden Hauwe, physiotherapist , with extensive experience in clinical assessment of 6MWD, timed tests, pulmonary function, .. both in clinic and clinical trials. In the absence of Mrs vanden Hauwe, some testings have been performed by Annelies van Impe, physiotherapist, as well experienced in the assessment of these tests both in clinic and in clinical research setting.

Name, date of birth, date of visit, age at visit, weight, height, BMI, pulmonary function tests, DEXA data, 6MWD, Timed tests (10m,stairs, Gowers,) steroid use, (start and stop date of steroid, dosage,) code for past or current participation in any investigational trial, Becker DMD. Columns with Geiger 's % pred 6MWD were added later on.

First data entered 11 august 2011, including retrospective data. Ongoing expansion and curation of data from then on.

Datacut for publication in Neuromuscular Disorders : September 2012

Datacut for sharing data with Sarepta: February 2015

Publication of data cut Sept. 2012: Ambulatory capacity and disease progression as measured by the 6-minute-walk-distance in Duchenne muscular dystrophy subjects on daily corticosteroids . *N. Goemans et al. Neuromuscular Disorders 2013*

## **Materials and methods**

### **Participants**

This study was an observational single center cohort study reporting 6MWD collected as part of routine follow-up clinics from genetically confirmed and corticosteroid treated DMD boys attending the Leuven Neuromuscular Reference Center (NMRC) for clinical care and management.

All DMD subjects up to 17.5 years of age attending the NMRC between January 2007 and September 2012 were assessed for eligibility. Inclusion criteria were genetically proven diagnosis of DMD and being on chronic daily treatment with corticosteroids. Subjects with known severe cognitive or behavioral disorder impairing compliance with the 6MWT procedure, subjects with a clinical picture of Becker muscular dystrophy and genetic diagnosis predicting a milder phenotype such as in frame deletions, as well as subjects that were involved in clinical trials or had participated in any trials with investigational products, were excluded.

Genetic data, treatment information (type of corticosteroid, dosage, duration of treatment and regimen) and anthropometric measurements (weight, height measured according to standard anthropometric methods) were collected.

This study was approved by the Institutional review board of the University Hospitals Leuven. Written consent was obtained from parents of all DMD boys to report their clinical assessment data anonymously in an observational study.

### **Assessments**

6MWTs, using a 25 meter linear marked course on a flat surface and a "safety chaser" to provide standardized encouragements and assist with falls, timed function tests and North Star Ambulatory Assessment were performed as part of the assessments at follow-up clinics by two trained and experienced evaluators according to the procedure currently used in clinical trials.

**Data analysis:**

-Summary statistics on all functional data from steroid treated DMD ("steroid" column x code 1)

-The remaining records are not included ( "code" column Y)

1="PTC on treatment"

1.5="PTC placebo"

2="PRO051"

3="PRO044"

3.5="Stop PRO44"

4="Beckers"

5="Intermediate"

6="Post PTC"

7="Poor cooperation"

8="Late referral" (*patients referred in a later stage from area's with poor standards of care-  
no physio, no steroids*)

9="GSK968"

9.5="Stop GSK968"

## Appendix 3: Protocol of the Italian DMD Registry

### Protocol GUP07009/ GUP09010/ P?

This study is designed as a large multicenter study. Patients who will fulfil the following inclusion criteria will be included in the study:–

**INCLUSION CRITERIA:**1. Patients with genetically confirmed diagnosis of DMD with age between 2 and 18 years;2. Good health at the time of the assessments. Assessments will be rescheduled for a later date in the event of any intercurrent illness that might affect performance. 3. If on any drug or dietary supplement, the dose must have been the same for the 90 days prior to entering the study.–

**EXCLUSION CRITERIA:**1. Mental retardation (IQ 7 years)2. Severe behavioural problems or frank psychiatric disease (pervasive developmental disorders, psychosis diagnosed according to DSM IV)3. Poor compliance with physicians' recommendations.4. Primary caregiving parent (who will accompany the child) who is, in the investigators opinion, mentally or legally incapacitated, preventing informed consent, or are unable to read and understand written material including the consent.5. Patients on steroids or other treatments will not be excluded but type, regime and duration of treatment will be noted.

**6MWT:** The test will be performed according to the guidelines provided by the American Thoracic Society (ATS, 2002) and modified for DMD as in the recently used PTC protocol.

**NSAA:**The NSAA has been developed in order to be used in a range of ambulant children. The scale has a manual with clear instructions and can be completed in approximately 10 minutes even in children with mild to moderate mental retardation.

**Timed items (walking 10 meters and getting up from the floor from sitting and from lying):**The tests will be performed according to standard procedures.

In each Centre the examiner involved in the previous study will be responsible for the assessments and will perform all the longitudinal evaluations. The participating groups will meet before the study will be started to:a. be trained on the scoring system and the administration procedures of the NSAA and the timed items,. b have a training session for the 6MWT by one dedicated physiotherapist who is familiar with the test and will follow the protocol provided by the American Thoracic Society adapted for DMD. The training sessions will involve a physiotherapist/neurologist from each participating centre and after a formal

training session, each physiotherapist will be asked to perform the 6MWT on a patient together with the trainer. 3. Application of the assessment to DMD boys: a. the NSAA and the timed items (walking 10 meters and getting up from the floor from sitting and from lying) and the 6MWT will be performed.

Each PT will be asked to perform a full examination in his/her own centre and a second training session will be organised to review possible mistakes and have a new interobserver reliability assessment on video

The assessment will be performed at baseline and after 6 and 12 months following the 6 month assessment schedule that is part of the routine follow up of these patients.

Steroids are routinely used in all centers but different regimes are used even within individual centers. We foresee to include at least 20% who will not be on treatment.

After 1 year follow up for each outcome measure we will establish the distribution of results and variability for each year of age and the changes observed over a 1 year period. Even if the cohort will be relatively large, the analysis of the longitudinal data will be affected by the number of variables related to age, type of treatments that may results in too small subgroups. These natural history data can be useful as background information for forthcoming trials in which the same outcome measures will be used. Taking also in consideration that the ongoing trials allow patients to continue the previously started treatment regime. We will also look at the correlation among the selected outcome measures. All the centers will meet one year after the recruitment has started to discuss the state of the recruitment and to plan further steps. A final meeting will be held after the results of the statistical analysis will be available.

#### **Time table**

Month 1: The participating groups will meet before the beginning of the study to discuss practical issues about training and to agree on outcome measures administration and scoring systems.

.Months 2–3: Training sessions.

Months 4–10: Enrollment of patients and baseline assessment. All patients in our centres are routinely seen every 6 months and we therefore foresee that we will be

able to complete the enrollment over a 7 month period. A second meeting will be held in order to discuss number of patients recruited and any difficulty met in the first phase. Months 10–22: Follow up assessments 6 and 12 months after the initial assessment. Month 23–24: Analysis of the data. A final meeting will be held to discuss results and possible further follow up.

**DATA RECORDING AND STORAGE:** A paper Case Report Form (CRF) will be used to record patients' details and performance on the functional tests.

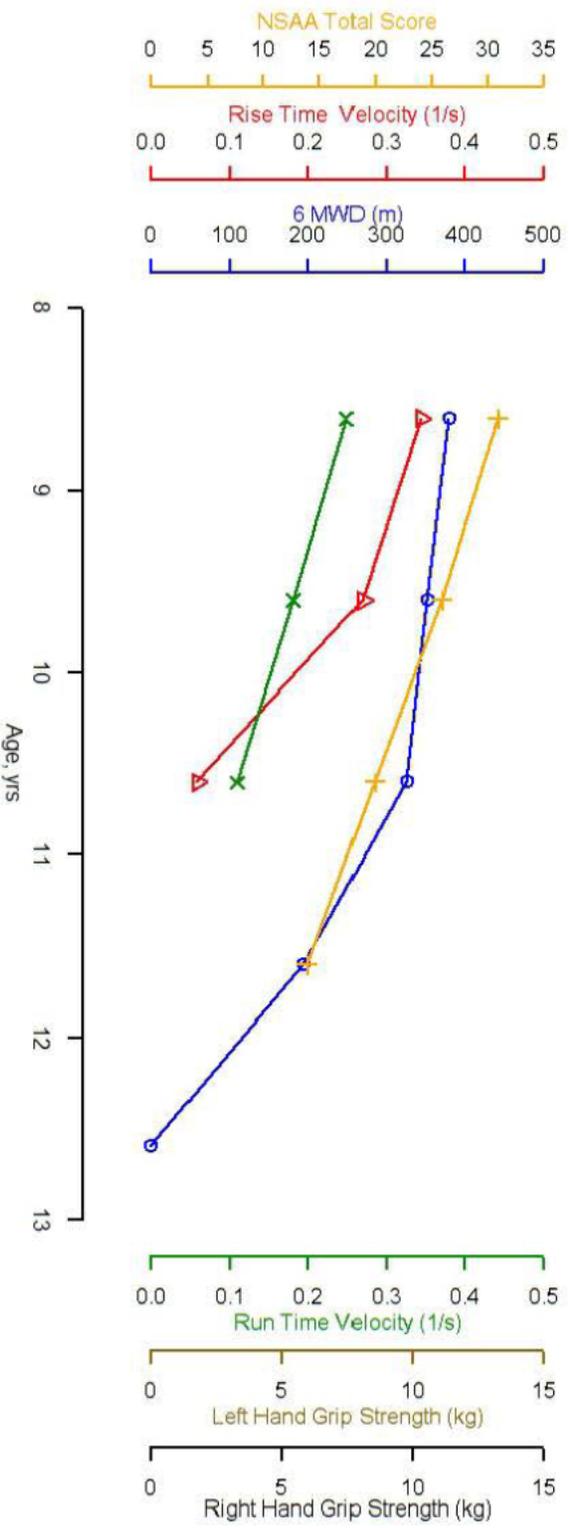
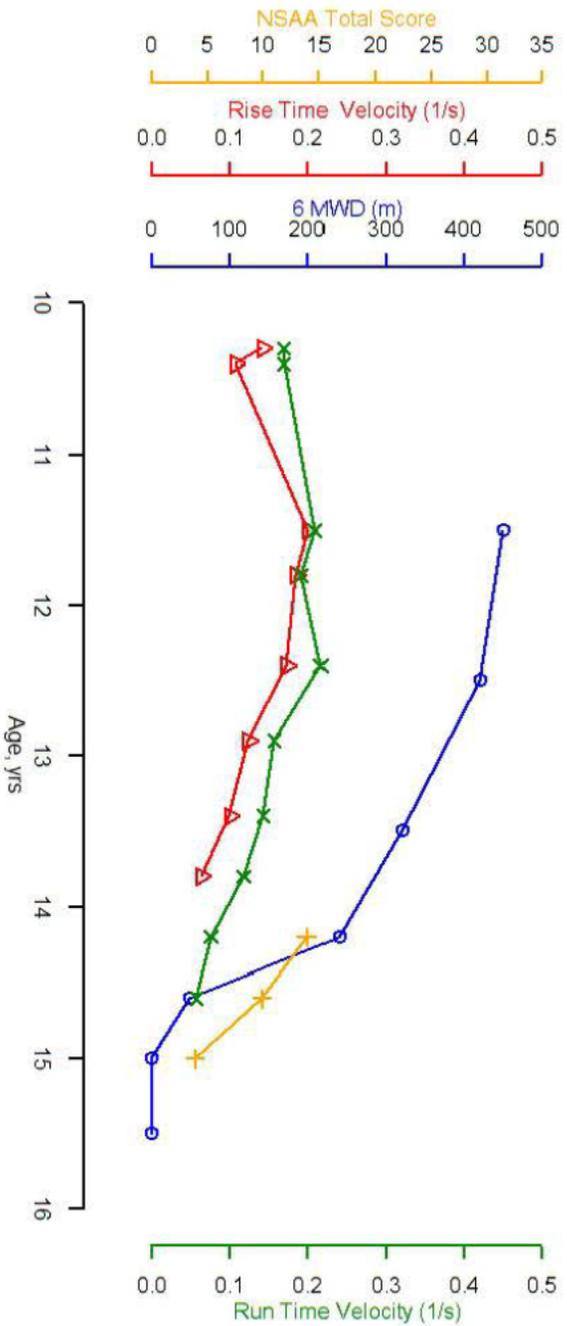
The patient CRF will be filled in by a part-time fellow in each centre. The CRF of each patient will be maintained on site under the responsibility of the P.I. in each institution. The CRF data of all centres will be then collected and entered in a dedicated database by the dedicated research fellow of the Coordinator Centre. Patients will be identified by an anonymous code number, with the master key available only by the P.I. in each institution.

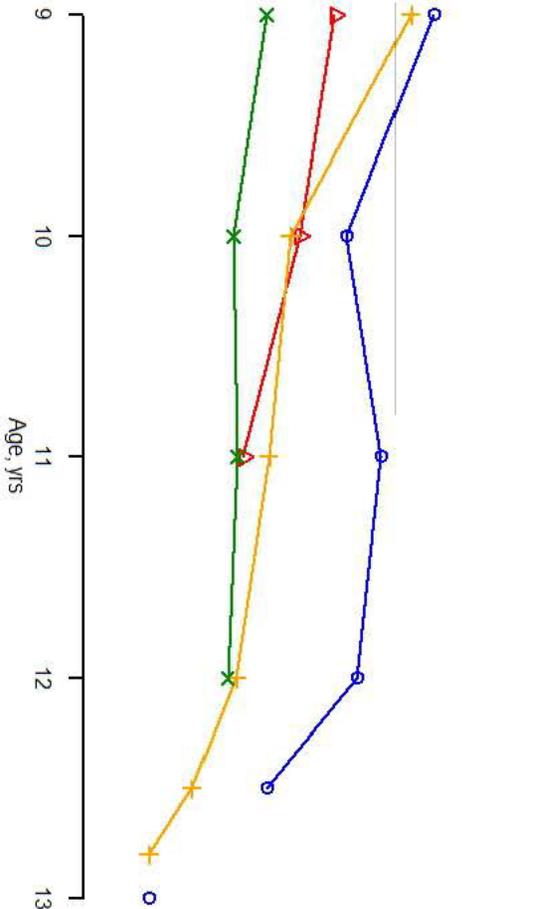
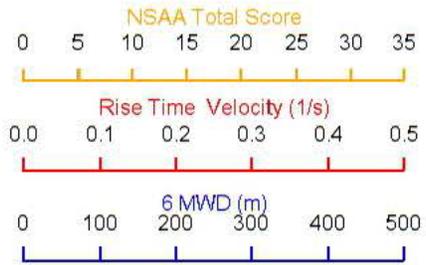
Maintenance of the study database will be the responsibility of the coordinator unit. The data will be stored and analyzed in accordance with Italian legislation. Only aggregate results will be disseminated.

**PLAN FOR STATISTICAL ANALYSIS:** The distribution of each of the outcome variables (6MWT, NSSA, timed items) will be assessed. Descriptive analysis will be carried out by computing means and medians of continuous variables (as appropriate according to the type of distribution, i.e. whether approximately normal or not), together with ranges, standard deviation and standard errors. Proportions and 95% confidence intervals will be computed for categorical variables. Test-retest reliability will be assessed using Intraclass Correlation Coefficients (ICCs) (Stanish et al. 1983). Appropriate statistics for repeated measures study design (parametric and nonparametric ANOVA and multivariable mixed-effects regression modeling) will be used to assess changes of outcome scores over time (baseline, 6 and 12 months) adjusting for covariates (e.g. steroid treatment). The relationship among outcome measure results will be assessed through the ICC; means and 95% CI of times will be computed separately for the individual outcome measure results.

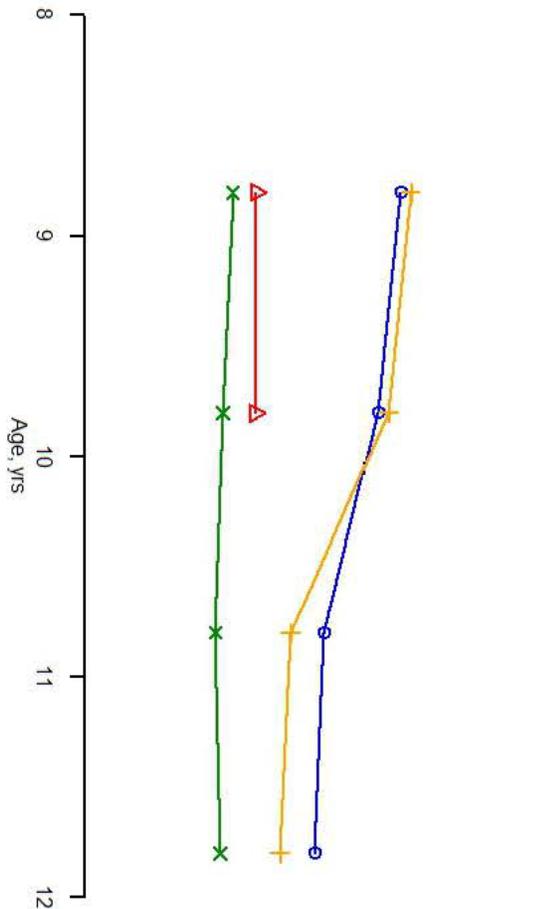
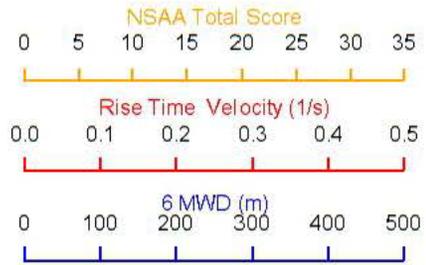
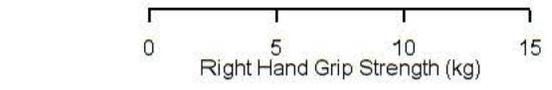
Verified: Date: 24 nov 2015  
Name: Prof. E. Mercuri  
Signature: 

## Appendix 4: Patient profiles from the Belgium and Italian Registries



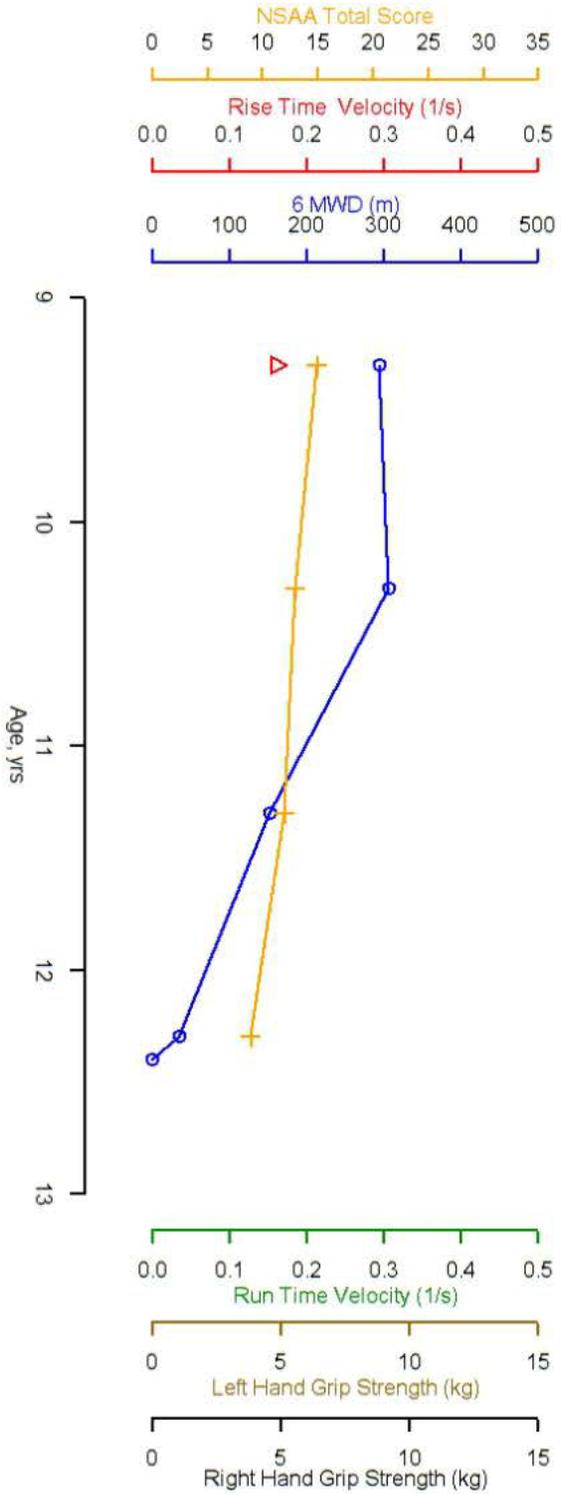
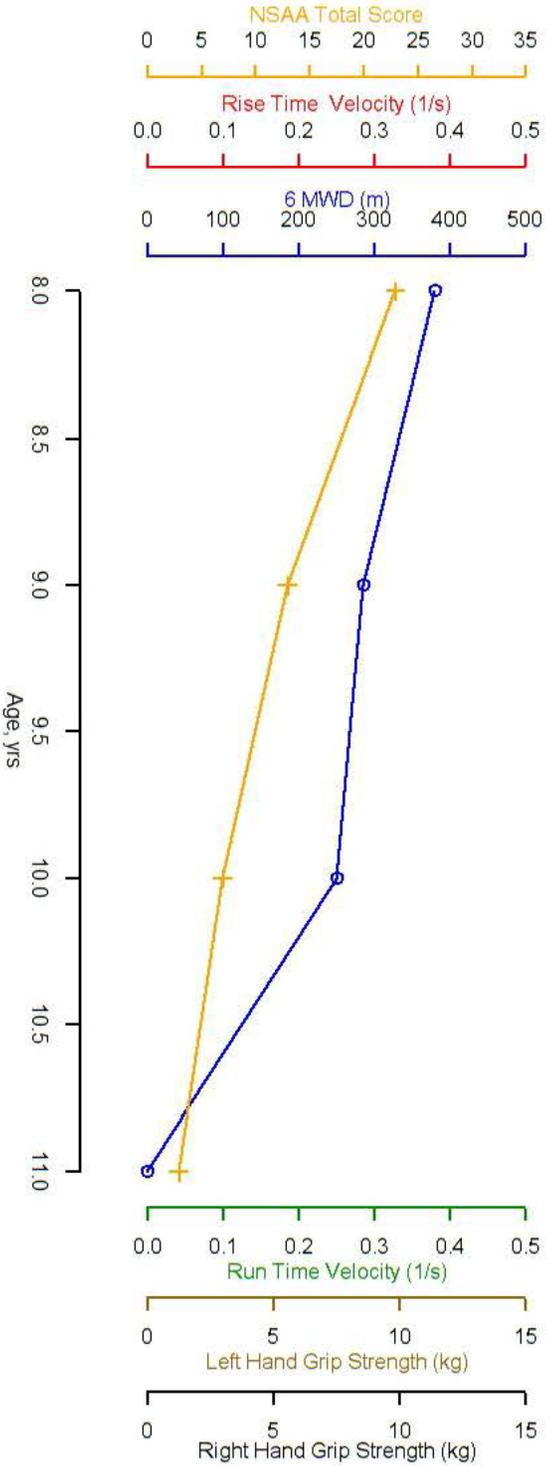


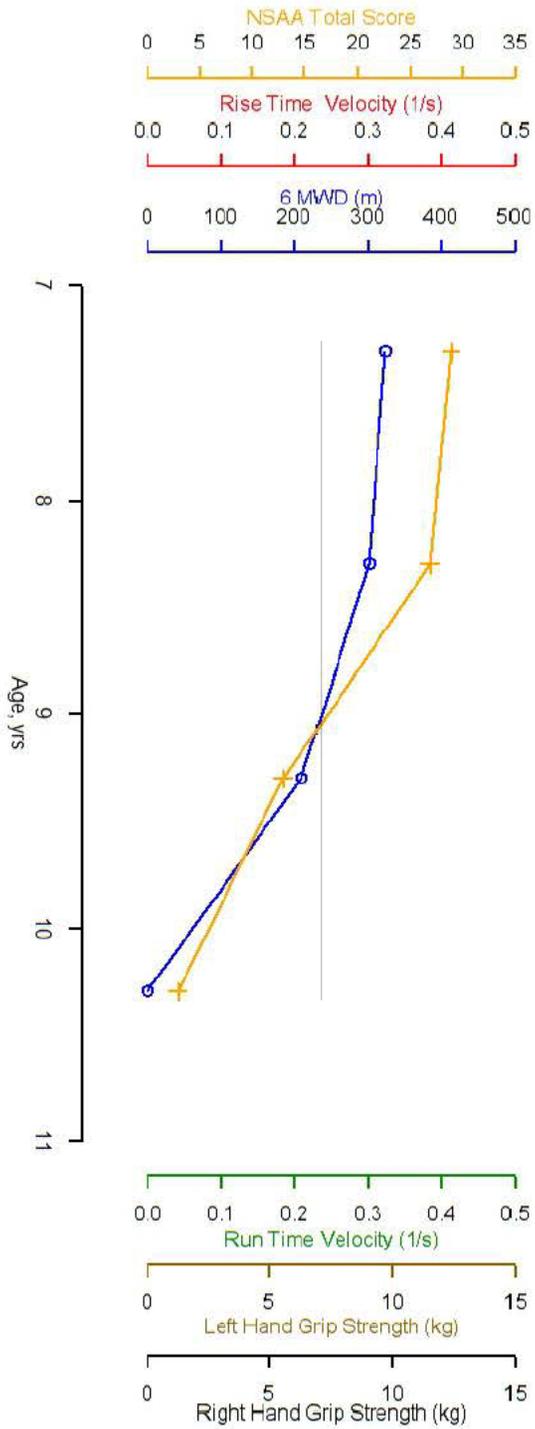
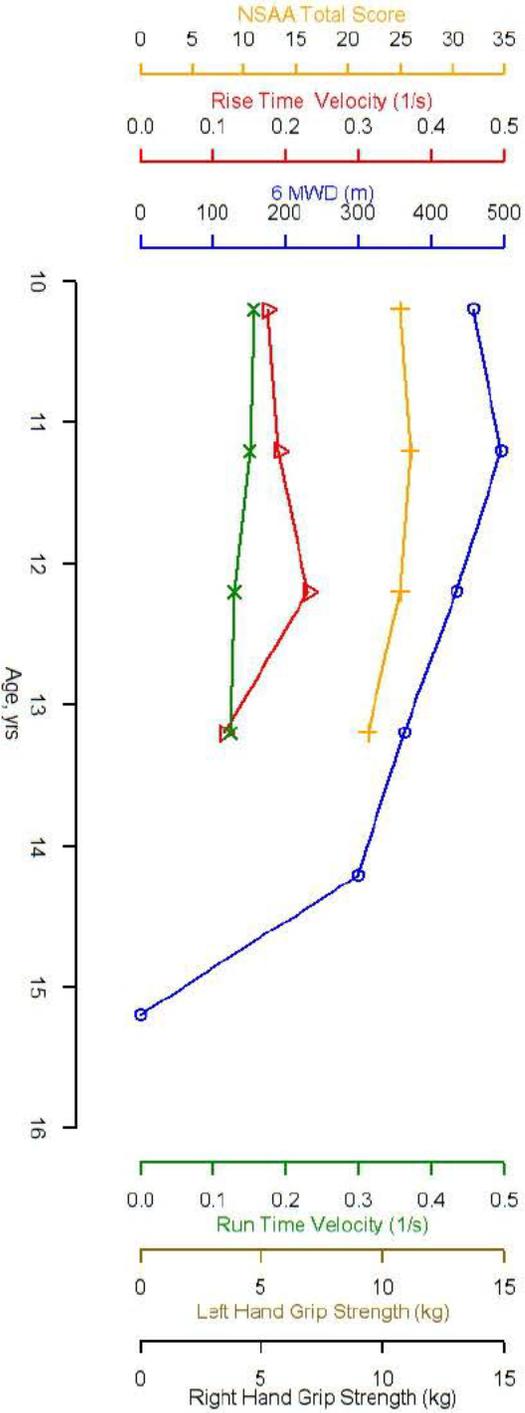
Mibesta8

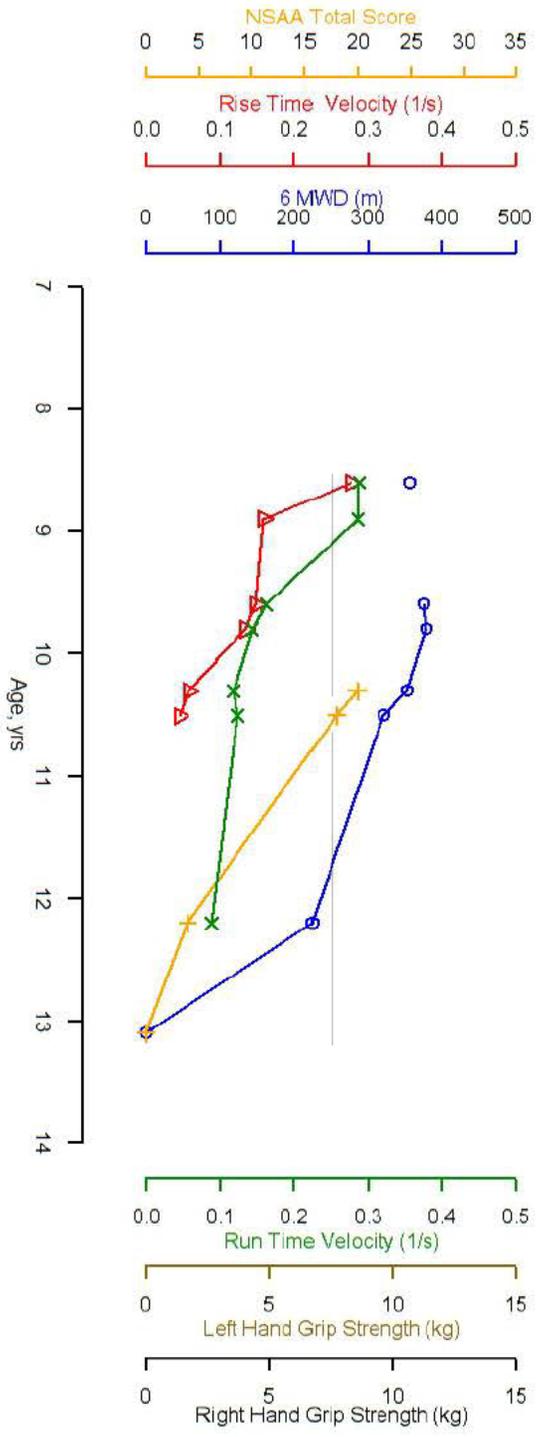
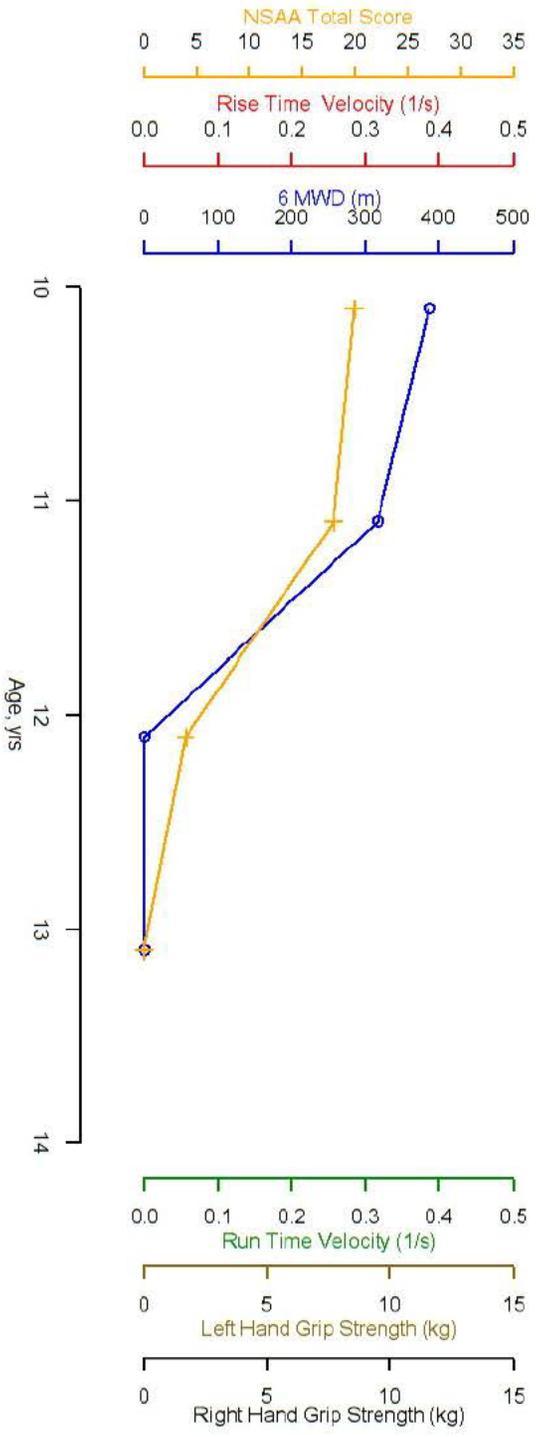


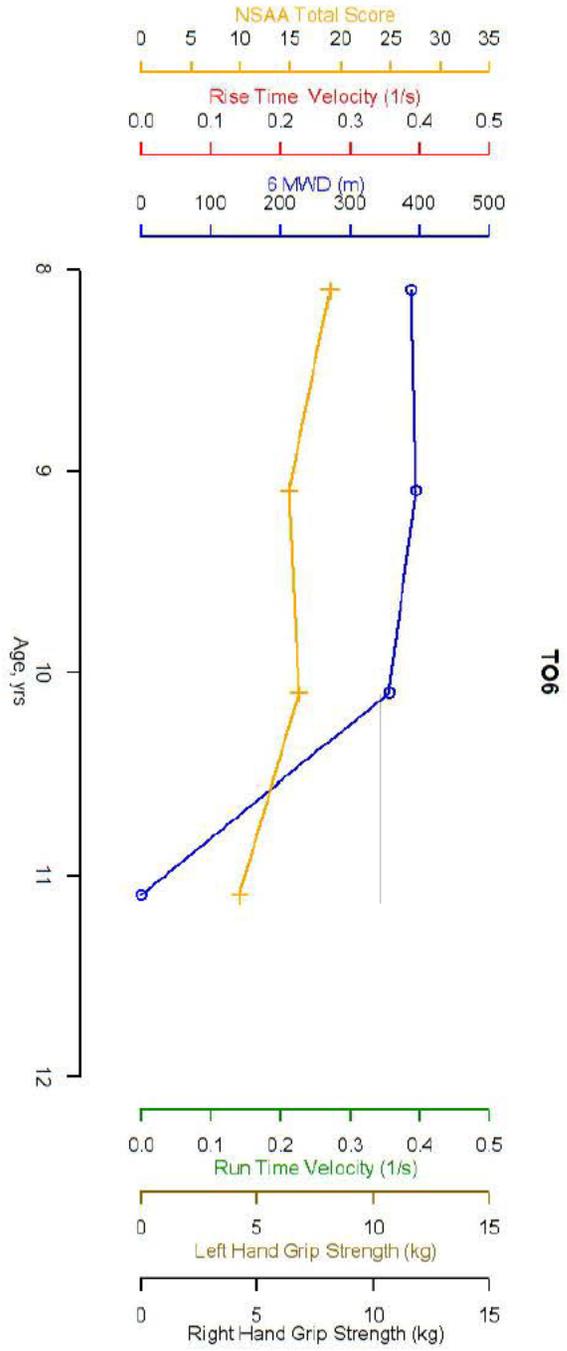
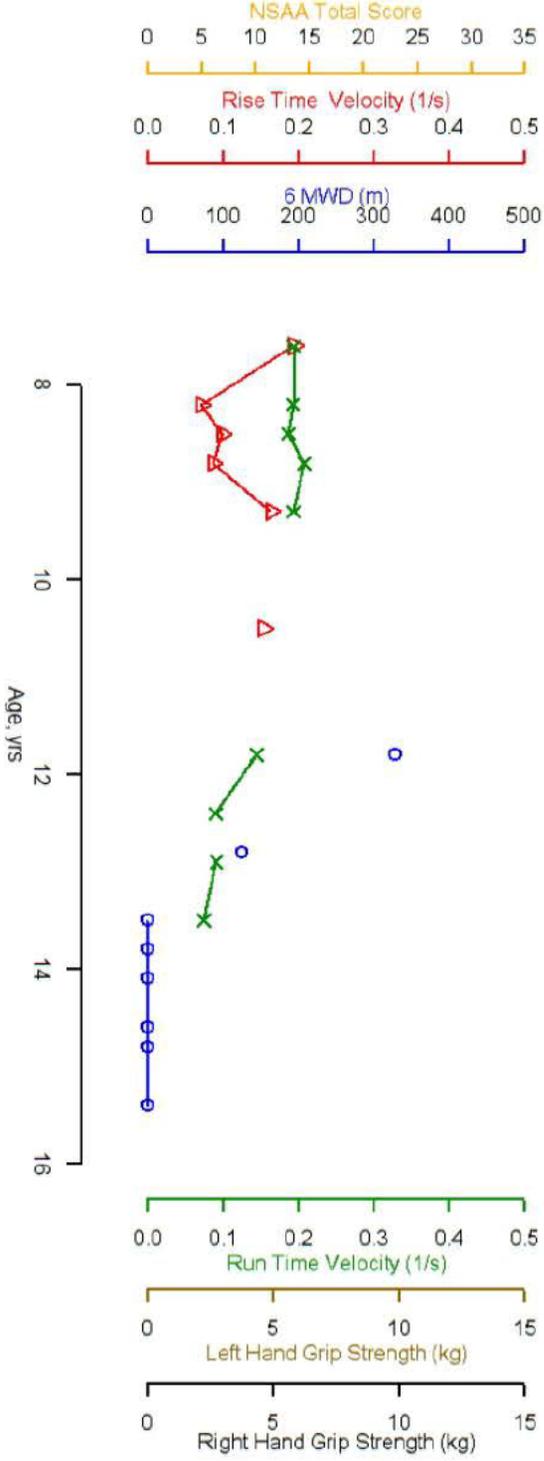
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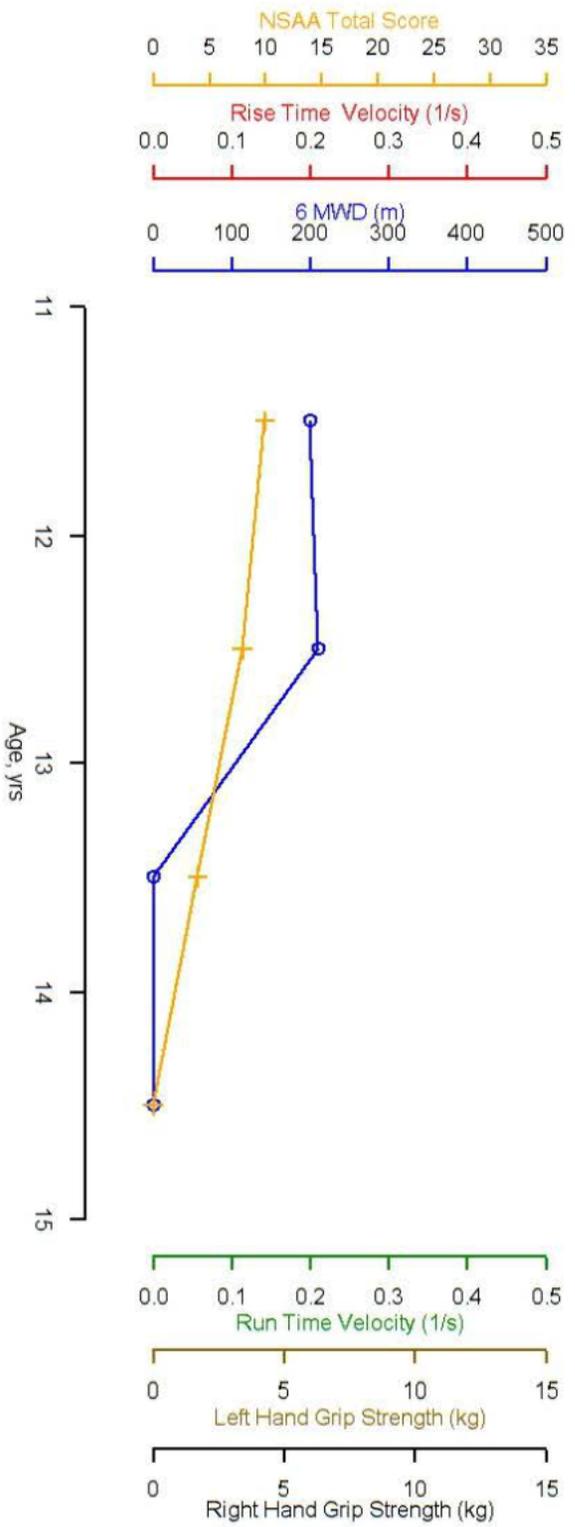








GE10



## Appendix 5: Week 180 Western Blot procedures

### Sarepta response:

By convention, the Study 201/202 Week 180 Western blot protocol (SR-CR-15-004) listed a Laboratory Director, Laboratory Technicians and a Contributing Scientist, Biometrics who was responsible for the statistical analysis.

- [REDACTED] (b) (4)
  - Role: In this project was responsible for review of protocol and final report
- Dr. Schnell and Cas Donoghue: Sarepta Laboratory Technicians
  - Role: Execution of technical laboratory aspects of Western blot method on blinded samples at NCH laboratories; no further involvement in the analysis of the data.
- [REDACTED] (b) (4): Contributing Scientist, Biometrics
  - Role: Provide initial statistical support for protocol development. Subsequent analysis was performed [REDACTED] (b) (4)

As defined in the Week 180 protocols, **rigorous and appropriate blinding of test article and control samples was maintained for the duration of the analytical process** (Table 1, Figure 1, Figure 2). Mr. James Shao (Director of Biostatistics, Sarepta) who was not involved in sample processing or analysis, assigned random blinding codes to each sample analyzed. He generated labels and forms containing the blinding codes and assembled blinding kits, which were QC'd by Stefan Seman (Sr. Associate, GCP Compliance, Sarepta). Stefan Seman sealed the blinding kits and provided them to Jon Voss (Sr. Director of Quality, Sarepta) who hand delivered the blinding kits to [REDACTED] (b) (4)

[REDACTED] (b) (4) allocated muscle biopsy tissue into tubes and onto slides for further processing (SR-CR-15-003). [REDACTED] (b) (4) opened the sealed blinding kits and applied labels to tubes and slides with the appropriate blinding code numbers and filled out the blinding code records. The blinding code records were kept in a secure location and all laboratory personnel performing sample processing, imaging, assay execution and analysis remained blinded to the patient identification and treatment status throughout the study until after database lock. The blinded tubes were then transferred by [REDACTED] (b) (4)

[REDACTED] (b) (4) handed the blinded tubes containing the samples to be processed to the laboratory technicians Fred Schnell (Sr. Scientist, Sarepta) and Cas Donoghue (Research Associate,

Sarepta), who executed the technical aspects of the Western blot assay at the NCH laboratory. The blinded labeled Western blot films were then provided to another scientist, (b) (4) to perform the scanning and the densitometry at NCH laboratories and record the raw blinded data into datasheets. This step was taken to ensure separation of duties and rigorous maintenance of the blind.

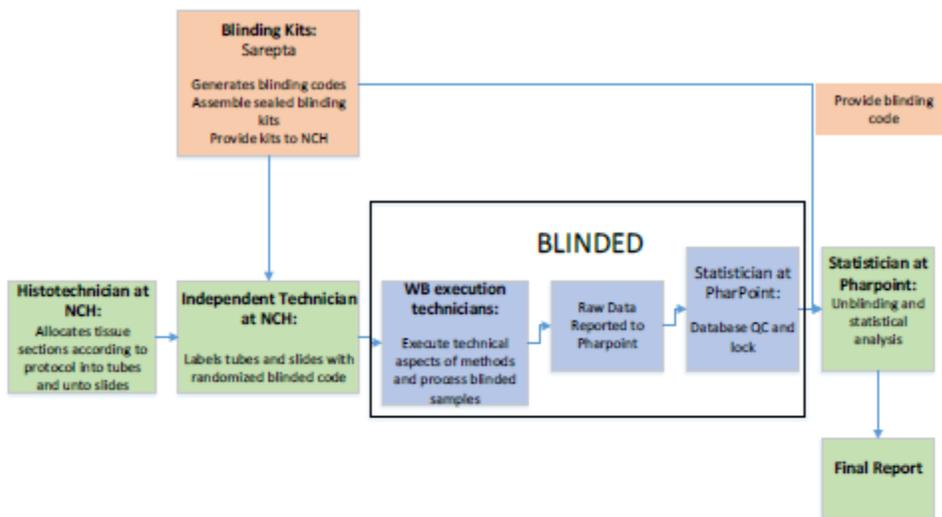
The original datasheets containing the blinded raw data of each sample were checked for completeness of data entry by Mr. Stefan Seman. The original datasheets were photocopied by him and provided as certified copies to (b) (4). (b) (4) entered the raw blinded data into a secure database, oversaw QC and locked the database. Mr. Shao provided the blinding key to (b) (4) to unblind the data, perform subsequent statistical analyses, and create unblinded tables, listings and figures.

The tables and figures below provide further information on:

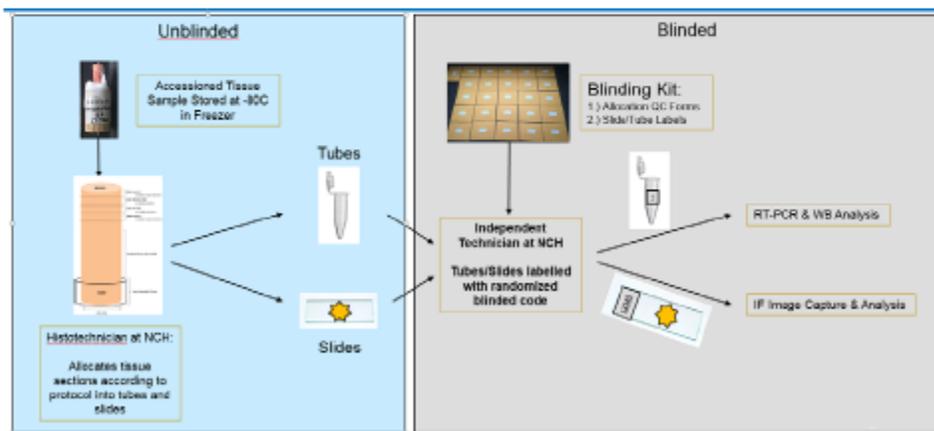
- A list of the personnel involved in the Week 180 Western blot blinding and analyses (Table 1)
- Flowchart depicting key stages of sample blinding, analysis and unblinding (Figure 1)
- Blinding for tissue allocation for Western blot analysis (Figure 2)
- NDA reports for tissue allocation and Western blot (Table 2)

**Table 1: Personnel Involved in Study 201/202 Week 180 Western Blot**

Name	Title	Role
<b>Nationwide Children's Hospital</b>		
(b) (4)	Laboratory Director	Protocol and final report review
	Laboratory Coordinator	Tissue allocation
	Histology technician	Assigned blinding codes to samples
<b>Sarepta Therapeutics, Inc.</b>		
James Shao	Director, Biostatistics	Generated and held blinding code
Stefan Seman	Sr. Associate, GCP Compliance	Assembled blinding kits, QA oversight and data QA
Dr. Fred Schnell	Sr. Scientist	Execution of WB laboratory work
Cas Donoghue	Research Associate	Execution of WB laboratory work
Johannes Dworzak	Scientist, Translational Research	Execution of WB laboratory work
Dr. Uditha DeAlwis	Director, Quality Control	QA oversight and data QC
Jon Voss	Sr. Director, Quality	Quality oversight
(b) (4)		
	Sr. Biostatistician	Data entry, database lock, statistical analysis, final data listings



**Figure 1: Flowchart Depicting Key Stages of Sample Blinding, Analysis and Unblinding**



**Figure 2: Blinding for Tissue Allocation for Western Blot Analysis**

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/s/  
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ERIC P BASTINGS  
07/15/2016

## Cross-Discipline Team Leader Review

<b>Date</b>	May 26, 2016
<b>From</b>	Ronald Farkas
<b>Subject</b>	Cross-Discipline Team Leader Review
<b>NDA/BLA #</b>	206488
<b>Supplement#</b>	
<b>Applicant</b>	Sarepta Therapeutics
<b>Date of Submission</b>	June 6, 2015
<b>PDUFA Goal Date</b>	May 26, 2015
<b>Proprietary Name / Non-Proprietary Name</b>	EXONDYS 51/eteplirsen
<b>Dosage form(s) / Strength(s)</b>	For intravenous infusion / 50 mg/mL
<b>Applicant Proposed Indication(s)/Population(s)</b>	Treatment of Duchenne muscular dystrophy in patients who have a confirmed mutation in the dystrophin gene that is amenable to exon 51 skipping
<b>Recommendation on Regulatory Action</b>	Complete Response
<b>Recommended Indication(s)/Population(s) (if applicable)</b>	N/A

## 1. Benefit-Risk Assessment

### Benefit-Risk Summary and Assessment

**Introduction:** Eteplirsen is a phosphorodiamidate morpholino oligomer with a sequence intended to bind to exon 51 of the human dystrophin pre-mRNA to cause skipping of exon 51 and result in production of an internally truncated but still partially functional dystrophin protein. In some patients with a similar but less severe form of muscular dystrophy, called Becker muscular dystrophy (BMD), this truncated dystrophin is produced as a result of the underlying mutation. The phenotype of these BMD patients is very heterogeneous, and premature death from cardiac involvement is common, but in many patients ambulation is preserved well into adulthood and, in other patients, symptoms are few and lifespan can be normal.

**Analysis of Condition and Treatment Options:** DMD is a sex-linked disease that occurs from lack of functional dystrophin. Structural weakness of the muscle cell membrane from lack of dystrophin leads to degeneration of both skeletal, respiratory, and heart muscle. Lack of dystrophin also affects other organs, including the brain, which can result in learning and behavioral problems in some patients. The disease is present at birth but often is not diagnosed until developmental delays become more apparent at several years of age. Degeneration of muscle and loss of strength leads to loss of ambulation by the teen years, and patients subsequently lose arm strength. Decline in respiratory and cardiac function is often apparent shortly after loss of ambulation, and death from respiratory or cardiac failure typically occurs in the second or third decade. About 13% of DMD patients, which corresponds to about 2,000 boys in the U.S., have mutations that could be treated by exon 51 skipping. There are no FDA approved treatments for DMD. Glucocorticoids have been shown to prolong function and survival by a few years, and improvements in supportive care, including physical therapy and assisted ventilation, have led to a slow but steady increase in survival over the past few decades. Chronic glucocorticoid use is associated with side effects typical for that class of drugs, including Cushingoid syndrome, hypertension, behavioral changes, etc. There is thus significant unmet medical need in DMD.

**Clinical Efficacy:** Substantial evidence of efficacy on clinical endpoints has not been presented for eteplirsen.

**Biomarker Efficacy Evidence:** Dystrophin protein could be considered under the accelerated approval provisions as a biomarker endpoint reasonably likely to predict benefit in DMD, but the amount, localization, and functionality would be key considerations. There is some evidence that eteplirsen increases the expression of a Becker-type dystrophin protein, to a level  $\approx 1\%$  of normal, but the evidence is less than the amount that is usually considered to be “substantial evidence.” This amount of Becker-type dystrophin is low enough that a conclusion that it was reasonably likely to predict clinical benefit would have to be based on a low threshold for reasonably likely.

**Risk:** No serious or severe adverse effects of eteplirsen were identified at the doses studied. The safety database is small such that low-frequency events may not have been identified.

**Analysis and Recommendations:**

- No serious or severe safety risks were identified at the doses studied. A small beneficial effect of eteplirsen, if present, would be acceptable to support approval based on risk-benefit.
- If eteplirsen is approved under the accelerated approval provisions, postmarketing requirements would be necessary to confirm clinical efficacy. The potential for any drug to produce clinical benefit, including molecularly-targeted drugs such as eteplirsen, is related to drug exposure. The proposed dose may be lower than necessary to produce clinical benefit. A study to determine the maximum tolerated dose (MTD), and the dystrophin production associated with that dose, is recommended.
- An externally controlled trial at the proposed does (30 mg/kg/wk IV) appears unlikely to yield interpretable evidence of clinical efficacy because of inability to adequately control or account for bias, combined with evidence suggesting that the effect size of eteplirsen is unlikely to be large enough to provide a clear result that could overcome the uncertainties inherent in such a study design.
- Confirmation of efficacy of eteplirsen could be provided by both statistically positive clinical findings and a large effect size in a randomized, double-blind, placebo-controlled study of a drug similar to eteplirsen but designed to skip other exons (e.g. an exon 45 and/or exon 53 skipping PMO). The levels of truncated dystrophin produced by the different drugs would, however, need to be adequately similar to enable the conclusion that the clinical efficacy of eteplirsen was similar.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
<p><a href="#"><u>Analysis of Condition</u></a></p>	<ul style="list-style-type: none"> <li>• Duchenne muscular dystrophy (DMD) is a severe pediatric neuromuscular disorder that occurs almost exclusively in males. DMD is caused by the absence, or near absence, of functional dystrophin protein that is thought to protect muscle fibers against contraction damage. Exon 51 skip-amenable DMD, a subgroup of DMD, is defined by the presence of exon 51 in the dystrophin gene and the deletion of one or more exons contiguous with exon 51, resulting in an out-of-frame deletion in which the reading frame is potentially restorable by the skipping (removing) of exon-51.</li> <li>• Lack of dystrophin results in degeneration of muscle fibers, inflammation, and ultimately replacement of muscle by fibrotic and adipose tissue. There is loss of muscle strength, and ultimately pulmonary and cardiac failure.</li> <li>• Loss of muscle strength is progressive, typically resulting in loss of ability to ambulate by age 8 to 18 years. Progressive scoliosis develops that further impairs pulmonary and cardiac function. Patients with DMD usually survive until late adolescence, but with current supportive care about 20 to 25 percent live beyond the</li> </ul>	<p>The loss of muscle strength in DMD is progressive, leading to loss of ambulation in the teens, followed by decline in respiratory and cardiac function, leading to death typically in the third decade.</p>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<p>twenty-fifth year.</p> <ul style="list-style-type: none"> <li>• Mutations that are treatable by skipping exon 51 are thought to comprise about 13% of the DMD population, resulting in a prevalence of about 2000 boys in the US.</li> </ul>	
<p><u>Current Treatment Options</u></p>	<p>There are no FDA-approved treatments for DMD.</p> <ul style="list-style-type: none"> <li>• The current standard of care is glucocorticoids (prednisone, prednisolone and deflazacort) administered either daily or intermittently, which has a modest beneficial effect on function and survival. In addition, supportive care, such as assisted ventilation and physiotherapy, is modestly effective in prolonging function and survival.</li> <li>• The risks of chronic use of glucocorticoids include increased infections, diabetes, Cushingoid appearance, delayed puberty, behavioral changes, obesity, osteoporosis, and increased frequency of long bone and vertebral fractures.</li> </ul>	<p>There is high unmet medical need for treatment of DMD to slow functional decline and prolong survival.</p>
<p><u>Benefit</u></p>	<ul style="list-style-type: none"> <li>• Clinical efficacy was evaluated in a single trial, Study 201/202, with a 24 week placebo-controlled period followed by long-term open-label treatment that was compared to external natural history controls. The placebo-controlled portion of the study was negative. The clinical course of patients on long-term (3+ years) eteplirsen was not reliably distinguishable from expected natural history.</li> <li>• There is some evidence from Study 201/202 that eteplirsen increased the expression of dystrophin protein to 0.9% of normal, but because of poorly matched controls and the fact that all data was from a single site, this would not ordinarily be considered to meet the threshold of substantial evidence.</li> <li>• 0.9% dystrophin is low enough that a conclusion that such an amount is reasonably likely to predict clinical benefit under accelerated approval provisions would have to be based on a low threshold for reasonably likely because the level is well within the range of dystrophin levels of untreated DMD patients, and appears to be substantially lower than dystrophin levels in patients with less severe</li> </ul>	<p>Substantial evidence of efficacy was not provided for clinical or biomarker (dystrophin) endpoints.</p> <p>A conclusion that the amount of dystrophin produced by eteplirsen was reasonably likely to predict clinical benefit would have to be based on a low threshold for reasonably likely.</p>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	forms of dystrophinopathy.	
<u>Risk</u>	<ul style="list-style-type: none"> <li>The clinical safety database for eteplirsen is small: 114 total patients exposed, with only 36 exposed for <math>\geq 24</math> weeks and 12 exposed for <math>\geq 1</math> year. Most of these exposures were outside of placebo-controlled studies, limiting ability to determine if adverse events were the result of drug effect or chance. However, the serious and severe adverse events that occurred were generally consistent with events expected in DMD. The 12 patients in Study 202 were exposed for <math>&gt;3</math> years, which provides some reassurance against delayed toxicity.</li> <li>In animal studies, the primary target organ was the kidney, with dose-dependent renal tubular cytoplasmic basophilia and/or vacuolation and, at the high dose, tubular degeneration/necrosis. In a mouse study, dilatation of the lateral ventricles of the brain was observed at the mid and high doses. The mechanism of this effect, and its relevance to humans, is unknown. Mean eteplirsen plasma exposures (AUC) at the NOAELs for monkey and juvenile rat were 20-fold and 6-fold, respectively, greater than that in patients dosed once weekly with 30 mg/kg IV eteplirsen.</li> </ul>	The safety database was small, but would be sufficient to support approval if there was demonstration of substantial evidence of efficacy.
<u>Risk Management</u>	<ul style="list-style-type: none"> <li>Safety risks have not been identified that would require risk management beyond standard pharmacovigilance.</li> </ul>	Standard pharmacovigilance is recommended

## 2. Background

Key manifestations of DMD include progressive degeneration of skeletal and cardiac muscle resulting in loss of function in childhood and adolescence and premature death from respiratory or cardiac failure in the second to fourth decade. DMD is caused by genetic mutations in the dystrophin gene that result in near absence of the dystrophin protein from muscle. Dystrophin is thought to maintain the structural integrity of the muscle cell membrane by connecting the cytoskeleton to the surrounding extracellular matrix, and to act as a scaffold for several signaling molecules that also contribute to normal muscle physiology. Immunological and inflammatory processes downstream of dystrophin deficiency contribute to muscle pathology in DMD, and corticosteroid therapy is considered standard of care, delaying loss of ambulation and respiratory decline by several years. No other drugs have been established as effective in DMD and, consequently, a large unmet medical need remains.

Because of the near total lack of dystrophin in DMD, one rational approach to therapy involves trying to restore dystrophin expression. In many patients with DMD, very small amounts of a shorter than normal “truncated” form of dystrophin are produced, due to what might otherwise be considered an error in mRNA splicing: an exon is left out, or “skipped”, which, in the setting of specific DMD-causing mutations, can result in restoration of the mRNA reading frame. Unfortunately, the small amount of exon skipping that occurs naturally in DMD patients does not appear to appreciably slow muscle degeneration. It was reasoned, however, that if exon skipping could be augmented by drug therapy, levels of the truncated dystrophin could be increased to a level high enough to confer clinical benefit. Eteplirsen was designed to bind to dystrophin mRNA at a specific site to cause the splicing machinery to skip exon 51, thus restoring the dystrophin reading frame in certain amenable patients, and increasing production of the truncated dystrophin. How much of the truncated dystrophin would be necessary to confer clinical benefit remains an open question, but a related form of muscular dystrophy, called Becker muscular dystrophy (BMD), provides a natural model of what exon skipping in DMD might achieve. In so-called “exon 51-model” BMD patients, the same truncated form of dystrophin that would be produced by eteplirsen in DMD patients occurs naturally. These BMD patients experience a mild, or in some cases asymptomatic, muscle disease. Importantly, however, the truncated dystrophin in these BMD patients is expressed at high levels, roughly 50- to 100% of what would be expected for normal dystrophin.

### *Presubmission Regulatory Activity*

There were extensive discussions and FDA guidance to the applicant during the eteplirsen development program, as detailed in the primary clinical review, and summarized below.

Clinical efficacy was examined in Study 201/202. Shortly after Study 201/202 passed 1 year duration, the applicant proposed a post-hoc analysis with a number of changes from the original analysis: a) data for 2 out of 8 patients treated with eteplirsen (patients who quickly lost ambulation) were dropped, b) the prespecified comparison of each dose arm to placebo was changed to comparison of the 6 remaining treated patients to the 4 placebo-treated patients, and c) the endpoint was taken to be Week 36, instead of Week 24. FDA explained in detail to the

applicant in March of 2013 why the proposed analysis was unreasonable even for hypothesis generation, and why Study 201 did not provide evidence of efficacy.

As the duration of exposure in Study 202 increased, the applicant proposed comparing the clinical course of treated patients to historical controls. FDA expressed strong reservations regarding the potential interpretability of the applicant's proposed comparison to historical controls and the use of 6MWT as the primary endpoint in such a historical comparison. Because of these concerns, FDA noted that a dramatic effect size would be necessary for any such analysis to be potentially interpretable.

FDA consistently and strongly encouraged the sponsor to conduct adequately powered randomized placebo-controlled trials, and expressed doubt about the interpretability of externally controlled trials. As early as October 2012, Sarepta and its academic associates announced that in the randomized controlled portion of Study 201/202 eteplirsen had demonstrated unparalleled effects on enabling dystrophin production and slowing the progression of the disease, with levels of dystrophin potentially as high as 50% of normal. In the context of an ongoing series of reports from the applicant and its academic associates describing continued striking and unprecedented stabilization of disease progression, many in the DMD community expressed strong reservations regarding the ethics of conducting another placebo-controlled trial, and informed FDA that performing such a study would be extremely difficult or impossible. In this context, and based on assertions that eteplirsen had been shown unequivocally to produce high levels of dystrophin, FDA expressed willingness to consider an externally controlled trial, although stating clearly that interpretation of the data could be difficult, and that the acceptability of the study would be a matter for NDA review.

FDA informed the applicant that if it were to pursue a comparison of patients in Study 201/202 to external controls, evaluating such a comparison would be difficult without submission of patient-level external data, including data from a number of different sources to understand variability across different datasets, which can be substantial in DMD.

### 3. Product Quality

The OPQ Integrated Quality Assessment concludes that from a CMC perspective, the NDA is recommended for approval. Additional considerations from the OPQ review are as follows:

- *General product quality considerations:* Per the OPQ review, eteplirsen belongs to a class of molecules known as phosphorodiamidate morpholino oligomers, or PMOs. The molecule is comprised of 30 linked (b) (4) phosphorodiamidate morpholino subunits each attached at the 1-position to one of the heterocyclic bases found in DNA (adenine, cytosine, guanine, and thymine). (b) (4)

The drug substance is manufactured (b) (4)

(b) (4). The applicant has provided adequate characterization of impurities and justification for the proposed acceptance criteria. Based on evaluation of stability data from primary and supportive batches, an expiration dating period of 18 months is established for eteplirsen injection when stored refrigerated (5°C).

- *Facilities review/inspection:* Per the OPQ review, an initial facility risk assessment indicated that an NDA 206488 pre-approval inspection would not be required because of the site history and low risk of the proposed API manufacturing process based on the drug substance reviewer's input. The facility is acceptable for the above listed drug substance responsibilities on the basis of its currently acceptable CGMP compliance status and recent relevant inspectional coverage.
- *Other notable issues (resolved or outstanding):* the following postmarketing commitments to which the applicant has agreed. The recommended time frame for fulfillment of the post-marketing commitments is no later than one year following NDA approval.
  1. Investigate the root cause of the increasing assay trend observed in the drug product stability study.
  2. Revalidate the accuracy of the in-process (b) (4) method used during drug product manufacture.
  3. Revalidate the robustness of the in-process (b) (4) method in terms of (b) (4).
  4. Investigate the consistent bias in the in-process (b) (4) results and the release (b) (4) results.

As noted in an addendum to the OPQ review, on 3/10/2016, a potential OAI alert for the DS manufacturing facility, (b) (4) was activated as a result of a routine GMP surveillance inspection from (b) (4). The inspection resulted in a 14-item FDA-483 and was initially classified OAI by the field investigators. The (b) (4) compliance branch conducted a review of the Establishment Inspection Report (EIR) for the inspection and firm's 483 responses, and concluded that the firm's responses are adequate and downgraded the classification from OAI (official action indicated) to VAI (voluntary action indicated). A recommendation of Approve Facility was made by the (b) (4) on 4/29/2016. The facility is acceptable for the above listed drug substance responsibilities on the basis of its currently acceptable CGMP compliance status and recent relevant inspectional coverage. There are no significant, outstanding manufacturing risks that prevent approval of this application.

#### 4. Nonclinical Pharmacology/Toxicology

The overall pharmacology/toxicology findings were that the nonclinical data submitted adequately support the approval of eteplirsen for the treatment of DMD in patients with mutations amenable to exon 51 skipping therapies.

In animal studies, the primary target organ was the kidney, with dose-dependent renal tubular cytoplasmic basophilia and/or vacuolation and, at the high dose, tubular degeneration/necrosis. Mean eteplirsen plasma exposures (AUC) at the NOAELs for monkey and juvenile rat were 20-fold and 6-fold, respectively, greater than that in patients dosed once weekly with 30 mg/kg IV eteplirsen

In a mouse study of AVI-4225, which has a different base sequence from eteplirsen that is specific to exon-skipping in the mdx mouse, dilatation of the lateral ventricles of the brain was observed at the mid and high doses. The mechanism of this effect, and its relevance to humans, is unknown.

No reproductive and developmental toxicity studies of eteplirsen were required because the DMD patient population is almost entirely male. No effects on reproductive organs or developmental parameters were observed in the pivotal toxicity studies conducted in adult monkeys or juvenile rats, with the exception of reductions in bone length, width, area, mineral content, and mineral density observed in juvenile rats at the HD, with dose-dependent trends noted in some parameters at lower doses.

Carcinogenicity studies have not been conducted. If eteplirsen is approved, the nonclinical review indicates that Carcinogenicity studies in two species should be conducted as a post-marketing requirement.

## 5. Clinical Pharmacology

The overall clinical pharmacology findings were that the clinical pharmacology data submitted adequately support the approval of eteplirsen for the treatment of DMD in patients with mutations amenable to exon 51 skipping therapies.

- **General clinical pharmacology considerations**
  - The bioavailability is assumed 100% because of the proposed route of drug administration (i.e., IV infusion).
  - The parent drug eteplirsen is the only known active moiety.
  - Following single or multiple IV infusion, plasma concentration-time profiles of eteplirsen were generally similar and showed multi-phasic decline, whereas the majority of drug elimination occurred within 24 hours.
  - In vitro investigation suggested that plasma protein binding of eteplirsen in human is relatively low, ranging 6.1~16.5% and is concentration-independent under the study condition. The volume of distribution (Vd) values obtained following single or multiple doses (e.g., approximately 601 mL/kg or 19 L/31.5kg after 30 mg/kg/week doses in Study 201) suggest the distribution or cellular uptake of eteplirsen into peripheral tissues.
  - The inter-subject variability of eteplirsen is considered to be moderate. The mean inter-subject variability for exposure measures (C<sub>max</sub> and AUCs) as well as other key PK parameters (such as CL and Vd) were generally in the range of 20~55%.

- **Pathway of elimination, including metabolism, half-life, and excretion.**
  - The 30 and 50 mg/kg/wk doses studied in the clinical trials resulted in 64.1% and 69.4% of mean percent of dose excreted in the urine. Elimination  $t_{1/2}$  was 3.3~3.5 and 3.2~3.8 hours on average for 30 and 50 mg/kg, respectively.
  - Eteplirsen was found to be metabolically stable in vitro with no evidence of metabolism or metabolite.
- **Intrinsic factors potentially affecting elimination: age, gender, hepatic impairment, and renal impairment.**
  - Intrinsic factors including age, gender, body weight, geographic region, hepatic impairment, renal impairment, and other potential significant covariate were not studied in Phase 1 program or via population analysis. Potential impact of race is not known since nearly all the patients in studies are Caucasians.

- **Drug-drug interactions**

Eteplirsen is expected to have a low potential for DDI in humans based on results of in vitro investigation on microsomal metabolism, plasma protein binding, inhibition or induction of major CYP isozymes or major drug transporters at the concentration range studied for clinical dosing regimen.

- **Genomics and Targeted Therapy Group Review**

Not all mutations amenable to exon 51 skipping were represented in the clinical development program. Some mutations amenable to exon 51 skipping are very rare, and would be difficult to study. Many unknowns remain about the quantity and functionality of dystrophin that might be produced by eteplirsen in different underlying exon-51 amenable mutations. However, it appears reassuring that patients with large in-frame deletions can still have mild BMD. There are no reasons to believe that the safety of eteplirsen would be different in patients with different underlying amenable mutations. In light of all the above factors, Dr. Rogers recommend that if eteplirsen is approved, any DMD deletions amenable to exon-51 skipping (i.e. theoretical restoration of the reading frame) should be eligible to receive eteplirsen.

CDTL Discussion: I generally agree with the conclusions of the Genomics and Targeted Therapy Group, and recommend that if approved eteplirsen be indicated in all patients with mutations amenable to exon 51 skipping. I'm more optimistic, however, that feasible studies could be conducted on the amount of skipped dystrophin produced in patients with different underlying mutations; single patients could contribute substantially to addressing questions of amount of skipped dystrophin present, even if questions of functionality or ultimate clinical outcome were more difficult to address because of the high inter-patient variability in disease course in DMD.

- The clinical pharmacology review concluded that due to lack of clear evidence of benefit from eteplirsen in Study 201/202, the sponsor should make efforts to evaluate doses greater than 50 mg/kg administered weekly or alternate regimens that would include loading and maintenance doses. This recommendation is based on the pharmacokinetics

of eteplirsen (3 to 4 hours plasma half-life, urinary excretion of 60-70% of the dose within 24 h post-dose) and no reports of major safety events at doses up to 50 mg/kg in clinical studies.

- In Study AVI-4658-28, patients had undetectable levels of anti-dystrophin antibody following treatment of eteplirsen. The development of anti-dystrophin antibodies can be further assessed in future clinical trials.

## 6. Clinical Microbiology

Not applicable

## 7. Clinical/Statistical- Efficacy

This section is based on the text of the Cross Disciplinary Team Leader (CDTL) Memorandum for the April 25, 2016 Peripheral and Central Nervous Systems Drugs Advisory Committee (PCNS AC) meeting. Additional figures from the PCNS AC presentation are also incorporated into this section of the review, as is discussion of findings in the primary clinical review conducted by Dr. Breder and the consultative review conducted by Dr. Rao.

The CDTL memorandum for the April 25<sup>th</sup> was revised from an earlier memorandum for the PCNS AC meeting for eteplirsen that had been scheduled for January 22, 2016. The revisions were based on additional data submitted by the applicant for both eteplirsen-treated and natural history patients, newly available natural history from the Cooperative International Neuromuscular Research Group (CINRG), new analyses of data previously submitted by the applicant, and comments from other interested parties subsequent to the release of the previous memorandum. Following release of the FDA briefing material the applicant stated in an addendum<sup>1</sup> that there were key inaccuracies in the FDA material regarding dystrophin analytical methodology and findings. FDA's responses to the applicant's statements were also included in the revised memorandum and the applicant's table of "Key Inaccuracies" is appended to this review. For clarity, the revised AC memorandum contained the previous text and figures, with new text in italics; this formatting has been retained in this review.

Information provided to FDA by the applicant at the PCNS AC meeting, and public testimony, both written and during the open public hearing at the PCNS AC, was also considered in drafting this section of the review, and is also discussed in **Section 9: Advisory Committee Meeting**.

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<sup>1</sup><http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/PeripheralandCentralNervousSystemDrugsAdvisoryCommittee/UCM481913.pdf>

## 1. Dystrophin Evidence

Dr. Ashutosh Rao, from the Office of Biotechnology Products, reviewed dystrophin methodologies and supporting assays. The effect of eteplirsen on dystrophin expression was examined in 3 clinical studies: Study 33, Study 28, and Study 201/202, as follows:

- a. **Study 33:** In this exploratory phase 1 study, small doses of eteplirsen (up to 0.9 mg total) were injected directly into a foot muscle in 7 patients with DMD. An increase in dystrophin expression was reported adjacent to the needle track, but it is not clear whether, or to what degree, this might reflect the activity of eteplirsen when given by the intravenous (IV) route, which does not produce similar high local concentrations or mechanical effects.
- b. **Study 28:** In this exploratory study, eteplirsen was administered intravenously once a week for 12 weeks at doses ranging from 0.5 to 20 mg/kg, with up to 4 patients per dose level. The methods for dystrophin quantification were not reviewed by FDA prior to the conduct of the study, and FDA has concerns about the reliability of the methods and procedures. In one response from the applicant to an information request from FDA about quality control methods, the applicant responded that “Study 28 was an exploratory phase 1b study which was only intended to generate proof of concept data to guide future studies. For this reason, quality controls for the dystrophin data in Study 28 were not properly optimized.” In addition, Study 28 examined dystrophin levels after 12 weeks of dosing, but it is necessary to understand dystrophin levels that are present with longer- term, more clinically relevant durations of therapy. Thus, as described below, FDA considers the 4<sup>th</sup> biopsy from patients in Study 201/202, which was taken after 180-weeks of treatment with eteplirsen, to be of greater potential clinical relevance.

*The results of Study 28 do not appear to be interpretable. Western blot bands were too saturated to allow reliable quantification. Study design and conduct issues were also a major concern. The study was unblinded and, according to the applicant, assays were repeated and reanalyzed. Repeating assays and analyses when unblinded to treatment can increase the risk of bias and false positive findings; results supportive of the preferred hypothesis may be preferentially selected, whereas ambiguous or non-supportive results may be discounted as having resulted from the types of technical failures that are common in laboratory research. The Study 28 report from the applicant states the following regarding repeated assays and analyses: “Of note, the laboratory performing the Western blot analyses used multiple samples from the same patients to re-analyze the results. Initially, the Western blot analyses reported the results from one sample per patient and any post-treatment increases in dystrophin protein level were reported as an ‘X’-fold increase from baseline. Subsequently, while preparing the Lancet publication, the laboratory repeated several Western blots to achieve publication standard results and also to test different pieces of muscle within a patient. These results were*

reported as the maximum amount of dystrophin per patient and were expressed as a percentage of normal.”

*As detailed in later sections of this memo, dystrophin levels in the 4<sup>th</sup> biopsies of Study 201/202, which were obtained after 180 weeks of eteplirsen treatment, were estimated to be about 0.9% of the amount in normal muscle. In contrast, Study 28 reported amounts 10- to 20-fold higher after only 12 weeks of eteplirsen treatment, in patients treated with doses of eteplirsen as low as 1/10<sup>th</sup> those used in study 201/202. In light of the issues noted above, however, FDA does not believe the dystrophin results from Study 28 are interpretable*

**Study 201/202, First 3 Biopsies:** Study 201/202 was a 3-arm, 12-patient study comparing the effects of 30 mg/kg or 50 mg/kg IV eteplirsen to placebo. Biopsies were taken at baseline, week 12 (for half the patients), week 24 (for the other half), and week 48 for all patients. During the development of eteplirsen FDA communicated to the applicant concerns about the biomarker studies on the first 3 biopsies.<sup>2</sup> With additional review following submission of the NDA, it is not clear that any of the dystrophin biomarker data from the first 3 biopsies are reliable or interpretable.

### **Immunofluorescence images (Study 201/202, first 3 biopsies)**

The measurement of total dystrophin immunofluorescence by Bioquant was first carried out on blinded baseline, Week 12, and Week 24 images, captured at 20x magnification. The results showed essentially no change in intensity for any patient. Negative results were obtained both when the study was conducted with MANDYS106 antibody or with Dys2 antibody. However, investigators attributed the negative results to the image magnification, and captured new images at 40x magnification after the blind was broken, with personnel reporting to FDA site inspectors that positive fields were uniquely selected for further quantitation. The images selected at 40x magnification showed roughly a doubling of immunofluorescence intensity for all patients between baseline and Week 12 (50 mg/kg patients) or week 24 (30 mg/kg patients). Because the analyses were intentionally targeted to fibers whose staining intensity exceeded a particular threshold, it is not clear whether these results are representative or interpretable.

The 20x immunofluorescence images on samples obtained through Week 24 were selected by an individual blinded to treatment group, but the microscopic fields to be photographed were selected manually by the operator, as opposed to a more automated method introduced for studies of the 4<sup>th</sup> biopsy. Bias in field selection may have resulted in preferential capture of bright fibers that appear similar to revertant fibers.

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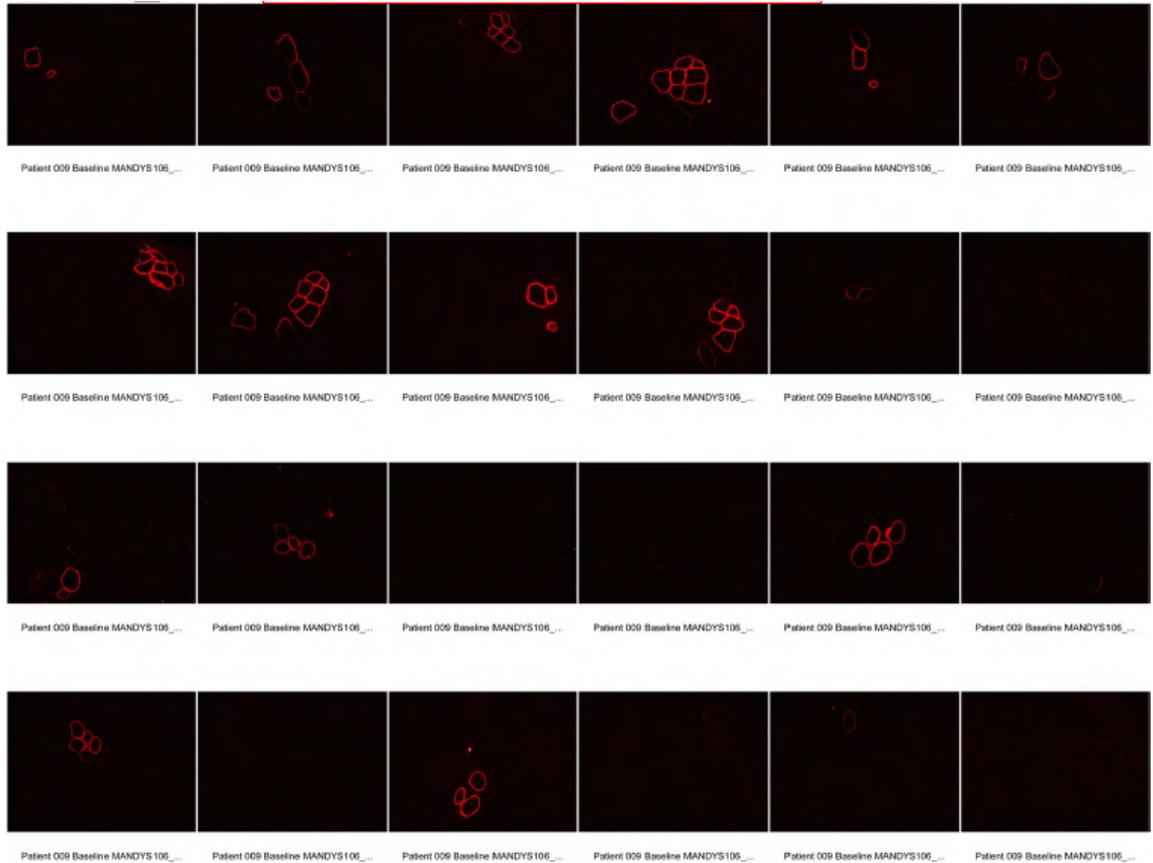
<sup>2</sup> e.g. at a meeting on March 13, 2013, FDA stated “while we do not believe that you have adequately characterized the quantity of truncated dystrophin produced by eteplirsen treatment (Western blot data is not available), the immunofluorescence data you presented suggest that a much lower quantity of truncated dystrophin is produced by eteplirsen treatment than is present in BMD.” In the April 15, 2014, advice letter in which potential pathways for approval were discussed, FDA stated “After examining the source data and images you provided in support of dystrophin protein expression from eteplirsen treatment, we remain skeptical about the persuasiveness of the data, and concerned about serious methodological problems explained previously.”

Figure 1 shows all 24 fields captured from a single patient at Week 24 in Study 201. Three of the fields show a cluster of what appear to be the same revertant fibers that appear to extend through multiple levels of the tissue sample. Similar apparent over-representation of bundles of likely revertant fibers occurred for many other patients and time points; for example, images obtained at baseline from a different patient are shown in Figure 2.

**Figure 1: Example of immunofluorescence fields, Study 201**



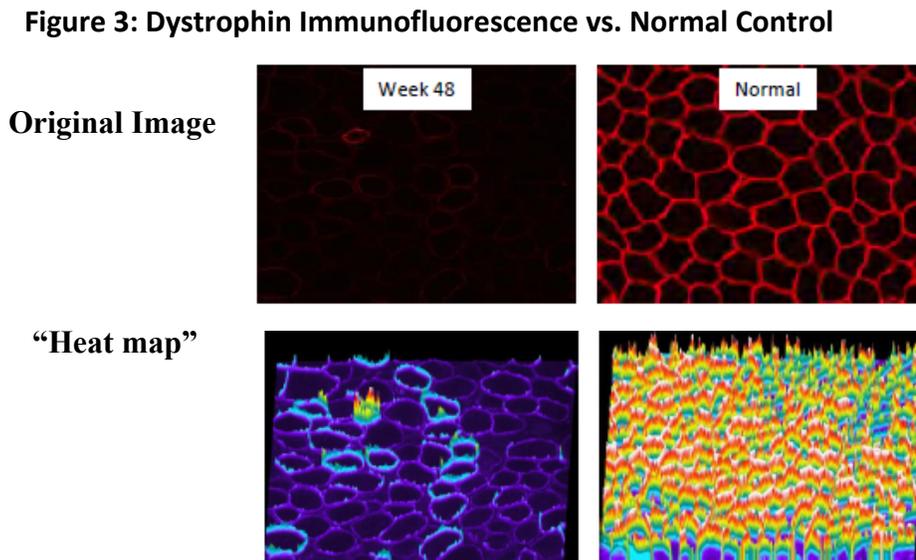
**Figure 2: Example of Baseline Dystrophin Immunofluorescence**



Week 48 samples were processed separately for dystrophin immunofluorescence from earlier samples, and had higher background staining. As a consequence, valid comparison is not possible with earlier time points for percent positive fibers or total immunofluorescence because the higher background staining, and not necessarily an effect of drug, could be responsible for any differences observed.

Importantly, the Week 48 immunofluorescence was still very low, and much less intense than normal controls, as shown in

Figure 3. The top two images show the intensity as originally captured, and the bottom two images show the intensity converted to “heatmap” images that represent the observed (unmodified) pixel intensity as color, from low intensity blue to high intensity red and white.



It is important to note that the applicant digitally processed<sup>3</sup> dystrophin images in their background material (images in Appendix 12) in such a way that low intensity values were preferentially increased to produce a higher intensity and higher contrast image.

*Note: following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the above paragraph as a key inaccuracy:*

*Sarepta: “The digitally processed images referenced by FDA in this statement were included in Sarepta’s briefing document for demonstration purposes only, and it is far more important to note that the referenced images were not used in the analysis of fiber intensity, nor to score dystrophin-positive fibers.”*

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<sup>3</sup> Per the applicant: To generate the enhanced inverted\_b base100 Image (InvertBase100), the algorithm produces a non-linear mapping of r,g,b fluorescent values that will specifically enhance low contrast objects in the image. It does this by scaling the r,g,b fluorescent values using the following formula:  $I' = 1 - 100^{(-I)}$  normalized by the max value of  $1 - 100^{(-1)}$  for each of the channels independently. This results in low intensity values being stretched and therefore perceived as having a higher intensity and a higher contrast

***FDA response:*** *FDA acknowledges that digitally manipulated images were not used in the applicant's numerical assessment of fiber intensity or percent positive fibers, but it is concerning that images used to provide evidence of an effect of eteplirsen greatly exaggerate the immunofluorescence signal from the muscle samples.*

### **Western blots (Study 201/202, first 3 biopsies)**

Western blots from the first 3 biopsies are not considered interpretable because of substantial technical shortcomings, including lack of a dilution-series of normal muscle as a comparative control, saturation of bands such that ratios of intensity are unreliable and, in many blots, multiple bands in the region of dystrophin immunoreactivity that decrease confidence that the correct band was identified for quantification. Additional potential for bias was introduced because multiple Western blots were performed, with a number of different antibodies (Mandys106, Dys1, Dys2), with negative findings on many blots attributed to technical issues, whereas positive findings were attributed to drug effect.

### **c. Study 201/202, 4th Biopsy**

Biomarker studies on the 4<sup>th</sup> biopsy obtained at Week 180 were conducted by the applicant with technical advice from FDA. However, the reliability of results remains questionable for a number of reasons, including the following:

- **Controls were not matched by muscle group:** Biopsies at Week 180 were taken from deltoid, one of the few muscle groups that, along with the calf muscle, can be hypertrophied in DMD.<sup>4</sup> In contrast, both the baseline samples available from eteplirsen-treated patients, and most of the new external controls from untreated patients, were obtained from biceps (except for one, which was obtained from deltoid). There is little human data on differences in dystrophin levels between muscle groups in DMD but, in nonclinical models of DMD, there is evidence that dystrophin levels vary between muscles,<sup>5</sup> which may affect the readout of experiments in which the effectiveness of the treatment is not particularly high.
- **Controls were not matched by patient:** There appears to be considerable inter-patient variability in dystrophin levels present in exon-51 skippable DMD. In Western blots from biopsies of extensor digitorum brevis (EDB),<sup>6</sup> dystrophin levels averaged about 0.3% of normal, but ranged from undetectable to  $\approx$  1% of normal or somewhat higher. The applicant obtained data from biopsies of 9 untreated patients, and reported an average dystrophin level of 0.08%.<sup>7</sup> However, such a small sample size may not provide a reliable

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<sup>4</sup> Pradhan S (2002) Valley sign in Duchenne muscular dystrophy: importance in patients with inconspicuous calves. *Neurol India*. 50,184-186.

<sup>5</sup> Pigozzo S et al (2013) Revertant fibers in the mdx murine model of Duchenne muscular dystrophy: an age- and muscle-related reappraisal. *PLOS ONE*. 8,e72147.

<sup>6</sup> FDA Advisory Committee presentation for drisapersen, slide 43.

<sup>7</sup> Noting, however, that values <0.25% were rounded to zero. Including those lower values leads to an average

estimate of baseline levels that were present in the eteplirsen-treated patients. The dystrophin level estimated in these biceps controls is lower than the estimate from the EDB biopsies, perhaps because dystrophin levels truly differ between these muscle groups, or perhaps only secondary to chance when a small number of observations with high variability are compared.

- *Stored baseline biopsy samples were available for 2 eteplirsen-treated patients who had a biopsy at Week 180 but, importantly, these baseline biopsies were from a different muscle group than the Week 180 samples, which introduces a potential source of confounding.*
- *Preferential survival and expansion of revertant fibers over time has been observed in experimental disease models,<sup>8</sup> and may occur in DMD<sup>9</sup> (one study in DMD did not find expansion of revertant fibers with age, but appears to have had low sensitivity for detecting change<sup>10</sup>). There is a concern that differences in dystrophin levels between baseline and Week 180 samples could also have been caused by preferential survival and/or expansion of revertant fibers in the eteplirsen patients over the time between the baseline sample and the biopsy at week 180, unrelated to eteplirsen treatment.*
- *The absence of detectable dystrophin in the 3 stored baseline samples from eteplirsen patients (1 baseline sample was available for a patient who did not undergo a Week 180 biopsy) also raises concern about differences that might have arisen due to sample handling, unrelated to an effect of eteplirsen. Experts in the quantification of dystrophin have suggested, in the context of a different study, that dystrophin degradation may be a concern in stored muscle samples.<sup>11</sup>*
- **Lack of independent confirmation:** The applicant has not obtained independent confirmation of dystrophin findings.<sup>12</sup>

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level about twice as high, but still half as much as in EDB.

<sup>8</sup> Yokata et al (2006) Expansion of revertant fibers in dystrophic *mdx* muscles reflects activity of muscle precursor cells and serves as an index of muscle regeneration. *Journal of Cell Science* 119:2679-2687.

<sup>9</sup> Fanin et al (1995) Dystrophin-positive fibers in Duchenne dystrophy: origin and correlation to clinical course. *Muscle and Nerve*. 18:1115-1120.

<sup>10</sup> Arechavala-Gomez, V et al (2010) Revertant fibres and dystrophin traces in Duchenne muscular dystrophy: implications for clinical trials. *Neuromuscul Disord*. 20:295-301.

<sup>11</sup> Taylor LE (2012) Quantification of dystrophin immunofluorescence in dystrophinopathy muscle specimens. *Neuropathology and Applied Neurobiology*. 38:591-601.

<sup>12</sup> For example, in the April 15, 2014, letter discussing data that would be filed with the NDA, FDA stated “We expect that the initial biomarker data from these [newly exposed patients] exposures will start becoming available at about the time of NDA submission and shortly thereafter.” Also, as early as the July 23, 2013 meeting FDA expressed concern that “all muscle biopsies were obtained and processed by a single technician at a single study center” and that in part because of concern about bias, “we also ask that you confirm, [biomarker results] by an independent laboratory.”

*Note: Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the above information as a key inaccuracy.*

***Sarepta:** “Methodology for dystrophin analyses of the fourth biopsy tissue samples, including confirmatory assessments of percent dystrophin-positive fibers (PDPF) analysis performed by 3 independent pathologists, were agreed with FDA prior to conducting any analyses of the fourth biopsy tissue samples. In accordance with the mutually agreed-upon protocols for the assessment of dystrophin-positive fibers in DMD muscle biopsy samples from the fourth biopsy obtained at Week 180, 3 independent pathologists performed a blinded assessment of the randomized muscle fiber microscopy images, which independently confirmed the results obtained by the pathologist at Nationwide Children’s Hospital (NCH).*

*Assessment of PDPF at NCH indicated a significant increase in PDPF score ( $p < 0.001$ ) relative to untreated control samples. This increase in PDPF score was confirmed by the 3 independent pathologists ( $p < 0.001$ ).*”

***FDA Response:** The FDA statement that biomarker studies on the 4<sup>th</sup> biopsy are considered of questionable reliability is correct. FDA explained to the applicant that it would be reasonable for them to perform the proposed analyses on the newly acquired biopsy tissue but that there were shortcomings and limitations to potential interpretability (communicated March 30, 2015):*

- ***Controls for 4<sup>th</sup> biopsy:** Prior to conduct of biomarker studies on the 4<sup>th</sup> biopsy, FDA provided the following advice about the shortcomings of the controls selected by the applicant and limitations the controls would place on interpretability:*
  - *“The control biopsy tissue that you propose to use is from a number of different muscle groups, such that differences that may exist in dystrophin expression among muscle groups may affect your results. However, in the context of other major sources of variability among biopsies (including both intra- and interindividual differences even within the same muscle group), it appears reasonable for you to proceed with these controls, with the understanding that dystrophin changes would need to be robust to be interpretable as a drug effect.”*
- ***Meaning of Percent Dystrophin Positive Fibers (PDPF):** FDA also reminded the applicant at that time of the importance of WB data for quantifying dystrophin:*
  - *“As proposed, your western blot method is likely to be more reliable for quantitative measurement of dystrophin.”*

**Meaning of independent confirmation of findings:** *Multiple readings of data from a single study, e.g., 3 independent readings of dystrophin-positive fibers, do not constitute an independent study. As early as the July 23, 2013 meeting FDA expressed concern with the applicant that “all muscle biopsies were obtained and processed by a single technician at a single study center.”*

## Exon Skipping

The applicant reported positive findings for all patients on detection of exon 51-skipped mRNA, as measured by RT-PCR. However, RT-PCR is highly sensitive to the presence of even a few molecules of mRNA, and does not indicate how much, or even whether, any dystrophin protein might have been produced.

## Western Blot, 4<sup>th</sup> biopsy

Western blot results for eteplirsen-treated patients are shown in

Table 1. Dystrophin levels in treated patients were, on average, about 0.9% of normal<sup>13</sup> (range <0.25% -2.5%) as measured by Western blot, the most quantitative method used by the applicant. At the low dystrophin levels present in the Week 180 biopsies, random measurement error can be large in comparison to the estimated amount of dystrophin. Consequently, little confidence can be placed on any individual patient value, and the data should not be considered as reliable evidence that some patients failed to produce any dystrophin from eteplirsen whereas others were more responsive.

**Note:** *Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statement, “Random measurement error can be large in comparison to the estimated amount of dystrophin” as a key inaccuracy.*

**Sarepta:** *“The random measurement error of our Western blot protocol for measurement of dystrophin levels was well below the observed difference between untreated and treated Week 180 biopsy samples. A rigorous validation of the Western blot method was reviewed by the FDA prior to Week 180 biopsy analysis. Validation data demonstrated a %CV of +/- 50% and a linear range ( $R^2 > 0.9$ ) of sensitivity extending as low as 0.25% of normal.”*

**FDA response:** *As quoted above, prior to analysis of the 4<sup>th</sup> biopsy, FDA explained to the applicant that major sources of random error were the results of both intra- and inter-individual differences, including differences in dystrophin that might occur within the same muscle group, or even within different regions of a single biopsy sample.<sup>14</sup> The applicant’s discussion of the variability of the Western blot method does not consider these potentially large sources of biological variability.*

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<sup>13</sup> The applicant notes that Week 180 samples were measured relative to a single normal individual’s deltoid muscle biopsy, which introduces additional uncertainty into the interpretation of fold increase vs. normal because dystrophin appears to vary about 2-fold among different normal individuals.

<sup>14</sup> Anthony et al (2014) Dystrophin quantification, biological and translational research implications. Neurology 83:1-8.

## Percent Positive Fibers

Table 1 shows the percent positive fibers in eteplirsen patients. On average, the percentage of fibers with any detectable staining was about 17%, versus about 1% in the controls selected by the applicant. It is important to stress, however, that the applicant's definition of a positive fiber was *not* based on a threshold amount of dystrophin or staining brightness, but rather only on "a majority of the fiber perimeter stain at an intensity judged by eye to be *above background* of the image." [emphasis added] Consequently, "17% positive fibers" does not correspond to 17% of normal dystrophin levels, or to 17% of fibers being as bright as in BMD. The percent positive fiber result is, instead, mainly useful for localization of dystrophin, not quantification.

*It is important to stress that 17% positive fibers does not represent 17-times more dystrophin compared 1% positive fibers, and is consistent with the estimate of 0.9% of normal dystrophin from Western blot. Most fibers counted as positive were faintly stained. The amount of dystrophin per fiber that would correspond to this faint immunofluorescence is unknown, but if it were 5% of normal, then 17% positive fibers with each fiber containing 5% of the normal level of dystrophin would contain  $17\% \times 5\% = 0.85\%$  of normal levels of dystrophin, essentially the same value that was obtained by Western blot.*

For dystrophin levels above the applicant's lower limit of reliable detection for Western blot, 0.25%, there was little correlation between Western blot and percent positive fibers, although the extent to which this represents a true inconsistency vs. random noise is not clear.

**Table 1: 4th Biopsy Western Blot and %Positive Fibers, Eteplirsen Patients**

Patient	Western Blot	% Positive Fibers
A	2.05	18.5
B	1.15	19.1
C	0.38	33.5
D	1.62	24
E	0.52	21.5
F	0.98	12.8
G	0	7.1
H	2.47	20.7
I	0.96	28.2
J	0	1.4
N	0.14	4.5

There was additional discussion of Percent Positive Fibers in the presentation to the PCNS AC. The table below shows results of Percent Positive Fibers from both the first 3 biopsies and, on the right, from the 4<sup>th</sup> biopsy.

	Nationwide Children’s Hospital analysis				Re-analysis by 3 blinded readers				
	Baseline	Week 12	Week 24	Week 48	Baseline	Week 12	Week 24	Week 48	Week 180 (n=11)
30 mg/kg (n=4)	18		41	70	14		27	23	17
50 mg/kg (n=4)	11	12		54	15	17		25	
Placebo to 30 mg/kg (n=2)	24		24	58	10		10	9	
Placebo to 50 mg/kg (n=2)	7	7		49	11	9		10	

The following are key observations about the Percent Positive Fiber data:

- It remains difficult to find consistency in the Percent Positive Fiber counts, even with the improved method of re-analysis by 3 blinded readers.
  - Percent Positive Fibers did not consistently increase at week 24 even within study 201/202, according to the re-analysis. The numbers of patients was small, but whereas the results for the 30 mg/kg arm suggest that dystrophin increased after 24 weeks in patients treated initially with the lower dose, Percent Dystrophin Positive Fibers did not increase after the 24 weeks of eteplirsen treatment which was dosed following an initial 24 weeks of placebo for the “Placebo to 30 mg/kg” arm or the “Placebo to 50 mg/kg” arm.
- Of concern, the 4th biopsy controls that were selected by the applicant had 1% dystrophin positive fibers, compared to much higher findings of 10-15% dystrophin positive fibers (as read by the 3 blinded readers) for the patient-matched original baseline samples. It is not clear if this inconsistency might have arisen from differences in methods or reading, or differences between the original patient-matched controls and the later, poorly matched controls.
- In contrast, it might be expected that there would be a substantial difference in the percentage of positive fibers between samples taken after 180 weeks of eteplirsen treatment, compared to their baseline. However, there was little difference in positive fibers between the patient-matched baseline samples (10- to 15 percent by the 3 blinded

readers) and treated samples taken from the same patients at 180 weeks of eteplirsen treatment (17%, as shown in the circle [point]).

## **Total Dystrophin Immunofluorescence Intensity**

There was about a 2-fold increase in overall immunofluorescence intensity in tissue sections as measured by semi-quantitative immunofluorescence (Bioquant). As discussed below (Section e), there is no simple or reliable way to compare estimates of dystrophin amount derived from overall immunofluorescence with estimates derived from Western blot.

*Note: Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statement, “There is no simple or reliable way to compare estimates of dystrophin amount derived from immunofluorescence with estimates derived from Western blot” as a key inaccuracy.*

*Sarepta: “Correlation between dystrophin quantification by Western blot and IHC methods has been demonstrated by multiple laboratories (Taylor, 2012; Anthony, 2011; Anthony, 2014; Hathout, 2015 FDA Workshop on Measuring Dystrophin).”*

*FDA response: WB is generally the more reliable method for dystrophin quantification, whereas IF is used primarily for localization of dystrophin. WB data is available, such that the strength of correlation between dystrophin quantification by the two methods is not a key issue for understanding whether or how much dystrophin may be produced by eteplirsen. Regarding the specific work cited by the applicant, the correlation between IF and WB is higher at dystrophin levels that are above those encountered in eteplirsen studies; however, the correlation is low at the low levels of dystrophin in eteplirsen treated patients.*

Importantly, the applicant digitally altered<sup>15</sup> dystrophin images in their background material (images in Appendix 12) such that low intensity values were increased to produce a higher intensity and higher contrast image. We are concerned that this type of image alteration makes dystrophin levels appear closer to those of BMD patients than they truly are.

### **d. Dystrophin in BMD**

**Quantity:** The minimum level of Becker-type dystrophin that might be reasonably likely to predict clinical benefit remains unknown, but experts in DMD,<sup>16</sup> including those directly involved in the development of eteplirsen,<sup>17</sup> have stated that levels less than 3% of that of normal

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<sup>15</sup> Per the applicant: “To generate the enhanced inverted\_b base100 Image (InvertBase100), the algorithm produces a non-linear mapping of r,g,b fluorescent values that will specifically enhance low contrast objects in the image. It does this by scaling the r,g,b fluorescent values using the following formula:  $I' = 1 - 100^{(-I)}$  normalized by the max value of  $1 - 100^{(-1)}$  for each of the channels independently. This results in low intensity values being stretched and therefore perceived as having a higher intensity and a higher contrast”

<sup>16</sup> Flanigan KM (2014) Duchenne and Becker muscular dystrophies. *Neurol Clin.* 32,1 671-688.

healthy muscle, as identified by Western blotting, are generally associated with the typical DMD phenotype, and have proposed, based on a wide range of scientific observations, that “induction of approximately 10% of normal dystrophin levels sets a minimum level to confer measurable clinical benefit.”<sup>18</sup>

Dystrophin levels in exon-51 model BMD patients have been observed to be much higher than these estimates, roughly 80% of normal on average.<sup>19</sup> The clinical phenotype in these patients is, however, generally much milder than DMD, and this should not be taken to suggest that such high levels would be necessary for any benefit.

*Since the discovery of revertant fibers and trace dystrophin in DMD, investigators have looked for, but generally not found,<sup>20</sup> a correlation between DMD severity and trace levels of dystrophin. However, interpretation of studies is limited by questions of reliability and comparability of methods, and lack of consistent and quantitative definition of “trace” or “low level” dystrophin. For example, in one report that found a relationship between low levels of dystrophin and clinical severity of DMD, the dystrophin levels that correlated with a milder course appeared to be substantially higher than 3%,<sup>21</sup> perhaps 15%, as measured by Western blot. Another report failed to find a correlation between the presence of reverted fibers and the clinical severity of DMD, and found a less severe clinical course only in a limited number of patients showing a faint dystrophin labeling in most fibers.<sup>22</sup> Patients who are amenable to exon 44 skipping have been reported to express higher levels of dystrophin than in DMD patients with other exon-skippable mutations, and to have a somewhat milder course, but it is not clear how much dystrophin is expressed in these patients [although see immediately below for additional discussion] (most reports have focused on immunofluorescence rather than Western blot<sup>23</sup>) or on the percentage of fibers staining for dystrophin (staining in nearly 100% of fibers occurs in at least some exon 44 skippable patients<sup>24</sup>). Possible differences in functionality of the truncated dystrophin species produced in patients with different mutations also confounds interpretation of possible effects on clinical course of differences in dystrophin levels.*

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<sup>17</sup> Lu QL, Cirak S, Partridge T (2014) What can we learn from clinical trials of exon skipping for DMD? *Mol Ther Nucleic acids*. 3, e152.

<sup>18</sup> Wilton SD, Veedu RN, Fletcher S (2015) The emperor’s new dystrophin: finding sense in the noise. *Trends in Molecular Medicine*. 21, 417-426.

<sup>19</sup> Anthony K et al (2011) Dystrophin quantification and clinical correlations in Becker muscular dystrophy: implications for clinical trials. *Brain*. 134, 3544-3556.

<sup>20</sup> Flanigan KM (2014) Duchenne and Becker muscular dystrophies. *Neurol Clin* 32:671-688.

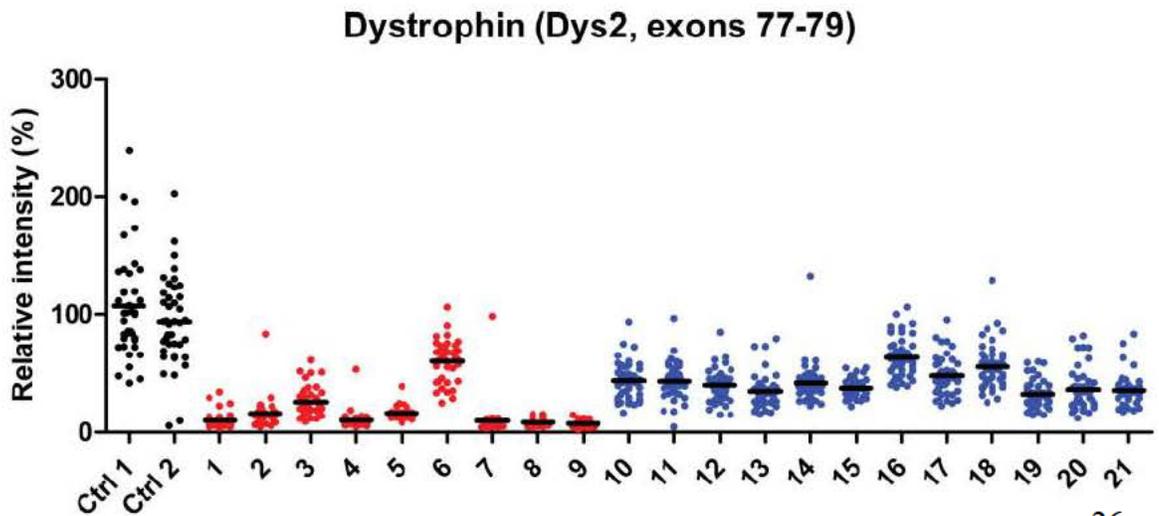
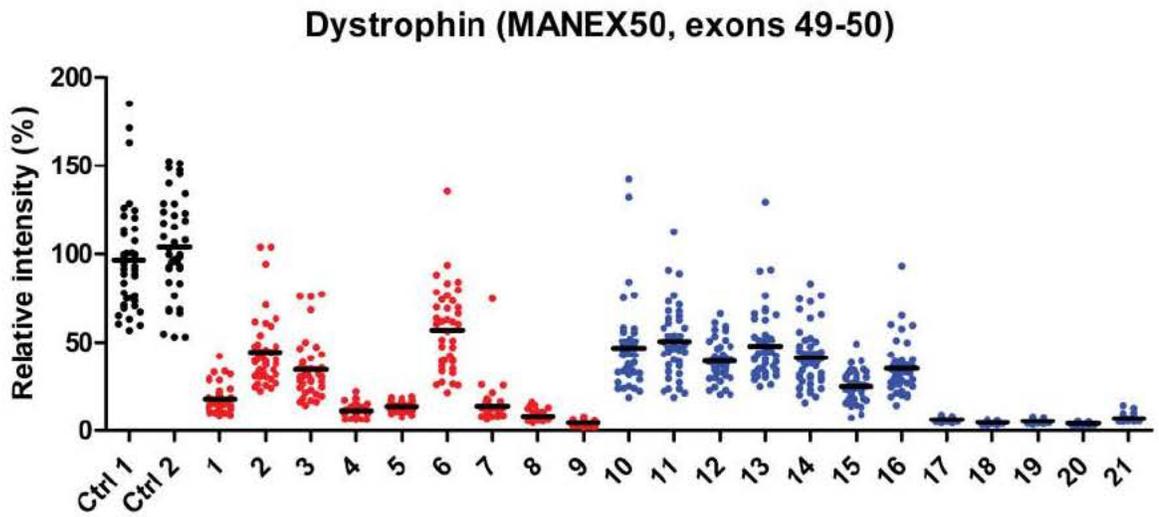
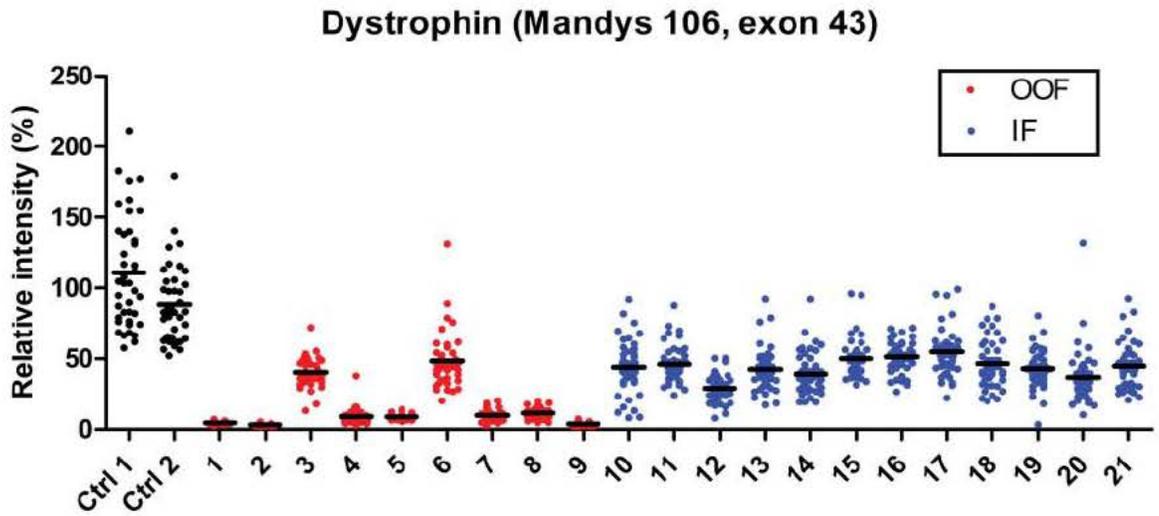
<sup>21</sup> Nicholson, LVB (1993) Functional significance of dystrophin positive fibers in Duchenne muscular dystrophy. *Archives of Diseases in Childhood*. 68:632-636.

<sup>22</sup> Fanin et al (1995) Dystrophin-positive fibers in Duchenne dystrophy: origin and correlation to clinical course. *Muscle and Nerve*. 18:1115-1120.

<sup>23</sup> Anthony K, et al (2014) Biochemical characterization of patients with in-frame or out-of-frame DMD deletions pertinent to exon 44 or 45 skipping. *JAMA Neurol*. 71:32—40.

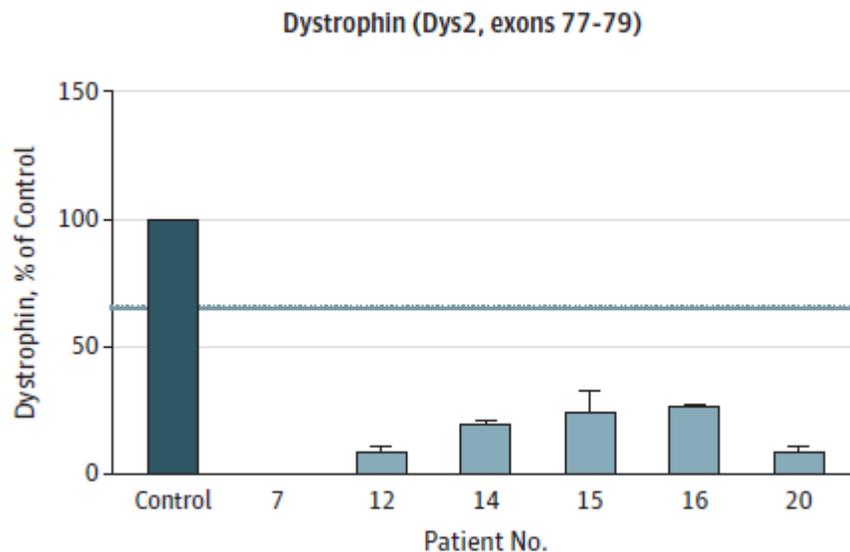
<sup>24</sup> Beekman et al (2014) A sensitive, reproducible and objective immunofluorescence analysis method of dystrophin in individual fibers in samples from patients with Duchenne muscular dystrophy. *PLOS ONE*. 9:e107494.

There was additional discussion in the FDA presentation to the PCNS AC of data suggesting that dystrophin levels in exon 44 skippable patients with less severe phenotypes may be substantially higher than 1% or normal. The figure below, from the supplemental material for Anthony et al (2014)<sup>23</sup> shows a comparative immunohistochemical analysis of dystrophin expression in patients with in-frame (IF) (blue) or out-of-frame (OOF) (red) deletions around exons 44 and 45. Patients 1 through 5 are exon 44 skippable, and patients 6 through 9 are exon 45 skippable. Exon 45 skippable patients, similar to exon 44 skippable patients, may be useful for understanding the relationship of dystrophin levels to DMD phenotype.



The following are key observations about this data:

- Among both exon 44 and 45 skippable patients, there is a wide range of relative dystrophin intensities by immunofluorescence. A number of antibodies were used with results that were generally directionally consistent.
- Patients 3 and 6 had the highest dystrophin expression and the mildest course of disease progression, with Patient 3 reported as ambulant at age 17 years, not running, with difficulty climbing stairs, and Patient 6 reported as ambulant at age 37 years.
- The 4 other exon 44 skippable patients had dystrophin intensities that appeared to be lower, although results were not entirely consistent across the different antibodies. Patients 1 and 2 were walking indoors at age 15 years and 14 years, respectively, whereas Patient 4 lost ambulation at age 11 years, and Patient 5 lost ambulation at age 12 years.
- Dystrophin levels in patients 3 and 6 (and perhaps patient 2 based on staining by the MANEX50 antibody) appear to be similar to dystrophin levels in the in-frame BMD patients. Western blot data for Patients 7, 12, 14, 15, 16, and 20 were presented in the publication, and are shown below (note that the blue horizontal line is the average dystrophin expression in exon 51 model BMD patients).



Importantly, because Patients 3, 6, and perhaps patient 2 have immunofluorescence levels similar to the in-frame BMD patients, as measured within the same study, it may be reasonable to conclude that dystrophin levels by Western blot might also have been roughly similar if measured, somewhere between about 10% and 25% of normal. Even considering the potentially large degree of error in such a “cross-method comparison” this data suggests that dystrophin levels may have been substantially higher than 1% of normal in the exon 44 patients with milder clinical course. Western blot data from additional exon 44 skippable patients with varying disease course would be highly desirable

to increase understanding of dystrophin levels that might be reasonably likely to predict clinical benefit.

**Timing:** Experts have cautioned that dystrophin is present in BMD from birth, and that “we should not conclude that dystrophin restitution in DMD patients with established dystrophic pathology will confer comparable benefits to the dystrophins in BMD patients”<sup>25</sup> for reasons including the pro-inflammatory environment that develops in DMD.<sup>26</sup>

**Functionality:** The exact dystrophin mutation affects the clinical phenotype in BMD,<sup>27</sup> and likely also in DMD, confounding interpretation of any possible clinical impact of small differences in dystrophin levels among DMD patients, with experts stressing that “it will be essential to account for different mutations when looking at other possible contributing factors to disease severity.”<sup>28</sup>

**Localization:** In BMD, dystrophin is typically present in all or most fibers<sup>29,30</sup> and, in addition to the total amount, this is thought to be important for function of the dystrophin. In contrast, in DMD many patients have no detectable dystrophin staining, while others have bright staining in a small percentage (1- to 5%) of “revertant” fibers in which exon skipping is thought to occur spontaneously. Some DMD patients can also show faint dystrophin staining in up to about 25% of fibers,<sup>31</sup> with the percentage of positive fibers appearing to depend in part on technical factors that affect assay sensitivity.

*Low level dystrophin immunofluorescence in almost 100% fibers has also been reported in DMD, including in exon-51 skippable patients.<sup>32</sup>*

**Unusual BMD Patients:** Rarely, patients with BMD are encountered who have dystrophin levels that are less than 1% of normal, which is as low as typical DMD patients. Importantly, however, rather than suggesting that very low levels of drug-induced dystrophin are likely to be beneficial, such patients highlight the complexity of the relationship between dystrophin levels and phenotype. The fact that such patients can have mild disease appears to be unrelated to, not necessarily the result of, low levels of dystrophin. In this context, the applicant selected three BMD patients as comparators for the Week 180 dystrophin studies, one of whom had low

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<sup>25</sup> Wilton SD, Fletcher S, Flanigan KM(2014) Dystrophin as a therapeutic biomarker: Are we ignoring data from the case? *Neuromuscular Disorder.* 24, 463-466.

<sup>26</sup> Rosenberg et al (2015) Immune-mediated pathology in Duchenne muscular dystrophy. *Sci Transl Med* 7,299rv4.

<sup>27</sup> Nicolas et al (2015) Becker muscular dystrophy severity is linked to the structure of dystrophin. *Human Molecular Genetics.* 24:1267-1279.

<sup>28</sup> Van den Bergen JC et al (2014) Dystrophin levels and clinical severity in Becker muscular dystrophy patients. *Neurol Neurosurg Psychiatry.* 85, 747-753.

<sup>29</sup> Arahata et al (1989) Dystrophin diagnosis: comparison of dystrophin abnormalities by immunofluorescence and immunoblot analysis. *Proc Natl Acad Sci.* 86,7154-7158.

<sup>30</sup> Morandi et al (1995) Dystrophin characterization in BMD patients: correlation of abnormal protein with clinical phenotype. *Journal of Neurological Sciences* 132,146-155.

<sup>31</sup> Arechavala-Gomez et al (2010) Revertant fibres and dystrophin traces in Duchenne muscular dystrophy: implications for clinical trials. *Neuromuscul Disord.* 20,295-301.

<sup>32</sup> Beekman et al (2014) A sensitive, reproducible and objective immunofluorescence analysis method of dystrophin in individual fibers in samples from patients with Duchenne muscular dystrophy. *PLOS ONE.* 9:e107494.

dystrophin level of about 2% of normal. However, the BMD patients selected by the applicant do not appear representative, and this patient may correspond to one of the rare BMD patients with very low dystrophin levels.

***Note:** Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statements, “In this context, the applicant selected three BMD patients as comparators for the Week 180 dystrophin studies, one of whom had low dystrophin level of about 2% of normal. However, the BMD patients selected by the applicant do not appear representative, and this patient may correspond to one of the rare BMD patients with very low dystrophin levels.” as a key inaccuracy.*

***Sarepta:** “BMD patient samples were not chosen to be representative; rather, they were selected in response to an FDA request to assess the relationship between dystrophin as measured by Western blot and immunofluorescence fiber intensity. Therefore, BMD samples were obtained that represented low, middle, and higher ranges of dystrophin expression. A comparable Western blot analysis - IHC correlation was presented by Hathout, et al. (MDA 2015 Scientific Conference poster, FDA - NIH workshop on measuring dystrophin, 2015), where BMD biopsies were chosen to represent low- and mid-level dystrophin expression. Consistently, their BMD low patient biopsy was 2% of normal.”*

***FDA response:** It isn't clear that there is any disagreement. The BMD patient selected by the applicant, who has dystrophin levels of about 2% of normal, is not representative of levels typically associated with BMD, and may correspond to one of the rare patients whose clinical course is milder than expected despite low levels of dystrophin typically associated with the DMD phenotype.*

*As further illustration, there are rare cases of siblings where both show a negative pattern of dystrophin immunostaining and scattered revertant fibers yet have highly discordant phenotypes. For example, Zatz et al<sup>33</sup> reported a case of nonsense mutation DMD in which the younger brother was wheelchair-bound at age 9 years, whereas his half-brother was reported to have some difficulties running and climbing stairs at age 15 years but normal walking ability.*

#### **e. Reviewer Discussion, Dystrophin Quantification Methods**

Considerable confusion can be created by the fact that a number of different methods have been used to quantify dystrophin expression, some more quantitative than others, and some producing higher absolute numbers than others. As discussed above, immunofluorescence is mainly informative of dystrophin localization, but is not a reliable measure of dystrophin amount (beyond perhaps the binary distinction between “undetectable” and “detectable”). For example, in many patients with typical DMD, only trace levels of dystrophin are present, yet these levels result in 25% or more of fibers being faintly dystrophin-positive.

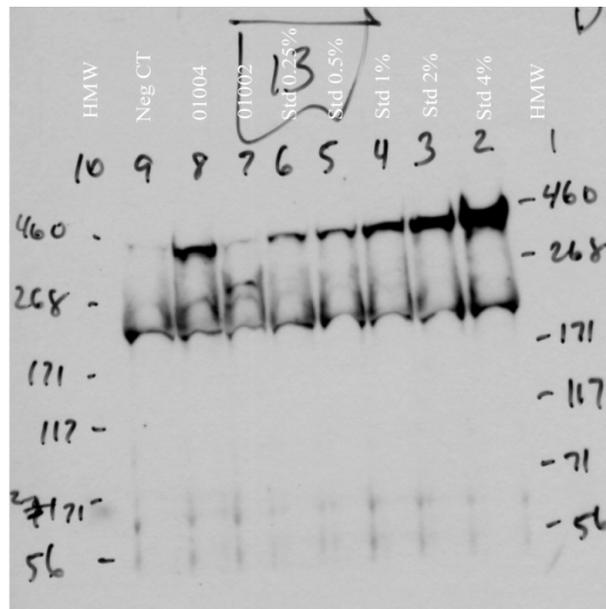
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<sup>33</sup> Zatz M et al (2014) Milder course in Duchenne patients with nonsense mutations and no muscle dystrophy. *Neuromuscular Disorders*. 24:986-989.

Western blot, in contrast, cannot provide information about dystrophin localization within the tissue, but does allow reasonable quantification through the use of internal controls with defined amounts of dystrophin (currently defined in terms of percent of dystrophin of a normal individual, not purified protein, which does introduce a small amount of uncertainty, but perhaps 2-fold or less). A dilution series control is shown in

Figure 4, near the “460” molecular weight marker, from right to left.

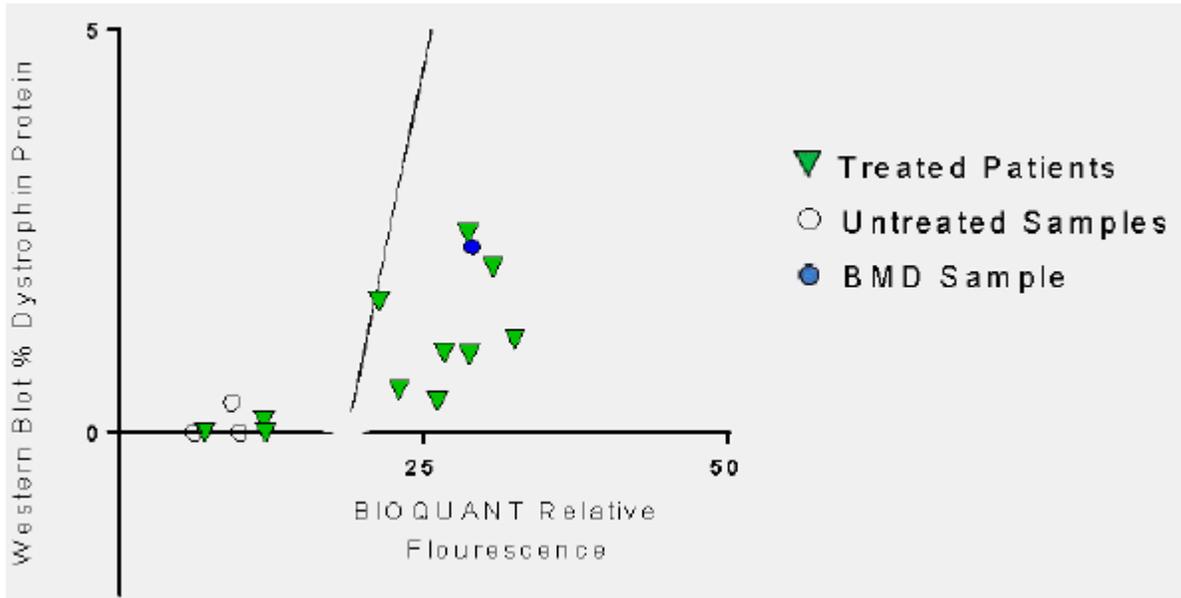
**Figure 4: Western blot, 4th Biopsy, Study 202**



In contrast, immunofluorescence methods lack similar internal controls, and as a consequence it is essentially impossible to correlate a certain amount of fluorescence to a certain amount of protein measured by Western blot, or relative to a normal control. There is no simple or reliable way to compare estimates of dystrophin amount derived from immunofluorescence with estimates derived from Western blot.

Figure 5 shows that at low levels of dystrophin (<5% by Western blot), immunofluorescence appears to overestimate the amount of dystrophin; for example, immunofluorescence shows about 25% intensity for samples with roughly 1- or 2% of normal dystrophin by Western blot, and shows about 10% of normal intensity for samples with <1% of normal dystrophin levels.

**Figure 5: Western blot vs. Bioquant**



Finally, a representation of the change in dystrophin levels in terms of percent change from baseline is problematic in this situation, because the trace baseline dystrophin levels in many patients are too low to be measured accurately, resulting in ratios that are imprecise, and that are greatly affected by small amounts of random variability in denominators that are close to zero.

*Expressing dystrophin levels as percent- or fold-change compared to controls exaggerates the difference:*

- *Dystrophin levels that were, in fact, detected but that were less than 0.25% were imputed as zero.*
- *The lower limit of reliable detection of the assay is 0.25%. It would be more accurate to consider undetectable dystrophin levels as <0.25%, not as zero.*

#### **f. FDA Review Team Preliminary Conclusions on Dystrophin Findings**

Adequate scientific methods appear to be available to measure dystrophin expression in DMD. As discussed in the recent FDA draft Guidance on DMD,<sup>34</sup> there is justifiable interest in

<sup>34</sup> Duchenne muscular dystrophy and related dystrophinopathies: developing drugs for treatment. <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>

dystrophin as a potential surrogate endpoint for accelerated approval in DMD. However, the Guidance also states that the potential for a biomarker to predict clinical benefit in DMD is inseparable from such factors as the magnitude of change of the biomarker. Regarding methodology, the Guidance stresses the importance of the performance characteristics of the biomarker assays, including quality-control measures.

Based on the data submitted by the applicant, considerable doubt remains about how much, or perhaps even whether, dystrophin levels were increased by eteplirsen. The degree of uncertainty about the dystrophin data hinders discussion of its use as surrogate endpoint for eteplirsen. However, to the degree that the dystrophin data may be interpretable, the amount and distribution of dystrophin in treated patients appears to be within the range typically associated with DMD, not BMD or intermediate forms of dystrophinopathy. Data suggesting that higher levels of dystrophin were produced by eteplirsen appear unreliable.

## Clinical Efficacy Evidence

The only study that evaluated clinical efficacy is Study 201/202. Dr. Xiang Ling, from the Office of Biometrics, provided a statistical review of that study. As described below, and in Dr. Ling's review, Study 201/202 was not designed in a way that allows reliable use of statistical hypothesis testing (i.e., "p-values"), and is only capable of providing interpretable evidence of efficacy if the beneficial effect of eteplirsen is so large that it is essentially self-evident, without the use of statistics.

### a. Design and analysis of Study 201/202

Clinical efficacy was examined in one single-center, 24-week, 3-arm controlled trial (Study 201) in 12 patients assigned 1:1:1 to 30 mg/kg eteplirsen, 50 mg/kg eteplirsen, or placebo. Study 201 was continued as an open-label extension, called Study 202, which has been ongoing for more than 3 years. Multiple functional endpoints were assessed both in the placebo-controlled and open-label extension periods, including 6 minute walk test (6MWT), North Star Ambulatory Assessment (NSAA), and a number of measures of pulmonary function. Analysis of clinical endpoints was not controlled for multiplicity, but in Study 201 the clinical endpoints were essentially uniformly negative, without trends supportive of efficacy.

*Note: Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statement in the statistical review that "the robustness of the study result is a concern since a single patient could change the results substantially"*

*Sarepta: "This statement is inaccurate. A comprehensive sensitivity analysis was performed in order to address any potential issue regarding robustness of the data. Specifically:*

- *Two patients were removed: the best performing eteplirsen and the worst performing external control patient.*

- *Results demonstrated a robust 6MWT treatment advantage of >100 meters with nominal significance.”*

***FDA Response:*** *This statement from the statistical review is in reference to the placebo-controlled portion of Study 201/202, which was small in size (N = 4 per arm), such that changes in the outcome measure for a single patient could change the overall results substantially. The statistical review also notes that a key limitation of the externally controlled open-label portion of Study 201/202 was dissimilarity of the groups being compared, along with differences in how the data were collected, as also detailed in this memo and other background information from the FDA. The applicant’s statistical approach to analysis of the externally-controlled portion of Study 201/202 does not address the key source of uncertainty in any externally-controlled trial: the presence of non-drug related differences between groups, some of which are known, and some of which are unknown. One of the applicant’s proposed sensitivity analyses, which removed the single best-performing eteplirsen patient and the single worst performing external control patient, does not address this fundamental issue.*

Shortly after Study 202 passed 1 year duration, the applicant proposed a post-hoc analysis with a number of changes from the original analysis: a) data for 2 out of 8 patients treated with eteplirsen (patients who quickly lost ambulation) were dropped, b) the prespecified comparison of each dose arm to placebo was changed to comparison of the 6 remaining treated patients to the 4 placebo-treated patients, and c) the endpoint was taken to be Week 36, instead of Week 24. FDA explained in detail to the applicant in March of 2013 why the proposed analysis was unreasonable even for hypothesis generation, and why Study 201 did not provide evidence of efficacy.

As the duration of exposure in Study 202 increased, the applicant proposed comparing the clinical course of treated patients to historical controls. FDA expressed strong reservations regarding the potential interpretability of the applicant’s proposed comparison to historical controls and the use of 6MWT as the primary endpoint in such a historical comparison. Because of these concerns, FDA noted that a dramatic effect size would be necessary for any such analysis to be potentially interpretable. Well-designed historically-controlled trials can, in certain circumstances, be considered adequate and well-controlled designs that can support FDA approval. However, Study 201/202 is not a well-designed historically-controlled trial. It is well established, as detailed in guidelines developed by U.S. and international regulatory bodies,<sup>35</sup> that “inability to control bias is the major and well-recognized limitation of externally-controlled trials, and it is always difficult, and in many cases impossible, to establish comparability of the treatment and control groups.” Furthermore “a consequence of the recognized inability to control bias is that the potential persuasiveness of findings from externally controlled trials depends on obtaining much more extreme levels of statistical significance and much larger estimated differences between treatments than would be considered necessary in concurrently controlled trials.”

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<sup>35</sup> Choice of control group and related issues in clinical trials, E10. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2000.

**Note:** *Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statement, “As the duration of exposure in Study 202 increased, the applicant proposed comparing the clinical course of treated patients to historical controls” as a key inaccuracy.”*

**Sarepta:** *“The proposal to compare with historical control patients originated from the FDA. Specifically, a requirement to compare the clinical course of treated patients in Study 202 to matched patient-level historical control data was made by the FDA at the March 2014 guidance meeting, and reiterated at the September 2014 pre-NDA meeting. Sarepta had proposed an open-label confirmatory study comparing treated patients to concurrent (not historical) untreated patients with exon deletions not amenable to skipping exon 51 (i.e., the PROMOVI study).”*

**FDA response:** *FDA consistently and strongly encouraged the sponsor to conduct adequately powered randomized placebo-controlled trials, and expressed doubt about the interpretability of externally controlled trials. As early as October 2012, Sarepta and its academic associates announced that in the randomized controlled portion of Study 201/202 eteplirsen had demonstrated unparalleled effects on enabling dystrophin production and slowing the progression of the disease,<sup>36</sup> with levels of dystrophin potentially as high as 50% of normal. In the context of an ongoing series of reports from the applicant and its academic associates describing continued striking and unprecedented stabilization of disease progression, many in the DMD community expressed strong reservations regarding the ethics of conducting another placebo-controlled trial, and informed FDA that performing such a study would be extremely difficult or impossible. In this context, and based on assertions that eteplirsen had been shown unequivocally to produce high levels of dystrophin, FDA expressed willingness to consider an externally controlled trial, although stating clearly that interpretation of the data could be difficult, and that the acceptability of the study would be a matter for NDA review.*

*FDA informed the applicant that if it were to pursue a comparison of patients in Study 201/202 to external controls, evaluating such a comparison would be difficult without submission of patient-level external data, including data from a number of different sources to understand variability across different datasets, which can be substantial in DMD. For example, Biggar et al<sup>37</sup> reported that about 75% of a population of DMD boys treated with deflazacort was ambulant at age 15 years (N = 40), whereas Bello et al<sup>38</sup> reported that in data collected by the Cooperative International Neuromuscular Research Group (CINRG) about 25% boys<sup>39</sup> similarly treated with deflazacort were ambulatory at age 16 years (N = 80).*

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<sup>36</sup> <http://investorrelations.sarepta.com/phoenix.zhtml?c=64231&p=irol-newsArticle&ID=1741044>; accessed March 17, 2016.

<sup>37</sup> Biggar WD et al., (2006). Long-term benefits of deflazacort treatment for boys with Duchene muscular dystrophy in their second decade. *Neuromuscular Disorders* 16:249-255.

<sup>38</sup> Bello L et al (2015) Prednisone/prednisolone and deflazacort regimens in the CINRG Duchenne natural history study. *Neurology*. 85, 1048-1055.

*After release of the previous version of this memo, CINRG provided additional unpublished analyses to FDA suggesting that exon-51 skippable patients follow a clinical course for age of loss of ambulation generally similar to that described for the broader DMD population described in Bello et al, with about 25% of boys maintaining ambulation to 16 years of age and about 15% of patients maintaining ambulation to 18 years of age. At the time this revised memo was written, CINRG was in the process of providing patient-level CINRG data to FDA that should enable more detailed comparison with eteplirsen-treated patients for both age at loss of ambulation and functional endpoints such as 6MWT and 10 m walk/run, based on a prespecified plan.*

**Note:** *Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statements, “Finally, as the natural history studies proceeded, some patients left to enter interventional clinical trials, further decreasing the similarity of the natural history cohort to the eteplirsen patients” as a key inaccuracy.*

**Sarepta:** *“Two types of missing data sensitivity analyses were performed, the results confirmed that the magnitude of difference remained over 100 meters and nominal statistical significance was maintained:*

- *MMRM using all the available data*
- *Last Observation Carried Forward imputation (conservative analysis assuming that the 2 control patients did not decline)”*

**FDA response:** *It should be stressed that for a variety of reasons the clinical course of patients in recent observational studies in DMD, including CINRG, might be expected to be worse than the clinical course of patients selected for studies of experimental drugs. Differences in patient selection, supportive care, motivation, and how loss of ambulation is defined and measured, among other factors, are likely to be important. Various analytical methods to impute missing data, such as mixed effect model repeat measurement (MMRM) and last observation carried forward (LOCF), do not address the key limitation of a comparison between an open-label treatment group in an interventional clinical trial and an independent group of patients who are in an observational study: non-drug-related differences between the groups being compared. Recent observational studies in DMD have been enrolling patients simultaneously with interventional trials of new drugs. Thus, patients in an observational cohort who were motivated to enroll in a drug study and could qualify for enrollment might have preferentially left the observational study. In other words, patients who remained in the observational study may have been less motivated or less able to participate in studies of experimental drugs. Moreover, patients in an observational study are likely to differ in other important ways. Specific evidence of this effect appears to be present in the historical data submitted by the applicant. A patient selected as a historical control for Study 201/202 lost ambulation after a single 6MWT measure, and stayed in the*

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<sup>39</sup> CINRG has subsequently provided FDA with unpublished analyses suggesting similar natural history in exon-51 skippable patients, as discussed elsewhere in this review.

*observational study for several years, long enough to be matched to eteplirsen patients. In contrast, two other exon-51 patients with similar baseline age and 6MW distance discontinued the observational study to participate in drug studies. These patients, doing reasonably well, were therefore not under observation for long enough to serve as historical controls for the eteplirsen study.*

*Many aspects of supportive care are important for prolonging function in DMD, yet difficult to quantify, and this appears to be particularly true for physical activity. Regular physical activity is necessary to maintain function in DMD and to avoid disuse atrophy.<sup>40</sup> Gentle exercise appears to provide additional benefit, including delay of functional deterioration.<sup>41</sup> Use of a wheelchair may justifiably be encouraged by caregivers for reasons of safety and independence, or even be required in settings such as school. In addition, although difficult to quantify, accounts by caregivers suggest that pessimism and resignation about prognosis in DMD may contribute to decreased time spent walking and less independent activities and self-care, whereas feelings of hope and optimism from enrolling in a drug study may lead to the opposite behavior. Particularly in muscular dystrophy, it therefore seems possible that hope and positive expectations might increase physical activity and decrease the risk of disuse atrophy, thus slowing functional decline. Slower decline or even improvement in function have been observed in placebo arms of controlled trials in other types of muscular dystrophy,<sup>42</sup> and potentially may be the result of some of the above mechanisms.*

FDA encouraged the sponsor at the March 2013 meeting to conduct an adequately powered placebo-controlled trial of eteplirsen, stating “if it is true that eteplirsen leads to remarkable clinical benefit in even some patients, there is no doubt that a feasible placebo controlled study can be designed to demonstrate that benefit.” FDA also stated that “there is considerable variation among individual patients with regard to clinical measures and important milestones” and that data from an open-label study “may only be interpretable if a relevant objective endpoint obviously insulated from bias demonstrated compelling data that are clearly outside the known variability range for DMD.” FDA further stated that, at that time, comparison of data from Study 202 did not provide interpretable evidence of benefit “given the limitations of the open-label design for protecting against bias on effort-dependent endpoints like 6MWT.” At a July 2013 meeting with the applicant, at which the possibility of NDA filing based on dystrophin production was discussed, FDA similarly expressed reservations about natural history controls “due to the usual difficulty in showing comparability between the study populations in natural history studies,” and reiterated that 6MWT was susceptible to bias in the proposed natural history comparison.

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<sup>40</sup> Bushby K et al (2009) Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. The Lancet. DOI:10.1016/S1474-4422(09)70271-6

<sup>41</sup> Jansen M et al (2013) Assisted bicycle training delays functional deterioration in boys with Duchenne muscular dystrophy: the randomized controlled trial “no use is disuse”. Neurorehabil Neural Repair. DOI: 10.1177/1545968313496326

<sup>42</sup> Statland JM et al (2013) Reevaluating measures of disease progression in facioscapulohumeral muscular dystrophy. Neuromuscul Disord. 23,306-12.

Discussions about comparison of Study 202 patients to natural history continued with the April 15, 2014, communication from FDA to the applicant which stated that, with additional data to support the efficacy and safety of eteplirsen, an NDA should be filable. FDA noted that patients in Study 202 appeared to be receiving optimal care, including intensive physical therapy and intensive steroid regimens, and again stated that “performance on the 6-minute walk test is strongly influenced by motivation and coaching, and open-label trials are susceptible to bias on the part of investigators, patients, and parents.” In a September 2014 communication, FDA explained its concern that, as noted by DMD experts, “preservation of ambulation and other skills is affected by the value that families and caregivers put on maintaining those skills, with such factors as risk of falls and injury from continued ambulation weighed against the safety and speed of allowing patients to use a wheelchair.” FDA further advised the applicant that it was not clear that such biases could be adequately controlled, and that the applicant should present data from measures of muscle strength in the NDA to assist in determining if measures of ambulation had been affected by these types of bias. As discussed below, results from rise time measures and the NSAA appear to be reasonable measures of muscle strength in this context, and thus important for interpreting the 6MWT results.

As stated by Mendell et al. (2007)<sup>43</sup> “Patients may differ in the value they put on maintaining certain skills. Take, for example, prolonging independent ambulation. Some may consider the burden of preserved activity (effort of walking, risks of falling, time required) inferior to the ease and comfort in getting from place to place in a wheelchair.”

FDA advice to the applicant was also informed by information provided by the Muscular Dystrophy Association and Parent Project Muscular Dystrophy, including the following:

- “Transition to a wheelchair usually is a gradual process”<sup>44</sup>
- “Children often experience renewed independence once they fully transition to a power wheelchair. For many parents and caregivers, it is painful to accept that a child needs help getting around, but it is better for the child to have mobility using help from braces, scooters, or wheelchairs—and the independence it gives the child—than not to be able to move as freely as possible”<sup>45</sup>

To interpret the applicant’s comparison of 6MWT results for eteplirsen patients to historical controls, it is also important to understand the progression of 6MWT as DMD patients near the time of loss of ambulation. At younger ages, during the period of relative stability or slow decline of 6MWT, a difference between two patients in 6MWT of 100 m is likely to predict a difference of several years in time to loss of ambulation, particularly if one patient is below about 300 meters and the other above. Differences between patients of 150- or 200 m on 6MWT have even larger prognostic implications, with patients who can walk in the range of 400- to 500 m on 6MWT unlikely to lose ambulation for many years. In contrast, however, large differences in 6MWT between patients near the time of loss of ambulation occur even when patients have generally similar prognoses.

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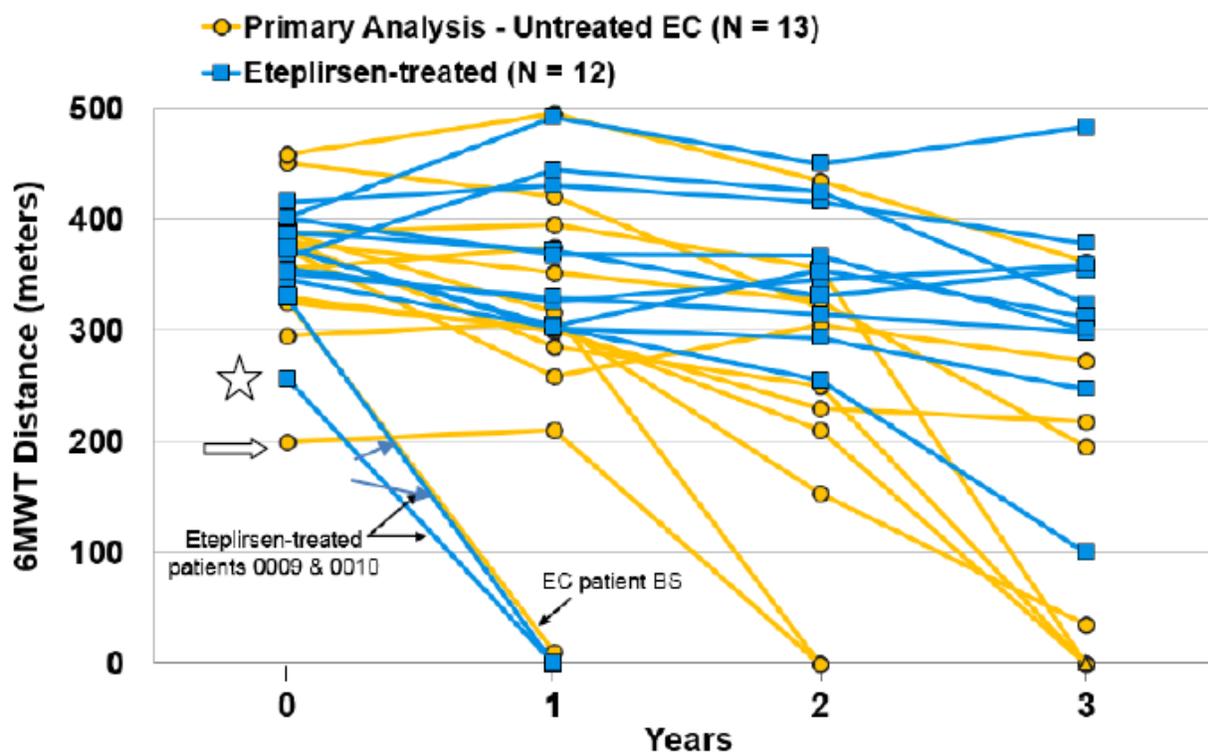
<sup>43</sup> Mendell et al. (2007) Challenges in drug development for muscle disease: a stakeholders meeting. *Muscle Nerve* 35:8-16.

<sup>44</sup> [www.mda.org/disease/duchenne-muscular-dystrophy/signs-and-symptoms](http://www.mda.org/disease/duchenne-muscular-dystrophy/signs-and-symptoms). Accessed 9 May 2016.

<sup>45</sup> [http://www.parentprojectmd.org/site/PageServer?pagename=Care\\_stage\\_nonambulatory](http://www.parentprojectmd.org/site/PageServer?pagename=Care_stage_nonambulatory).

Figure 6, taken from the applicant’s NDA, shows patient-level data for eteplirsen and historical controls. Consider two patients in their final year or two of ambulation: the historical control patient with a baseline of about 200 m (arrow), and the eteplirsen patient with a baseline of about 260 (star). At Month 12, the eteplirsen patient has lost ambulation, whereas the 6MWT for the historical control patient remains at about 200 m, such that the difference in 6MWT has increased from 60 m at baseline to about 200 m. By Month 24, the historical control patient has also lost ambulation, such that the difference between patients has become zero. Thus, in contrast to younger patients, the 200 m difference near the time of loss of ambulation corresponded to about 1 year difference in age at loss of ambulation. The general pattern and size of this effect is typical, with many DMD patients decreasing from about 300 m on 6MWT to loss of ambulation over 1- to 2 years, leading to brief but very large differences in 6MWT between patients whose disease course is otherwise generally similar. This does not imply that a difference of 150- or 200 m on 6MWT would not be clinically meaningful, but does suggest that even modest differences between study arms in poorly controlled studies such as Study 202 can exaggerate differences in certain functional measures near the time that patients lose ambulation.

**Figure 6: 6MWT in Patients Using Steroid, Age ≥ 7 Years, Amenable to Exon 51 Skipping by Treatment Status – Individual Patient Data**



**b. Rate of progression of 6MWT in eteplirsen-treated patients is consistent with expected natural history**

*Data reliability is a major concern in the comparison of eteplirsen-treated patients from Study 201/202 to external controls. It has been suggested to FDA by a number of outside individuals and groups that ambulation is a reliable efficacy endpoint in historically-controlled trials in DMD because it is a “hard” endpoint, i.e., an objective, invariant state indicating inability to walk independently. However, near the time of loss of ambulation factors such as effort and motivation on the part of both patient and examiner can have very large effects on ambulatory endpoints, such that loss of ambulation cannot be considered a “hard” endpoint in this setting. A 6-minute walk distance of 0 meters, or isolated or even consecutive zero values resulting, for example, from an injury from which the patient recovers, does not necessarily represent irreversible inability to walk.*

*Subsequent to the release of the previous version of this memo, FDA has determined that for at least two or three<sup>46</sup> of the 13 exon-51 skippable natural history patients selected by the applicant as controls, a value of zero was recorded for 6-minute walk distance apparently prior to loss of ambulation as documented by ability to perform the 10 meter walk/run test. Similar discordance between 6MW distance and 10 m walk/run was identified for at least 6 patients in the group of external control patients. Importantly, for both the exon-51 skippable patients and larger group of external controls, 10 m walk/run data were not available for many patients, limiting ability to assess discordance of results.*

- *At age 12, one exon-51 skippable control patient from Belgium was recorded as having a 6MW distance of 327 m, and a 10 m walk time of 7 s. At the next exam about 6 months later, 6MW distance was recorded as zero, but the patient was able to complete the 10 m walk in 11 s. This pattern continued with the next two exams over the following year, with 10 m walk values of 11s and 13 s, yet a 6MW distance of zero.*

*The applicant has recently provided FDA with source documents from the clinical sites for this patient and the other historical controls. These documents appear to indicate that at a follow-up visit 6 months later, 6MWT was not attempted because the patient was judged to be unable to walk. At the next visit 6 months later (1 year after the 327 m was recorded), a 6MWT was attempted, with the patient walking 125 m in about 3½ minutes. The examiner at the time noted that the patient “no longer wanted to continue (could still continue, had back pain).” The examiner’s comment appears to underscore the importance of motivation in 6MWT.*

- *At age 10, one exon-51 skippable control patient from Italy was recorded as having a 6MWT of 356 m, and a 10 m walk time of 10 s. One year later, at age 11, 10 m*

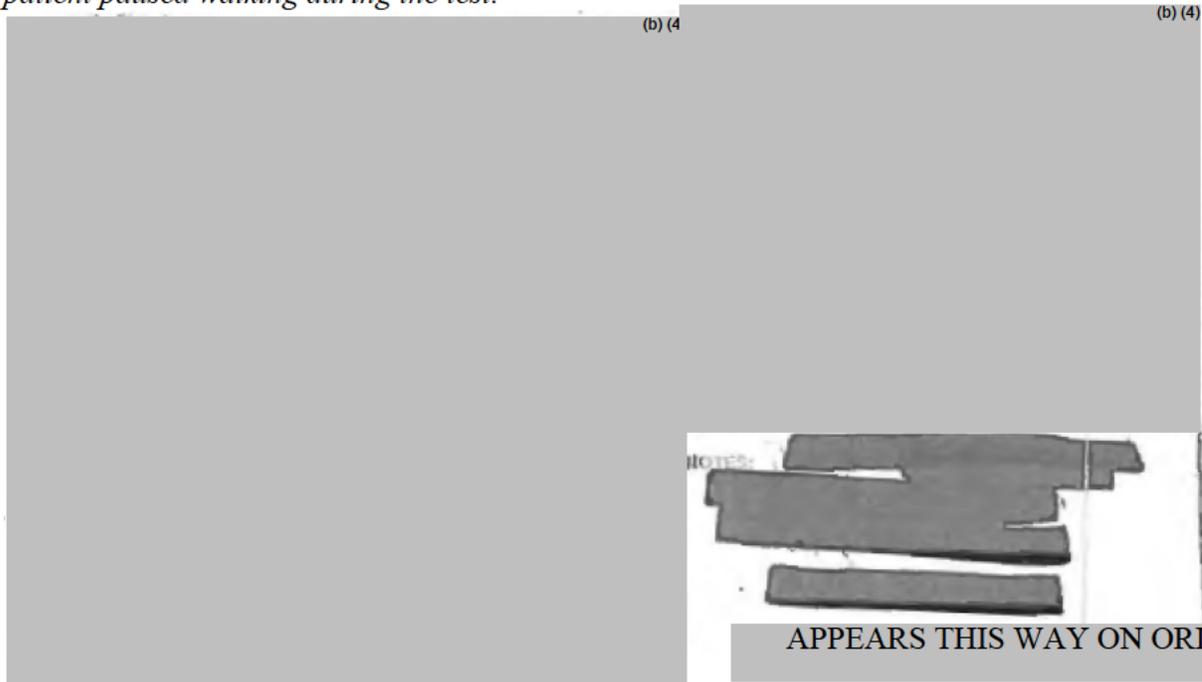
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<sup>46</sup> An additional exon-51 skippable patient had a 10 m walk time of 35s, and 6MWT of zero. Under some conventions, 6MWT would not be measured if the 10 m walk time is >25s, but it is not clear that consistent conventions were adopted across the natural history studies and Study 201/202.

*walk/run time was 12 s, but 6MWT was apparently not attempted and was recorded as zero (source documents state “not executable”).*

*Similar concern about reliability exists for 3 additional<sup>47</sup> exon-51 skippable natural history patients for whom 6MW distance was reported as zero but apparently not measured. Initial review of source documents recently received by FDA suggests the applicant asked the investigators in December 2015 if patients who had been last recorded in the clinic several years previously had maintained ambulation 4 years post-baseline.*

*There are, in addition, low 6MW distance values recorded for natural history controls that appear atypical for reasons that are not well documented. The image below shows a source document from a historical control patient who walked for only about 1½ minutes during the 6 minute test, and was recorded as having a final distance of 35 m (note: 50 m appears to have been the total distance, but due to an apparent error the value for “1 minute distance” of 35 m was transcribed). The notes section appears to have been blackened out. For other patients, this section of the document contained important information about patient performance during the test, such as “good cooperation” or the number of times that the patient paused walking during the test.*



*It should also be noted that eteplirsen-treated patients had two opportunities on consecutive visit days to perform functional tests, whereas natural history patients had only one. This systematic difference speaks to the dissimilarity in how the patients were managed and the level of attention given to the 6MWT in the eteplirsen study.*

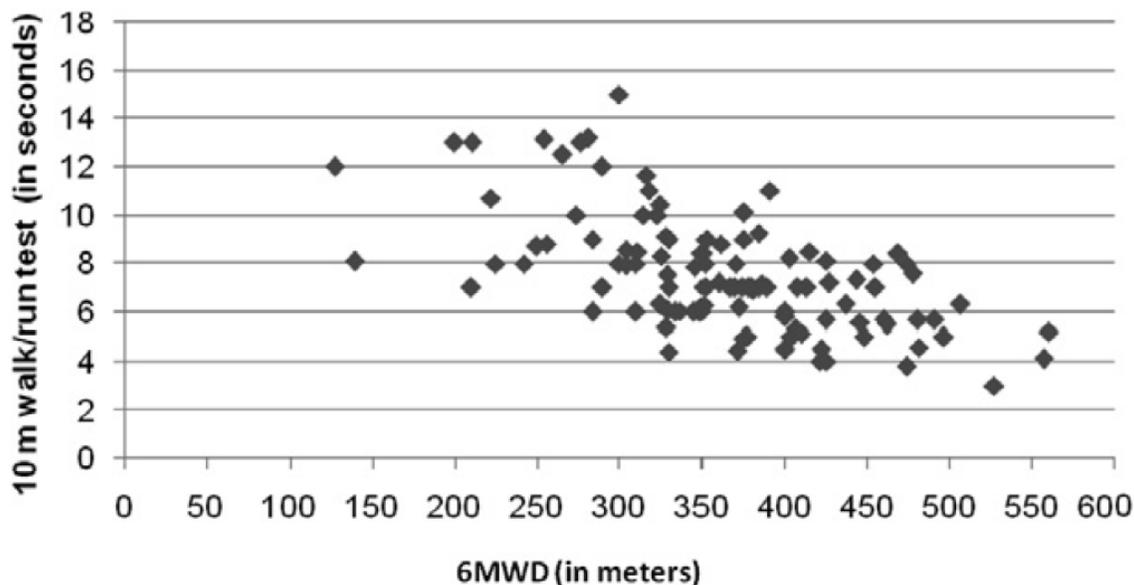
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<sup>47</sup> Per applicant addendum for February 23, 2016 AC meeting: “patients (b) (6) were subsequently reported to have loss of ambulation with “0” meters on the 6MWT at ~4.5 years. In addition, external control patient (b) (6) was known to have lost ambulation with a 6MWT of “0” at 4.8 years”

*Datasets from the natural history studies and the eteplirsen study were examined in more detail to characterize the typical relationship between 6MWT and 10 m walk/run values that might have been expected for control patients. The investigators for the Italian natural history cohort previously reported<sup>48</sup> an average 6MW distance of approximately 150 to 375 m for DMD patients with 10 m walk/run values between 11 and 13 s (*

Figure 7).

**Figure 7: 10 m walk/run vs 6MWD, by individual patient, Italian natural history cohort**



*There appeared to be a generally similar relationship between 6MWT and 10 m walk/run in eteplirsen-treated patients, for example, with values of 11 s to 12 s on 10 m walk/run corresponding to roughly 200 to 300 m on 6MWT, and 13 s to 15 s corresponding to roughly 150 to 200 m. One patient who walked 50 m on 6MWT had a 10 m walk/run time of 20 s.*

Patients from the placebo arm of randomized double-blind trials are likely to be better matched to patients in eteplirsen trials for factors that are difficult to measure, such as motivation and compliance with supportive therapy, compared to patients from registries. Placebo-controlled trials have recently been conducted with patients with DMD amenable to exon-51 skipping. Data from patients from the placebo group from some of these studies are publically available, and were used for a comparison with eteplirsen-treated patients.<sup>49</sup> The figures below show the clinical course on 6MWT of eteplirsen-treated patients from Study 201/202 (colored lines) compared to patients treated with placebo in other controlled studies in exon-51 skippable

<sup>48</sup> Mazzone et al (2010) North Star ambulatory assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. *Neuromuscular Disorders*. 20,712-716.

<sup>49</sup>

<http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/PeripheralandCentralNervousSystemDrugsAdvisoryCommittee/UCM475956.pdf>

patients with DMD (grey lines). Patients are divided by baseline rise from floor time (an important prognostic variable), and by steroid treatment (deflazacort,

Figure 8), or prednisone (Figure 9), because some evidence suggests deflazacort may be more effective than prednisone at preserving ambulation in DMD.

A few observations about these data follow:

- Clinicians expert in the care of DMD patients often perceive that, even in patients treated with corticosteroids, decline of 6MWT after about age 7 is steady, and that periods of stability or improvement, particularly after periods of decline, do not occur. However, the placebo data show that while decline ultimately occurs, many exon-51 patients experience periods of stability or even substantial improvement. This occurs in patients older than 10 years of age, and in patients who, at least as measured by 6-minute walk distance, have experienced substantial earlier declines. This complicates the interpretation of treatment trials in DMD that may not be well-controlled.
- The figures below divide patients by baseline rise time and steroid treatment,<sup>50</sup> but each can be interpreted as a continuum of disease progression, from top to bottom, because the loss of ambulatory ability in DMD almost always proceeds in sequence, with rise time steadily worsening (increasing), followed by loss of ability to rise from the floor but retained ability to walk, then loss of ability to walk, which often occurs with a sharp decline when 6MWT decreases below about 300 m. Thus, even though each placebo patient was followed for only 1 year, whereas eteplirsen patients were followed for more than 3 years, there can be reasonable confidence that most placebo patients would follow a stepwise progression through higher rise times prior to loss of ambulation, such that their clinical course can be extrapolated beyond the 1 year period of observation.
- The course of 6MWT for eteplirsen patients was generally similar to the course of placebo patients across all rise time categories, and for both types of corticosteroid, with some of the placebo patients having higher (better) 6MWT than matched eteplirsen patients, and some worse. This appears to be expected given the known wide variability of progression in exon-51 DMD, and the small numbers of patients available for comparison.
- Finally, decline in 6MWT is also a reliable predictor of loss of ambulation. At the most recent study visit, 6MWT was less than 250 m for the 7 out of 10 eteplirsen patients who had maintained ambulation past the first months of the study, which also predicts a high probability of loss of ambulation in a timeframe of 1 to 2 years.

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<sup>50</sup> Patient 7 was switched from prednisone to deflazacort in 2013, and is shown in the prednisone figure

In the figures below, many of the eteplirsen patients appear to have few or no matches to the placebo patients in the most recent year of treatment, but this is a result of the division of the figures into categories based on baseline rise time. Most eteplirsen patients are currently in the >15 s rise time category (10 of the 12 eteplirsen patients, including at least 5 who lost ability to rise), and can be compared to the >15 s rise time group of control patients. In general, the course of eteplirsen-treated patients in Study 201/202 is similar to the course in these control patients, as shown in

Figure 10, which combines all eteplirsen and control patients.

**Figure 8: 6MWT, Deflazacort-treated patients**

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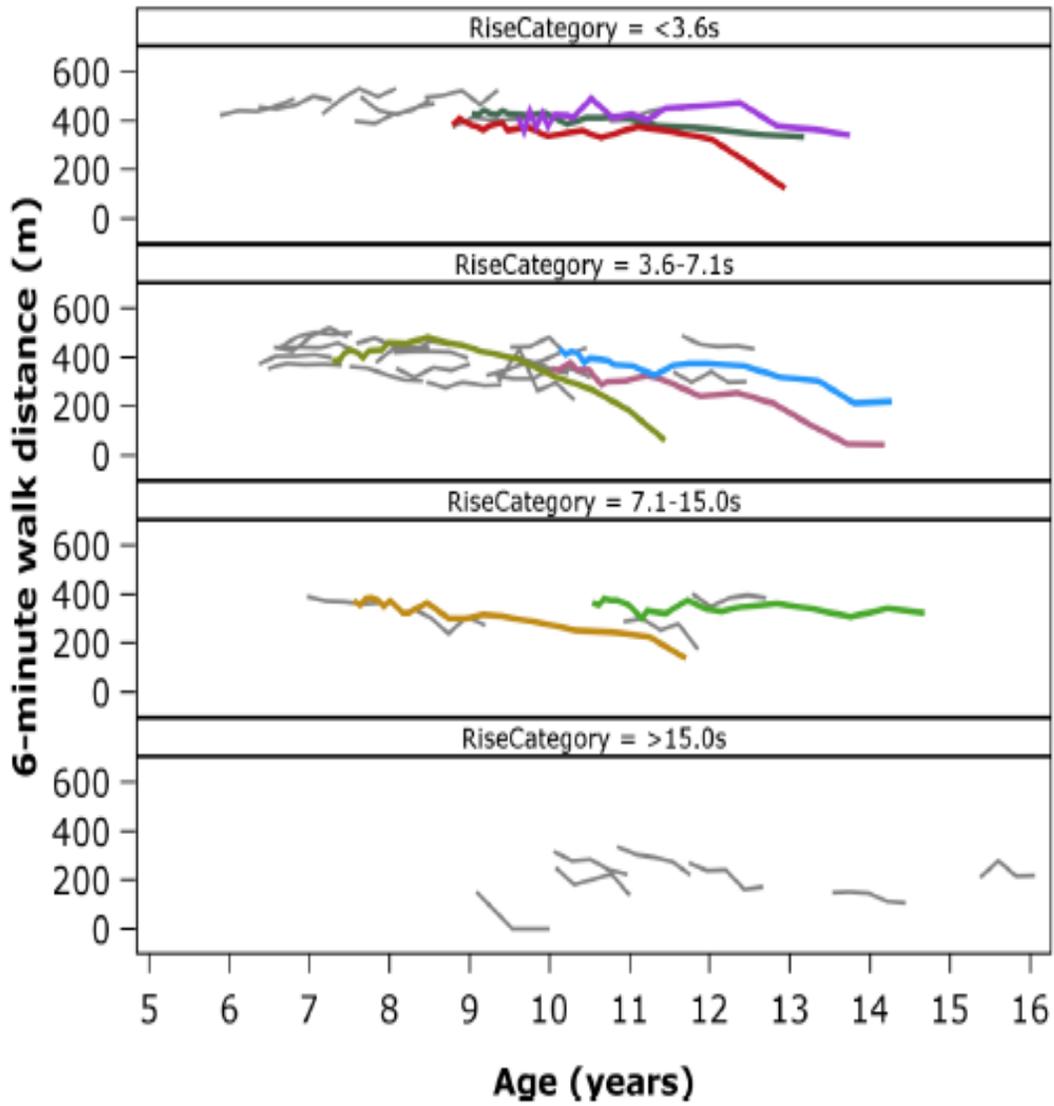


Figure 9: 6MWT, Prednisone-treated patients

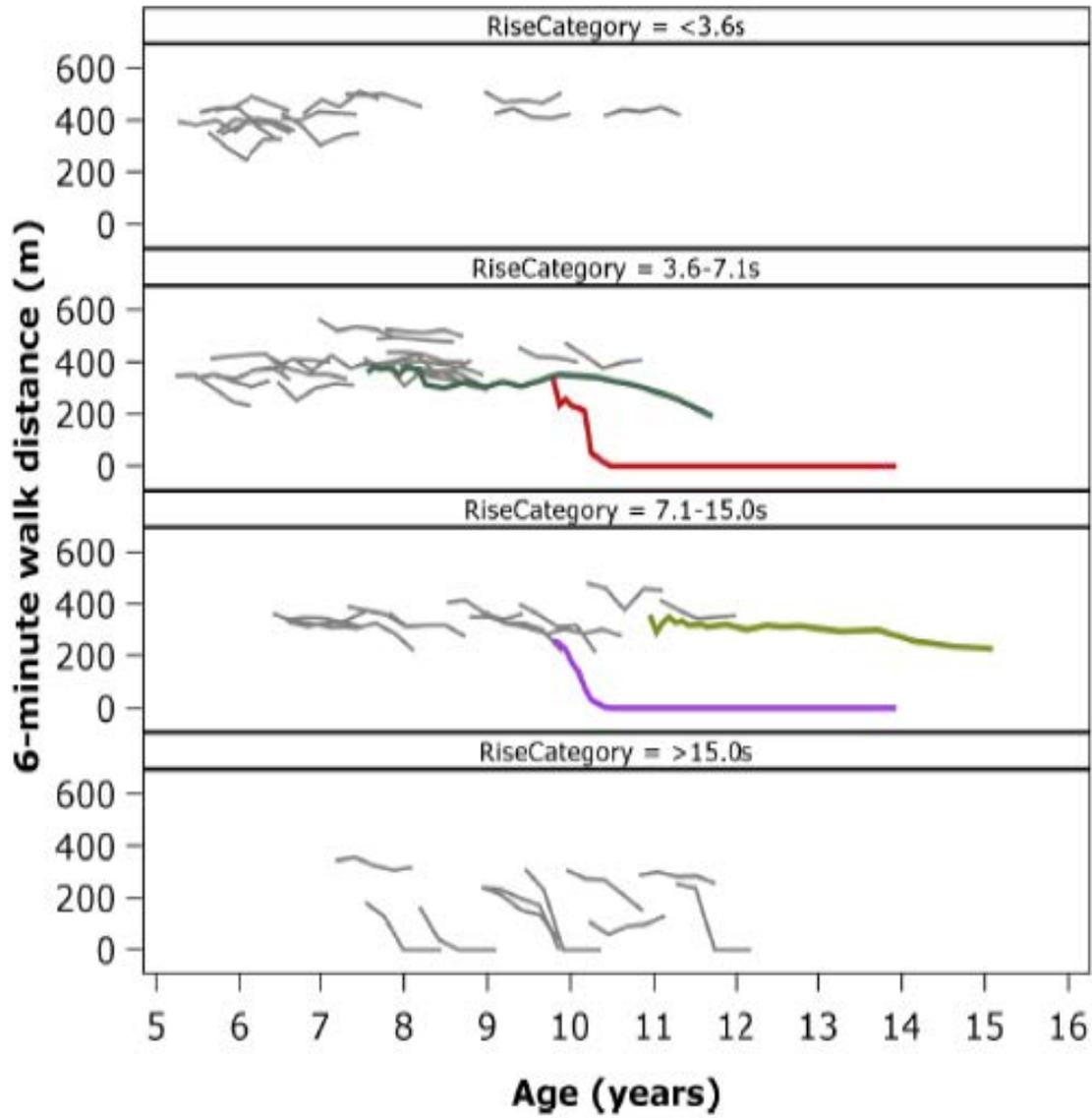
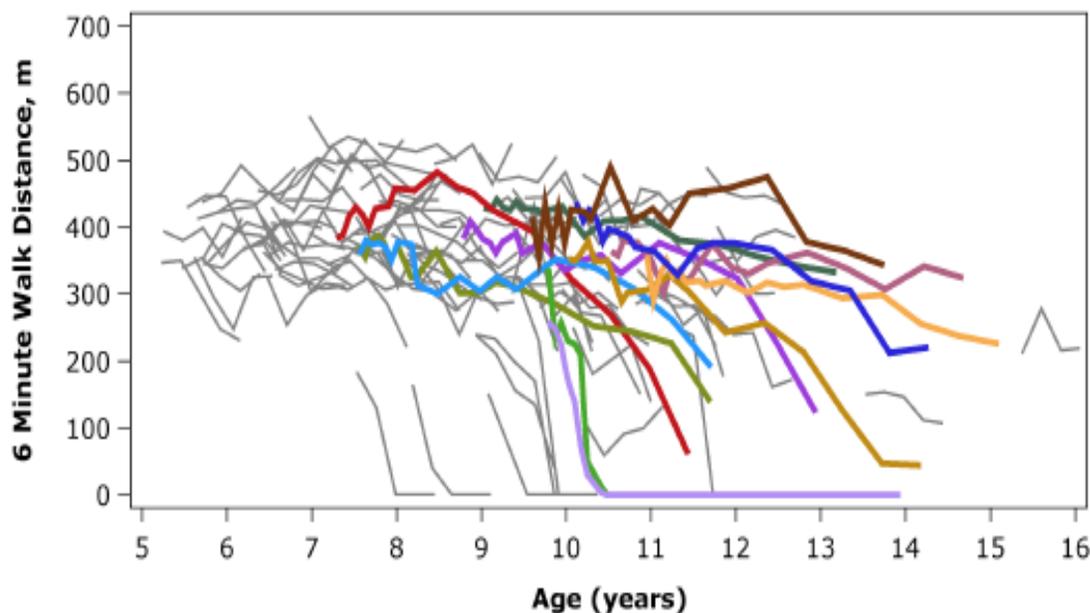


Figure 10: 6MWT, eteplirsen vs controls on placebo, all patients



**Note:** Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statements, “Arguably, placebo-treated patients who were blinded to treatment assignment from other controlled trials are more appropriate as matched controls than registry patients, as they may receive special care and attention as trial participants, and may be more highly motivated” as a key inaccuracy.

**Sarepta:** “The placebo patients from another study as referenced by the FDA are not appropriate for comparison with the eteplirsen-treated patients: Baseline characteristics are not comparable between eteplirsen and the proposed placebo group:

- Placebo group included boys <7 years old
- Placebo group included many patients with baseline 6MWT >440 meters which is outside the eteplirsen trial’s inclusion criteria.”

**FDA response:** The FDA figures match patients with comparable baseline characteristics to eteplirsen-treated patients. Control patients with similar baseline characteristics to eteplirsen patients can be readily identified by examining the figures, as can the control patients who do not match the eteplirsen patients, for example those who are younger or had a baseline 6MWT >440 meters.

**Sarepta:** “By virtue of the ambulatory requirement at study entry, older placebo patients (e.g.,  $\geq 11$  years) were a group of pre-selected, better performing subjects”

**FDA response:** The drisapersen placebo control patients are informative of the variability and range of function in exon-51 skippable patients. A key observation is that

*exon 51-skippable patients can maintain ambulation, and experience a relatively slow decline in ambulation, through an older age than is sometimes recognized.*

**Sarepta:** *“The first year of an 11-year-old-at-baseline placebo patient (i.e., 11-12 years old) to the third year of a 9-year-old boy with 3 years of eteplirsen treatment (i.e., 11-12 years old) is not a valid comparison due to the difference in duration of observation, as well as the biased selection of the 11-year-old ambulatory placebo by, irrespective of both patients having the same age at last assessment”*

- **FDA Response:** *FDA did not make this comparison. The drisapersen control patients can be used to show the presence of exon-51 skippable patients who are similar to eteplirsen-treated patients. The earlier version of this memo explained that most eteplirsen patients are currently in the >15 s rise time category and can be compared to the >15 s rise time group of control patients. This comparison is now explicitly shown in*
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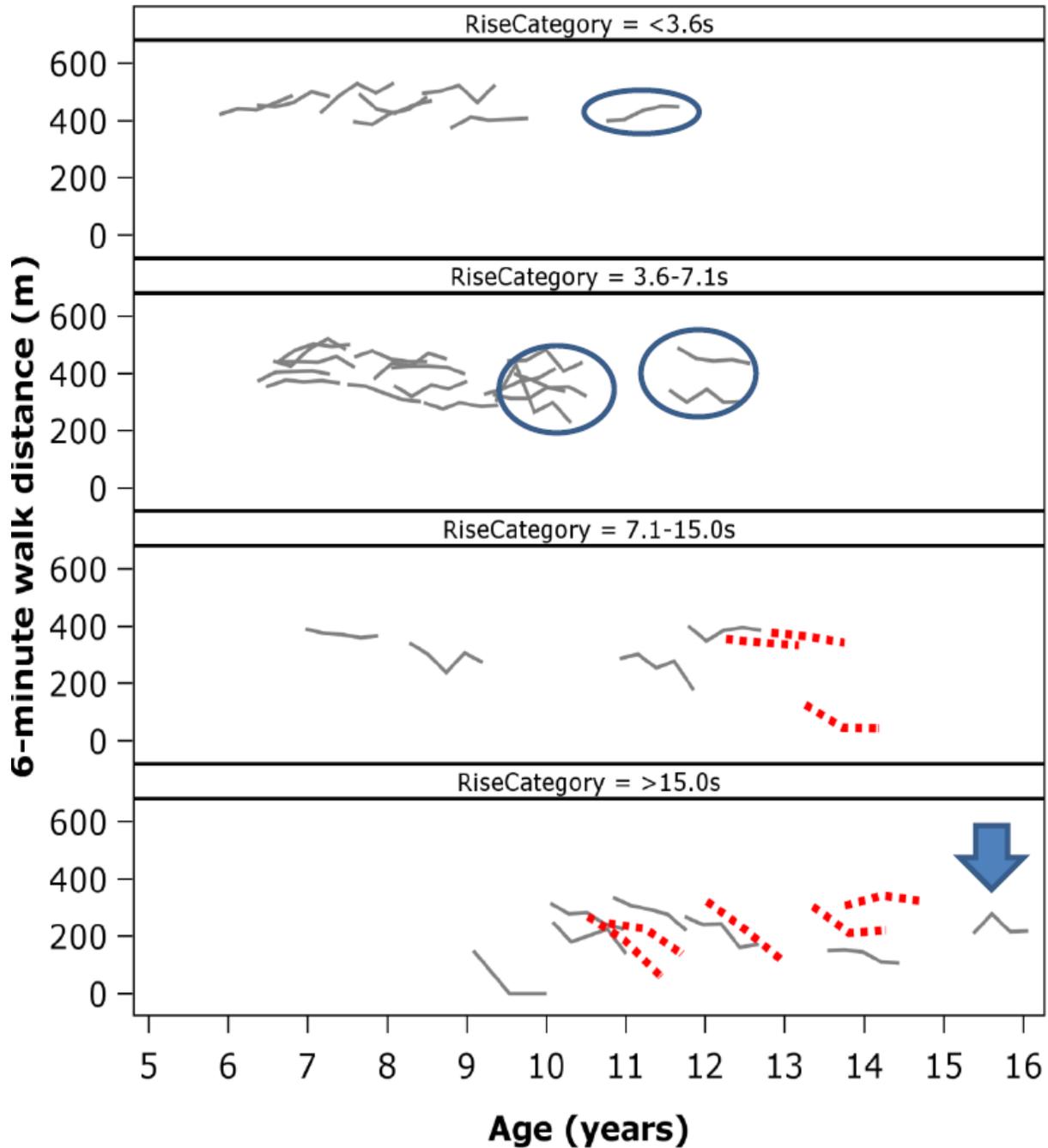
Figure 11, which overlays the third year of data from eteplirsen patients (red dashed lines) with placebo patients matched on the basis of rise time at the beginning of the third year of treatment (grey lines; for clarity, only deflazacort-treated patients are shown). The following are some notable observations:

- *Many placebo patients in the highest (worst) rise time category show a relatively slow decline in ambulation similar to that seen in many of the eteplirsen patients in their third year of treatment, including placebo-treated patients who are as old or older than the eteplirsen-treated patients (e.g., Figure 11, arrow).*

- *Increase in rise time generally occurs prior to loss of ambulation. Many placebo patients in lower (less advanced) rise time categories would be predicted to maintain ambulation for several years (Figure 11, circles).*

**Figure 11: Third-Year Eteplirsen 6MWT (Deflazacort-treated patients)**

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**Sarepta:** “Comparison of eteplirsen-treated patients to the appropriately matched external control shows that more than one year is required to observe a divergence in disease progression between the two groups”

**FDA response:** The comparison to placebo controls incorporates the full duration of eteplirsen treatment and all potential cumulative effects. After 3+ years of treatment,

*eteplirsen patients are still within the range of clinical condition that occurs in the natural history of exon-51 DMD.*

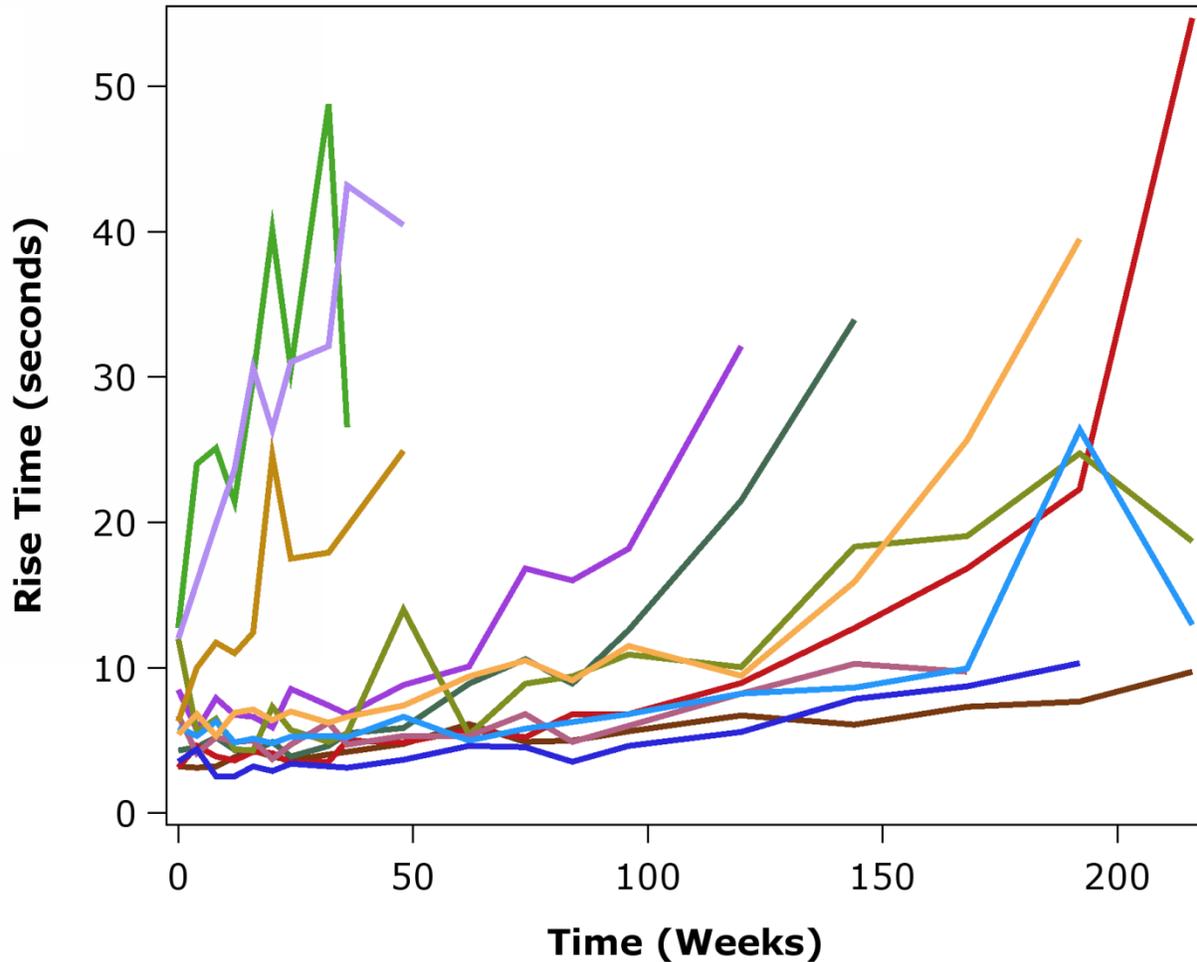
Because evidence that even a few eteplirsen patients might have progressed markedly differently than expected by natural history would be of interest, a few additional observations about these data are important. Assignment of eteplirsen patients to rise-time category is affected by random noise in the baseline measure. Specific patients may appear to progress faster or slower than “matched” controls, but the noise inherent in matching needs to be considered. For example, the patient indicated by the bright green line in

Figure 8 was placed in the 7.1- to 15-second rise time category, but had large variability for rise time values, and a more accurate estimate of rise time for this patient might be closer to 5 seconds, suggesting that matching to a less advanced group of historical controls might have been as, or more, appropriate. In addition, a number of other factors can confound efforts to match treated with historical patients. For example, the sponsor has argued that loss of muscle, as measured by MRI, was more severe at baseline in two patients than suggested by functional tests, decreasing the interpretability of the rapid loss of ambulation experienced by these patients after starting eteplirsen.

**c. Increases in rise time in eteplirsen-treated patients predict a high likelihood of sequential loss of ambulation within 1 or 2 years**

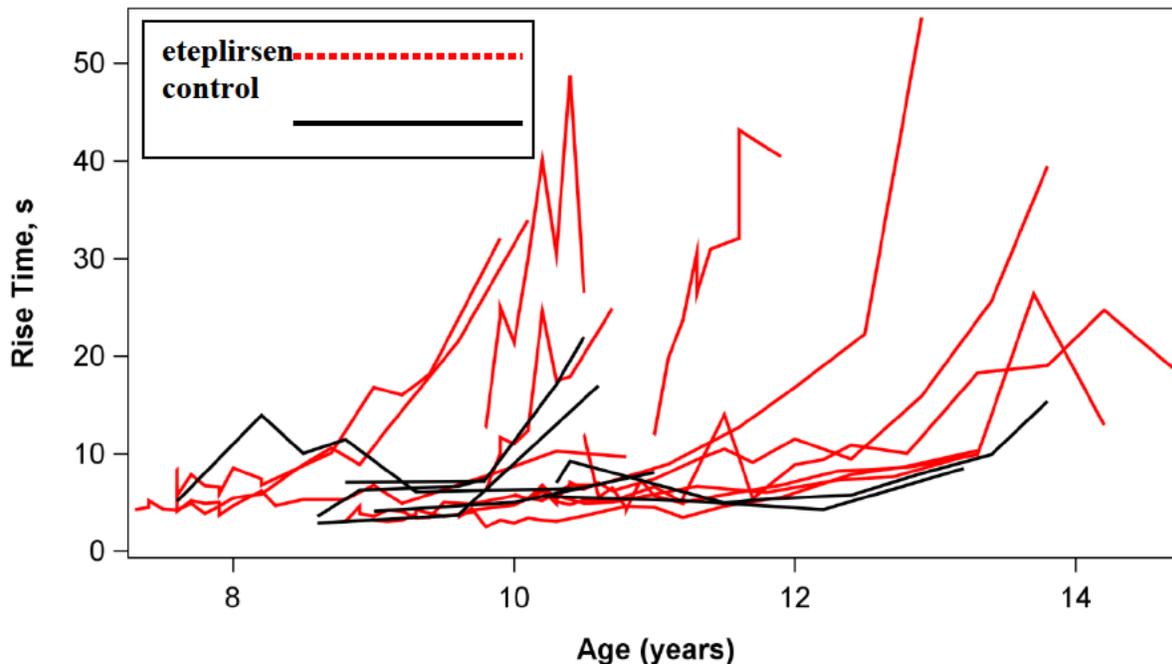
Figure 12 shows rise time from floor for the eteplirsen patients. Three eteplirsen patients lost the ability to rise from the floor in the first year of Study 201. The applicant has, at times, proposed that after an initial time period in which dystrophin levels from eteplirsen accumulated, disease progression largely stabilized in treated patients. All patients in Study 202 have continued to progress steadily while taking eteplirsen, as indicated by rise time from floor, without any discernible stabilization or slowing. Most have now become unable, or nearly unable, to rise from the floor which, in the typical clinic setting, predicts a high likelihood of sequential loss of ambulation within 1 or 2 years.

**Figure 12: Rise Time, Study 201/202**



*Rise-time data were submitted by the applicant for 8 of their 13 natural history patients, and new FDA analyses are shown in Figure 13 for the comparison with rise time data in eteplirsen-treated patients. In the graph, a more horizontal slope indicates a slower rate of progression, whereas a faster rate of progression is indicated by a more vertical slope. Progression of rise time was marked by a high level of inter-patient variability, but was generally similar for eteplirsen and natural history patients. Note that two of the patients with the most preserved rise time were historical control patients, and that no eteplirsen treated patient declined slower (more horizontal course) than the range set by the natural history patients.*

Figure 13: Rise Time, Eteplirsen in Study 201/202 and External Controls



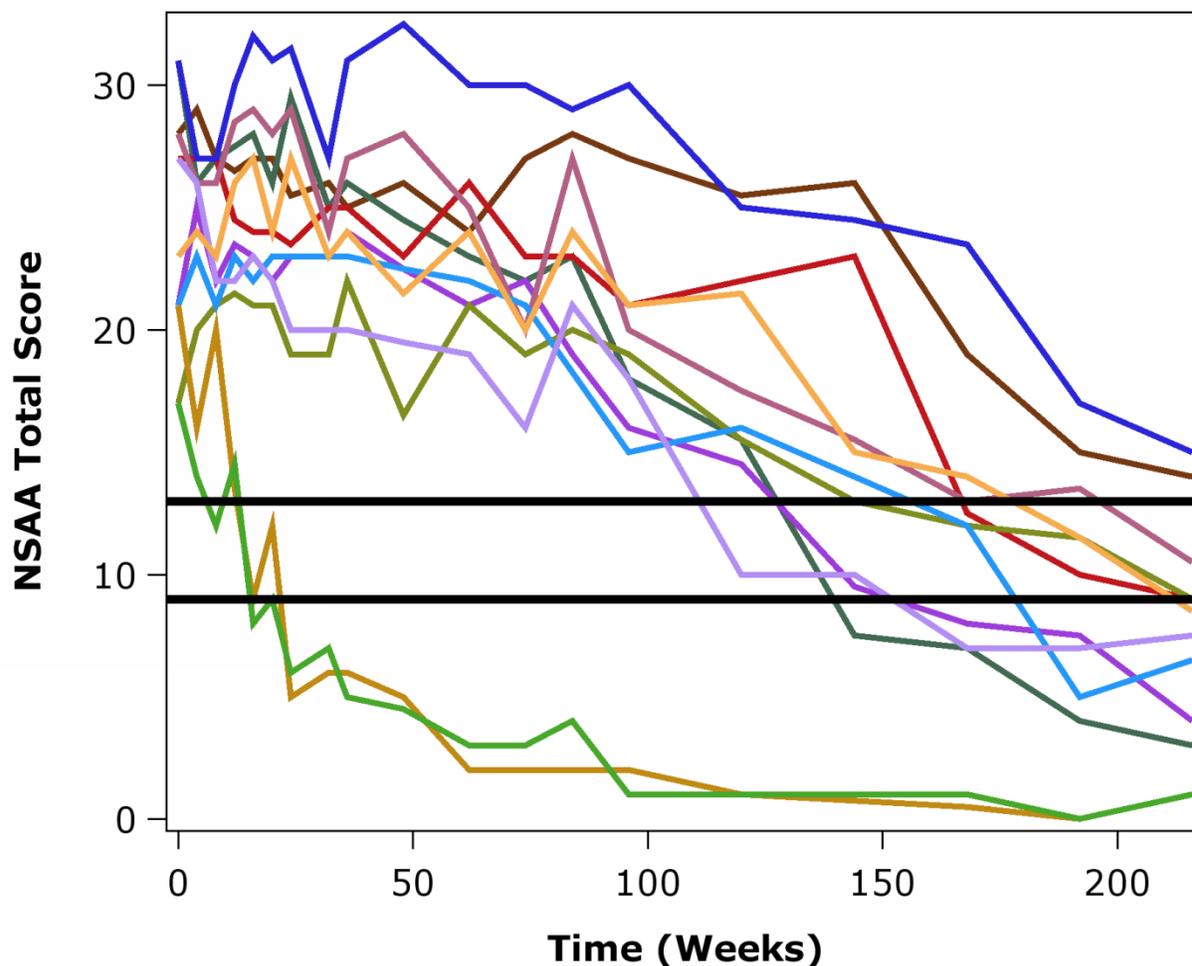
*The applicant has emphasized a time-to-loss analysis for rise time but, similar to 6MWT, the recording of when a function is lost is partly subjective, and may be substantially affected by the level of disability at which the examiner concludes that attempting the test of function is no longer warranted. The data in Figure 13 suggest that rise time may have been measured through a higher degree of disability for eteplirsen-treated patients, through rise times into the 40- and 50-second range, whereas above a rise time of about 20 to 25 seconds, control patients may have been considered unable to perform the task by the examiner.*

Notably, FDA recently learned that use of an external support was allowed in testing of rise time in eteplirsen-treated patients, which may have differed from the testing of rise time for external controls. FDA reviewed the Study Operations Manual for Study 202 to understand testing conditions, but the procedures for measurement of rise time were not discussed in the Manual.

Similar observations (indicating steady progression) were noted for NSAA, which measures broader abilities related to muscle strength that are important for walking, including standing from a chair and ability to climb on and off a box step. As NSAA score decreases, patients may still be able to walk, but are at greater risk of falls, less able to assume a safe position if a fall occurs, and less able to stand up after falling. Eteplirsen patients declined by roughly 5

points/year on average (Figure 14), similar to patients in the NorthStar network. The two horizontal lines in Figure 14 indicate NSAA scores of 9 and 13 that have been reported to be associated with being either 1 or 2 years, respectively, from loss of ambulation.<sup>51</sup> Combined with loss of ability to rise from the floor, the NSAA scores suggest that the eteplirsen patients, who are currently 11 to 14 years or age, are at, or close to, a level of muscle strength often associated with use of a wheelchair.

**Figure 14: NSAA, Study 201/202**



**d. Issues with comparison of eteplirsen-treated patients with applicant’s proposed historical controls**

Untreated historical control groups tend to have worse outcomes than apparently similar control groups in randomized studies. Patients in randomized studies need to meet certain criteria to be entered that generally select a less sick population than is typical of external control groups. Such

<sup>51</sup> Ricottii et al (2015) The NorthStar Ambulatory Assessment in Duchenne muscular dystrophy: considerations for the design of clinical trials. J. Neurol Neurosurg Psychiatry. 0,1-7.

concerns appear to apply to muscular dystrophy, although the magnitude of this effect is difficult to quantify. In patients with fascioscapulohumeral muscular dystrophy Statland et al.<sup>52</sup> observed that “whereas natural history data showed a *decrease* in strength over 1 year, there was an apparent *increase* in strength at 6 months in 2 of the 3 clinical trials in both the placebo and treatment groups.” [emphasis added] The authors concluded that this type of bias should be taken as a reminder of the importance of placebo groups when measuring strength in muscular dystrophy.

Supportive care can prolong ambulation in DMD by several years, but its effectiveness is dependent on both type and intensity of care, which is likely to differ substantially between patients enrolled in observational studies or registries versus interventional treatment studies. DMD care guidelines specify that corticosteroid efficacy needs to be balanced with side effects in the context of the individual patient’s goals. Patients enrolled in efficacy trials would likely be more interested in maximizing steroid efficacy compared to patients enrolled in observational natural history studies. This appears to have been the case for the eteplirsen patients compared to the controls selected by the applicant. A higher proportion, 69% vs. 8%, of the natural history controls vs. eteplirsen patients were on regimens other than daily dosing that are often selected to decrease side effects but that are thought to be associated with lower efficacy. Doses of corticosteroids also appear to have been lower in the applicant’s natural history patients, which included those “in whom the dose had not been always completely adjusted to the current weight.”<sup>53</sup> Adherence to treatment guidelines is difficult to measure, but adherence in the eteplirsen study was reported to be exceptional, while there is evidence that care received in the regions of origin of many of the sponsor’s historical control patients was likely of lower intensity.<sup>54</sup> Finally, as the sponsor’s natural history study proceeded, some patients left to enter interventional clinical trials, further decreasing the similarity of the natural history cohort to the eteplirsen patients.

*Note: Following public release of the original FDA briefing document on 1/15/16, the applicant provided an addendum to their briefing document that highlighted the statements, “Finally, as the natural history studies proceeded, some patients left to enter interventional clinical trials, further decreasing the similarity of the natural history cohort to the eteplirsen patients” as a key inaccuracy.*

*Sarepta: “Two types of missing data sensitivity analyses were performed, the results confirmed that the magnitude of difference remained over 100 meters and nominal statistical significance was maintained:*

- *MMRM using all the available data*

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<sup>52</sup> Statland JM et al (2013) Reevaluating measures of disease progression in facioscapulohumeral muscular dystrophy. *Neuromuscul Disord.* 23,306-12.

<sup>53</sup> Mazzone E et al (2010) North Star ambulatory assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. *Neuromuscular Disorders.* 20,712-716.

<sup>54</sup> Landfeldt E et al (2015) Compliance to care guidelines for Duchenne muscular dystrophy. *Journal of Neuromuscular Diseases.* 2,63-72.

- *Last Observation Carried Forward imputation (conservative analysis assuming that the 2 control patients did not decline)”*

***FDA response:*** *The applicant’s response, describing two types of analyses used to impute missing data, suggests that they construed FDA’s concern to be the problem of missing data, i.e., missing data from patients who left the natural history study. But FDA did not make this point to highlight missing data as an issue. FDA’s intent was to underscore the inherent and profound difference between patients in the interventional eteplirsen trial and patients in the observational study.*

*There are many reasons to conclude that there were meaningful differences between the groups, both at baseline and during the conduct of the study. Some additional examples of specific concerns are listed below.*

- *Important aspects of supportive care were incompletely and/or incorrectly recorded for both Study 201/202 patients and historical controls:*

*After FDA noted there were potentially clinically meaningful differences in steroid treatment between eteplirsen treated and control patients, the applicant revised the raw data for historical control patients, stating that it was incorrect and/or incomplete as originally submitted to FDA: one patient was changed from “intermittent” to “continuous” treatment, and 3 were changed from “unknown” to “continuous.” The reliability of data revised in this way is questionable. In the setting of knowledge of treatment arm, changing source data can introduce bias in favor of drug-treated patients. Applicants may be more likely to selectively question and revise data to support the apparent drug effect. For example, FDA recently received from the applicant source documents containing data on steroid use by the natural history patients in Belgium, indicating that one patient was initiated on only 6 mg/day deflazacort, apparently due to a misunderstanding, but this was not brought to FDA’s attention. There remains reason to be concerned that the differences in steroid treatment may have impacted prognosis. For example, steroids were reported to have been initiated in eteplirsen treated patients at a younger age than for historical controls (on average, over one year earlier). The possible impact of that difference on clinical outcomes is impossible to assess, which again highlights the limitations of the comparison to historical controls.*

- *Supportive care was not well documented for the eteplirsen-treated patients in Study 201/202. In response to an FDA request of 20 August 2015 for additional details about supportive care, the applicant responded “the study 368-us-201 and 4658-us-202 protocols did not include collection of supportive measures such as the use of night splints, physical therapy, etc., in the study population.”*

*Patient compliance with clinical recommendations is not expected to be complete, and there is a concern that it would be higher in interventional compared to observational studies. In the limited source documentation available for the historical control patients, some difficulty gaining patient compliance is documented.*

- *In a recently published correction,<sup>55</sup> the investigators of the Italian natural history study that contributed 10 of 13 historical control patients reported substantial changes in accounting for basic aspects of the patient registry – e.g., patient numbers, duration of enrollment, dropouts, survival, etc. Such changes raise concern about the reliability of the data, and that efforts to correct the data may have been influenced by investigator expectations about the disease course. In addition, the revised numbers indicate a high percentage of assessments were not carried out at 36 months (about 40%), increasing concern that the data collected might not have been representative of the original population.*

*The original and corrected statements are as follows [emphasis added]:*

- *ORIGINAL: Of 113 patients who fulfilled the inclusion criteria and entered the study, **96** also had an assessment at 36 months. One died, **2 were lost at follow up** and the other **14** entered interventional clinical trials*
  - *CORRECTED: Of 113 patients who fulfilled the inclusion criteria and entered the study, **70** also had an assessment at 36 months and another 26 were new patients, enrolled with the same criteria. Of the 43 patients excluded from the second year, 17 had not reached the 3 year assessment, 4 had assessments at different times but not at 3 years because they entered natural history clinical studies, 5 were younger than 5 years at baseline, **9 were lost at follow up** and **8** entered into a clinical study*
- *Study protocols for the Italian and Belgian observational DMD registries were brief and lacked detail, including the criteria by which it would be determined whether a patient should be deemed unable to complete an endpoint measure without attempting the test.*

Recent evidence from the Cooperative International Neuromuscular Research Group (CINRG) reinforces the observation that seemingly small differences in steroid treatment and clinical care may have relatively large effects, up to *several years*, on age at loss of ambulation.<sup>56</sup> The CINRG investigators caution that “differences in standards of care and dosing complicated

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<sup>55</sup> Pane et al. (2015) Correction: long term natural history data in ambulant boys with Duchenne muscular dystrophy: 36-month changes. PLOS ONE 10(12):e0144079.doi:10.1371/journal.pone.0144079

<sup>56</sup> Bello L et al (2015) Prednisone/prednisolone and deflazacort regimens in the CINRG Duchenne natural history study. Neurology. 85, 1048-1055.

interpretation...this study emphasizes the necessity of a randomized blinded trial of GC [glucocorticosteriod] regimens in DMD.” This is an important conclusion for DMD drug studies more broadly because differences of several years in age of loss of ambulation among different groups of patients may not be large enough to determine reliably the contribution of a drug versus other factors.

*The table below shows some of the numerical data from the CINRG study that is referred to in the paragraph above. There is a difference of about 3 years in median age of loss of ambulation between two large groups of patients, one treated with prednisone and the other with deflazacort. Also notable is that loss of ambulation differed by 2 years between patients on differing deflazacort dosing schedules, perhaps reflecting a combination of factors including random effects from small sample size (N = 8 for one group). Bello et al also note that “DFZ [deflazacort] is not commercially available in the United States, where many CINRG sites are located, and it is more expensive than prednisone, implying that its use may have been associated with higher standards of care and possibly adherence.”*

Steroid/Regimen*	Median loss of ambulation (years)	N
Prednisone/Daily	11	94
Deflazacort/Daily	14	80
Deflazacort/Switched	16	8

\*daily vs. weekly

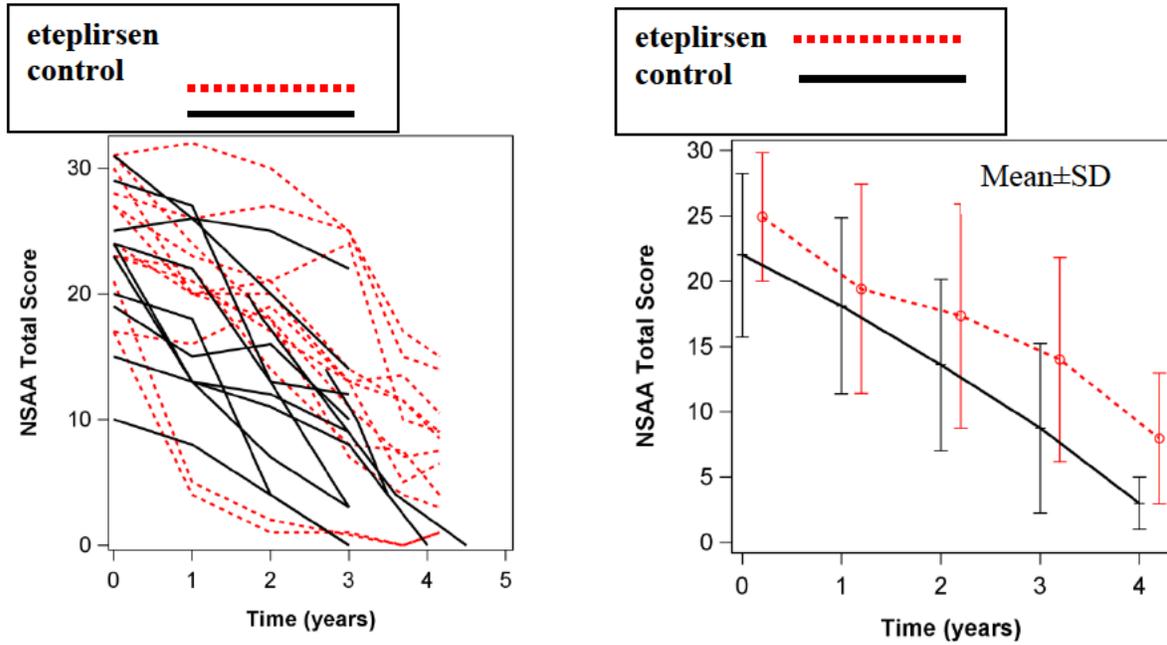
**e. NSAA, Eteplirsen vs. Applicant’s Controls**

Comparison of eteplirsen patients (red) to the applicant’s historical controls (black) is shown for NSAA in Figure 15 for individual patients (left) and mean for each group (right).

*In source documents recently received from the applicant, there appears to be documentation that NSAA was, in a number of instances, recorded as zero for the applicant’s historical control patients without being measured, potentially underestimating the patient’s actual abilities. The applicant identified 2 instances, and initial FDA review suggests there may have been more.*

As discussed above, the effects of bias can be considerable in historically-controlled trials, with many factors potentially favoring the treatment arm. The similarity of the clinical course of patients is therefore notable. The similarity between the groups on NSAA and, in particular, the large magnitude of the standard deviations, suggest that eteplirsen does not have the type of large beneficial effect that would be possible to reliably detect in even a well-designed historically-controlled trial.

Figure 15: NSAA, eteplirsen vs applicant's historical controls

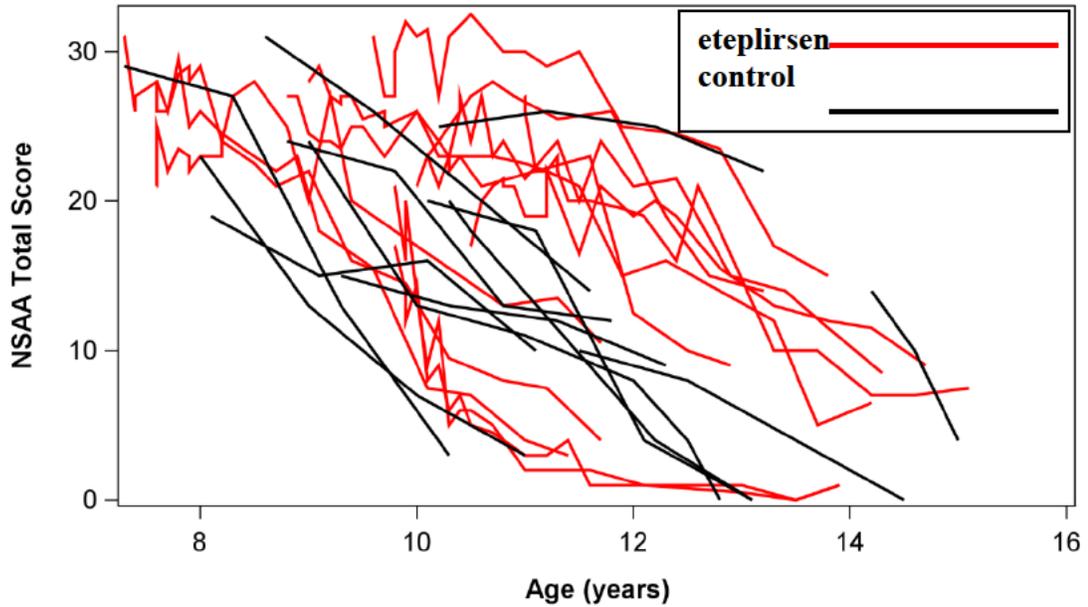


*Because muscle function is strongly correlated with age in DMD,*

*Figure 16 displays NSAA vs. age (in contrast to vs. years on treatment) to provide a better matched comparison of patients. NSAA values for control patients occur over the entire range of values for eteplirsen patients, e.g., two of the patients with the most preserved NSAA score at both age 13 and 14 years are external control patients.*

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Figure 16: NSAA by Patient Age



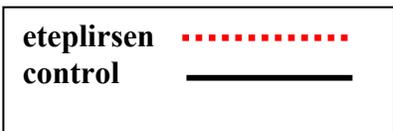
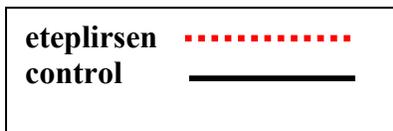
**f. 6MWT, Eteplirsen vs. Applicant's Controls**

Comparison of eteplirsen patients (red) to the applicant's historical controls (black) is shown for 6-minute walk distance in

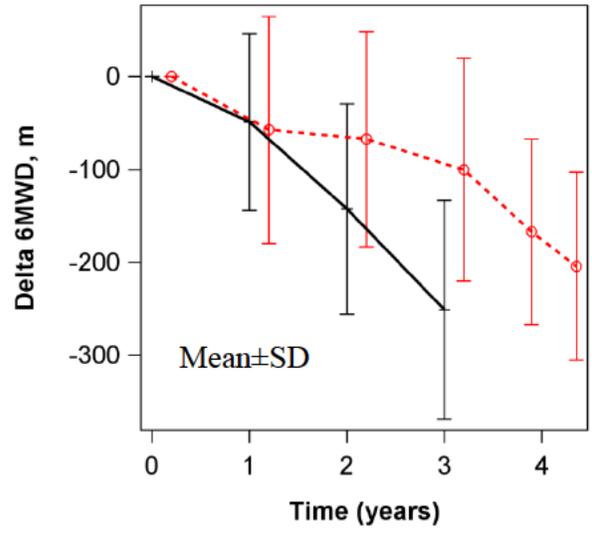
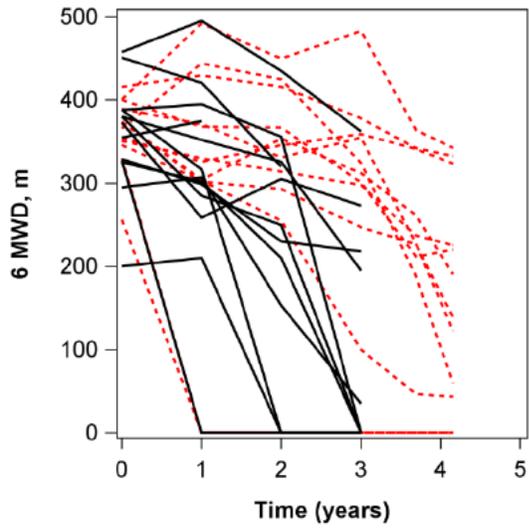
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**Figure 17**, for all patients (left) and mean for each group (right). As discussed above, FDA has long expressed concern to the applicant that the 6MWT is particularly susceptible to bias, and unreliable in Study 202. Importantly, whereas the difference in 6-minute walk distance shown would be of clinical importance if observed in a double-blind, placebo-controlled trial, the finding is extremely difficult to interpret given all of the limitations of historically-controlled trials noted above.

**Figure 17: 6MWT, eteplirsen vs applicant's historical control**

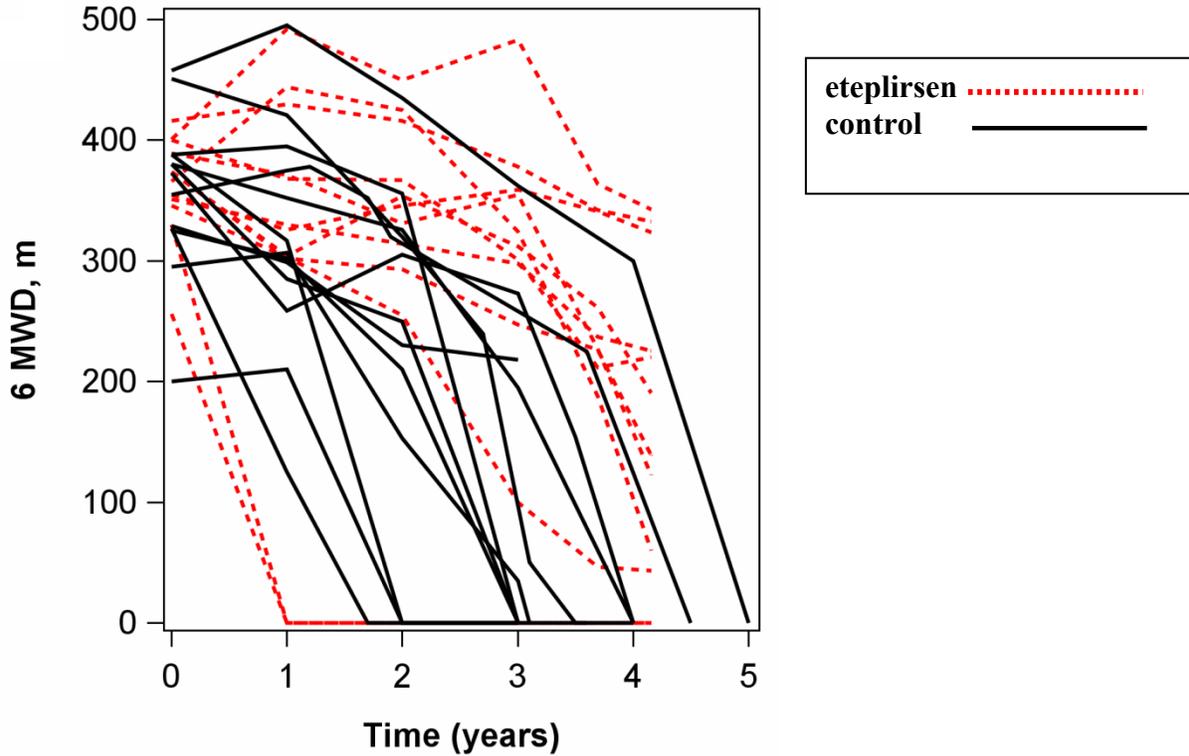


Cross Discipline Team Leader Review



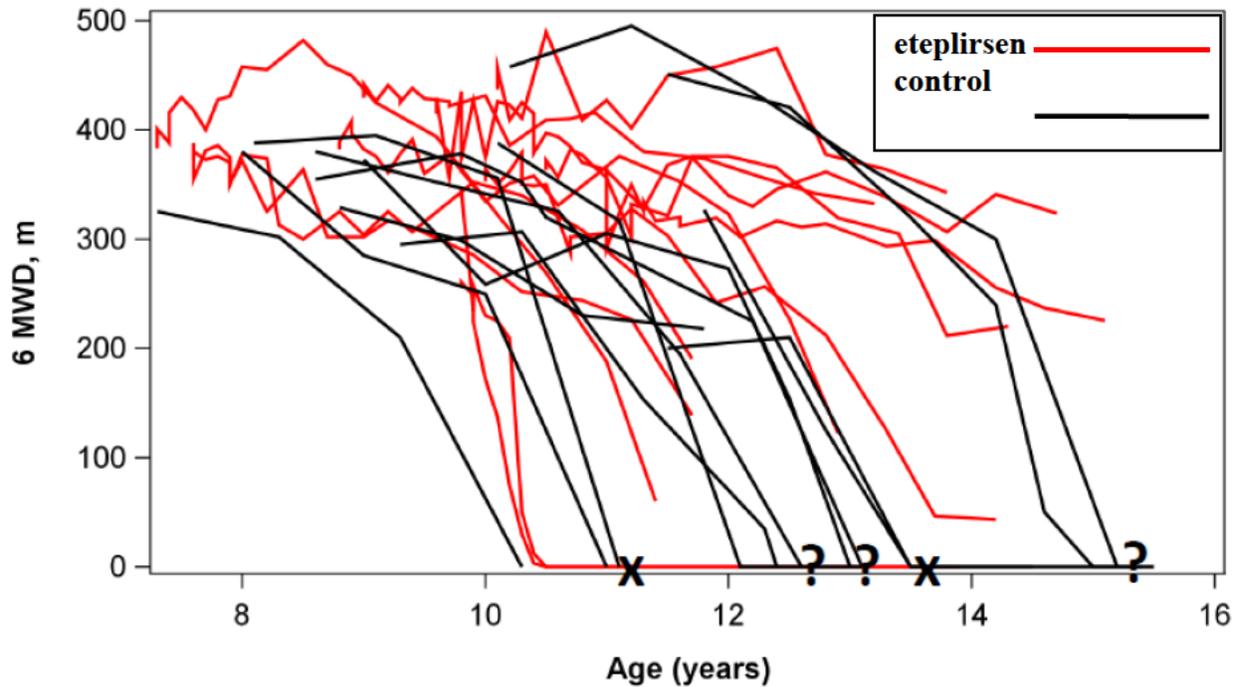
An updated version of 6MWT vs. time on treatment/observation is shown in Figure 18.

**Figure 18: 6MWD vs. Years Observed**



*Because function is strongly correlated with age in DMD, Figure 19 displays 6MWT values vs. age (as opposed to years on treatment) to provide a better-matched comparison of patients. A majority of eteplirsen patients (red) are declining in close parallel to the paths of historical control patients of similar age (black). For the patients older than 14 years, several eteplirsen patients are ambulating at a time when control patients of similar age have 6MWT values of zero, but as noted above, a number of these values appear not to represent the true ambulatory abilities of the patients (in the figure “x” marks patients who were ambulatory but recorded as having 6MWT of zero, and “?” indicates patients who were reported, but seemingly not measured, to have 6MWT of zero).*

Figure 19: 6MWT by Patient Age



**Week 240 6MWT Data**

6MWT data for Week 240 of Study 201/202 was submitted on 9 May 2016, and is shown in the table below. All of the patients declined on 6MWT except Patient 8, who improved from 55 meters to 103 meters. The applicant indicates that the 6MWT is “unknown” for patient 12 because the patient recently experienced a femur fracture and the week 240 assessment has not been performed at this time. In natural history studies, such a patient may have been deemed to be unable to perform 6MWT. Patient 4 walked 7 meters on Day 1 and 22 meters on Day 2, and is considered by the applicant in some analyses to have lost ambulation. Patient 3 walked 12 meters on Day 1 and 31 meters on Day 2, and is considered by the Applicant to have maintained ambulation. In natural history studies, both patients may have been deemed unable to perform 6MWT.

Notably, the patients who started eteplirsen treatment at younger ages appear to be declining more rapidly than patients started at older ages. For example, the youngest patient, patient 3, has essentially lost practical ability to ambulate prior to age 12 years, and the second and third youngest patients, who are 12.2 years old, are now walking about 100 meters (98 m and 125 m). Each of these patients had baseline 6MW distances >350 meters, such that decline in 6MWT seemingly could not be attributed to initiating treatment beyond a level of muscle loss that would have prevented the potential for benefit on ambulation. Age of loss of ambulation for these patients is thus similar to the mean age of loss of ambulation predicted by natural history (e.g. the applicant indicates a mean age of loss of ambulation of about 13 years for the Italian and Belgian external controls). There are not enough observations for any reliable conclusions, but

the limited available data do not appear to support the hypothesis that initiating eteplirsen at younger ages would lead to an increased potential for benefit. The observation that the 14 and 15 year old eteplirsen-treated patients, in contrast, are generally performing better on 6MWT than the 12 year old patients may be consistent with selection bias. Preserved function at younger ages in DMD is known to predict preserved function at older ages. Patients enrolled into Study 201/202 at older ages were known, based on ability to meet the enrollment criteria, to have relatively better preserved function through ages at which many patients would have declined or become unable to meet these criteria. The fact that such patients continue to perform better than average is thus expected. In contrast, the patients enrolled into Study 201/202 at younger ages were more typical of the average DMD exon-51 skippable DMD patient, and their clinical course on eteplirsen treatment has continued to follow the predicted natural history.

**Table 1: Study 201/202: 6MWT Results<sup>1</sup> through Study Week 240**

Patient	Treatment	Baseline 6MWT	Study Week 192 6MWT	Study Week 216 6MWT	Study Week 240 6MWT	Age at Study Week 240
2	30 mg/kg	416	349	346	325	13.7
6	30 mg/kg	355	332	313	236	15.2
9	30 mg/kg	330	0	0	0	14.4
10	30 mg/kg	256	0	0	0	14.4
3	50 mg/kg	366	192	71	31	11.9
4	50 mg/kg	389	221	120	7	13.5
12	50 mg/kg	351	237	228	Unknown <sup>2</sup>	~15.7
15	50 mg/kg	401	400	355	344	14.2
7	Pbo to 30 mg/kg	370	257	197	98	12.2
8	Pbo to 30 mg/kg	341	50	55	103	14.7
5	Pbo to 50 mg/kg	357	225	143	125	12.2
13	Pbo to 50 mg/kg	418	208	230	210	14.8

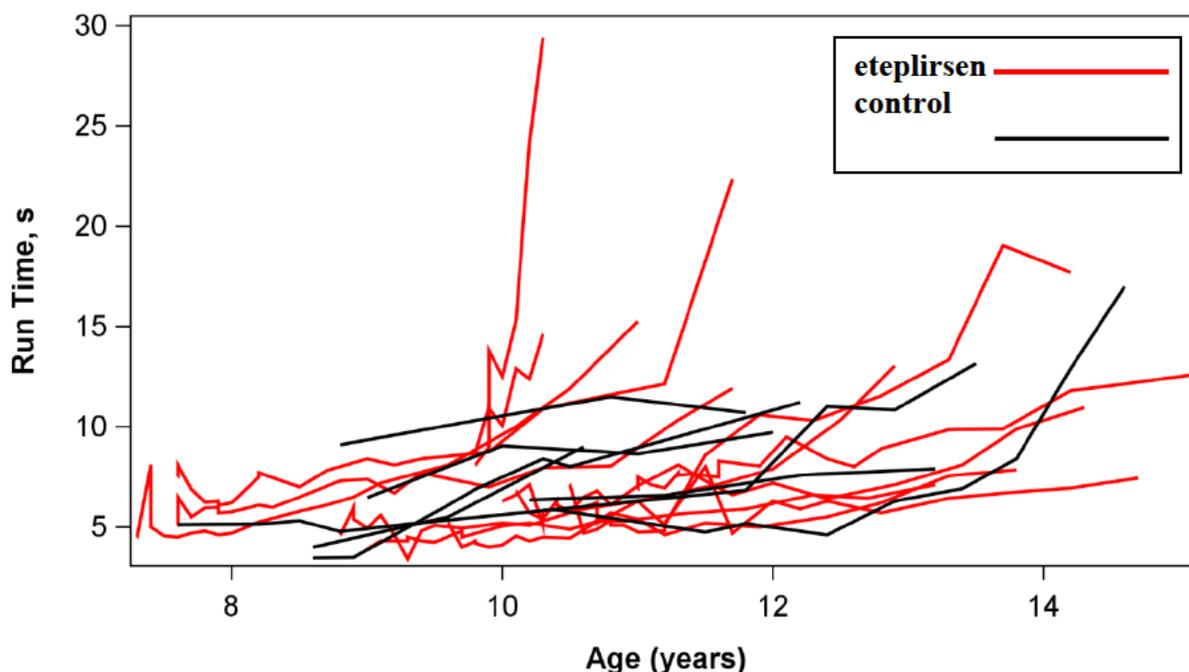
Pbo = placebo

<sup>1</sup>Day 1 6MWT results (meters)

<sup>2</sup>Patient 12 recently sustained a left femur fracture; Study Week 240 assessment has not been performed at this time.

*Data for 10 m walk/run were submitted by the applicant for 7 of their 13 natural history patients, with new FDA analyses comparing eteplirsen and natural history patients shown in Figure 20. In the figure, a more horizontal slope indicates a slower rate of progression, whereas a more vertical slope indicates a faster rate of progression. Progression as measured by 10 m walk/run was marked by a high level of inter-patient variability, but was generally similar for eteplirsen and external control patients.*

Figure 20: 10 m walk/run, eteplirsen and natural history patients



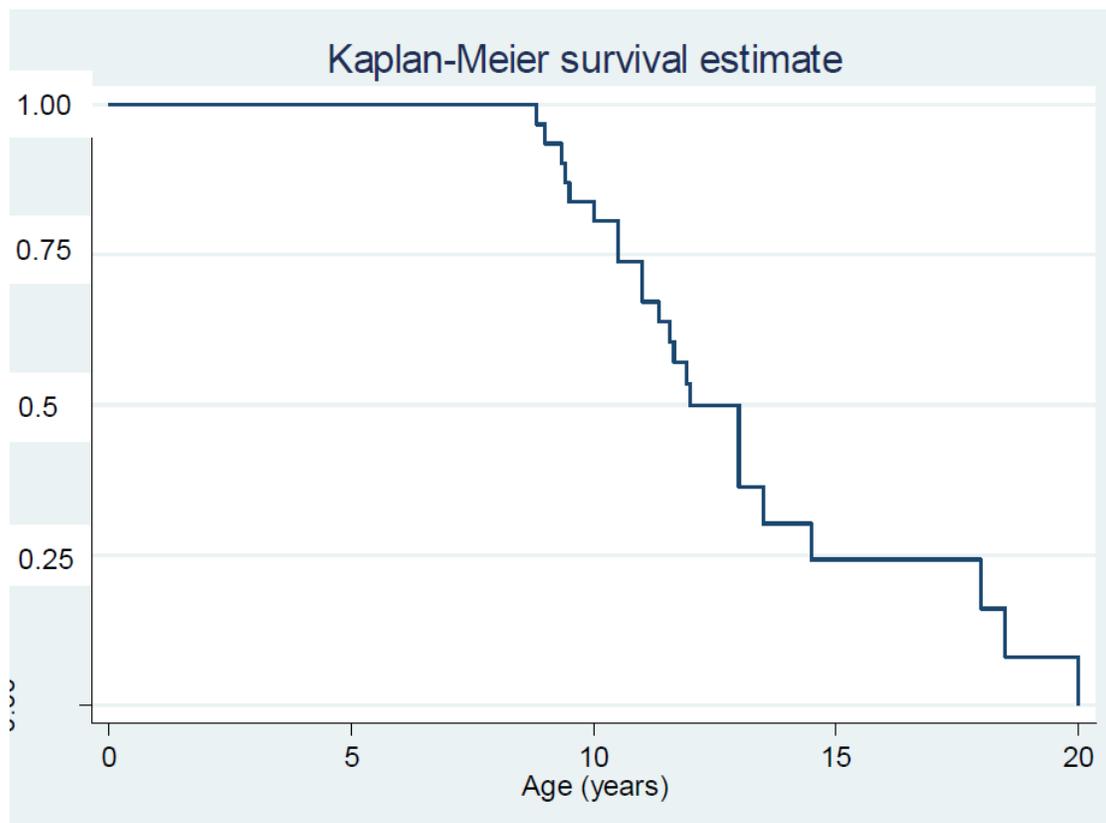
FDA recently received source documents that increase concern that 10m walk/run may have been measured differently for eteplirsen-treated patients compared to natural history patients. Eteplirsen patients appear to have been recorded for the time to **run** 10 m whereas at least some external control patients were recorded for the time specifically to **walk** 10 m, e.g., for one patient, a time of 7 s was recorded to **run** 10 m, and a time of 11 s was recorded to **walk** 10 m, with only the slower time submitted to the NDA as the 10 m run/walk time. Patients in the external control group who walked, rather than ran, for the test would tend to improve the results in the eteplirsen group relative to the external control group.

#### **New CINRG Data: Age Range of Ambulation of Exon-51 amenable patients**

*As noted above, after the release of the original version of this memo, CINRG provided additional unpublished analyses to FDA for age of loss of ambulation of exon-51 amenable patients (*

Figure 21; analysis as provided by CINRG). CINRG is additionally providing FDA with patient-level data that should enable a more detailed comparison with eteplirsen treated patients.

Based on the CINRG data, about 25% of exon-51 skippable patients maintain ambulation to age 16, and about 15% of patients to age 18. The oldest eteplirsen-treated patients are currently about 15 years old, such that it cannot be concluded that the ambulatory function of any eteplirsen-treated patient exceeds the expected range of natural history. This is an important point because some of the applicant's analyses give the impression that some, or most, of the eteplirsen patients have maintained ambulation longer than could have been expected compared to natural history.

**Figure 21: Age at Loss of Ambulation, Exon 51 Skippable Patients**

*Importantly, any comparison of the eteplirsen data to the CINRG data needs to account for the fact that eteplirsen patients, upon enrolment in Study 201, had to meet criteria based on a specific level of ambulation at an age at which some patients would have already declined to a point where they would not have met these criteria. The eteplirsen patients, therefore, represent a population enriched for patients with a better prognosis than the overall exon-51 skippable population. Therefore, the percentage of eteplirsen-treated patients who would be expected to maintain ambulation would be higher than 25% at age 16 years and 15% at age 18 years, even before considering other potential sources of difference between the groups.*

### **MD STARnet**

After release of the FDA memo but prior to the PCNS AC meeting additional natural history data became available from the Muscular Dystrophy Surveillance Tracking and Research Network (MD STARnet). MD STARnet is a population-based surveillance program for individuals with Duchenne and Becker muscular dystrophy (DBMD) in six states in the United States. Starting in 2004 MD STARnet identified all patients born with DBMD from 1982-2011 in the surveillance areas. Cases were identified retrospectively before 2004, but new cases were identified after that date and follow-up abstraction was conducted. Findings of age of ambulatory abilities from MD STARnet appear to be consistent with the CINRG natural history data and the placebo-arm of the drisapersen controlled trials:

- 612 DBMD patients in 3 three MD STARnet sites (Colorado, Arizona and Georgia), and 510 (83%) had testing for deletion mutation
  - 47 patients (9.3%) with mutations amenable to exon 51 skipping.
  - 26 patients with mutations amenable to exon 51 skipping and have taken or are taking steroids for at least one day prior to loss of ambulation or if they are still walking, prior to their last mobility entry
  - Of these 26 patients, there are 15 patients who are still ambulant.
  - Of these 15 patients who are still ambulant there are 3 patients walking at or beyond 14 years
  - 2 of these 3 patients walking at or beyond 16 years

MD STARnet limitations include the following:

- MD STARnet primarily captured individuals who sought clinical care at neuromuscular clinics.
- Cases born in the early to mid-1980's were less likely to have DNA testing in their records.
- Some patients may have been part of previous clinical trials

### **CINRG Timed Test Data**

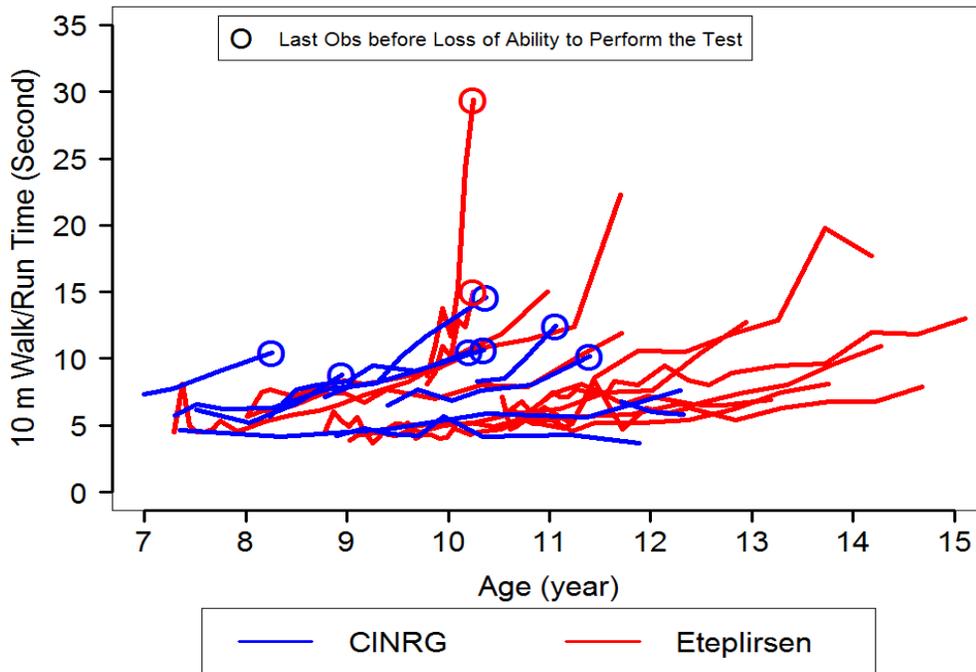
After release of the FDA memo prior to the PCNS AC meeting additional analyses were done on data that had been recently received from CINRG for 10 meter run/walk, rise time, and 4 step climb.

Prior to receipt of the CINRG data, FDA pre-specified a plan for matching CINRG patients to the eteplirsen-treated patients, and identified FDA statisticians from outside the division to conduct the matching. Patients were matched based on the following baseline characteristics:

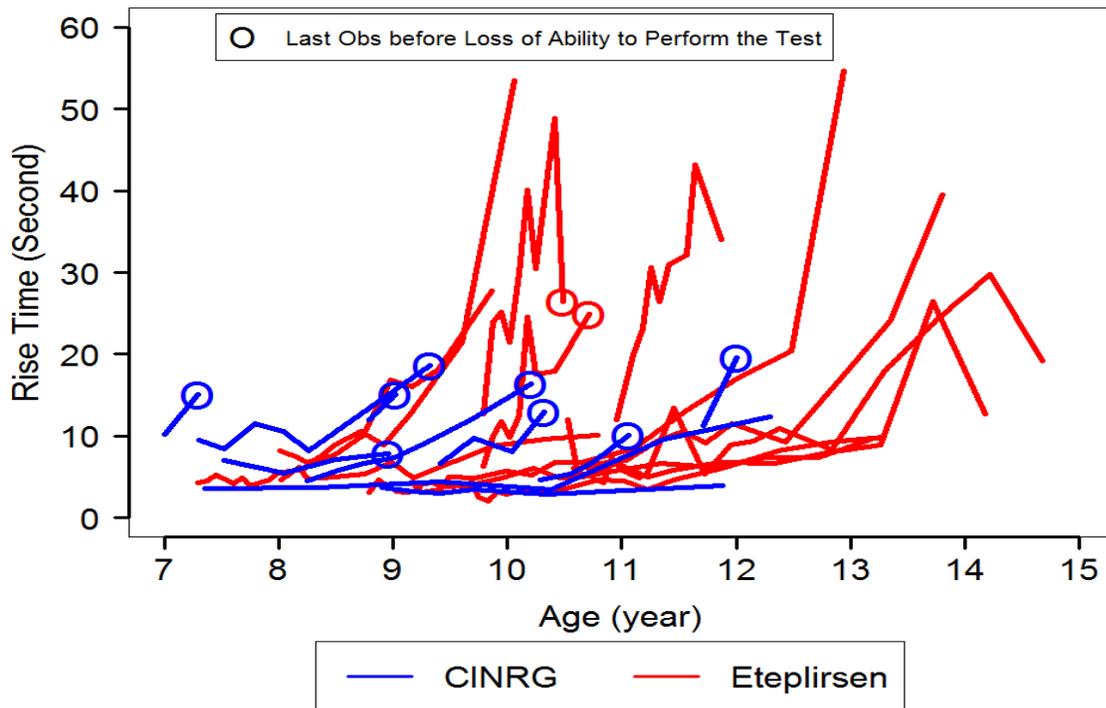
- Exon-51 skippable
- Ambulatory at baseline
- Baseline age 6-12 years
- 10m run/walk time less than 10 seconds

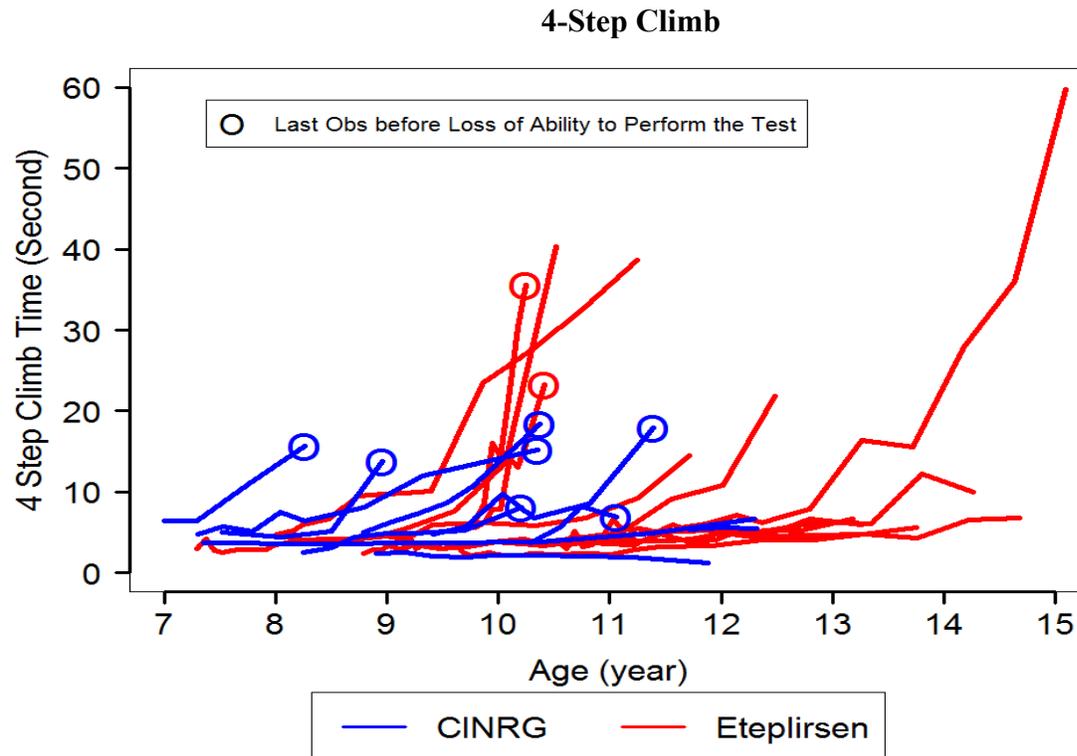
10m run/walk was considered the primary comparison because few long-term 6MWT data are currently available in the CINRG database. In the figures below, the lines show results for tests that were attempted, that is, had a numerical value, whereas circles indicate patients in whom the next value was imputed as “unable.”

### 10 Meter Run/Walk



### Rise Time





### Pulmonary Function

Figure 22 shows the comparison of percent predicted forced vital capacity (%FVC) in eteplirsen-treated patients (colored lines) with patients on placebo (grey lines) in controlled trials of another drug investigated in exon-51 skippable DMD patients. The course of both groups of patients is generally similar, marked by general stability or slow decline, as expected in steroid-treated DMD patients in this age range.<sup>57,58</sup>

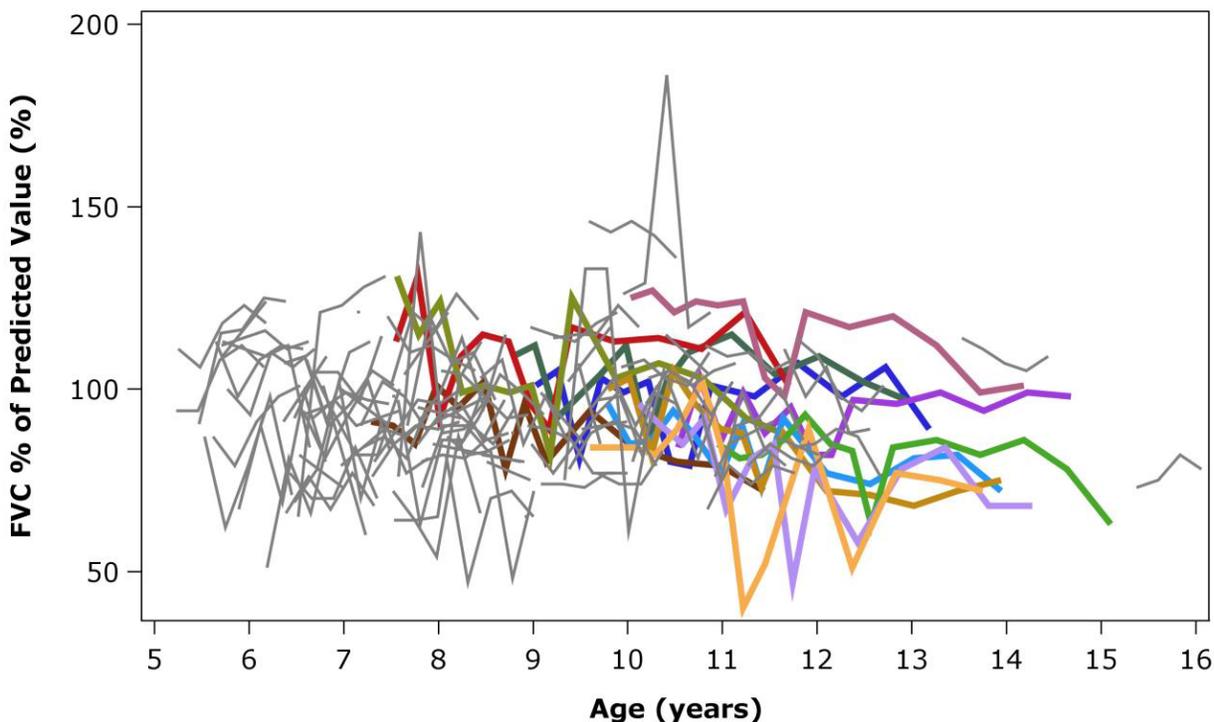
*The applicant compares eteplirsen-treated patients to natural history patients who were either not treated with steroids, or who were treated for shorter periods of time. The applicant suggests steroids have little or no effect on pulmonary function, but this does not appear to be supportable.<sup>59</sup> The applicant's analyses regarding pulmonary function therefore appear to be confounded and uninterpretable.*

<sup>57</sup> Biggar WD et al (2001) Deflazacort treatment of Duchenne muscular dystrophy. *Journal of Pediatrics*. 138, 45-50.

<sup>58</sup> Machado DL et al (2012) Lung function monitoring in patients with Duchenne muscular dystrophy on steroid therapy. *BMC Research Notes*. 5,435

<sup>59</sup> Gloss D et al (2016) Practice guideline update summary: corticosteroid treatment of Duchenne muscular dystrophy: report of the guideline development subcommittee of the American Academy of Neurology. *Neurology* 86:465-72.

**Figure 22: Forced vital capacity, eteplirsen-treated patients (colored lines) vs patients on placebo in other controlled trials in exon-51 skippable DMD patients (grey lines)**



### **g. Conclusions, Clinical Endpoints**

In the context of the above, the major conclusions with regard to clinical endpoints are listed below:

1. The natural history of DMD in patients amenable to exon 51 skipping has been characterized in a number of observational natural history studies and controlled trials, and the range of age at loss of ambulation is very wide, currently between about 8 and 18 years for most patients. Eteplirsen patients have experienced a sequential loss of ambulatory abilities and increasing muscle weakness, as measured by rise time from floor, NSAA, 6MWT, and other tests. In the context of this considerable variability among patients, the clinical course of eteplirsen patients over more than 3 ½ years of treatment with eteplirsen has been generally similar to expected natural history of patients provided with intensive supportive care.

*As noted above, recently available data from CINRG and MD STARnet suggest a higher percentage of exon-51 skippable patients maintain ambulation to older*

*ages than previously realized, to 18 years or perhaps even older. As discussed in a recent editorial by Dubovitz<sup>60</sup> in Duchenne dystrophy “there have been striking advances” and “the ‘natural history’ of Duchenne has now become a shifting target and has to be redefined as “DMD-with-all-the-interventions-to-date”. This introduction of shifting goalposts has a number of major implications. It immediately introduces a difference in “natural history” in different countries in relation to the support services available, and from centre to centre in relation to specialised services available, and indeed from one specialized centre to another depending on the regimes being followed and such important major factors as the age of diagnosis and commencement of therapy.”*

2. There are important differences between patients enrolled in observational natural history studies and patients enrolled in interventional drug efficacy studies, some of which are quantifiable, and some of which are not. Near the time when patients lose ambulation, decisions are made by patients and caregivers about whether weakness has progressed to the point that it is in the patient’s best interest to use a wheelchair to avoid the risk of falls and injuries and to decrease the effort and time required for mobility. Differences in individual care decisions, therefore, seemingly could produce large differences in 6MWT and time to loss of ambulation between eteplirsen patients and natural history controls. NSAA results, potentially representing a more direct measure of strength, suggest that differences in DMD progression between eteplirsen patients and the applicant’s natural history controls were too small and variable, in the context of a poorly-controlled trial, to be reliably attributed to drug treatment.

*New data and analyses described in the updated PCNS AC memo increase concerns about the reliability, completeness, and comparability of the clinical data for eteplirsen-treated patients and external controls. For example, differences in the way that key endpoints were measured, including the apparently large role of judgments of study personnel about when patients were deemed unable to perform an endpoint, may have underestimated the abilities of external controls. The applicant has emphasized newly submitted data on time to loss of ambulation and other functions, but such analyses appear to be particularly unreliable in the context of the differences between study arms.*

*Additional analyses of ambulatory functions such as rise time and 10m walk/run appear to suggest that, in the context of a poorly-controlled trial, the rate of DMD progression in eteplirsen-treated patients and external controls was generally similar. Assessing patient function in the context of age, which correlates strongly with function in DMD, may be more appropriate than by years of treatment/observation given the range of patient age enrolled in Study 201/202. Natural history data emerging from the CINRG study suggest that a substantial percentage of exon-51 skippable patients maintain ambulation beyond 16 years, at least to 18 years of age. The oldest eteplirsen-treated patients are currently*

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<sup>60</sup> Dubovitz V (2015) Unnatural natural history of Duchenne muscular dystrophy. *Neuromuscular Disorders* 25:936.

*about 15 years old, such that it cannot be concluded that the ambulatory function of eteplirsen-treated patients, either as a group or considered individually, exceeds the expected range of natural history.*

3. With regard to future efficacy studies, any beneficial effects of eteplirsen are unlikely to be large enough to be detectable outside of a placebo-controlled trial.

*It is important to note that the exposure-response relationship of eteplirsen is not well characterized. Dose-limiting toxicity was not observed, such that higher doses of eteplirsen, with potentially greater likelihood of efficacy, could be studied in the future.*

### **Overall Conclusions**

The overall conclusion of this review is that the applicant has not provided the substantial evidence of effectiveness required by law [see 21 CFR 314.126(a)(b)] to support approval, based on either endpoints measuring clinical benefit, or biomarker endpoints that might be considered reasonably likely to predict benefit under accelerated approval provisions.

Dystrophin protein could be considered under the accelerated approval provisions as a biomarker endpoint reasonably likely to predict benefit in DMD, but the amount, localization, and functionality would be key considerations. There is some evidence that eteplirsen increases the expression of a functional Becker-type dystrophin protein, to a level  $\approx 1\%$  of normal, but the evidence is less than the amount that is generally considered “substantial evidence.” Additional independent substantiation of dystrophin production would be necessary to reach the level of evidence generally considered substantial evidence.

The amount of Becker-type dystrophin that may be produced by eteplirsen,  $\approx 1\%$  of normal, is low enough that a conclusion that the amount would be reasonably likely to predict clinical benefit would have to be based on a low threshold for reasonably likely. The level is well within the range of dystrophin levels of untreated DMD patients, and appears to be substantially lower than dystrophin levels in patients with less severe forms of dystrophinopathy.

## 8. Safety

- **Adequacy of the drug exposure experience (i.e., the safety database)**

The clinical safety database for eteplirsen is small: 114 total patients exposed, with only 36 exposed for  $\geq 24$  weeks and 12 exposed for  $\geq 1$  year. Most of these exposures were outside of placebo-controlled studies, limiting ability to determine if adverse events were the result of drug effect or chance. However, the serious and severe adverse events that occurred were generally consistent with events expected in DMD. The 12 patients in Study 202 were exposed for  $>3$  years, which provides some reassurance against delayed toxicity.

- **Adequacy of the clinical safety assessments, including data integrity and submission quality, categorization of adverse events and clinical assessments**

I agree with Dr. Breder that, other than small size of the safety database, the clinical safety assessments were adequate.

- **Key safety results, including deaths, serious adverse events (SAEs), discontinuations due to adverse events, other adverse events, results of laboratory tests, and immunogenicity**

### Deaths

No deaths occurred through the 120-Day cutoff.

### Serious Adverse Events

There were 4 serious adverse events in patients treated with eteplirsen. I agree with Dr. Breder's assessment that there does not appear to be a causal relationship between eteplirsen treatment and these SAEs.

1. wound infection at muscle biopsy site
2. post-operative vomiting
3. ankle fracture secondary to fall, which is common in the natural history of DMD
4. femur fracture secondary to falling out of wheelchair in vehicle incident

### Severe Adverse Events

There was one patient in Study 28 who experienced cardiomyopathy with left ventricular dysfunction, a 10 year old boy treated with 4 mg/kg/wk eteplirsen for 7 weeks. A retrospective review of echocardiograms showed that the patient had pre-existing cardiomyopathy. The event was judged by the investigator as "possible related" to eteplirsen.

Cardiomyopathy is common in DMD, and this patient may have had pre-existing cardiomyopathy, decreasing concern that the event was drug-related.

### **Discontinuations**

The only discontinuation was the patient noted above with cardiomyopathy.

### **Common Adverse Events**

Dr. Breder identified common adverse events from the controlled portion of Study 201/202 that occurred in more than 1 eteplirsen-treated patient and at a higher incidence than in the placebo group, noting the following:

- Bleeding-related events: 2 patients in the 50 mg/kg/wk arm had prolonged activated partial thromboplastin time, vs. zero patients in the 30 mg/kg/wk arm and placebo arm
- Accident and injury: 11 events of non-serious injury occurred in the combined drug-treated arms, with no clear dose-relatedness, vs. zero events in the placebo arm.
- Infections: 9 events of upper respiratory infection occurred in treated patients vs. 1 in placebo.

### **Laboratory and other Monitoring Findings**

- Dr. Breder identified a number of abnormalities in cardiac monitoring results that also appear consistent with the cardiac effects of DMD.
- Anti-dystrophin antibodies were not assessed in any multiple-dose study
- Reference ranges for laboratory findings were different in different study periods, decreasing overall interpretability.
- There was no laboratory indication of renal toxicity

### **Other Safety Issues**

- CSS concluded that eteplirsen does not have the profile of a drug with abuse because it:
  1. Does not produce central nervous system behaviors in either animals or humans
  2. Has a mechanism of action that is limited to effects on mRNA
  3. Does not distribute into the brain after intravenous administration

## **9. Advisory Committee Meeting**

The application was presented to the Peripheral and Central Nervous System Drugs Advisory Committee on April 25, 2016.

### **Presentation by Christine McSherry, B.S.N.**

During the presentation time allotted to the Applicant, Ms. McSherry presented “Patient and Caregiver-Reported Outcomes of Patients in Clinical Trials of Eteplirsen for Treatment of Duchenne.” Information was obtained from 8 of 12 patients treated in Study 202, and 3 patients treated in Study 204. The summary below is not intended to be a complete representation of the statements made or of FDA’s consideration of those statements.

- Spontaneous Falls: Daily spontaneous falls were reported by caregivers to decrease substantially in Study 202 patients, in one case from a baseline level of greater than 5 to a level at 3+ years of near zero. For Patient C in Study 204, the time course of change in spontaneous collapses was shown. Collapses decreased from about 2.5 per day to close to zero after about 12 weeks of treatment

- Walking after fractures: Four ambulatory boys suffered fractures during the trial and all four boys regained the ability to walk.

CDTL Discussion: Persistent loss of ambulation following fracture has been reported to occur in 13% to 50%<sup>61,62, 63</sup> of independently mobile males with DMD, with a recent report of recovery of ambulation in 7 of 7 boys with DMD for whom early post-fracture rehabilitation was recommended between 9 and 15 years of age recovering ambulation after femur fracture.<sup>64</sup> Recovery of ambulation of eteplirsen-treated patients therefore appears to be within the range expected from natural history.

- Fatigue: 2 boys reported decreased levels of fatigue, 3 remained stable, and 3 experience increased levels of fatigue.
- Ability to participate in life, including activities of daily living (ADL's): ADL's were reported to be retained in 2 non-ambulatory boys.

### **Open-Public Hearing Speakers**

The summary below is not intended to be a complete representation of the statements made or of FDA's consideration of those statements.

- Mike Fitzpatrick
  - Need for innovative and flexible approach for DMD under FDASIA
- Kaaren Jurack
  - Need for access to eteplirsen
- Carlo Basile
  - Need for FDA flexibility in applying statutory standards and avoiding type 2 error
- Malanie Minor
  - The natural history of DMD is more severe than represented by the data available to FDA
- Christine McSherry
  - Criteria for accelerated approval have been met
- Brady and Martha Williams
  - Eteplirsen treatment maintained walking at age 15 years, decreased falling, improved strength, stabilized cardiac and pulmonary function, and was without side effects
- Chris Dunn, Kris Paschal, Dennise Taborski, Sadie Anderson
  - Eteplirsen treatment (72 weeks) results in fewer falls, more stamina, increased strength

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<sup>61</sup> McDonald et al. (2002) Fracture prevalence in Duchenne muscular dystrophy. *Dev Med Child Neurol.* 44:695-8.

<sup>62</sup> Vestergaard et al (2001) Fracture risk in patients with muscular dystrophy and spinal muscular atrophy. *J Rehabil Med* 33:150-155.

<sup>63</sup> King WM et al (2007) Orthopedic outcomes of long-term daily corticosteroid treatment in Duchenne muscular dystrophy. *Neurology.* 68:1607-1613.

<sup>64</sup> McCormick et al (2013) Recovery of ambulation and functional mobility in boys with Duchenne muscular dystrophy following femoral fractures. *Abstracts Neuromuscular Disorders* 23:738-852, p.7.12.

- Jodi Nicols, Jenn Dumm
  - Eteplirsen led to regained abilities, such as carrying tray, and to stronger arms and legs and improve activities of daily living
  - Natural history data in FDA briefing document does not appear correct
- Austin LeClaire
  - Eteplirsen led to increase in upper body strength
- Neera Gulati
  - Eteplirsen meets standards for accelerated approval
- Manni Scarso, Louise Crow-Arnold and James Arnold
  - Eteplirsen led to increased strength, less frequent falls, stronger grip, better stamina
- Cole and Kim Eichelberger
  - Eteplirsen led to stable 6MWT
- Billy and Terri Ellsworth
  - Eteplirsen led to increased independent activities of daily living and to less heel walking
- Debra Miller
  - Drug combinations need to be tested in DMD
  - 19 year old son is ambulatory with 3% of normal dystrophin
- Jordan McSherry
  - Improvement in strength from eteplirsen
- Tracy Secker, Valerie Pappas Llauro, Amy Martin, Scott Griffin and Lisa Lee
  - Loss of ambulation delayed by eteplirsen; deviation from natural history for loss of ambulation
- Max LeClaire and Jenn McNary
  - Increased grip strength and stability walking from eteplirsen
- Caden Bower and Beth Perez
  - Increased abilities from eteplirsen, fewer falls, increased endurance, in setting of therapy as advised for any child with DMD
- Susan Patterson and Wendy Kelly
  - Improvement from eteplirsen, increased strength, increased ambulatory ability,
- Mitch Leffler
  - Difficult or infeasible to conduct additional trials of eteplirsen; unethical to conduct placebo controlled trials
- Keith Wesley
  - Clear effect of eteplirsen on ADL's
- Ryan and Ana Vaish
  - Stable function outside of predicted natural history
  - Recommended level of physical therapy, not more intensive
- Jack Willis, Nolan Willis, Alison Willis and Alec Hoke
  - Increase in upper arm strength, less fatigue, stable heart and lung function, preserved arm strength from eteplirsen
- Alex Smith, Alex Johnson and Andrew Johnson, Emily Crossley, Lisa Kuhwald, Zoe Ward, Alasdair Robertson and Robyn Pete

- Clinical course of eteplirsen-treated patients differs from natural history, including CINRG findings
  - Exon 44 skippable patients provide additional evidence that eteplirsen is effective
- Pat furlong
  - Need for FDA flexibility
- Brian Denger, Trina Stelly, Mel and John Kelly and Katy Pease
  - Ambulation in eteplirsen-treated patients is outside the range of natural history
- Bill and Kim Procko
  - Ambulation maintained on eteplirsen longer than predicted by natural history; relaxed muscles, fewer contractions, fewer to no falls, better digestion; recovered ambulation after fracture; physical therapy not intensive
- Marissa Penrod, Catherine Jayasuriya, Anessa Fehsenfeld, Dave Schultz, Kelly Maynard and Natalie Gaudenzi
  - The evidence that eteplirsen works is strong, and FDA should be flexible
- Rose A. Juhasz
  - Eteplirsen is effective and should be approved without delay
- Kadee Roden, Christina Burrell, Ethan Marquez, and Sandra Katzin
  - Improved endurance from eteplirsen, reduction in falls, improved quality of life, increased strength, independent ADL's
- Mindy Leffler
  - Improvement in spontaneous collapses from eteplirsen; regained strength, regaining lost milestones
- Chelsey Hickman on behalf of Shannon DeMatteo
  - Greater stability of function from eteplirsen than expected from natural history
- Aidan Leffler
  - Increased abilities from eteplirsen, e.g. getting into the car
- Laura McLinn on behalf of Senator Joe Donnelly
  - Call for FDA flexibility
- Sue Fletcher, PhD (b) (4)
  - The fold-increase in dystrophin is the key measure because dystrophin levels can vary across normal samples used as controls.
  - In mouse, mouse-specific sequence induces dystrophin in all muscle fibers, and leads to reduced pathology.

CDTL: Some of Dr. Fletcher's observations appear concordant with those discussed by Dr. Rao about variability across normal individuals used as controls. Dr. Fletcher stressed the difference in dystrophin levels between week 180 samples and the controls, but it is not clear that this addresses FDA concerns about matching.

Regarding studies in mice, it appears that dystrophin expression in mice may be higher than in eteplirsen-treated patients, particularly at doses in mice that are several-fold higher, based on human equivalent doses, than doses studied in patients. The nonclinical data thus supports the recommendation to study higher doses of eteplirsen in patients.

- Barry Byrne, MD [REDACTED] (b) (4)
  - Eteplirsen findings were discussed in the context of other externally-controlled trials in rare pediatric diseases that led to marketing approval.

CDTL: Dr. Byrne's comments are concordant with FDA's statements that interpretable externally-controlled trials are capable of supporting FDA approval.

- Laura Gottschalk, PhD (National Center for Health Research)
  - Additional data from ongoing studies should have been submitted for consideration.

CDTL: FDA had expressed in the April 15, 2014 advice letter to the applicant that additional biomarker data from studies subsequent to Study 201/202 would be expected to be submitted with, or shortly following, submission of the NDA. Such data may help to clarify the degree to which eteplirsen might induce expression of truncated dystrophin.

- Linda Lowes PT, PhD [REDACTED] (b) (4)
  - Training was the same for personnel collecting data from eteplirsen-treated and control patients.
  - Boys in the eteplirsen study who were deemed unable to complete the 6MWT could only take a few steps.

CDTL: Similar training of study personnel can increase the potential for similar conditions across arms in externally controlled trials, but it is not clear the degree to which this alleviates concerns of meaningful differences between study arms. As discussed in this review, the boys who were deemed unable to complete the 6MWT had a level of ambulatory function, as indicated by 10 meter run/walk time, that would seemingly correspond to a substantial potential distance walked over 6 minutes.

- Catherine Wagner, MD (Physician for several boys in eteplirsen studies)
  - Patient 6, and other patients, are progressing more slowly than can be accounted for by natural history.
- Peter Heydemann, MD [REDACTED] (b) (4)
  - Unexpected stability in eteplirsen-treated boys
- John Day, MD, PhD [REDACTED] (b) (4)
  - By personal experience, exon 51 skippable boys are unlikely to walk beyond 12 years of age
  - The course of eteplirsen-treated patients differs from natural history

CDTL: As described in this review, several independent sources of natural history data indicate that the course of eteplirsen-treated patients is within the range of untreated patients.

- Anne Connolly, MD (b) (4)
  - Positive fibers in eteplirsen-treated patients are histopathologically distinct from revertant fibers, and show less pathology than expected in DMD.

CDTL: Evidence of altered muscle structure in eteplirsen-treated patients has not been presented by the applicant but, if present, could be helpful in determining whether eteplirsen is effective.

- Terrence Partridge, PhD
  - Dystrophin is irregularly distributed in eteplirsen-treated patients

CDTL: Irregular distribution of dystrophin could increase random variability.

- Carrie Miceli, PhD
  - 2 patients had pre-treatment biopsies that allow for validation of the internal controls.
  - Some muscle fibers have protective levels of dystrophin

CDTL: The pre-treatment biopsies available for 2 patients were from a different muscle group, a potential confounder. There may also be concerns arising from the long storage period of the pre-treatment biopsies, as described above in this review.

- Stanley Nelson, MD
  - Loss of ambulation is a hard endpoint

- Perry Shieh, MD (b) (4)
  - 6MWT is a hard endpoint.

CDTL: Loss of ambulation would be an acceptable endpoint if measured the same way across study arms in randomized, double-blind trials, in patients who were otherwise treated the same except for treatment with the investigational drug.

- Elizabeth McNally, MD (No consulting relationship with the Applicant)
  - Even a small increase in dystrophin is beneficial

- Jeff Chamberlain, PhD (b) (4)
  - Very low levels of dystrophin can be beneficial.
  - Dystrophin levels, including Becker-type dystrophins, at levels as low as 10% can prevent and reverse dystrophin pathology
  - A single dystrophin positive fiber is protective for adjacent dystrophin negative fibers. Patchy dystrophin is widely protective.

CDTL: FDA is receptive to reviewing any data that might support these claims. As discussed in this review, it is not clear that dystrophin levels in the range of 1% of normal have a measurable effect on the rate of progression of DMD. Regarding protective effects of dystrophin positive fibers on dystrophin negative fibers, there appears to be evidence

that this is dependent on a high enough proportion of surrounding fibers being dystrophin positive.<sup>65</sup>

- Louis Kunkel (b) (4)
  - 0.9% dystrophin does not occur in untreated DMD patients

CDTL: In Western blots from biopsies of extensor digitorum brevis (EDB),<sup>66</sup> in exon-51 skippable patients, dystrophin levels averaged about 0.3% of normal, but ranged from undetectable to  $\approx$  1% of normal or somewhat higher.

### ***Questions to the Committee:***

The Applicant is proposing approval based primarily on a post hoc comparison of 12 patients with Duchenne Muscular Dystrophy (DMD) amenable to exon 51 skipping from the open-label portion of a single study (Study 201/202) to 13 patients from an external untreated control group. The Advisory Committee will be asked to discuss and vote on whether the application has met the statutory requirements for substantial evidence of effectiveness, based on that comparison. The Advisory Committee will also be asked to discuss the evidence provided by the Applicant on dystrophin expression with eteplirsen treatment, and vote on whether the Applicant has provided substantial evidence from adequate and well-controlled studies that eteplirsen induces production of an amount of dystrophin that is reasonably likely to predict clinical benefit.

### **Statutory standards for approval**

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<sup>65</sup> Dunant et al. (2003) Expression of Dystrophin Driven by the 1.35-kb MCK Promoter Ameliorates Muscular Dystrophy in Fast, but Not in Slow Muscles of Transgenic Mdx Mice. *Molecular Therapy*. 8:80-89.

<sup>66</sup> FDA Advisory Committee presentation for drisapersen, slide 43.

Although drug approval ultimately reflects a benefit-risk assessment, the statutory standards for approval are applied stepwise, with the law first requiring substantial evidence that the drug is effective. If the standard for substantial evidence of effectiveness is met, a determination must be made that the drug is safe for its intended use, i.e., that its benefits outweigh the risks, given the nature of the disease and available treatment options.

### **Standard Approval**

Sponsors of marketing applications are required to establish a drug's effectiveness by providing "substantial evidence" of effectiveness from "adequate and well-controlled investigations." Positive findings on clinically meaningful endpoints in two adequate and well-controlled trials are typically required, but a single highly persuasive positive trial or a positive trial combined with independent findings that substantiate efficacy (confirmatory evidence) can also support approval in some cases. The intent of the statutory requirements is to reduce the chance of an incorrect conclusion that a drug is effective when, in fact, it is not effective. In making its determination on whether the statutory standards for approval have been met, the Agency considers all the available data.

### ***Accelerated Approval***

Under the Accelerated Approval provisions, an effect on a surrogate marker that is determined by FDA to be reasonably likely to predict clinical benefit can support approval, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments. An effect on an intermediate clinical endpoint - a clinical endpoint that can be measured earlier than irreversible morbidity or mortality (IMM) and that is reasonably likely to predict an effect on IMM or other clinical benefit - can also serve as a basis for accelerated approval.

Importantly, accelerated approval does not change the statutory requirement for substantial evidence; rather, it allows FDA to utilize a demonstrated effect on an endpoint other than clinical benefit as the basis for showing effectiveness if the sponsor provides substantial evidence from adequate and well controlled trials that the drug has an effect on a surrogate or intermediate clinical endpoint. The Agency's decision on whether to grant accelerated approval is based both on the appropriateness of the endpoints selected (surrogate marker or intermediate clinical endpoint), and on whether there is substantial evidence of an effect on these endpoints. Accelerated approval cannot be used to compensate for weak or inconsistent clinical findings (i.e., approval based on marginal data, to be buttressed with better data post-approval). When accelerated approval is used, post-approval studies to verify the expected clinical benefit are generally required.

### **Biomarker Evidence**

For DMD, there is obvious interest in dystrophin expression as a potential surrogate marker to support accelerated approval. Whether an effect on a biomarker such as dystrophin is reasonably likely to predict clinical benefit in DMD depends on a number of factors including, but not limited to, the reliability of the data, the magnitude of the effect on the biomarker, and confidence that the dystrophin produced is functional.

Eteplirsén's putative mechanism of action is to increase production of a truncated form of dystrophin. By Western blot, the most accurate quantitative method used by the Applicant, mean dystrophin levels after 180 weeks of eteplirsén treatment are  $0.93\% \pm 0.84\%$  of normal (mean  $\pm$  standard deviation). The Applicant reported a control (untreated) value of  $0.08\%$  dystrophin based on retained samples from the pre-treatment biopsy in 3 patients from Study 201/201, combined with data from six patients with DMD who were not enrolled in any study. FDA identified, however, some important limitations with respect to interpretation of the results of the untreated controls (e.g., limits of assay detection, different muscles sampled).

1. **DISCUSSION:** Discuss the evidence presented about dystrophin production, including the following:
  - a. The strength of evidence that eteplirsén increased the amount of dystrophin in muscles of treated patients, relative to their baseline.
  - b. Clinical meaning of the amount of dystrophin observed in the muscles of eteplirsén-treated patients, taking into consideration the range of amounts of dystrophin known to be typically present in patients with DMD and in patients with Becker muscular dystrophy.

**Committee Discussion:** *The committee members did not reach a consensus on either the strength of evidence that eteplirsén increased the amount of dystrophin in muscles of treated patients relative to baseline, or the clinical meaning of the amount of dystrophin observed in the muscles of eteplirsén-treated patients.*

- a. *Production of dystrophin: About half of the committee members thought that there was evidence that eteplirsén increased the amount of dystrophin produced in the muscles of the treated patients. Among those who were not convinced, two members cited issues with the controls (lack of pre- and post-treatment biopsies in the same patients; differences in muscle groups biopsied), two had concerns about inconsistencies between dystrophin levels and clinical response, and one cited concerns about the lack of a dose-response. The Chair found it surprising that there wasn't more scientific consensus.*
- b. *Clinical meaning: Only four Committee members had explicit comments with respect to the clinical meaningfulness of the amount of dystrophin observed in treated patients, and their opinions were split. One opined that the amount of dystrophin needed to impart clinical benefit is unknown, but could be very low, or very low in a subset of patients. One of the Patient representatives felt strongly that dystrophin was produced, and that*

*the amount was sufficient to produce clinical benefit. One committee member, having opined that some dystrophin was produced, stated that we have no idea how much dystrophin would be clinically significant, or whether the dystrophin is functionally active. Another committee member, one who had not opined on whether dystrophin was produced, noted that whatever the amount of dystrophin produced, it was not clinically meaningful, based on a lack of correlation between dystrophin results and clinical results. Please see the transcript for details of the committee discussion.*

2. **VOTE:** Has the Applicant provided substantial evidence from adequate and well controlled studies that eteplirsen induces production of dystrophin to a level that is reasonably likely to predict clinical benefit?

**Vote Result:** YES: 5 NO: 8 ABSTAIN: 0

***Committee Discussion:** One panel member stated that he had pressed the wrong voting button and stated that his vote should be changed to “Yes” for the record, which would make the vote 6 “Yes” and 7 “No.” Thus, 7 committee members voted “No” that the Applicant did not provide substantial evidence from adequate and well controlled studies that eteplirsen induces production of dystrophin to a level that is reasonably likely to predict clinical benefit. In explaining their “No” votes, 5 committee members opined that the studies were not adequate and well controlled; they questioned the techniques used to measure dystrophin as well as the appropriateness of the controls. Four committee members expressed concern about the lack of correlation between the dystrophin levels and clinical measures. They agreed that even if some dystrophin was produced, there was no evidence that dystrophin production was at a level that would be reasonably likely to predict clinical benefit. The 6 members who voted “Yes” included the consumer representative and both patient representatives. They believed that there was some difference in dystrophin production and some evidence of improvement in endpoints. One of the members who voted “Yes” stated that he was very troubled by not understanding what constitutes a clinically significant amount, but was impressed by the patients’ observations. Please see the transcript for details of the committee discussion.*

### Clinical evidence

Study 201/202 began as a 24-week randomized controlled study comparing three groups of 4 patients each, treated weekly with eteplirsen 50 mg/kg, eteplirsen 30 mg/kg, or placebo (Study 201). Study 201, when analyzed according to the pre-specified intent-to-treat (ITT) methods, did not show an advantage of eteplirsen over placebo on the 6-minute walk test (6MWT) after 24 weeks of treatment.

After the randomized placebo-control phase, all patients entered an open-label extension phase beginning at Week 28, i.e., Study 202. The primary clinical endpoint of Study 202 was a comparison of Week 48 6MWT results for patients originally randomized to eteplirsen vs placebo. When analyzed according to the pre-specified ITT methods, Study 202 did not demonstrate an advantage of eteplirsen over placebo on the 6-minute walk test.

The Applicant then continued open-label treatment with eteplirsen in Study 202, which is still ongoing, and is seeking approval primarily based on a post hoc comparison of 12 patients from Study 201 to 13 patients from an untreated external control group amenable to exon 51 skipping (from two DMD patient registries, the “Italian Telethon DMD Registry” database and the “Leuven Neuromuscular Reference Center” database).

Because of difficulty of controlling bias in historical control studies, important issues to consider include: 1) whether there are identified or possible differences between the treatment and control groups, at baseline or during treatment, that may have had an impact on clinical course; 2) whether the endpoint(s) used to assess benefit was (were) objective and assessed in a sufficiently similar way in the treatment and control groups to allow a valid comparison; and 3) whether the reported effect size is large enough to conclude that the course of patients in Study 201/202 is clearly different from the usual course of patients with DMD.

3. **DISCUSSION:** Discuss the strengths and weaknesses of the clinical evidence of efficacy provided by Study 201/202, with particular consideration of the design of the study, sample size, statistical methods, general concerns regarding a comparison to a historical control group, specific concerns with respect to the comparability of these two groups (in particular, how motivational factors and differences in assessment of physical performance outcomes may have affected the 6-minute walk endpoint and other endpoints), and any other issues that you think may be important.

***Committee Discussion:** Overall, the majority of the committee agreed that there were weaknesses to Study 201/202. One committee member noted that although placebo controlled trials can have flaws, studies with historical controls can have even more flaws and was uncomfortable with the study design of Study 201/202. Another committee member added that, considering the testimonies provided by the public, Study 201/202 might have been successful if the patient-reported results had been included. Other committee members noted that they would have liked to see a measurement of upper limb strength, which was reported to be improved in the testimonies from the public but was not captured in the North Star Ambulatory Assessment, 10-meter run/walk and 6-minute walk tests. Please see the transcript for details of the committee discussion.*

4. **VOTE:** Were decisions to administer the 6-minute walk test (vs. conclusions that the patient could no longer walk) sufficiently objective and free of bias and subjective decision-making by patients, their caregivers, and/or health care professionals to allow for a valid comparison between patients in Study 201/202 and an external control group?

**Vote Result:** YES: 5 NO: 7 ABSTAIN: 1

**Committee Discussion:** *A slight majority of the committee voted “No” i.e., that decisions to administer the 6-minute walk test (vs. conclusions that the patient could no longer walk) were not sufficiently objective and free of bias and subjective decision-making by patients, their caregivers, and/or health care professionals to allow for a valid comparison between patients in Study 201/202 and an external control group. These members explained that there were difficulties in assessing historical controls, that there were problems with the primary endpoints, which measured only lower body strength, and they questioned the objectivity of the conclusion that the people in the external control group were actually unable to perform the 6-minute walk test. The members who voted “Yes” agreed that the 6-minute walk test was sufficiently objective to be meaningful, and that there was no evidence of real bias. One committee member chose to abstain, explaining that the 6-minute walk, although subjective, could be a valid endpoint, but had trouble with the context in which it was used and therefore had difficulty interpreting the question to make a firm decision. Please see the transcript for details of the committee discussion.*

5. **VOTE:** What is the impact of the North Star Ambulatory Assessment results on the persuasiveness of the findings in Study 201/202?
- a. Strengthen
  - b. Weaken
  - c. No effect

**Vote Result:** Strengthen: 2 Weaken: 5 No Effect: 6

**Committee Discussion:** *Six members of the committee voted that the results of the North Star Ambulatory Assessment (NSAA) had no effect on the persuasiveness of the findings in Study 201/202. One panel member stated for the record that he wanted to change his vote from “Strengthen” to “No Effect.” These members agreed that, overall, there was no evidence of difference between the two groups on either measure. The members who voted that the impact of the NSAA results weakened the persuasiveness of the findings in Study 201/202 noted that NSAA is a more comprehensive measure of functional assessment and explained that the persuasiveness was weakened because there were no statistically significant differences between the treated vs. the control groups. Please see the transcript for details of the committee discussion.*

6. **VOTE:** What is the impact of the other tests of physical performance (e.g., rise time, 10-meter run/walk) on the persuasiveness of findings in Study 201/202?
- a. Strengthen
  - b. Weaken
  - c. No effect

**Vote Result:** Strengthen: 1 Weaken: 2 No Effect: 10

**Committee Discussion:** *The majority of the committee voted that the impact of the other tests of physical performance (e.g., rise time, 10-meter run/walk) had no effect on the persuasiveness of findings in Study 201/202. These members noted that the FDA and Applicant are in disagreement in assessing rise time. They agreed that overall, physical performance measures in the other tests were secondary outcomes and that there was no evidence of difference between the two groups, probably because of the small sample size of the studies.*

7. **VOTE:** Do the clinical results of the single historically-controlled study (Study 201/202) provide substantial evidence (i.e., evidence from adequate and well-controlled studies or evidence from a single highly persuasive adequate and well-controlled study that is accompanied by independent findings that substantiate efficacy) that eteplirsen is effective for the treatment of DMD?

**Vote Result:** YES: 3 NO: 7 ABSTAIN: 3

**Committee Discussion:** *The majority of the committee voted “No,” i.e., that the clinical results of the single historically-controlled study (Study 201/202) did not provide substantial evidence that eteplirsen is effective for the treatment of DMD. These members agreed that Study 201/202 was not a well-controlled study and based on statistical and scientific findings, substantial evidence regarding the efficacy of eteplirsen was not evident. Most who voted “No” cited problems with the controls. One noted that a historically-controlled study could provide evidence of effectiveness, but that this trial did not. Two committee members noted that the original placebo-controlled portion of the study was negative. One member, noting the disconnect between the trial data and the patient testimonies, suggested that the patient community should be more willing to participate in controlled trials. One member who cited problems with the controls also noted that a single trial is insufficient. The members who voted that “Yes” said that substantial evidence did exist, adding that the study correlated with the testimonies presented by the public. With respect to the members who abstained, one member stated he was torn between the data presented by the FDA and the testimonies presented by the public. One felt uncomfortable with what he thought was a leading question. Another stated that the study was not adequate and well controlled, but that he was moved by the patients’ testimony. Please see the transcript for details of the committee discussion.*

## 10. Pediatrics

- Pediatric exclusivity board review - Proposed Pediatric Study Requests (PPSR)/Written Request (WR)

The applicant did not submit a PPSR and a WR was not issued.

- Pediatric Review Committee (PeRC) Review Outcome-Post Marketing Commitments (PMCs), deferrals, waivers, pediatric plan, pediatric assessment

Eteplirsen is an orphan product, to which certain waivers for pediatric studies apply.

## 11. Other Relevant Regulatory Issues

- Office of Scientific Investigations (OSI) audits

As described in the primary clinical review, the US-201 and 4658- 202 studies were inspected at Dr. Mendell's site at Nationwide Children's Hospital. The review included an inspection of the IRB records, sponsor and monitor audit activities, financial disclosures, adverse events reporting, Informed Consent Documents for all subjects, the medical records/source data for 8 subjects enrolled, and observation of four subjects performing their individual subject level 6-Minute Walk Test (6MWT), individual subject level data for other functional assessments such as North Star Ambulatory Assessment (NSAA), Maximum Voluntary Isometric Contraction Test (MVICT), Rise Time, 10- Meter Run Time, Timed 4-Step Test, and pulmonary function tests. There was no evidence of inaccuracy of the data captured on the above metrics.

DNP consulted OSI for inspection of the sites in Belgium and Italy from which natural history data was derived. These inspections were ongoing at the time of writing of this review.

## 12. Labeling

### Prescribing Information

- INDICATIONS AND USAGE section:
  - As discussed in Section 5, Clinical Pharmacology, an indication for all DMD patients amenable to exon-51 skipping appears reasonable.
- DOSAGE AND ADMINISTRATION section:
  - As discussed in Section 7, Efficacy, it is not clear that the proposed dosage, route of administration, and dosing regimen of eteplirsen is effective.
- Safety information in the BOXED WARNING, CONTRAINDICATIONS, or WARNINGS AND PRECAUTIONS sections:
  - A BOXED WARNING is not recommended
  - Situations were not identified for which the risk from use clearly outweighs any possible benefit; no CONTRAINDICATIONS are recommended.
  - There are no additional clinically significant adverse reactions or risks that are recommended should be included in the WARNINGS AND PRECAUTIONS section of labeling.
- CLINICAL STUDIES section:
  - Clinical data does not appear interpretable for inclusion in labeling

- If eteplirsen is approved based on dystrophin expression, levels by Western blot for the 4<sup>th</sup> biopsy are the most reliable and interpretable values.

#### Other Labeling

- Proprietary name
  - The proprietary name was deemed acceptable by DMEPA.
- Patient labeling (i.e., Medication Guide, Patient Information, Instructions for Use)
  - Patient labeling is not deemed necessary by this review.

### **13. Postmarketing Recommendations**

#### Risk Evaluation and Management Strategies (REMS)

A REMS is not recommended.

#### Postmarketing Requirements (PMRs) and Commitments (PMCs)

In an advice letter to the applicant on April 15, 2014, FDA described its view of the clinical and biomarker data available at that time for eteplirsen and proposed a strategy to consider regarding the submission of an NDA for eteplirsen. FDA stressed that it had not determined whether an application for eteplirsen would be approved under Subpart H, but noted that in such a case confirmatory studies should be underway at the time of approval.

If eteplirsen is approved under Subpart H, the applicant is proposing to conduct 2 confirmatory studies:

- Study 4658-301 (also referred to as PROMOVI) in exon 51-skippable patients.
- 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.

The conclusions of this review regarding PMRs and PMCs are as follows:

- If eteplirsen is approved under the accelerated approval provisions, postmarketing requirements would be necessary to confirm clinical efficacy. The potential for any drug to produce clinical benefit, including molecularly-targeted drugs such as eteplirsen, is related to drug exposure. The proposed dose may be lower than necessary to produce clinical benefit. A study to determine the maximum tolerated dose (MTD), and the dystrophin production associated with that dose, is recommended.
- An externally controlled trial at the proposed dose (30 mg/kg/wk IV) appears unlikely to yield interpretable evidence of clinical efficacy because of inability to adequately control or account for bias, combined with evidence suggesting that the effect size of eteplirsen is unlikely to be large enough to provide clear results that can overcome the

uncertainties inherent in such a study design.

- Confirmation of efficacy of eteplirsen could be provided by both statistically positive clinical findings and a large effect size in a randomized, double-blind, placebo-controlled study of a drug similar to eteplirsen but designed to skip other exons (e.g. an exon 45 and/or exon 53 skipping PMO). The levels of truncated dystrophin produced by the different drugs would, however, need to be adequately similar to enable the conclusion that the clinical efficacy of eteplirsen was similar.

## **14. Recommended Comments to the Applicant**

The potential for any drug to produce clinical benefit, including molecularly-targeted drugs such as eteplirsen, is related to drug exposure. Because the currently proposed dose, regimen, and route of administration, exposure to eteplirsen may be lower than required for efficacy, additional data on exposure-response appears necessary. A randomized, double-blind, exposure-response study of eteplirsen may be a scientifically appropriate design for a subsequent trial. The results of such an exposure-response trial would be necessary to inform subsequent drug development decisions.

**Appendix: Applicant’s table of “Key Inaccuracies in the FDA Briefing Document”**

*Note: The first issue listed by the applicant in the table titled “Potential Clinical Impact” regards text from the memo from the Division and Office, and is addressed in that revised memo.*

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**Dystrophin Analytical Methodology:**

FDA Statement	Sarepta Clarification
<p><i>“It is important to note that the applicant digitally processed dystrophin images in their background material (images in Appendix 12) in such a way that low intensity values were preferentially increased to produce a higher intensity and higher contrast image.”</i> (FDA BD page 29 of PDF)</p>	<p>The digitally processed images referenced by FDA in this statement were included in Sarepta’s briefing document for demonstration purposes only, and it is far more important to note that the referenced images were not used in the analysis of fiber intensity, nor to score dystrophin-positive fibers.</p>
<p><i>“Biomarker studies on the 4th biopsy obtained at Week 180 were conducted by the applicant with technical advice from FDA. However, the reliability of results remains questionable for a number of reasons, including the lack of independent confirmation.”</i> (FDA BD page 30 of PDF)</p>	<p>Methodology for dystrophin analyses of the fourth biopsy tissue samples, including confirmatory assessments of percent dystrophin-positive fibers (PDPF) analysis performed by 3 independent pathologists, were agreed with FDA prior to conducting any analyses of the fourth biopsy tissue samples.</p> <p>In accordance with the mutually agreed-upon protocols for the assessment of dystrophin-positive fibers in DMD muscle biopsy samples from the fourth biopsy obtained at Week 180, 3 independent pathologists performed a blinded assessment of the randomized muscle fiber microscopy images, which independently confirmed the results obtained by the pathologist at Nationwide Children’s Hospital (NCH).</p> <p>Assessment of PDPF at NCH indicated a significant increase in PDPF score (<math>p &lt; 0.001</math>) relative to untreated control samples. This increase in PDPF score was confirmed by the 3 independent pathologists (<math>p &lt; 0.001</math>).</p>
<p><i>“Random measurement error can be large in comparison to the estimated amount of dystrophin.”</i> (FDA BD page 31 of PDF)</p>	<p>The random measurement error of our Western blot protocol for measurement of dystrophin levels was well below the observed difference between untreated and treated Week 180 biopsy samples.</p> <p>A rigorous validation of the Western blot method was reviewed by the FDA prior to Week 180 biopsy analysis. Validation data demonstrated a %CV of +/- 50% and a linear range (<math>R^2 &gt; 0.9</math>) of sensitivity extending as low as 0.25% of normal.</p>

FDA Statement	Sarepta Clarification
<p><i>“There is no simple or reliable way to compare estimates of dystrophin amount derived from immunofluorescence with estimates derived from Western blot.”</i> (FDA BD page 35 PDF).</p>	<p>Correlation between dystrophin quantification by Western blot and IHC methods has been demonstrated by multiple laboratories (Taylor, 2012; Anthony, 2011; Anthony, 2014; Hathout, 2015 FDA Workshop on Measuring Dystrophin).</p>
<p><i>“In this context, the applicant selected three BMD patients as comparators for the Week 180 dystrophin studies, one of whom had low dystrophin level of about 2% of normal. However, the BMD patients selected by the applicant do not appear representative, and this patient may correspond to one of the rare BMD patients with very low dystrophin levels.”</i> (FDA BD page 34 of PDF)</p>	<p>BMD patient samples were not chosen to be representative; rather, they were selected in response to an FDA request to assess the relationship between dystrophin as measured by Western blot and immunofluorescence fiber intensity. Therefore, BMD samples were obtained that represented low, middle, and higher ranges of dystrophin expression. A comparable Western blot analysis-IHC correlation was presented by Hathout, et al. (MDA 2015 Scientific Conference poster, FDA-NIH workshop on measuring dystrophin, 2015), where BMD biopsies were chosen to represent low- and mid-level dystrophin expression. Consistently, their BMD low patient biopsy was 2% of normal.</p>

**Potential Clinical Impact:**

FDA Statement	Sarepta Clarification
<p><i>“With these two comparisons of eteplirsen to placebo, there was a positive finding for only the lower dose (30 mg/kg) and for just one of the two time points (the later time point). The lack of an effect with the higher dose group tends to undermine the finding in the lower dose group and the lack of even a positive trend at the earlier time point (with a higher dose) sheds doubt on the finding at a later time point.”</i> (FDA BD page 7 of PDF)</p>	<p>The study was designed to see whether dose (50 mg/kg vs. 30 mg/kg) or duration was the most important criterion to enable consistent dystrophin production.</p> <ul style="list-style-type: none"> <li>• Duration of therapy was observed to be the critical variable when interpreting dystrophin levels. 12 weeks does not represent a clinically relevant duration of therapy (FDA BD page 26 of PDF).</li> <li>• Significant dystrophin levels were by measured at Week 24 for the 30 mg/kg dose, and, importantly, at Weeks 48 and 180 for both the 30 and 50 mg/kg doses by all dystrophin assay methods.</li> </ul>
<p><i>“Arguably, placebo-treated patients who were blinded to treatment assignment from other controlled trials are more appropriate as matched controls than registry patients, as they may receive special care and attention as trial participants, and may be more highly motivated.”</i></p>	<p>The placebo patients from another study as referenced by the FDA are not appropriate for comparison with the eteplirsen-treated patients (FDA BD pages 8, 9, 40-44, and 50 of the PDF):</p> <p>Baseline characteristics are not comparable between eteplirsen and the proposed placebo group:</p> <ul style="list-style-type: none"> <li>• Placebo group included boys &lt;7 years old</li> </ul>

FDA Statement	Sarepta Clarification
(FDA BD page 13 of PDF)	<ul style="list-style-type: none"> <li>Placebo group included many patients with baseline 6MWT &gt;440 meters which is outside the eteplirsen trial's inclusion criteria</li> </ul> <p>Placebo patients were followed for only one year, whereas eteplirsen-treated patients were followed for 3 or more years:</p> <ul style="list-style-type: none"> <li>By virtue of the ambulatory requirement at study entry, older placebo patients (e.g. ≥11 years) were a group of pre-selected, better performing subjects.</li> <li>The first year of an 11-year-old-at-baseline placebo patient (i.e. 11-12 years old) to the third year of a 9-year-old boy with 3 years of eteplirsen treatment (i.e. 11-12 years old) is not a valid comparison due to the difference in duration of observation, as well as the biased selection of the 11-year -old ambulatory placebo boy, irrespective of both patients having the same age at last assessment.</li> <li>Comparison of eteplirsen-treated patients to the appropriately matched external control shows that more than one year is required to observe a divergence in disease progression between the two groups.</li> </ul>
<p><i>"The robustness of the study result is a concern since a single patient could change the results substantially."</i></p> <p>(FDA BD page 69 of PDF)</p>	<p>This statement is inaccurate. A comprehensive sensitivity analysis was performed in order to address any potential issue regarding robustness of the data. Specifically:</p> <ul style="list-style-type: none"> <li>Two patients were removed: the best performing eteplirsen and the worst performing external control patient.</li> <li>Results demonstrated a robust 6MWT treatment advantage of &gt;100 meters with nominal significance.</li> </ul>
<p><i>"Finally, as the sponsor's natural history study proceeded, some patients left to enter interventional clinical trials, further decreasing the similarity of the natural history cohort to the eteplirsen patients."</i></p> <p>(FDA BD page 47 of PDF)</p>	<p>Two types of missing data sensitivity analyses were performed, the results confirmed that the magnitude of difference remained over 100 meters and nominal statistical significance was maintained:</p> <ul style="list-style-type: none"> <li>MMRM using all available data</li> <li>Last Observation Carried Forward imputation (conservative analysis assuming that the 2 control patients did not decline)</li> </ul>

**Regulatory Feedback:**

FDA Statement	Sarepta Clarification
<p><i>"As the duration of exposure in Study 202 increased, the applicant proposed comparing the clinical course of treated patients to historical controls."</i></p> <p>(FDA BD page 38 of PDF)</p>	<p>The proposal to compare with historical control patients originated from the FDA. Specifically, a requirement to compare the clinical course of treated patients in Study 202 to matched patient-level historical control data was made by the FDA at the March 2014 guidance meeting, and reiterated at the September 2014 pre-NDA meeting. Sarepta had proposed an open-label confirmatory study comparing treated patients to concurrent (not historical) untreated patients with exon deletions not amenable to skipping exon 51 (i.e. the PROMOVI study).</p>

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RONALD H FARKAS  
05/17/2016

## 14 Appendix 3 Financial Disclosure

Three individuals had financial disclosures.

1. [REDACTED] (b) (6)
  - As of 18 Jan 2013, [REDACTED] (b) (6) own[ed] 12332 shares of Sarepta Common Stock
2. [REDACTED] (b) (6)
  - Outcome measures consulting agreement with Sarepta in the amount of \$485,000 over 36 months in support of quality and standardization of functional assessments across multiple investigational sites [REDACTED] (b) (6).
  - Grant in the amount of \$50,700 from October 2014 – September 2017 [REDACTED] (b) (6) for the 100-meter walk run test in healthy male controls between age 4 and 12.
  - 150,000 give to [REDACTED] (b) (6) to provide training of clinical evaluators and provide data quality checks for upcoming studies
3. [REDACTED] (b) (6)
  - Disclosed the receipt of \$310,000 as per the research service agreement effective 08 February 2010 [REDACTED] (b) (6) [REDACTED] (b) (6) agreement was to evaluate lead compounds for the skipping of Exons 44, 45 and 53.

**Covered Clinical Study (Name and/or Number): AVI-4658-28; AVI-4658-33; 4658-us-201; 4658-us-202; 4658-204; 4658-301; SR-15-028**

Was a list of clinical investigators provided:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request list from Applicant)
Total number of investigators identified: <u>260</u>		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>3</u>		
If there are investigators with disclosable financial interests/arrangements, identify the number		

Clinical Review Christopher Breder, MD PhD  
 NDA 206488 (Eteplirsen)

of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: <u>2</u> Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in Study 1 Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) <u>0</u>		
Is an attachment provided with the reason:	Yes <input type="checkbox"/>	No <input type="checkbox"/> (Request explanation from Applicant)

Form FDA 3454, box 3 is not checked or filled in but the form 3455 was submitted with the information on the 3 individuals as noted above.

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CHRISTOPHER D BREDER

05/11/2016

Omitted previously from primary review

RONALD H FARKAS

05/11/2016

## CLINICAL REVIEW

<b>Application Type</b>	New Drug Application
<b>Application Number(s)</b>	206488
<b>Priority or Standard</b>	Priority
<b>Submit Date(s)</b>	June 26, 2015
<b>Received Date(s)</b>	June 26, 2015
<b>PDUFA Goal Date</b>	May 26, 2016 (1 extension for Major Amendment)
<b>Division/Office</b>	Division of Neurology Products/ Office of Drug Evaluation 1 / Office of New Drugs (CDB) Laboratory of Applied Biochemistry, Division of Biotechnology Review and Research III, Office of Biotechnology Products (AR)
<b>Reviewer Names</b>	Christopher D. Breder, MD PhD Ashutosh Rao, PhD (Dystrophin bioassays)
<b>Review Completion Date</b>	May 06, 2016
<b>Established Name (Proposed) Trade Name Applicant</b>	Eteplirsen EXONDYS 51 Sarepta Therapeutics Inc.
<b>Formulation(s)</b>	Solution
<b>Dosing Regimen Proposed</b>	Intravenous Injection
<b>Indication(s) Intended Population(s)</b>	Treatment of Duchenne Muscular Dystrophy Patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping
<b>Recommendation on Regulatory Action</b>	Complete Response
<b>Recommended Indication(s) (if applicable)</b>	N/A

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## 1 Executive Summary

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### 1.1. Product Introduction

Drug: This is a review of a new molecular entity, Exondys51 (eteplirsen) intended to restore the mRNA reading frame and induce dystrophin protein production. The proposed indication is for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping.

(b) (4)

Dosage form: EXONDYS 51 is supplied in single use 2 mL vials containing a 100 mg (50mg/mL) and single use 10 mL vials containing a 500 mg (50 mg/mL) preservative-free concentrated solution of eteplirsen. EXONDYS 51 is intended for intravenous infusion at a dose of 30 mg/kg in a total volume of 100-150 mL 0.9% sodium chloride solution.

### 1.2. Conclusions on the Substantial Evidence of Effectiveness

This review concludes that there is not substantial evidence of effectiveness. The placebo-controlled study was clearly negative. As I will describe in the body of the review, the evidence from tests of clinical function (e.g., the six minute walk test (6MWT); Northstar Ambulatory Assessment (NSAA), and pulmonary function tests (PFTs)) that are supposed to be controlled by the natural history<sup>1</sup> is uninterpretable for many reasons, including because the natural history cohort is not adequately matched to the active treatment group in aspects related to demographics and disease progression. In the biomarker data, the evidence supports that this drug has the effect of exon skipping, but the amount of dystrophin that may be produced is very low, <1% of normal. There does not seem to be clear evidence or even consensus in the literature on what percent of normal protein would translate to a useful level; however, the concept that this is reasonably likely to correlate with clinical benefit is inadequately supported by the evidence in this application.

I have considered this issue from the perspective of applying “flexibility” as described by FDASIA. The flexibility does not mean that the threshold for Substantial Evidence is lowered. I believe that considerable flexibility was afforded the application through the review team accepting studies that were not formally powered, by considering data where the standards of execution were evolving even through the review cycle, and by considering the patient and family testimony from the Advisory Committee. Despite these considerations, I still do not consider the threshold for Substantial Evidence to have been met.

An expanded executive summary of the efficacy results is found **Section 7.1.1**.

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<sup>1</sup> Subjects from the natural history cohorts of Mercuri and Goemans were used as controls for clinical function in Studies 201 / 202 and the Applicant references studies by [Khirani *et al.* 2014] and [Mayer *et al.* 2015] as controls for their PFT studies.

- **Benefit-Risk Assessment**

EXONDYS 51, or eteplirsen, is a phosphorodiamidate morpholino oligomer with a sequence designed to bind to exon 51 of the human dystrophin pre-mRNA. It is intended to cause the skipping of exon 51 and generate an internally truncated dystrophin.

Duchenne Muscular Dystrophy (DMD) is a fatal, degenerative, X-linked recessive genetic disorder associated with mutations in the dystrophin gene diagnosed between the ages of 3 to 5 years, when toddlers develop a waddling gait and inability to jump which progresses to loss of ambulation. While pulmonary and cardiac function are generally normal during early childhood, muscles of the heart and diaphragm progressively weaken during adolescence, and patients often die from cardiac or respiratory failure in their early 20s.

Currently there are no drugs approved for the treatment of DMD; Corticosteroids, both approved for other indications and those still in the investigational status, are used in an attempt to lessen the inflammation and slow disease progression. Central to the care of children with DMD is a rigorous program of respiratory therapy, adjunctive drug therapy (e.g., ace-inhibitors to decrease afterload), and non-medical therapy such as orthoses and physical therapy.

With respect to the evaluation of **Benefit**, the conclusion of this review is that substantial evidence of clinical efficacy was not established for eteplirsen in the treatment of DMD subjects amenable to exon 51-skipping. Similarly, this review concludes that there is no substantial evidence that any effect on the biomarker as evaluated by the Applicant is reasonably likely to predict clinical benefit. An expanded executive summary of the analysis of efficacy is found in **Section 7**.

With respect to the evaluation of **Risk**, The extent of patient exposure to eteplirsen was small and the studies were not designed to control for evaluating long-term safety. To date there have not been deaths in the program and a few serious adverse events and severe AEs that are consistent with DMD however they seem to occur more in the active treatment arms, which may reflect the trial design as noted above. Two key investigations, the test for urinary myoglobin and anti-dystrophin antibodies, were only reported for early studies (Labs for myoglobinuria in 28 without Myoglobinuria AEs and Myoglobinuria AEs in Study 33 without the reported lab. Anti-dystrophin antibody results only from Study 33) despite having a signal of concern for Myoglobinuria in those studies. These observations are made in the context of my recognizing that DMD is a fatal disease with no approved treatments. The deficiencies in safety assessments would not likely be an issue for approvability on their own but should be considered for the design of future trials.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
<p><b>Analysis of Condition</b></p>	<ul style="list-style-type: none"> <li>• Duchenne Muscular Dystrophy (DMS) is a fatal, degenerative, X-linked recessive genetic disorder associated with mutations in the dystrophin gene diagnosed between the ages of 3 to 5 years, when toddlers develop a waddling gait and inability to jump which progresses to loss of ambulation. While pulmonary and cardiac function are generally normal during early childhood, muscles of the heart and diaphragm progressively weaken during adolescence, and patients often die from cardiac or respiratory failure in their early 20s.</li> </ul>	<p>DMD is a serious and life threatening disease where a therapy with a true and meaningful treatment effect would be beneficial.</p>
<p><b>Current Treatment Options</b></p>	<ul style="list-style-type: none"> <li>• Currently there are no drugs approved for the treatment of DMD; Corticosteroids, both approved for other indications and those still in the investigational status, are used in an attempt to lessen the inflammation and slow disease progression. Central to the care of children with DMD is a rigorous program of respiratory therapy, adjunctive drug therapy (e.g., ace-inhibitors to decrease afterload), and non-medical therapy such as orthoses and physical therapy.</li> </ul>	<p>There is a substantial unmet need for therapies in DMD.</p>
<p><b>Benefit</b></p>	<ul style="list-style-type: none"> <li>• The development program consisted of one trial (Study 201/202) with a relatively short (24 week) placebo controlled portion (Study 201) and a segment which followed which was compared to a natural history cohort obtained by the Applicant (Study 202). The following are this Reviewer's concerns with the key endpoints:             <ul style="list-style-type: none"> <li>○ Comparisons to placebo in the 24 week portion of Study 201 / 202 were negative on tests of clinical function (6MWT) and biomarker data.</li> <li>○ Data from the long-term biomarker data seemed biased in terms of selection of controls. The effect after 36 Weeks of producing &lt; 1% of normal does not seem reasonably likely to me to predict a clinical benefit.</li> <li>○ Comparisons to natural history cohorts or literature references for the 6MWT, NSAA and PFTs did not show an improvement at the level discussed prior to NDA submission, i.e., greater than the variability associated with DMD and sufficient to overcome the uncertainty</li> </ul> </li> </ul>	<p>The placebo controlled portion of the clinical development program was uniformly negative. The long-term natural history comparisons were not adequately matched to the eteplirsen-treated subjects. It is therefore not clear that any differences between active and control in the long term open label portion of the program are due to treatment effect.</p>

	<p>inherent in historically controlled trials, and motivational factors that can affect the results. The cohorts were also not well matched showing more advance signs of disease progression (e.g., a higher percent of subjects with less than 350 M at baseline for the 6MWT or had fewer subjects on steroid therapy, in the case of the PFT comparator cohort than their eteplirsen counterparts).</p>	
<p><b>Risk</b></p>	<ul style="list-style-type: none"> <li>• Most of the events are also consistent with disease progression in DMD. The possibility that these signals appear disproportionately higher in the actively treated subjects may be related to the small sample size of actively treated subjects and the inadequate size and exposure duration of the comparator database. From my perspective, the deficiencies in safety assessments would not likely be an issue for approvability in their own right, but should be considered for the design of future trials.</li> </ul>	<p>The safety database is too small in terms of patient numbers exposed at the intended dose, the size of the placebo database, and the quality in matching of the natural history database. Several key safety investigations were not performed throughout the development program.</p>
<p><b>Risk Management</b></p>	<ul style="list-style-type: none"> <li>• If eteplirsen is approved, the following risk management approaches are recommended:           <ul style="list-style-type: none"> <li>○ Future clinical trials should be adequately designed to evaluate the safety profile of eteplirsen.</li> <li>○ The maximal tolerated dose should be determined and evaluated in a controlled clinical trial.</li> <li>○ A patient registry as a post-marketing requirement will help to evaluate the safety risks noted above in the postmarketing setting. An issue is that the premarket safety database was not adequate to ensure the type and magnitude of these risks is well defined. Labeling should be clear about the uncertainties and deficiencies of the eteplirsen clinical program.</li> <li>○ The potential for immunogenicity and rhabdomyolysis must be evaluated.</li> </ul> </li> </ul>	<p>If approved, a post marketing surveillance plan should be in place and re-evaluated on a regular basis to determine whether they are adequate to their purpose.</p>

## 2 Therapeutic Context

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### 2.1. Analysis of Condition

Duchenne Muscular Dystrophy (DMS) is a fatal, degenerative, X-linked recessive genetic disorder associated with mutations in the dystrophin gene. The mutations that cause DMD disrupt the mRNA reading frame and prohibit production of dystrophin, a critically important part of the protein complex that connects the cytoskeleton of a muscle fiber to the muscle cell membrane and extracellular matrix. In the absence of dystrophin, the stress of muscle contraction causes progressive muscle damage.

Duchenne muscular dystrophy is usually first diagnosed between the ages of 3 to 5 years, when toddlers develop a waddling gait and inability to jump which progresses to loss of ambulation [Emery 2002]. While pulmonary and cardiac function are generally normal during early childhood, muscles of the heart and diaphragm progressively weaken during adolescence, and patients often die from cardiac or respiratory failure in their early 20s.

Currently there are no drugs approved for the treatment of DMD; Corticosteroids, both approved for other indications and those still in the investigational status, are used in an attempt to lessen the inflammation and slow disease progression [Griggs *et al.* 2013]. Central to the care of children with DMD is a rigorous program of respiratory therapy, adjunctive drug therapy (e.g., ace-inhibitors to decrease afterload), and non-medical therapy such as orthoses and physical therapy [Birnkrant *et al.* 2010; Bushby *et al.* 2010; Sejerson and Bushby 2009]. An important aspect to the analysis of any study is careful documentation and reporting of the actual adjunctive therapies that were provided to the subjects.

## 3 Regulatory Background

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### 3.1. U.S. Regulatory Actions and Marketing History

#### 3.1.1. Summary of Presubmission/Submission Regulatory Activity

Sarepta is developing eteplirsen for the treatment of Duchenne Muscular Dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping.

- The Agency granted orphan drug designation and fast track designation for eteplirsen for the treatment of DMD on October 23, 2007, and November 27, 2007, respectively.
- The principle pre-NDA meetings/communications for this application were an advice letter dated April 15, 2014 and pre-NDA meetings on September 18, 2014, and May 19, 2015.

- The chronology of submissions from the applicant after and including the original NDA submission is detailed in **Appendix 1. Submissions from the Applicant**
  - **April 15, 2014** (Minutes: April 15, 2014) – The Agency provided the sponsor with a guidance letter describing FDA’s view of the clinical and biomarker data currently available for eteplirsen and proposed a strategy to consider regarding the submission of an NDA for eteplirsen. Two potential pathways to accelerated approval were outlined.
    - The first used the 6MWT data from 201/202 as an intermediate endpoint; however, the Division had serious concerns that this data did not demonstrate a significant treatment effect.
    - The second pathway involved the use of dystrophin quantification; however, the Division was also skeptical about the persuasiveness of the data and was concerned about serious methodological problems explained previously.

The possibility of a fourth biopsy demonstrating a robust effect was also discussed. The Division noted that if the accelerated pathway to approval was appropriate, confirmatory studies demonstrating a clinical benefit would be needed and that these should be underway at the time of approval. If the study were historically controlled, the effect size would have to be sufficient to overcome the uncertainty inherent in historically controlled trials, and motivational factors that can affect the results.

- **September 18, 2014** (Minutes: October 17, 2014) – A Type B, pre-submission meeting was held to discuss the strategy and content of an NDA submission for eteplirsen. Sarepta proposed to provide CSRs and integrated summaries of safety and efficacy for the complete (AVI-4658-33, AVI-4658-28, and 4658-us-201) and ongoing (4658-us-202) eteplirsen clinical studies.

The Division noted that the following issues needed to be resolved before considering an application for filing:

- The extent of patient exposure to eteplirsen was insufficient to adequately characterize the safety profile in patients with DMD, and we urged you to begin exposing additional patients as soon as possible, including patients both older and younger than those enrolled in previous eteplirsen studies
  - Regarding the conduct of a Natural History study, the Applicant needed to identify historical patients who are appropriately matched to the study 202 patients in measures such as *rise time and/or similar timed tests (e.g., NSAA), baseline factors including duration and dose of steroids, and intensity of physical therapy and other ancillary care that affect physical function*. Some of the analyses from study 201/202 were based on selecting the higher of two measurements, and comparison to historical data obtained from single measurements or average measurements would not be a valid comparison.
  - The Division has significant concerns about the ability of either your clinical or biomarker data to support approval. The overall persuasiveness of the efficacy data is more important than any single endpoint.
- **May 19, 2015** (Minutes: June 9, 2015) – A Type C, pre-NDA guidance meeting was held (Meeting Minutes 6/9/15) which focused on the content of the NDA. Topics discussed were the content and format of biomarker data and the difficulty in obtaining natural history data for clinical endpoints.

- Other key communications/milestones were the following:
  - **June 14, 2011** (Minutes: July 20, 2011) – A Type B (End of Phase 1) meeting was held after the proof of concept study, AVI-4658-33, and Phase 2 Study, AVI-4658-28, were completed (Minutes – July 20, 2011). A phase 2 study AVI-4658-201 was discussed to a target effective and well-tolerated dose is determined based on the Phase 1 and Phase 2 studies, further studies, including a pivotal registration study (or studies) are planned in patients in whom treatment based on exon 51 skipping may be efficacious. Key Points of this meeting are noted below:
    - Reliance on a single study and confirmatory evidence is generally limited to situations in which a trial has demonstrated strong evidence of clinically meaningful benefit
    - In general a placebo-controlled design using multiple fixed-doses is reasonable for supporting phase 3 development; however, it wasn't clear how much support could be provided by such a small and limited study
    - Since study 4658-US-201 is a phase II study and only 12 subjects will be randomized into three treatment groups (30 mg/kg, 50 mg/kg, and Placebo), the study results cannot be considered as pivotal efficacy evidence for the study drug. There was no further discussion on this point.
  - **July 12, 2012** – Receipt of change of Sponsor from AVI Biopharma, INC to Sarepta Therapeutics, Inc.
  - **March 13, 2013** (Minutes: April 12, 2013) – The Sponsor requested this meeting to seek the Division's opinion on the suitability of filing a New Drug Application (NDA) under Subpart H for eteplirsen to treat DMD (Minutes – April 12, 2013). Key Points of this meeting are noted below:
    - The Division commented that the specific quality and quantity of dystrophin produced by a drug is central to the question of if the effect can be considered reasonably likely to predict clinical benefit. Eteplirsen, by design, can only increase the production of truncated dystrophin, some of which may not be functional or result in conversion to the BMD phenotype.
    - The immunofluorescence data suggests that a much lower quantity of truncated dystrophin is produced by eteplirsen treatment than is present in BMD.
    - The Division did not find that Study 201 provides any interpretable evidence of benefit on 6MWT, as there was essentially no difference between drug and placebo based on the intent-to-treat population (even without consideration of multiple testing). Similarly, data from study 202 did not provide interpretable evidence of benefit given the limitations of the open-label design for protecting against bias on effort dependent endpoints like 6MWT. In fact, data from study 202 suggests that decline of 6MWT was similar to that expected from natural history (Mazzone [Mazzone *et al.* 2011]:  $42.3 \pm 73.9$  m/year; McDonald [McDonald *et al.* 2010]:  $57 \pm 104$  m/year). The Division expressed that there was no correlation between the dystrophin data and the 6MWD data through Week 62 and that they did not believe that an NDA filing for eteplirsen under Subpart H could be supported by available data.
    - To support filing of a Subpart H NDA for eteplirsen, the Applicant would have to provide adequate evidence that data collected on the biomarker is of sufficient quality to support meaningful regulatory review. In particular, they would need to document before filing an NDA that adequate steps were taken to minimize bias, and that a reliable quantitative

- assessment of drug effect was provided. The Division did not believe that information submitted to date provides adequate reassurance that an NDA would be fileable.
- If it is true that eteplirsen leads to remarkable clinical benefit in even some patients, there is no doubt that a feasible placebo controlled clinical study can be designed to demonstrate that benefit, and we remain eager to discuss such a possibility.
  - Up to this point Western blots had not been performed, the sponsor stated that although they believe that dystrophin assessment using the Western Blot was not as informative as the IHC, such assessment could be done.
  - Data from a confirmatory long-term open-label study may only be interpretable if a relevant objective endpoint obviously insulated from bias demonstrates compelling data that is clearly well outside the known variability range for DMD. For modest effects on clinical endpoints including the 6MWD, placebo-controlled data would seemingly be necessary to provide interpretable data. Upon further discussion on this point, the Division noted that a placebo-controlled design for the pivotal confirmatory trial appears justifiable and practicable. If that study proves impracticable, an open label study could be interpretable if the effect is large, well outside the known variability of the disease.
- **July 23, 2013** (Minutes: August 22, 2013) – A type C Meeting was held as a continuation of the discussion from the March 13, 2013.
- The truncated dystrophins may vary in both quality and quantity depending upon the particular mutation skipped; the functionality of each of these dystrophins in vivo is unknown, so the potentially functional dystrophin is reasonably likely to predict clinical benefit will be a review issue.
  - All of the muscle biopsies were obtained and processed by a single technician at a single study center, and immunofluorescence was quantified by a single muscle pathologist. Since image interpretation is susceptible to bias, and analyses of medical images require scrupulous attention to, and documentation of, blinded analysis. The Sponsor was also asked to confirm, by an independent laboratory, the immunohistochemical findings for dystrophin and associated proteins in the previously collected tissue blocks.
  - The Division raised concerns about the use of fluorescence intensity since without precise means of calibration; it is not a reliable quantitative method. The Division reiterated that that Western blot data with appropriate calibration would be useful to quantify the dystrophin produced by eteplirsen, and that the Division would work closely with the Sponsor to agree on a protocol for conducting these analyses.
  - The overall safety database at this time included only 38 patients exposed to eteplirsen by any route, dose, or duration.
- **November 6, 2013** (preliminary comments from planned teleconference) – Further concerns were discussed on the following issues:
- The specificity of the antibody proposed for quantification of truncated dystrophin protein
  - The correlation between protein levels and skipped transcript levels. Poor correlation may exist between mRNA and protein levels. Recent findings by suggest that antisense-mediated exon skipping in DMD may result in lower amounts of complete transcripts
  - Considerable doubt is also cast on the efficacy support provided by your ongoing open-label study (4658-us-202, 96-week data submitted), in which baseline 6MWT was >350 meters for all patients, as the intent-to-treat analysis showed no difference between drug and placebo, and the expected variability of 6MWT values appears sufficient to explain differences between arms on which the post-hoc analysis was based

- The Division believed that a placebo-controlled trial would be the most likely method for developing interpretable evidence of efficacy for eteplirsen, because efficacy endpoints in DMD are effort-dependent and susceptible to bias, and the natural history is highly variable and has recently improved with steroid use and advances in ancillary care. The Division stated that they would like to discuss the perceived barriers to conducting such a trial with the Applicant. To increase the feasibility and acceptability to patients of a randomized placebo-controlled trial if drug supply is not otherwise limiting, the Division proposed an ‘early exit’ provision for patients who meet a primary endpoint based on clinical progression, so as to limit an individual patient’s exposure to placebo.
- **December 17, 2013** – (Minutes: December 17, 2013) Further concerns were discussed on the following issues:
  - Sarepta stated that they had reevaluated the feasibility of a placebo-controlled study in light of all Agency feedback received to date, and remained convinced that an open-label study versus an untreated age- and eligibility-matched control group can provide the necessary evidence required for eteplirsen’s marketing approval. The Division stated that they continued to have reservations about the Applicant’s proposed clinical trial design.
- **April 23, 2014** – (Meeting Minutes: May 02, 2014) Further concerns were discussed on the following issues:
  - FDA commented that the raw data did not seem to fully support the qualitative and quantitative conclusions submitted by Sarepta.
  - Dr. Rao said the Western Blot data submitted by the sponsor contributed to our lack of confidence in the overall dystrophin conclusions presented by the sponsor. Issues with the data included over-filled protein gels. The sponsor agreed that the Western Blot data were inadequate.

### 3.2. Foreign Regulatory Actions and Marketing History

Eteplirsen is not marketed outside of the USA.

## 4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

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### 4.1. Office of Scientific Investigations (OSI)

The US-201 and 4658- 202 studies were inspected at the Dr. Mendell’s site at Nationwide Children’s Hospital. The review included an inspection of the IRB records, sponsor and monitor audit activities, financial disclosures, adverse events reporting, Informed Consent Documents for all subjects, the medical records/source data for 8 subjects enrolled, and observation of four subjects performing their individual subject level 6-Minute Walk Test (6MWT), individual subject level data for other functional assessments such as North Star Ambulatory Assessment (NSAA), Maximum Voluntary Isometric Contraction Test (MVICT), Rise Time, 10- Meter Run Time, Timed 4-Step Test, and pulmonary function tests. There was no evidence of inaccuracy of the data captured on the above metrics.

#### 4.2. **Office of Regulatory Affairs / Investigations**

A limited High Priority Data Validation Inspection [FACTS #8771400] was done in accordance with a CDER memo dated 5/12/2014 and CP 7348.001. This was a joint inspection conducted by Karen M. Kondas, ORA Investigator. The following individuals from CDER also participated in the inspection: Richard Moscicki, MD, Deputy Director, CDER; Ellis Unger, MD, Director, OND/ODEI; Young Moon Choi, PhD, Pharmacologist, DBGLP/OSI; Ashutosh Rao, PhD, Pharmacologist, OPS/OBP. The laboratories at Nationwide Children's Hospital Research Institute analyzed muscle tissues and blood samples that were collected during AVI-4658-US-201/202 to the quantify dystrophin expression and immunity from studies 201 / 202. This inspection focused mainly on the laboratory practices and procedures related to muscle biopsy collections and immunofluorescence histochemistry methods and analysis. At the end of the inspection an FDA 483 was not issued. Details of observations from this inspection are presented and discussed in **Section 4.1**.

## **5 Sources of Clinical Data and Review Strategy**

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### **5.1. Table of Clinical Studies**

The Table of Studies (**Table 1**) follows on the next page.

### **5.2. Review Strategy**

One primary medical review will be performed for this NDA that combines efficacy and safety evaluation. Where applicable, comments and the review opinions of Dr. Ashutosh Rao will be included on matters related to the methodology and technical interpretation of biomarker experiments and are prefixed with **[AR]**.

**Table 1 Completed and Ongoing Studies included in the 120-Day Safety Update**

Descriptor	Study Number						
	4658-33	4658-28	4658-us-201	4658-us-202	4658-203	4658-204	4658-301
Study Design	Investigator-sponsored, single-blind, placebo-controlled, dose-escalation, proof-of-concept	Open-label, multiple-dose, dose-ranging, safety, tolerability, efficacy, and PK	Randomized, double-blind, placebo-controlled, multiple-dose, efficacy, safety, & PK	Multi-center, open-label, multiple-dose extension study to Study 201	Multi-center, open-label, multiple-dose safety & efficacy in pts with early stage DMD	Multi-center, open-label, multiple-dose safety & efficacy in pts with advanced stage DMD	Multi-center, open-label, multiple-dose efficacy & safety
Study Status	Completed	Completed	Completed	Ongoing	Enrolling	Enrolling	Enrolling
No. Pts. Planned	7	18-24	12		40	20	160 (80 treated and 80 untreated)
No. Pts. Enrolled	7	19	12	12	4	24	48 treated and 15 untreated
No. Pts. Completed	7	18	12	NA	NA	NA	NA
No. Pts. Ongoing	0	0	0	12	4	24	48 treated and 15 untreated
Study Population	Male, non-ambulatory DMD patients amenable to exon 51 skipping	Male, ambulatory <sup>a</sup> DMD patients amenable to exon 51 skipping	Male, ambulatory DMD patients amenable to exon 51 skipping	Patients who successfully completed Study 201	Male, DMD patients amenable to exon 51 skipping	Male, non-ambulatory <sup>b</sup> DMD patients amenable to exon 51 skipping	Male, ambulatory DMD patients amenable to exon 51 skipping
Required Age at Entry	10 to 17 yrs	5 to 15 yrs	7 to 13 yrs	NA	4 to 6 yrs	7 to 21 yrs	7 to 16 yrs
Actual Age at BL	10 to 16 yrs	6 to 13 yrs	7 to 11 yrs	NA	4 to 6 yrs	8 to 19 yrs	7 to 16 yrs
Treatment & Regimen	<u>Eteplirsen</u> : 0.09 mg or 0.9 mg (IM) in the EDB muscle of 1 foot <u>Placebo</u> (IM) in the EDB muscle of the opposite foot Single dose	<u>Eteplirsen</u> : 0.5, 1, 2, 4, 10, or 20 mg/kg Once weekly IV infusion for 12 wks	<u>Eteplirsen</u> : 30 or 50 mg/kg, or <u>Placebo</u> * *Placebo: 24 wks, followed by 4 wks of eteplirsen at 30 or 50 mg/kg Once weekly IV infusion for 28 wks	<u>Eteplirsen</u> : 30 or 50 mg/kg Once weekly IV infusion for up to an additional 212 weeks after completing Study 201	<u>Eteplirsen</u> : 30 mg/kg Once weekly IV infusion up to 96 wks <u>Untreated control</u> : DMD patients not amenable to exon 51 skipping	<u>Eteplirsen</u> : 30 mg/kg Once weekly IV infusion up to 96 wks	<u>Eteplirsen</u> : 30 mg/kg Once weekly IV infusion up to 96 wks <u>Untreated control</u> : DMD patients not amenable to exon 51 skipping
CSR / Original NDA: Data Cut-off Date	01 Apr 2009	08 Jun 2010	29 Feb 2012	01 Apr 2015 (Week 185 °)	NA	16 Apr 2015	NA
D120 Update: Data Cutoff Date				12 Aug 2015 (Week 208 °)	14 Aug 2015	14 Aug 2015	14 Aug 2015

Source NDA 206488 S0018, Summary of Clinical Safety, Table 2, p 16 of 184

## **6 Review of Relevant Individual Trials Used to Support Efficacy**

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### **6.1. 4658-us-201: Randomized, Double-Blind, Pbo-Controlled, Single and Multiple-Dose, Dose-Escalation Safety, Tolerability, Pharmacokinetic, and Efficacy Study of AVI-4658, a Phosphorodiamidate Morpholino Oligomer, Administered Over 12 Weeks in the Treatment of Ambulant Subjects with Duchenne Muscular Dystrophy**

#### **6.1.1. Study Design**

##### **Overview and Objective**

The stated objectives of this study are to assess the safety, efficacy, and tolerability of 12 once-weekly intravenous (i.v.) doses of AVI-4658 in ambulant subjects with DMD. A secondary objective is to explore the pharmacokinetic (PK) profile of different i.v. doses of AVI-4658 in subjects with DMD.

##### **Trial Design**

- Basic study design

This is a single-center, randomized, double-blind, PBO-controlled intended to assess the safety, tolerability, PK, and exploratory efficacy of 12 once-weekly i.v. doses of AVI-4658 in subjects with genotypically confirmed DMD. Activities in Weeks 25-28 (Visits 26-29) were limited to safety assessments (Weeks 25-28) and PK at Week 25/Visit 26.

Study 202 is an open label multiple dose (30 and 50 mg/kg/week) extension of an additional 212 weeks (and currently ongoing) for subjects who completed Study 201. Results from the 201 study (placebo-controlled) will be discussed together followed by a discussion of results from Study 202, which includes comparisons to natural history and untreated cohort.

- Population
  - Key Inclusion / Exclusion Criteria

##### **Inclusion**

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)
2. Be between the ages of 7 and 13 years, inclusive.
3. Have stable cardiac function and stable pulmonary function (forced vital capacity [FVC]  $\geq 50\%$  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.
5. Have intact right and left biceps muscles or an alternative upper arm muscle group.
6. Achieve an average distance within 200 m and 400 m  $\pm 10\%$  (i.e. within 180 m and 440 m) while walking independently over 6 minutes.

7. Have a left ventricular ejection fraction (LVEF) of >40% based on the echocardiogram (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  $\beta$ -blockers for at least 24 weeks before study entry.

#### Exclusion

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 or planned surgery at any time during the study.
  5. Presence of other clinically significant illness at the time of study entry, including significant renal dysfunction (as measured by urinary cystatin C, kidney injury molecule (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  $\beta$ -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).
- Study Treatments
    - Dose Selection

According to the Applicant, the doses of eteplirsen administered in this study, 30 or 50 mg/kg/wk, were expected to be well tolerated based on preclinical data in non-human primates and mice

- Schedule of Events

**Table 2 Schedule of Key Events for Study 201/202**

Visit	1	2	3	4	5	6	7	8	9	10	11	12	13		14	15	16	17	18	19	20	21	22	23	24	25		
Week	--	1	2	3	4	5	6	7	8	9	10	11	12	12.5	13	14	15	16	17	18	19	20	21	22	23	24	24.5	
Randomization		X																										
Dosing		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	
<b>Efficacy and Pharmacodynamics</b>																												
PedsQL	X												X														X	
Muscle biopsy	X <sup>s</sup>													X <sup>1,t</sup>														X <sup>2,t</sup>
Functional tests <sup>#</sup>	X <sup>v</sup>				X				X				X <sup>v</sup>					X					X				X <sup>v</sup>	
9-Hole Peg Test	X																										X	
<b>Pharmacokinetics</b>																												
PK – blood		X <sup>p</sup>											X <sup>q</sup>														X <sup>p</sup>	
PK – urine													X <sup>r</sup>															
<b>Safety Assessments</b>																												
Physical exam	F <sup>c</sup>	B <sup>de</sup>	B <sup>df</sup>	B <sup>df</sup>	B <sup>df</sup>	B <sup>df</sup>	F <sup>cf</sup>	B <sup>df</sup>	F <sup>cf</sup>		B <sup>df</sup>	F <sup>cf</sup>	B <sup>df</sup>	F <sup>cf</sup>														
Vital signs <sup>g</sup>	X	X <sup>i</sup>	X	X <sup>i</sup>	X <sup>i</sup>	X																						
Weight <sup>h</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																										X	
ECG	X												X <sup>k</sup>														X <sup>k</sup>	
ECHO (EF, FS)	X	X <sup>l</sup>											X <sup>k</sup>														X <sup>k</sup>	
ELISPOT	X						X						X							X							X	
Safety lab tests <sup>n</sup>	X	X <sup>w</sup>	X		X		X		X		X		X <sup>w</sup>				X			X			X				X <sup>w</sup>	
AE questioning <sup>o</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PFTs	X <sup>j</sup>												X <sup>j</sup>														X <sup>j</sup>	

Source: Clinical Study Report 4658-us-201; Event; # - 6MWT, Timed4-Step Test, NSAA, MVIC

in which maximum feasible doses (320 mg/kg/wk and 960 mg/kg/wk, respectively) were described as well tolerated when administered for 12 weeks. In addition, in study AVI-4658-28, the highest dose of eteplirsen tested, 20 mg/kg/wk for 12 weeks, was described as well tolerated by all 4 patients dosed. Moreover, 1 patient in this dose group was said to have shown an increase in dystrophin-positive fibers from 3% at baseline to 55% at Week 14. This same patient was reported to have had approximately 50% greater  $C_{max}$  (maximum observed concentration) and AUC (area under the concentration curve) of eteplirsen than the remaining 3 patients in that group, suggesting to the Applicant that higher doses of eteplirsen could lead to a more consistent response in dystrophin expression.

– Assignment to Treatment

Dosing assignment for the 12 subjects in this study is demonstrated in **Table 3**.

**Table 3 Treatment Sequence Assignment in Study 201/202**

Group	Treatment/Dose of Eteplirsen	N
1	50 mg/kg/wk eteplirsen for 28 weeks	4
2	30 mg/kg/wk eteplirsen for 28 weeks	4
3a	Pbo for 24 weeks followed by 50 mg/kg/wk eteplirsen for 4 weeks	2
3b	Pbo for 24 weeks followed by 30 mg/kg/wk eteplirsen for 4 weeks	2

Source: Clinical Study Report 4658-us-201

Following Week 28, subjects continued on their therapy through Week 196 in Study 202.

– Blinding

The patients, Sponsor, and all research personnel were blinded to treatment assignment during the first 24 weeks of this study, except for:

1. 1 unblinded statistician and 3 statistical programmers who produced data presentations for the DSMB
2. 2 unblinded site personnel who verified dose and dispensed study treatment
3. 1 unblinded clinical study monitor.

Beginning Week 25, all parties were aware that all patients were receiving 50 or 30 mg/kg/wk eteplirsen during the last 4 weeks of the study. Moreover, laboratory assessments, electrocardiograms (ECGs), echocardiograms (ECHOs), vital signs, and pulmonary function tests (PFTs) were generally stable over the course of both studies.

– Concomitant Medications

All patients, regardless of treatment assignment, were required to be on a stable dose of corticosteroids at study entry and to remain on that dose (as clinically indicated) for the duration of the study.

- The following concomitant medications were allowed however, attempts to keep the dosage constant throughout the treatment period were to be made:

- Oral corticosteroids including, but not limited to, prednisolone, prednisone, and deflazacort
- Oral ACE inhibitors including, but not limited to, perindopril and Lisinopril
- Oral  $\beta$ -blockers (stable dose for 24 weeks) including, but not limited to, carvedilol and Atenolol
- Angiotensin-receptor blockers including, but not limited to losartan, irbesartan, valsartan, and candesartan
- Oral laxatives including, but not limited to, lactulose, Senokot, and Movicol
- Vitamin D and calcium supplements
- Over-the-counter herbal preparations, including herbal supplements, vitamins, minerals, and homeopathic preparations, provided the patient had been on stable doses for 24 weeks before enrollment in this study (e.g., bisphosphonates or other non-RNA antisense medications)
- The following concomitant medications were not allowed
  - Initial prescription of intranasal and/or inhaled and topical steroids for a condition other than DMD in the week before enrollment in this study or during the study period
  - Investigational agents for the treatment of DMD within 12 weeks of entry into this study;
  - Use of the shock training system or STS or planned use during this study
  - Previous exposure to eteplirsen, BMN-195, or PRO051
  - Any medication with the potential to affect muscle mass, strength, and/or function, such as, but not limited to, growth hormone, within 12 weeks before enrollment in this study
  - Immunosuppressants (other corticosteroids) during the screening period or while on study
  - Use of aminoglycoside antibiotic during the study (unless discussed and agreed with the Principal Investigator and Medical Monitor)
- Study Endpoints
  - Primary Efficacy Endpoint

The primary efficacy endpoint is the change from Baseline in percent of dystrophin positive fibers as measured in the muscle biopsy tissue using immunohistochemistry (IHC) at Week 12 for groups 1 and 3a and at Week 24 for groups 2 and 3b<sup>2</sup>.

### Methodology

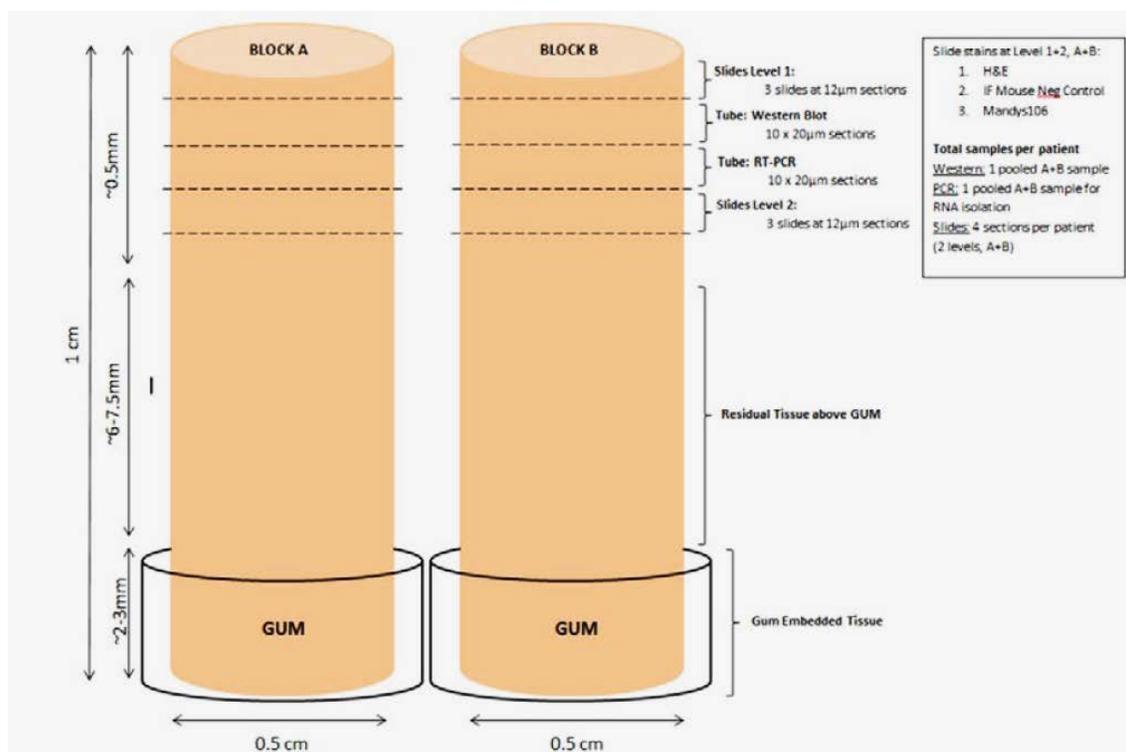
During the **biopsy procedure**, sample tissue of approximately 5 mm<sup>3</sup> in size was removed from the patient's biceps muscle. A pre-treatment muscle biopsy was taken from the biceps of all subjects who were enrolled in study 201 prior to treatment. While on treatment, biopsy samples were collected from subjects in study 201 at week 12 or 24 depending on the dose of Eteplirsen that was administered in the study. Four subjects who were administered a dose of 50 mg/kg and two subjects on Pbo in the 50 mg/kg cohort were biopsied at week 12 in the study. Four subjects who were administered a dose of 30 mg/kg and two subjects on Pbo in the 30 mg/kg cohort were biopsied at week 24. In study 202, biopsy samples, i.e., block A and B, were collected from the deltoid muscle of each subject at Week 20. The week 20 of study 202 was 48 weeks after the subject started treatment in study 201. Slides were prepared after tissue sectioning and labeled by [a staff member] based on the blinding key that [the staff member] created.

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<sup>2</sup> Protocol 4658-US-201 Statistical Analysis Plan 20 February 2012, p. 20 of 25

**Digital capture of images**, per the applicant, was originally performed on the same microscope using the 20X objective from four areas of each stained muscle tissue section, one image from each quadrant of the section, to ensure broad sampling of tissue. Areas of the tissue section that contained processing artifacts and the edges of sections were avoided. A total of 24 images were captured for each patient biopsy time point-4 images from each of 3 section levels for the 2 biopsy segments. Digital camera exposure was controlled and normalized across all batch processing days by setting optimal digital camera exposure conditions from positive control (normal muscle) slides that were included in each batch of stained slides.

**Figure 1 Tissue Biopsy Processing for Study 201/202**



Source – 4658-us-202sr-cr-15-002, p 9 of 23.

These exposure conditions were supposed to be used for image acquisition of all samples in the same batch. Area lighting was turned off and window shades were drawn over all windows in hallway. Analysis was said to be avoided during times of day when light from shaded windows affected viewing of monitor. Original captured RGB images were analyzed in ImageJ (NIH software) using the Cell Counter plug-in.

**Scoring of positive fibers** was performed using unadjusted images (e.g. no adjustment to intensity). Criteria for scoring a fiber as positive included:

- Minimum fiber diameter of roughly 5 µm
- Fibers at image edges were scored if adequate circumference was judged to be visible by eye (typically at least 10-20% of circumference)
- Majority of fiber perimeter intensity was judged by eye to be above background of image

After positive fibers were scored for an image, the image contrast was increased to levels that allowed visualization of all fibers. This adjustment varied between images and was based on the judgment of the analysts that allowed for visualization of fiber perimeters without generating so much pixelization that it obscured fibers. The analyzed image including an image mask that indicated fibers scored as positive or negative was saved as .jpg RGB file with the enhanced contrast level as analyzed for negative fibers. This image documented the scoring state (e.g. positive, negative) for every image that was analyzed.

The **Data Analysis** steps performed by Sarepta were reported as:

- For each image, percent positive fibers calculated as (# positive fibers / (#neg fibers+#pos fibers))\*100
- Average % positive fibers and standard deviation was calculated for 24 images of each biopsy.
- Summary results reported as cumulative results from A and B biopsy.
- Final effects of Eteplirsen treatment on positive fibers calculated to account for revertant fibers present in pre-treatment biopsies.
  - Final effect of treatment on % positive fibers = (% positive on-treatment) – (% positive pre-treatment)

**Reanalysis of Immunohistochemical Data** After breaking the blinding code on 4/5/2012, the 2<sup>nd</sup> Bioquant analysis of the 30 mg dose cohort (i.e., subjects 01002, 01006, 01009, and 01010) with the exception of PBOs (i.e., subjects 01007 and 01008) was performed using images taken at 40x magnification. For each subject, eight images at 40x magnification were captured between 4/12/2012 to 4/17/2012 (i.e., 4 images from block A and 4 images from block B). Similarly, the 2<sup>nd</sup> Bioquant analysis of 50 mg dose cohort was performed on 10/31/2012 including all four Pbo subjects (i.e., subjects 01003, 01004, 01005, 01007, 01008, 01012, 01013, and 01015).

**Reviewer’s Analyses and Comments on Methodology from Biopsies 1 to 3** – The following passages were extracted from the Agency’s ORA Inspection Report of the Nationwide Children’s Hospital facility<sup>3</sup> (see **Section 4.2 Office of Regulatory Affairs / Investigations**)

- *The blinding procedure was not ideal because (1) the same analyst designed the blinding key and performed the field selection on the microscope, (2) the 48-week biopsies were processed and analyzed after unblinding of the NML laboratory and there was no documentation that confirmed that the analysts remained blinded, (3) reacquisition and analysis of all images at 40x for Bioquant analyses was done post unblinding and, as per Dr. Mendell, positive fields were uniquely selected for further quantitation, and (4) the pre/post treatment samples were paired as Scarlet or Red leaving the possibility that one could try to guess the other sample after reviewing one of the paired samples.*
- *The data that were obtained from the images taken at 20x magnification were obtained before the blind was broken; these data were not used.*

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<sup>3</sup> Report FEI#(#3007522723) (Dates of Inspection: 5/29/2014-5/30/2014)

- *During the inspection, [the staff member] stated that before the allocation key was received on 7/17/2012, several tissues from subjects 01002, 01003, 01004, 01005, 01006, and 01008 had already been sectioned and the slides were prepared without an allocation number to maintain the blind. Photographs of slide images for subjects 01002, 01003, and 01004 were captured without using allocation numbers to maintain the blind.*
- *It was noted that the workstation was located in a hallway outside the main laboratory room and adjacent to non-darkened glass windows and an entryway door with traffic between the main laboratory and staff offices. The ambient lighting was quite variable, depending on the angle of the sun and cloud cover. At certain times of the day, [the rater] would simply have to discontinue [their] analyses because of room lighting.*
- *Although digital images were analyzed, they were not subjected to digital image analysis per se; they were analyzed by visual inspection of a single observer [the staff member] explained that the entire tissue section was first manually and virtually divided into 4 quadrants. One image was acquired from each quadrant. The [staff member] stated that she first viewed the entire slide. Then she started from the left-hand top edge of the slide and proceeded to find a continuous field of fibers without any debris or artifacts.*
- *The laboratory made no attempt to assess inter- or intra-observer variability.... [For example], on the first day of the inspection, [The rater] demonstrated the process by re-analyzing 2 images she had previously examined for Study 201. The immunostaining was red in color. For the first image, [the rater] counted 61 positive and 68 negative muscle fibers. Previously, [the rater] had recorded 8 positive and 135 negative fibers. For the second image, [the rater] counted 11 positive and 90 negative muscle fibers. Previously, [the staff member] had counted 50 positive and 80 negative fibers.*

Revised methodology after FDA input was used more recently for the 4<sup>th</sup> biopsy at week 180 of exposure, as described below:

**Additional Methodology for the 4th biopsy (not included in inspection discussed above)**

Per the revised protocol, tissue was to be collected from untreated DMD, Becker' Muscular Dystrophy (BMD) and non BMD/DMD patients to serve as control for the fiber counts and immunohistochemistry intensity assay and the Western blots. Images for immunohistochemical intensity and fiber counts were to be obtained in a different manner than for previous samples. A systematic random sampling method (raster grid), utilizing unbiased sampling rules was to be employed to select microscope fields for image capture. The area of tissue section was divided by the number of desired images, in this case four images per section. The microscope stage was repeatedly stepped using a series of systematic steps following a raster pattern.



- (b) (4) *that the lights were not always turned off during image analyses because other users needed to use the room... The considerable variation in image brightness and contrast could have been avoided by using a modern computer monitor (LED or better) with a wide viewing angle in a windowless room.*
- *The thresholding of images in Bioquant was not pre-validated to minimize variability and subjectivity at the time of each analysis depending on the healthy control or first DMD sample examined at that time.*
- *FDA asked how fields were chosen for the images used for Bioquant. (b) (4) that images were acquired by (b) (4). During subsequent discussions with (b) (4) (b) (4) stated that the 40x images were acquired from fields that were preferentially selected for their high fluorescence intensity to clearly show the increased intensity. (b) (4) explained that the images were re-acquired at 40x because, based on discussions with the Sponsor, the 20x images were deemed not suitable for thresholding because magnification at 20x did not allow for optimal differentiation of the muscle fibers for quantitation.*
- *The inspection team and NML scientists acknowledged that the procedures for study 201/202 were intended for a phase 2 study and not intended as a pivotal study.*

#### 4. Total Dystrophin Protein (assessed by Western blot analysis)

For each biopsy sample, at least ten, 10- mcM frozen sections were pooled and homogenized using a standard SDS buffer solution, then stored at -20C until use. 75 mcg total protein was loaded onto a denaturing polyacrylamide gel. Sample loading efficiency was confirmed by uniform signal of muscle specific actin internal loading control. Following electrophoresis, protein was transferred from the gel to a PVDF membrane. The membrane incubated with MANDYS106, DYS2, or the DYS1 antibody. The membrane was washed again and incubated with a secondary antibody conjugated to horseradish peroxidase and visualized by standard enhanced chemoluminescent techniques. The proper size of the dystrophin protein (~427kD) was verified through the use of standard size markers, and intensity was compared to a homogenate from normal muscle tissue.

To test for non-specific binding and signal, the applicant used their secondary antibody only (a sheep-derived, anti-mouse IgG (b) (4)) as a negative control. During their Bioquant analysis, the applicant similarly uses a negative control for the Mandys106 primary antibody that has samples probed with secondary antibody only (no primary). In case of Bioquant, the background signal is subtracted from the average field density values for the test and positive control sample images (per protocol SR-CR-15-007).

#### ***Reviewer's Analyses and Comments***

The following passages were extracted from the Agency Inspection Report of the Nationwide Children's Hospital facility<sup>3</sup>:

- *Dr. Rao suggested that lowering the concentration of the healthy and DMD samples might provide cleaner results and better resolve the high molecular weight bands. If there are many high molecular bands around 427 kDa to allow an objective resolution of the dystrophin band(s), they could consider running an electrophoresed sample on a second gel or on a second dimension on the same gel to allow better resolution based on size or charge of the high molecular weight proteins close to or related to dystrophin.*
  - (b) (4) *acknowledged that the quality of the Western blots performed so far was not optimal.*
  - *No testing or confirmatory assays had been performed to confirm that the ~427 kDa band labeled as dystrophin on the membranes in regulatory submissions was truly full-length dystrophin. Dr. Rao advised (b) (4) to include healthy, DMD, and BMD samples as positive, negative, and intermediate control samples for validating the specificity of assay and identifying the dystrophin-specific bands.*
5. Exon skipping (assessed by reverse transcriptase-polymerase chain reaction [RT-PCR]) at Week 12 for groups 1 and 3a and Week 24 for groups 2 and 3b

The presence or absence of exon skipping expected to be induced by eteplirsen (deletion of exon 51) was assessed in RNA isolated from tissue sections from the same muscle biopsies using a gel-based nested reverse transcriptase polymerase chain reaction (RT-PCR). The applicant sequenced the amplified mRNA to identify and confirm an exon 51-skipped sequence pattern. Primers were designed specific to each patient's genotype and for their ability to detect a product of specified size for the exon 51-skipped and non-skipped sequence. The band size was visualized using agarose gel electrophoresis.

6. Changes from Baseline in the following clinical assessments:
- a. Other NSAA components (i.e., those not included among the key endpoints listed above).
  - b. 9-hole Peg Test results.
  - c. Pediatric Quality of Life Inventory (PedsQL), (including the neuromuscular module) results.
  - d. Pulmonary function testing (PFT) measurements (forced vital capacity [FVC], percent predicted FVC, forced expiratory volume in 1 second [FEV1], FEV1%, FEV1/FVC ratio, maximum inspiratory pressure [MIP], and maximum expiratory pressure [MEP]).
- Methodology for Selecting Historical Controls
    1. Tests
      - A. 6MWT – To serve as historical control data for comparison with eteplirsen-treated DMD patients (n=12), individual patient data for the 6MWT and NSAA in untreated patients were obtained by the Applicant from Professor Eugenio Mercuri, MD, PhD, from the Catholic University in Rome on behalf of the Italian DMD Registry database (n=97) and from Professor Nathalie Goemans, MD, from the University Hospitals in Leuven (n=89). From these 186 patients (all with genetically-confirmed diagnosis of DMD), 50 patients had a genotype amenable to exon skipping therapy, were using corticosteroids at Baseline, had available 6MWT data at baseline, and were age  $\geq 7$  years. Among these 50 patients, there were 13 patients with a genotype specifically amenable to exon 51

skipping therapy.

- B. NSAA – As with the 6MWT, NSAA total score data from eteplirsen-treated patients in combined Studies 201/202 were compared with longitudinal data from matched historical control cohorts. The most closely matched DMD patients were those amenable to exon 51 skipping (N=10).
- C. Endpoints for this study included 6 MWT and NSAA. As part of the routine assessments in all centers, patients are seen at least once every 12 months, and all centers performed the NSAA followed by the 6MWT for 36 months.

## 2. Population

- A. Mercuri – Italian Patients were recruited between January 2008 and June 2010 and were to be followed for at least 3 years. Patient inclusion criteria at baseline were:
  - Genetically proven DMD diagnosis
  - Still ambulant and able to walk independently for at least 75 meters
  - No severe or moderate learning difficulties or behavioral problems.
  - Registry participants were categorized based on the respective corticosteroid regimen they had received at baseline:
    - No steroids: boys who had never been on steroids and others who had used them for less than a year and had stopped treatment at least one year before the study;
    - Intermittent regimen: patients who had been, at the time of the study, on alternate days or alternate weeks or 10 days on/10 days off of either 0.75 mg of prednisone or 0.9 mg/kg/day of deflazacort for at least a year;
    - Daily regimen: patients who had been, at the time of the study initiation, on daily treatment of 0.75 mg of prednisone or 0.9 mg/kg/day of deflazacort for over a year, also including those in whom the dose had not been always completely adjusted to the current weight. A small number of patients who took deflazacort on alternate days but with a dose of approximately 2 mg/kg were also included in this group as their monthly dose was similar, if not higher, to those with a standard daily dose of steroids.
- B. Leuwen - All DMD subjects up to 17.5 years of age attending the NMRC between January 2007 and September 2012 were assessed for eligibility. Genetic data, treatment information (type of corticosteroid, dosage, duration of treatment and regimen) and anthropometric measurements (weight, height measured according to standard anthropometric methods) were collected from patients enrolled into the registry. Key inclusion criteria were:
  - Genetically proven diagnosis of DMD
  - Age <17.5 years
  - Being on chronic daily treatment with corticosteroids

Sixty-five DMD patients meeting the inclusion criteria for the registry were identified. All were on daily corticosteroids, with 90% of patients treated with deflazacort

### ***Reviewer's Comment's***

The Applicant has reported to apply filters such as (A) Age>7 y (B) Genotype (Exon 51 skipping) (C) Steroid Use (D) Sufficient longitudinal 6MWD data. However as may be seen in Figures 3-7 below, these did not produce “matched” cohorts. Rather the active treatment (eteplirsen) group

seemed at an advantage when subjects were categorized by age and baseline measures.

- Safety Assessments
  1. The frequency and severity of adverse events (AEs), serious adverse events (SAEs), and discontinuations due to AEs
  2. Safety laboratory tests including hematology, coagulation, and serum chemistry assays (including serum cystatin C) and urinalysis (including urinary cystatin C and KIM-1)
  3. Immune response to dystrophin as assessed by enzyme-linked immunosorbent spot assay.
  4. Vital signs
  5. Physical examinations
  6. 12-lead electrocardiograms (ECGs)
  7. ECHO
  
- Pharmacokinetics

Plasma and urine samples were collected over 24 hours post-end of infusion on Week 12 and at 5 post-end of infusion on Weeks 24 and 25. The PK parameters characterized included time (T<sub>max</sub>) and value of maximum plasma concentration (C<sub>max</sub>), the apparent volume of distribution at steady state (V<sub>ss</sub>), the elimination half-life (t<sub>1/2</sub>), areas under the plasma concentration-curve (AUC), total clearance (CL), mean residence time (MRT), and renal (i.e., urinary) clearance (C<sub>IR</sub>).

- Statistical Analyses
  1. Populations
    - **Full Analysis Population** – Efficacy analyses were performed using the full analysis population, which included all 12 patients.
    - **Safety population** – Safety analyses included all 12 patients.
    - **PK population** – Pharmacokinetic analyses included all 12 patients.
  2. Pre-specified methods of handling missing data

No imputation of values for missing data was performed.

3. Statistical methodology used to adjust for multiplicity

No method to adjust for multiplicity was found in the statistical analysis plan<sup>4,5</sup>.

4. Interim analysis (if applicable) and statistical corrections

A blinded interim safety analysis was performed by an independent Data Safety Monitoring Board (DSMB) after the patients in Groups 1 and 3a completed the Week 12 muscle biopsy, and again after Groups 2 and 3b completed the Week 24 muscle biopsy.

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<sup>4</sup> 201 / 202 Clinical Study Report, 4658-us-201-e3-16-1-01, P 132 of 637

<sup>5</sup> SAP 4658-201-e3-16-1-09.pdf, from February 20, 2012

## 5. Primary Analysis

The primary efficacy endpoint was analyzed by comparing the 50 mg/kg/wk eteplirsen treatment group (Group 1) at Week 12 to the combined Pbo treatment group (Groups 3a and 3b), and the 30 mg/kg/wk eteplirsen treatment group (Group 2) at Week 24 to the combined Pbo treatment group using the change from baseline values.

### **Protocol Amendments**

Significant protocol amendments for Study 201 / 202 are found in **Table 4**. The first treatment was administered on August 15<sup>th</sup>, 2011<sup>6</sup>, so Amendments 5 and those after occurred while the treatment phase of the trial was underway.

### ***Reviewer's Comments***

From this Reviewer's perspective, changes in bold, italics had the most impact on the originally designed protocol. The study was essentially redesigned from its original state to a different type of study.

### **Disposition of Subject**

12 subjects participated in this trial through approximately Week 196.

### **Protocol Violations/Deviations**

Protocol Violations and deviations were reviewed from Protocol Listing 16.2.2 Protocol Deviations Safety Population. From this list the following deviation was considered significant but did not have an apparent effect on the analysis of efficacy or safety

- The protocol states that only 2 pharmacists are to be designated as unblinded personnel. Due to the timing requirements of IP preparation and storage, all pharmacy staff were trained on the unblinded process.

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<sup>6</sup> Randomization scheme for Study 201 / 2024658-us-201-e3-16-1-07.pdf

**Table 4 Significant Protocol Amendments for Study**

Amendment	Date	Significant Changes in Conduct or Analysis of Study
1	21 Apr 2011	<ul style="list-style-type: none"> <li>• <b>Changed dosing regimen from 50 or 100 mg/kg/wk eteplirsen administered for 12 weeks to 30 or 50 mg/kg/wk for 24 weeks.</b></li> <li>• Changed the overall duration of the study from 30 to 28 weeks.</li> <li>• <b>Changed the design of the study from a dose escalation study to a randomized, double-blind, Pbo-controlled, multiple dose, efficacy, safety, tolerability, and PK study.</b></li> <li>• <b>Changed the number of patients from 5 patients each in 4 groups to 4 patients each in 3 groups (30 mg/kg/wk, 50 mg/kg/wk, and Pbo), i.e., from an N of 20 to an N of 12</b></li> <li>• <b>Changed the age range for patient enrollment from 5 to 15 years of age to 7 to 13 years of age.</b></li> <li>• Added requirement that patients be able to walk between 200 and 350 meters on 6MWT to entry criteria.</li> <li>• Changed the entry requirement that participants be on a stable dose of corticosteroids for at least 12 weeks before study entry to at least 24 weeks before study entry.</li> <li>• Added several assessments including the NSAA, PedsQL, the 9-Hole Peg Test, inflammatory biomarkers (CD3, CD4, and CD8 in muscle biopsies), MIP and MEP, and removed the timed 4-Step Test and DEXA.</li> <li>• Added post-treatment muscle biopsies to the list of required assessments.</li> <li>• Specified that the primary efficacy endpoint would be dystrophin production (versus a general collection of data as per the original protocol)</li> </ul>
2	25 May 2011	<ul style="list-style-type: none"> <li>• Added the Timed 4-Step Test to the efficacy assessments.</li> <li>• <b>Expanded the maximum distance on the 6MWT inclusion criterion from 350 to 400 meter.</b></li> </ul>
3	22 Jun 2011	<ul style="list-style-type: none"> <li>• Clarified and added urine biomarker testing</li> </ul>
4	10 Aug 2011	<ul style="list-style-type: none"> <li>• Clarified that 6MWT would be administered twice during screening visit and that mean of 2 assessments <math>\pm</math> 10% of the lower or upper limit (200 m, 400 m) would be value used to determine qualification.</li> <li>• Specified that the screening Holter monitor recording would be reviewed prior to the patient undergoing a muscle biopsy, and that if the average heart rate during the recording exceeded 100 bpm, the patient would either be started on <math>\beta</math>-blockers and rescreened in 4 weeks or excluded from the study.</li> <li>• Added the DSMB to the protocol.</li> </ul>
5	8 Sep 2011	<ul style="list-style-type: none"> <li>• Clarified that MIP and MEP would be measured, not % predicted MIP and MEP.</li> <li>• <b>Deleted the 24-hour total urine protein collection from the protocol, because the results from the initial collection were confounded by the presence of nitrogen in eteplirsen.</b></li> </ul>

Amendment	Date	Significant Changes in Conduct or Analysis of Study
6	04 Nov 2011	<ul style="list-style-type: none"> <li>• <b>Made the 6MWT a secondary endpoint.</b></li> <li>• Modified statistical method to Wilcoxon rank-sum test, because it was more appropriate for the sample size of this study.</li> <li>• Removed peak inspiratory and expiratory flow from the list of PFT assessments, because these tests are measures for pulmonary obstruction, not intercostal or diaphragmatic muscle function.</li> <li>• Updated planned statistical analyses.</li> <li>• Removed the “modified intent to treat” and “per protocol” populations from the list of analysis populations and added a “full analysis population”, which, like the safety population, included all patients who received any study medication.</li> </ul>
8	07 Jan 2012	<ul style="list-style-type: none"> <li>• Extended the duration of the study from 24 to 28 weeks.</li> <li>• <b>Specified that beginning Week 25, patients who received Pbo for the first 24 weeks of the study would begin receiving the same dose of eteplirsen to which they were Pbo-matched while those who received 50 or 30 mg/kg/wk eteplirsen for the first 24 weeks would continue to receive the same dose regimen of eteplirsen without interruption.</b></li> </ul>
Mentioned as changed in the Clinical Study Report (Section 9.7.9.2); Date not specified.		<ul style="list-style-type: none"> <li>• A single blood sample for PK determination was drawn at 5 ± 2 minutes after the end of study drug administration at Week 1. However, these samples were lost during shipping and therefore, were not available for analysis.</li> <li>• <b>For the purpose of the efficacy analyses of functional endpoints, the maximum observed value of any 2 consecutive days of assessment was to be used in the analysis. As the intent for this plan was to use the patient’s best score as a reflection of best effort made, the minimum value (representing best value) was used for the following assessments: the Timed 10-meter run and Rise Time from the NSAA.</b></li> <li>• <b>Specific conditions for the Western blot and RT-PCR analyses were altered after the initial analyses were performed because of higher than expected concentrations in total protein and RNA extracted from the tissue samples, respectively. Results from the initial and follow-up analyses are included in the summary tables and listings. Results from the follow-up analyses are reported in the body of this study report.</b></li> </ul>

Source: CSR 4658-us-201-e3 Sections 9.7.9.1 and 9.7.9.2 (pp 52-56) and Listing 16.1.1

### Table of Demographic Characteristics

Demographic properties of the study subjects are shown in **Table 5** and disease characteristics in **Table 6**.

In the disease characteristics, the mean findings are consistent between treatment groups, though the range in the 50 mg/kg group with respect to duration of disease was slightly longer. Baseline data for the functional measures is discussed further in the description of clinical endpoints results.

**Reviewer’s Comments:** Overall, the demographic factors seemed balanced between the treatment groups. The main observation with this data is that the numbers of subjects is very few and that it difficult to make well-founded interpretations based on the sample size.

**Table 5 Summary of Demographic Characteristics (Safety Population)**

Parameter		Placebo N = 4	Eteplirsen			All Patients N = 12
			30 mg/kg/wk N = 4	50 mg/kg/wk N = 4	All Eteplirsen N = 8	
<b>Gender n(%)</b>	Male	4 (100)	4 (100)	4 (100)	8 (100)	12 (100)
<b>Age, years</b>	Mean	8.5	9.3	8.5	8.9	8.8
	Median	8.5	9.0	8.5	9.0	9.0
	SD	1.73	0.50	1.29	0.99	1.22
	Min, Max	7, 10	9, 10	7, 10	7, 10	7, 10
<b>Height, cm</b>	Mean	119.3	130.5	121.3	125.9	123.7
	Median	118.5	133.5	117.5	124.5	118.5
	SD	3.40	9.47	7.85	9.45	8.40
	Min, Max	116, 124	117, 138	117, 133	117, 138	116, 138
<b>Weight, kg</b>	Mean	30.65	34.85	29.05	31.95	31.52
	Median	32.15	37.40	27.10	31.25	32.15
	SD	6.035	7.050	6.376	6.952	6.411
	Min, Max	22.1, 36.2	24.8, 39.8	23.7, 38.3	23.7, 39.8	22.1, 39.8
<b>BMI, kg/m<sup>2</sup></b>	Mean	21.51	20.23	19.57	19.90	20.44
	Median	22.02	20.68	19.80	20.23	20.47
	SD	3.980	1.470	1.918	1.622	2.573
	Min, Max	16.4, 25.6	18.1, 21.5	17.0, 21.7	17.0, 21.7	16.4, 25.6
<b>Race, n(%)</b>	Asian	0	1 (25)	0	1 (12.5)	1 (8.3)
	White	4 (100)	3 (75)	4 (100)	7 (87.5)	11 (91.7)

Source: [Table 14.1.2](#)  
Abbreviations: BMI = body mass index; max = maximum; min = minimum; SD = standard deviation.

Source: CSR Study 4658-us-201, Table 10-3, p. 60 of 107

**Table 6 Baseline Disease Characteristics (Safety Population)**

Parameter		Eteplirsen				All Patients N = 12
		Placebo N = 4	30 mg/kg/wk N = 4	50 mg/kg/wk N = 4	All Eteplirsen N = 8	
Mutation	45-50 n (%)	0	2 (50)	1 (25)	3 (37.5)	3 (25)
	48-50 n (%)	0	1 (25)	0	1 (12.5)	1 (8.3)
	49-50 n (%)	3 (75)	0	2 (50)	2 (25)	5 (41.7)
	50 n (%)	1 (25)	0	0	0	1 (8.3)
	52 n (%)	0	1 (25)	1 (25)	2 (25)	2 (16.7)
Time Since DMD Diagnosis, months	Mean	50.3	52.5	66.5	59.5	56.4
	Median	51.0	57.0	68.0	57.0	57.0
	SD	13.74	14.06	44.29	31.33	26.40
	Min, Max	36, 63	32, 64	18, 112	18, 112	18, 112
Duration of Steroid Use, months	Mean	44.875	49.875	52.825	51.350	49.192
	Median	45.550	53.800	52.050	53.800	53.800
	SD	21.6297	13.4812	35.3952	24.8455	23.0344
	Min, Max	21.7, 66.7	30.4, 61.5	15.5, 91.7	15.5, 91.7	15.5, 91.7
Holter Monitor Average Heart Rate, bpm	Mean	96.8	96.8	93.8	95.2	95.8
	Min, Max	91, 102	86, 102	86, 102	86, 102	86, 102
6MWT <sup>a</sup> , meters	Mean	394.5	355.3	396.0	375.6	
	Median	379.0	359.0	395.0	380.5	
	SD	42.25	74.78	26.61	56.34	
	Min, Max	364, 456	261, 442	365, 429	261, 442	
%FEV <sub>1</sub> (%)	Mean	111.000	92.750	94.000	93.375	
	Median	109.500	92.000	98.500	95.500	
	SD	11.9722	7.7190	23.2236	16.0351	
	Min, Max	98, 127	85, 102	62, 117	62, 117	
%FVC (L)	Mean	116.3	95.3	92.3	93.8	
	Median	119.0	98.0	88.0	93.5	
	SD	15.44	7.80	11.59	9.29	
	Min, Max	96, 131	84, 101	84, 109	84, 109	

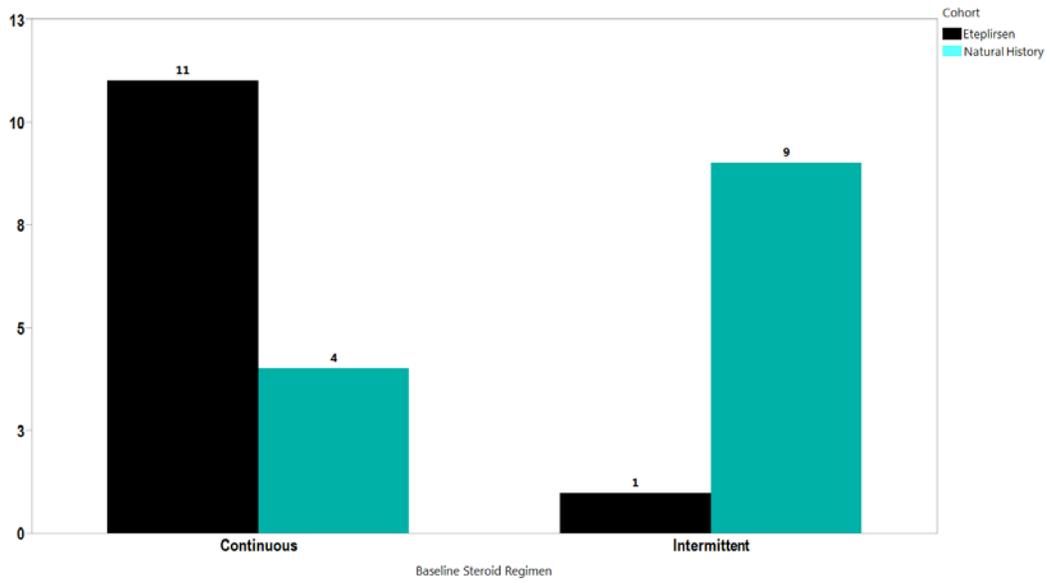
Sources: [Table 14.1.2](#) (mutations and time from DMD diagnosis to screening); [Listing 16.2.8.5](#) (24-hour Holter Monitor findings at screening); [Table 14.2.5.1](#) (6MWT results); [Table 14.2.8.1](#) (PFT results); [Listing 16.2.5.2](#) (Duration of steroid use at baseline).  
<sup>a</sup> 6MWT results are maximum observed value of 2 tests administered on 2 consecutive days at screening.  
Abbreviations: 6MWT = 6-Minute Walk Test; %FEV<sub>1</sub> = percent predicted forced expiratory volume in 1 second; %FVC = percent predicted forced vital capacity; bpm = beats per minute; DMD = Duchenne muscular dystrophy; max = maximum; min = minimum; SD = standard deviation.

Source: CSR Study 4658-us-201, Table 10-3, p. 60 of 107

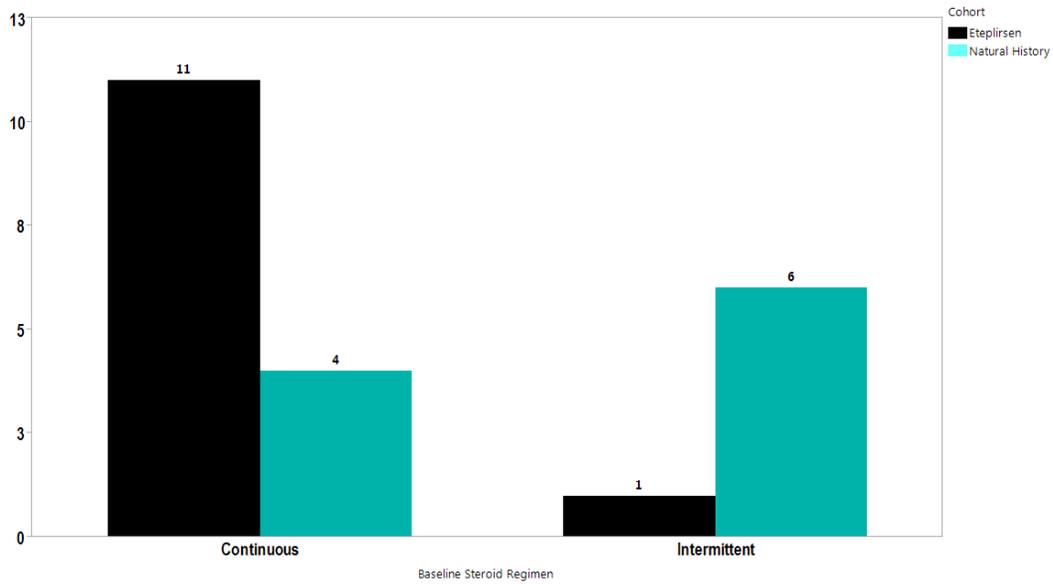
Baseline Characteristics of Natural History Cohort Versus Placebo and Active Treated subjects in Study 201/201

*Reviewer's Analyses and Comments:* Information on baseline metrics were provided and I graphed the steroid use for the cohorts participating in the 6MWT (**Figure 3**) and the NSAA (**Figure 4**). I also graphed the baseline performance for the 6MWT (**Figure 5** and **Figure 6**), NSAA Total Score (**Figure 7**), and Rise Time (**Figure 8**) as a function of age between the Eteplirsen treated subjects and the Natural History Cohort. Overall, it appeared that differences between the eteplirsen and natural history subjects in terms of steroid use may have been a factor in the clinical course. The Eteplirsen subjects also seemed, at least numerically, to be meaningfully different from the Natural History subjects, especially on such metrics as the proportion of subjects above 350 meters at baseline for the 6MWT and on the baseline rise time.

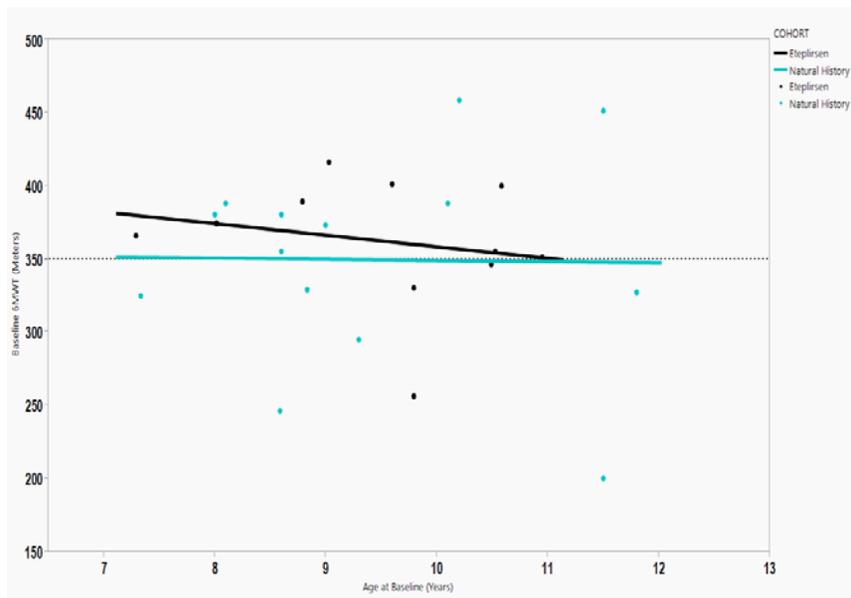
**Figure 3 Baseline Steroid Use 6MWT Exon 51 Amenable Population**



**Figure 4 Baseline Steroid Use NSAA Exon 51 Amenable Population**

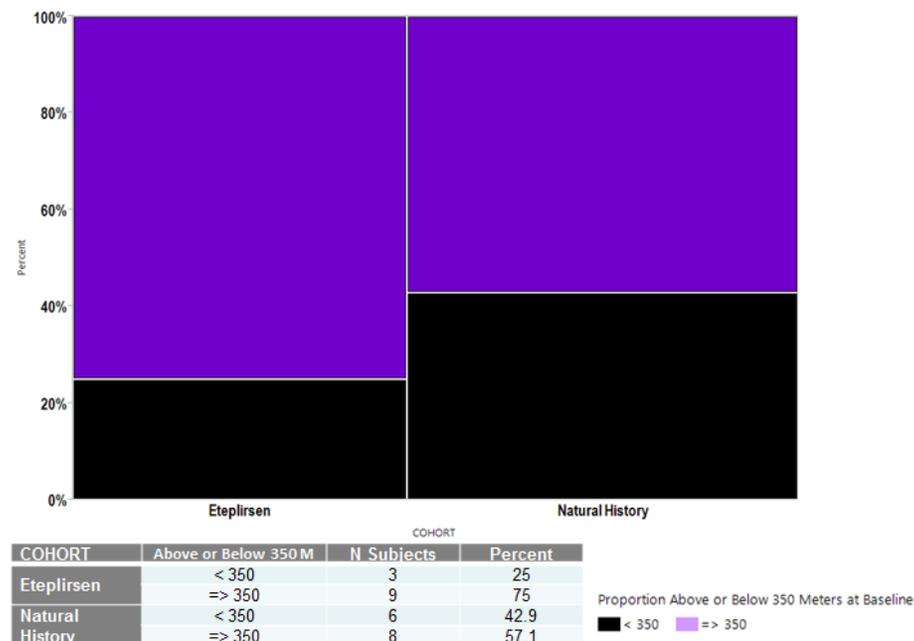


**Figure 5 Baseline Six Minute Walk Test by Age and Treatment Group**



Source: Medical Reviewer Analysis of 6MWTDER

**Figure 6 Proportion of Subjects with Baseline Six Minute Walk Above and Below 350 Meters**



Source: Medical Reviewer Analysis of 6MWTDER

Figure 7 Baseline NSAA by Age and Treatment Cohort

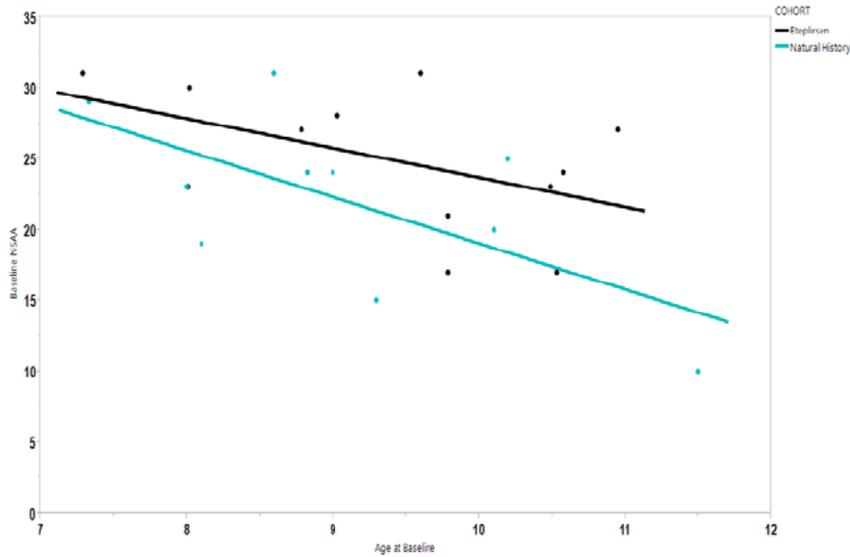
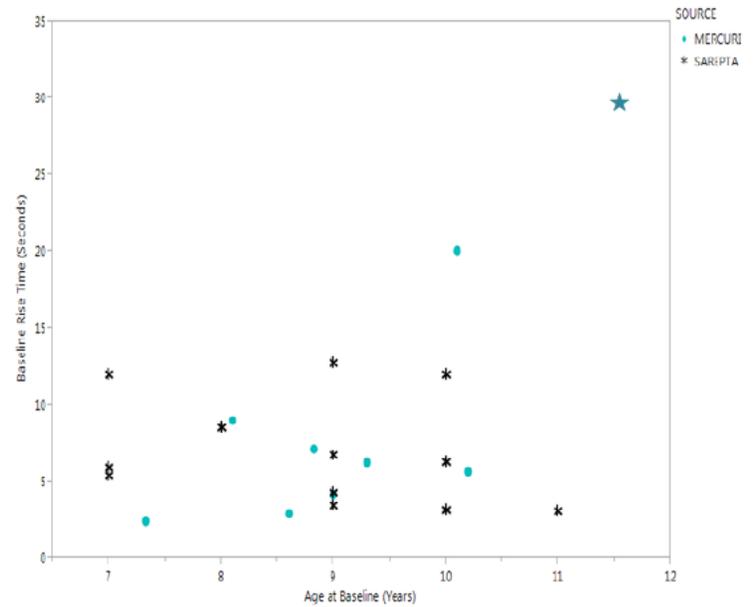


Figure 8 Baseline Rise Time by Age and Treatment Cohort



Source: Medical Reviewer analysis of NSADER dataset

Mercuri patient OBG20 missing baseline rise time

★ = Subject unable to rise; Imputed at 30 sec for display purposes

## Efficacy Results

The Efficacy Results section is divided in 3 sections,

1. The Primary Endpoint and Related, Secondary Endpoints of Biomarker Data,
2. Clinical Function Data, and
3. Additional Efficacy Endpoints.

The first two sections are divided into parts describing the placebo-controlled portion of Study 201 and the second portion describing Long-term data and data compared to Natural History cohorts.

### Primary Endpoint and Related Secondary Endpoints of Biomarker Data

#### Study 201 and 202 up to the 3<sup>rd</sup> Biopsy

This section describes the results from the evaluation of biomarker data up to and including the third biopsy (Week 48 on treatment; Week 20 of Study 202). These data are largely uninterpretable because of bias, and for the other reasons described in my comments below. Consequently, I do not find supportive evidence from the biomarker data of an amount of dystrophin that would reasonably predict a clinical benefit.

The primary efficacy endpoint was the change from baseline in percent of dystrophin positive fibers as measured in the muscle biopsy tissue using immunohistochemistry (IHC) at Week 12 for groups 1 and 3a and at Week 24 for groups 2 and 3b<sup>7</sup>. In this first section of results, I will discuss the biomarker data from the 201 Study (up to the 3<sup>rd</sup> biopsy). The biomarker data from the fourth biopsy is presented separately in this Section.

The Applicant provided the following summary of the primary endpoint in their study report (**Table 7**). They reported  $p = 0.002$  for 30 mg/kg/wk eteplirsen vs. PBO based on ANCOVA model for ranked data with treatment (Pbo, 30 mg/kg/wk, 50 mg/kg/wk) as a fixed effect and [baseline value] and [time since DMD diagnosis] as covariates. The change for the 50 mg treatment group was not significant (treatment effect = -0.1%, ( $P = 0.958$ ; 95%CI -3.6, 3.5)) nor was the comparison between placebo and the combined eteplirsen dose groups (treatment effect = 3.6%, ( $P = 0.143$ ; 95%CI -1.5, 8.8))

**Table 7 Effect of Eteplirsen on MANDYS106-immunoreactive Positive Fibers (Full Analysis Population)<sup>a</sup>**

Time point		Pbo N = 4	30 mg/kg/wk Eteplirsen N = 4	50 mg/kg/wk Eteplirsen N = 4
Baseline	Mean	15.64	18.19	11.00
	Median	15.58	17.80	11.51

<sup>7</sup> Protocol 4658-US-201 Statistical Analysis Plan 20 February 2012, p. 20 of 25

Time point		Pbo N = 4	30 mg/kg/wk Eteplirsen N = 4	50 mg/kg/wk Eteplirsen N = 4
	SD (SE)	10.74 (5.37)	5.50 (2.75)	4.67 (2.33)
	Min, Max	3.2, 28.2	11.9, 25.3	5.4, 15.6
On-Treatment <sup>b</sup>	Mean	11.59	41.14	11.79
	Median	9.44	38.77	11.81
	SD (SE)	7.13 (3.57)	10.10 (5.05)	4.46 (2.23)
	Min, Max	5.7, 21.7	32.7, 54.3	6.4, 17.2
Change from Baseline	Mean	-4.05	22.95 <sup>c</sup>	0.79
	Median	-6.13	23.46	2.52
	SD (SE)	5.83 (2.92)	5.79 (2.90)	7.10 (3.55)
	Min, Max	-8.5, 4.5	15.9, 29.0	-9.3, 7.4

Source: CSR Study 4658-us-201, Table 10-3, p. 60 of 107

<sup>a</sup>Results expressed as a percentage of total fibers counted.

<sup>b</sup>On-treatment samples are from Week 12 for all 4 patients in the 50 mg/kg/wk eteplirsen group and 2 patients in the Pbo group, or from Week 24 for all 4 patients in the 30 mg/kg/wk eteplirsen group and 2 patients in the Pbo group.

**Abbreviations:** max = maximum; min = minimum; SD = standard deviation; SE = standard error.

### Reviewer's Analyses and Comments

#### Percent Positive Fibers

Because the issues of bias and other of the methodological concerns are previously described, I do not present further extensive analyses of the fiber counts from the first 3 biopsies because I believe the numbers are not meaningful data except to point out certain issues in the fiber counting. However, I do note that in my own analysis of the data supplied by the Applicant, I arrived at a different set of baseline values from the Applicant. The mean percentage of fibers at baseline derived from two different datasets<sup>8</sup> was the same (**Table 8**) but differed from that reported in **Table 7**.

**Table 8 Baseline Percent Positive Fibers from Study 201 and 202 Datasets**

Treatment	Mean % Positive Fibers (SD) from both datasets
Eteplirsen 30 mg/kg	13.6 (8.6)
Eteplirsen 50 mg/kg	14.9 (4.7)
Placebo	10.5 (5.4)

Source: Medical Reviewer Analysis of ADRB and FIBERS2 dataset

My rationale for not believing that the data for the Percent Positive Fibers from Biopsies 1 – 3 is sound may be found in the description of the methodology (c.f., my comments between **Figure 1** and **Figure 2**) and in the bullet points below.

<sup>8</sup> The datasets were (ADRB) reporting the 1<sup>st</sup> through 3<sup>rd</sup> biopsy data in the original NDA submission and from the dataset (FIBERS2) with the 1<sup>st</sup> through 4<sup>th</sup> biopsy data received September 16, 2015

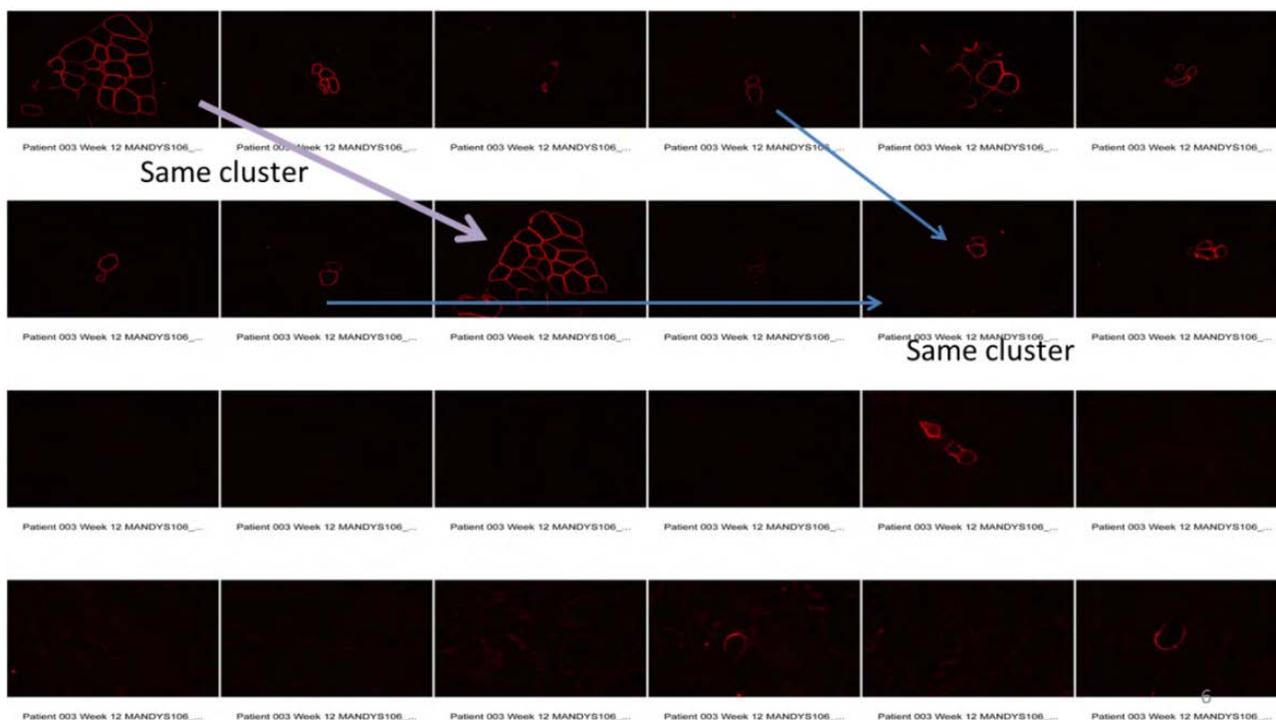
- Different muscle groups are used for different biopsies.

The first and second biopsies are from the biceps and the third biopsy is from the deltoid muscle. Different muscle groups may undergo different rates of pathological decline, including, fatty infiltration during the course of disease progression [Hollingsworth *et al.* 2013].

- The acquisition of fields on the slides to analyze from the first 3 biopsies was biased.

According to the site inspection the acquisition of fields for image analysis was biased. The fields appear to have been selected preferentially of revertant fibers, which are cells in which the reading frame for dystrophin has spontaneously modified to generate a truncated version of dystrophin. Our own inspection of the images selected suggests that in several cases, the same fiber clusters were selected for analysis, albeit at different levels of tissue slicing (**Figure 9**).

**Figure 9 Example of Where Several Microscopic Fields Containing the Same Cluster of Revertant Fibers has Been Selected for Quantification**



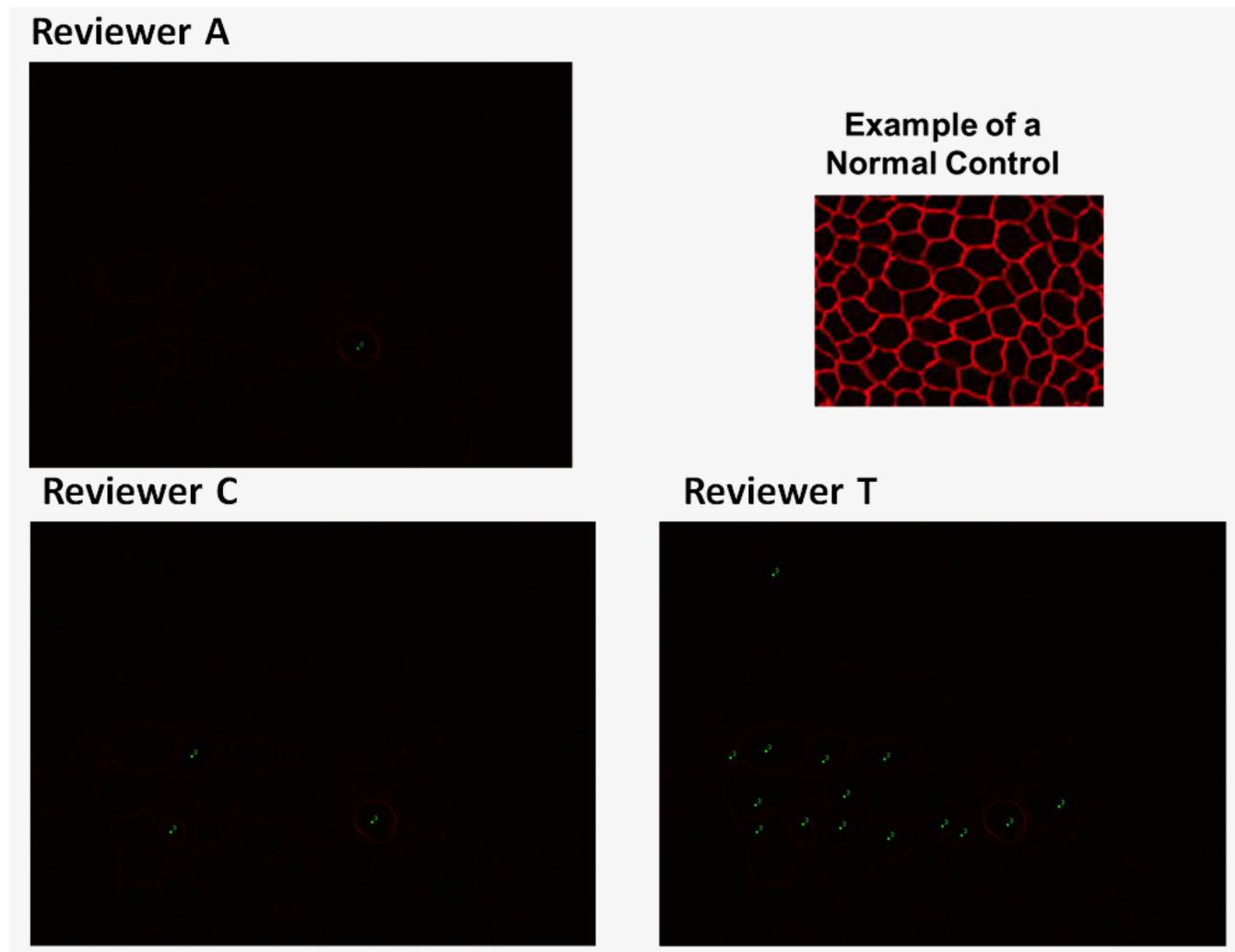
Source: Images from Study 201, Patient 003, Week 12

- The fiber count analysis gives the erroneous impression that the treatment reconstituted dystrophin expression (or at least dystrophin –immunoreactivity) to the same level in the fibers counted as positive; however, the intensity of what was considered positive in the reanalysis is far below the typical Becker or normal case. This issue is present in all biopsies (first through fourth).

Clinical Review Christopher Breder, MD PhD  
NDA 206488 (Eteplirsen)

Three pathologists at Flagship Biosciences were provided identical images of MANDYS106-ir fibers to score positively stained fibers (**Figure 10**).

**Figure 10 Images of MANDYS106-immunoreactive fiber counts from the Flagship Biosciences CRO from Biopsy 1 (Study 201/202 ITT Population)**

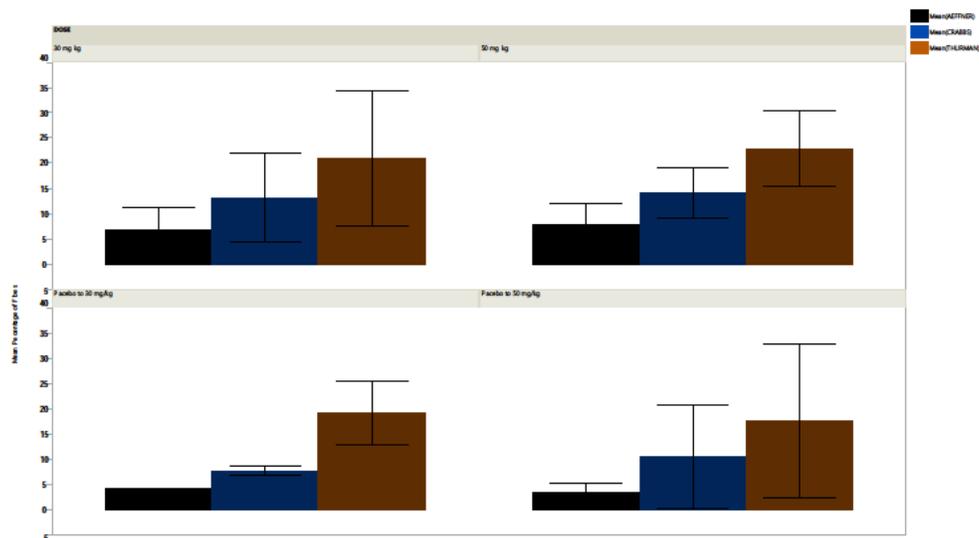


Source: Images for Reviewers A, C, T: 1005\_V001\_BL3im4; Image for Normal Control: 01006\_30TT\_48\_MD\_AL2im2.tif

Abbreviations Reviewer A = Reviewer Aeffner, Reviewer C = Crabbs, Reviewer T = Thurman. Each “green dot (actually a “•3”) represents a fiber scored as being positively stained.

Each pathologists assessments appear as green dots (actually seen as “•3” at much higher magnification) in the figure where a “positive fiber” was observed. This demonstration of the data make several of my points evident. First, what is counted as positive represent levels of dystrophin, or at least MANDYS106-ir that is far below normal. The expression levels are so low that it seems the expert pathologists often do not agree on which fibers are actually expressing dystrophin, except for revertant fibers, where the staining is most obvious. This is graphically demonstrated by histograms of the mean baseline data (**Figure 11**)

**Figure 11 Mean Number of MANDYS106-immunoreactive fibers at Baseline by Reviewer and Treatment Sequence (Biopsy 1; Study 201/202) (ITT Population)**



Each error bar is constructed using 1 standard deviation from the mean.

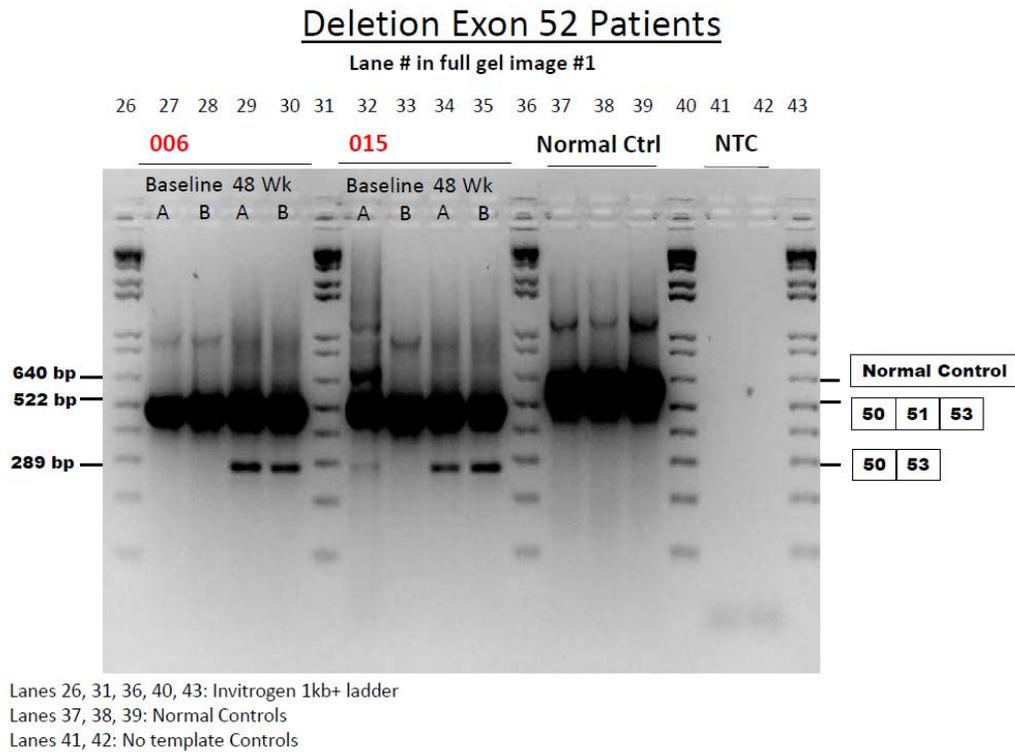
This variance and the level of discordance in the Pathologist's assessment is further analyzed in the discussion of the 4<sup>th</sup> Biopsy.

### Exon Skipping for Biopsies 1 to 3

**[AR]** Images of skipped product that were compared between the treated and baseline samples (**Figure 12**). "A" and "B" correspond to different biopsy blocks from the same patient. Some baseline samples also showed a skipped mRNA band, likely due to revertant or trace dystrophin mRNA. An appreciably pronounced band for the skipped band was apparent in each of the eleven post-treatment samples compared to the baseline on each gel. A skipped product appears in at least one of the two replicate samples for each of the post-treatment subjects.

**[AR] Reviewer's comment:** It is noted, however, that the applicants nested RT-PCR is not quantitative due to a lack of a reference gene. The presence of an exon skipped band also does not indicate that the mRNA was translated into a functional protein.

**Figure 12 Exon Skipping in Subjects 006, 015 and a Normal Control from Study 201 / 202**



Immunofluorescence Intensity for Biopsies 1 to 3 (Bioquant)

As I have previously described, the applicant deemed their original analysis with 20x images not suitable because this magnification did not "...allow for optimal differentiation of the muscle fibers for quantitation," and so they discarded this analysis. After the blind was broken and the original analysis discarded, the samples were reanalyzed at 40x. The applicant did not do inferential statistics on these data but commented on the numerical superiority of the eteplirsen treatment arms over placebo (**Table 9**).

**Table 9 Average Intensity of MANDYS106-IR by Treatment in the Study 201/202 (ITT Population)**

Time point		Placebo N = 4	30 mg/kg/wk Eteplirsen N = 4	50 mg/kg/wk Eteplirsen N = 4
Baseline	Mean	9.11	12.13	9.01
	Median	9.11	11.62	8.78
	SD (SE)	0.208 (0.104)	1.550 (0.775)	0.563 (0.281)
	Min, Max	8.9, 9.3	10.9, 14.4	8.7, 9.9
On-Treatment <sup>b</sup>	Mean	9.18	21.20	19.47
	Median	9.04	19.54	19.69
	SD (SE)	0.526 (0.263)	4.711 (2.356)	1.990 (0.995)
	Min, Max	8.7, 9.9	17.6, 28.1	17.0, 21.5
Change from Baseline	Mean	0.07	9.07	10.45
	Median	-0.08	8.15	10.91
	SD (SE)	0.640 (0.320)	3.304 (1.652)	2.505 (1.252)
	Min, Max	-0.5, 1.0	6.2, 13.8	7.2, 12.8

Source: Table 14.2.1.1.1 and Table 14.2.1.1.2

<sup>a</sup>Results are expressed as a percentage of normal.

<sup>b</sup>On-treatment samples are from Week 12 for all 4 patients in the 50 mg/kg/wk eteplirsen group and 2 patients in the placebo group, and from Week 24 for all 4 patients in the 30 mg/kg/wk eteplirsen group and 2 patients in the placebo group.

**Abbreviations:** max = maximum; min = minimum; SD = standard deviation; SE = standard error.

Source: Table 11-2, 4658-us-201-body.pdf, p. 64 of 107

### Reviewer's Analysis and Comments

I have several concerns with the Applicants Intensity analysis by Bioquant in this application

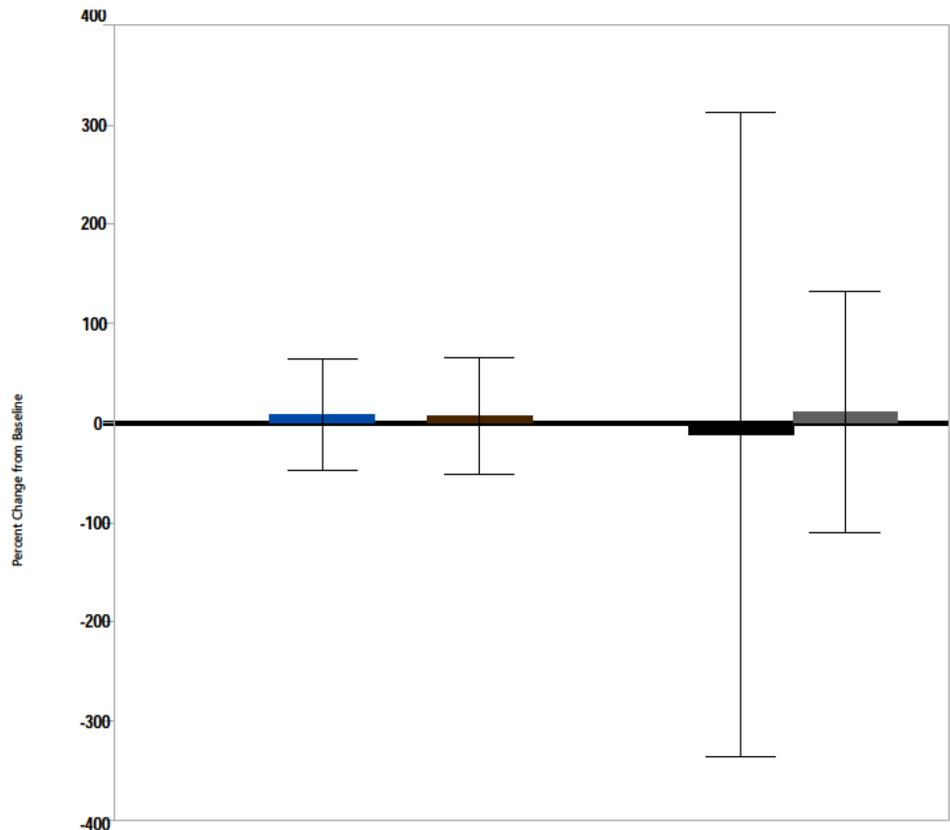
- The Applicant chose to disregard the results taken at 20X for Bioquant, which were negative.

**[AR]** The applicant claimed that the 20x data set was not used for Bioquant because “the background staining appeared to confound analysis at lower magnifications”. They also state that “to have a more precise reading of the membrane intensity change induced by eteplirsen treatment, images at higher magnification were used to capture the precise area(s) expressing dystrophin.” [Sarepta responses to information requests on 12-Feb-15 and 10-Oct-2014, filed under IND77429 / Sequence 0106]. From a methodological perspective, a study should have validated protocol and predefined acceptance criteria. If the 20x method was originally validated, reanalysis of the same images at 40x would qualify as an unplanned deviation and should have been reproduced with a fresh set of blinded samples and revalidated/revised protocol at 40x magnification.

**[AR]** In the absence of a comparative study at 20x and 40x using the same blinded samples and clearly demonstrating why a “precise reading of membrane intensity” could not be determined at 20x, in my opinion, it would not be a good scientific practice to dismiss the 20x set of data.

Reviewer [CDB] analyses from the applicant's 20x image data are presented below ( **Figure 13**). There were no significant differences between treatment arms in the original, placebo controlled analysis.

**Figure 13 Original Analysis of the Percent Change from Baseline in Intensity of MANDYS106-IR by Treatment and Visit during the placebo-Controlled portion of Study 201 / 202**



Source: Medical Reviewer analysis of ADBI dataset

Each error bar is constructed using a 95% confidence interval of the mean.

- Mean(Percent Change from Baseline\_Week 24) (TRT01P=Eteplirsen 30 mg/kg)
- Mean(Percent Change from Baseline\_Week 12) (TRT01P=Eteplirsen 50 mg/kg)
- Mean(Percent Change from Baseline\_Week 12) (TRT01P=Placebo)
- Mean(Percent Change from Baseline\_Week 24) (TRT01P=Placebo)

- Discrepancy Between The Relative Reported Intensity Between Normal And Eteplirsen Stained Tissue

The intensity of stained tissue from pdf's or even tiffs reproducing what is observed under the microscope is difficult to judge. However, numerous image fields are reported with such high relative intensity to normal that do not seem anywhere close to the reported relative intensity (see **Figure 17**, an example of the same issue from the 4<sup>th</sup> biopsy).

2. Total dystrophin protein (assessed by Western blot analysis)

[AR] The Applicant commented on an increase in the MANDYS106 immunoreactivity detected by Western blot (**Table 10**) however, the Western blots from the first 3 biopsies had oversaturated bands, did not have appropriate controls or quality control metrics and were essentially uninterpretable.

**Table 10 Effect of Eteplirsen on Dystrophin Protein as Measured by Western Blot in Study 201/201 (ITT Population)**

Time point		Placebo N = 4	30 mg/kg/wk Eteplirsen N = 4	50 mg/kg/wk Eteplirsen N = 4
Baseline	Mean	0.38	0.17	0.15
	Median	0.39	0.15	0.05
	SD (SE)	0.157 (0.079)	0.197 (0.099)	0.243 (0.121)
	Min, Max	0.2, 0.5	0.0, 0.4	0.0, 0.5
On-Treatment <sup>b</sup>	Mean	0.45	2.02	0.59
	Median	0.44	1.03	0.21
	SD (SE)	0.170 (0.085)	2.708 (1.354)	0.841 (0.420)
	Min, Max	0.3, 0.7	0.1, 6.0	0.1, 1.8
Change from Baseline	Mean	0.07	1.86	0.44
	Median	0.05	0.69	0.20
	SD (SE)	0.315 (0.157)	2.801 (1.401)	0.916 (0.458)
	Min, Max	-0.3, 0.5	0.1, 6.0	-0.4, 1.7

Source: 4658-us-101 CSR Table 11-3, p 65 of 107 from Table 14.2.1.1.1.

A Results are expressed as a percentage of normal.

B On-treatment samples are from Week 12 for all 4 patients in the 50 mg/kg/wk eteplirsen group and 2 patients in the Pbo group, and from Week 24 for all 4 patients in the 30 mg/kg/wk eteplirsen group and 2 patients in the Pbo group.

Abbreviations: max = maximum; min = minimum; SD = standard deviation; SE = standard error.

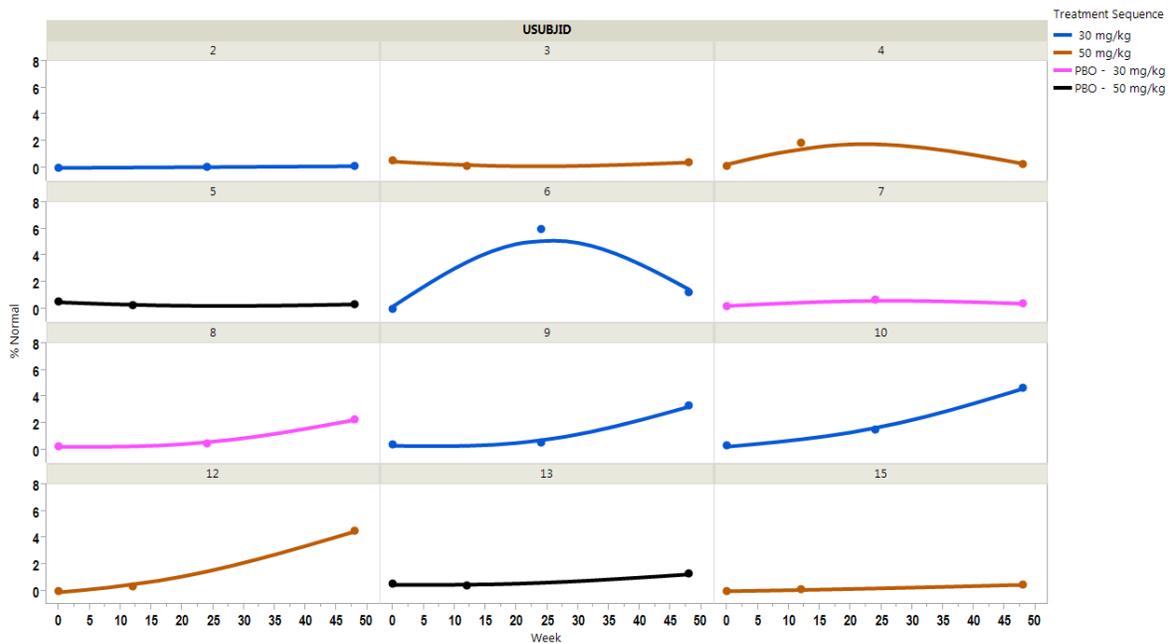
**Figure 14** shows the reported percent of normal expression of MANDYS106-immunoreactivity in Western Blots from Biopsies 1 to 3 in Study 201/202. In these Western blots, the quantification was not done using a serial dilution so the actual percentages are not certain. The applicant appears to have compared their test samples to the one or two healthy control samples run on the same gel. However, the intensity of those positive control bands was saturated, preventing reliable quantitation of the MANDYS106-immunoreactive bands in studies in 201/202 (c.f., **Figure 15**).

**Table 11 Percentage of Normal for MANDYS106-immunoreactivity in the Western Blot Analyses of the First Three Biopsies**

Treatment Sequence	USUBJID	Week 0	Week 12	Week 24	Week 48
30 mg/kg	002	0	NA	0.06	0.12
	006	0	NA	5.98	1.23
	009	0.37	NA	0.53	3.32
	010	0.3	NA	1.52	4.67
50 mg/kg	003	0.51	0.12	NA	0.42

Treatment Sequence	USUBJID	Week 0	Week 12	Week 24	Week 48
	004	0.1	1.84	NA	0.25
	012	0	0.31	NA	4.52
	015	0	0.08	NA	0.48
[Placebo] - [ 30 mg/kg]	007	0.22	NA	0.67	0.38
	008	0.28	NA	0.46	2.31
[Placebo] - [ 50 mg/kg]	005	0.52	0.26	NA	0.35
	013	0.51	0.42	NA	1.28

**Figure 14 Percent of Normal Expression of MANDYS106-immunoreactivity in Western Blots from Biopsies 1 to 3 in Study 201 / 202 (ITT Population)**



Source: Medical Reviewer's analysis of dataset ADBI

**Figure 15 Western Blot analysis of Subject 6 at Baseline and Week 24 in Study 201**



Source: 01006\_30TT\_BL\_24\_MD\_WB.tif

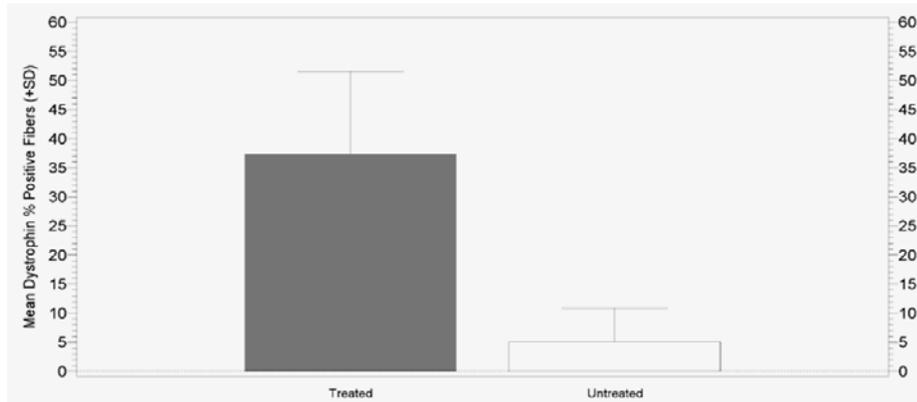
#### 4<sup>th</sup> Biopsy Data

[AR] The methodologies used by the Applicant were relatively improved for the 4<sup>th</sup> biopsy. For example a systematic method was specified to select microscopic fields for analysis of fiber counts and immunofluorescence intensity. The Western blots for the 4<sup>th</sup> biopsy used more standardized serial dilutions for calibration. However, it is not clear exactly how much dystrophin or if any was made based on a drug effect at the time of the fourth biopsy. This is largely due to not having matched baseline controls stained in the same subject for all treated samples, with the same antibody, and with tissue of comparable quality (i.e., fresh versus frozen for about 3 years). I will return to this issue with my discussion of the Western blot of the 4<sup>th</sup> biopsy since it was the only technique that was calibrated with enough rigor to begin to address this issue.

#### Percent Positive Fibers

The applicant reported that the 4<sup>th</sup> biopsy, Week 180 muscle biopsy samples treated with eteplirsen had a statistically significant increase in Percent Dystrophin-Positive Fiber (PDPF) score ( $p < 0.001$ ) relative to the untreated control samples selected by the applicant.

**Figure 16 Applicants Comparison of the Percent MANDYS106-immunoreactive Fibers in Eteplirsen treated Subjects to Untreated Controls**

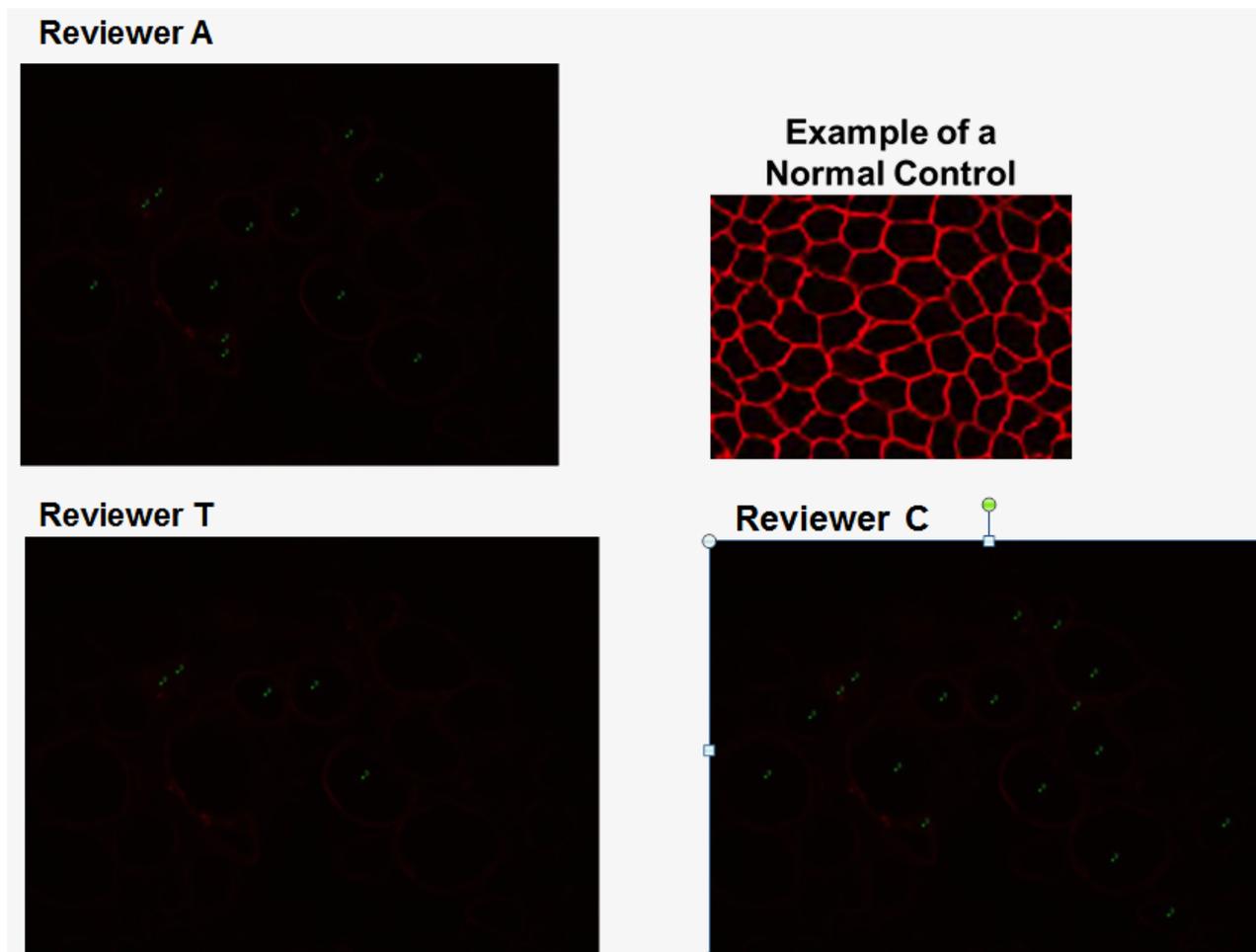


Source: Report 4658-us-cr-15-008

***Medical Reviewer Comments and Analyses Specific to the 4<sup>th</sup> Biopsy***

- The principle concern I have with the analysis of the fourth biopsy (which extends to the intensity and Western blot analyses) is that there are not matched controls from the same patients and muscle groups for all treated samples. Importantly, it is not clear how similar the external controls were to the treated patients, and it is not clear that the applicant selected the external controls completely at random, so bias may have been introduced.
- The dystrophin immunostaining was very faint in the 4<sup>th</sup> biopsy, and variability of the rater for the assessment of Percent Positive Fibers, originally described in my review of Biopsies 1 – 3, persisted through the study including the evaluation of the 4<sup>th</sup> biopsy (**Figure 17**).

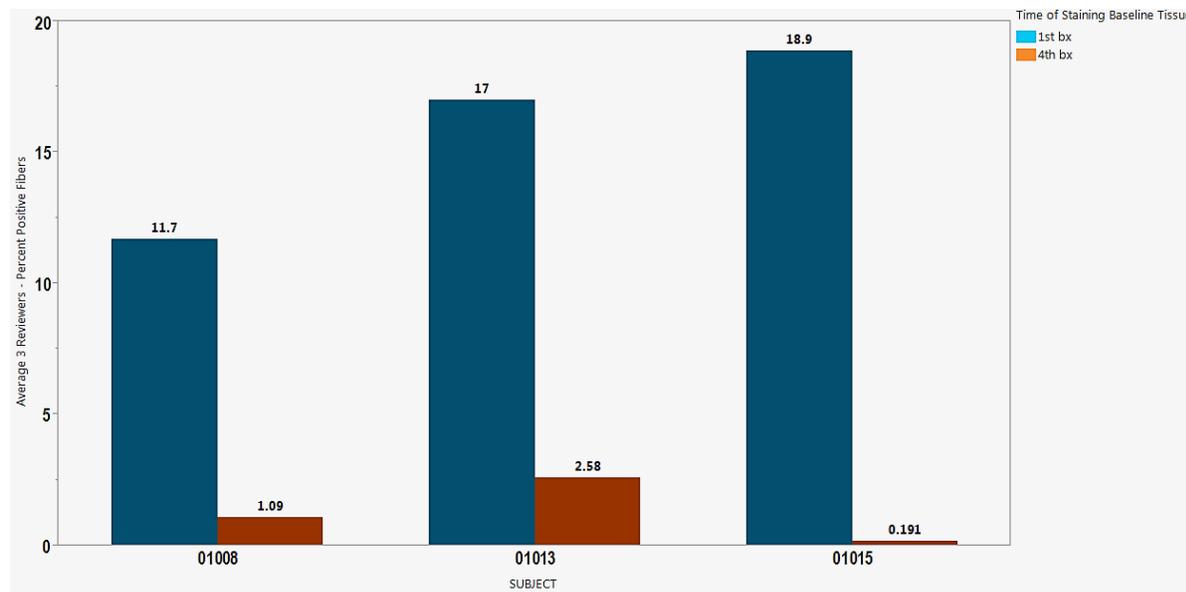
**Figure 17 Images of MANDYS106-immunoreactive fiber counts from the Flagship Biosciences CRO from Biopsy 4 (Study 201/202 ITT Population)**



Source: Images for Reviewers A, C, T: 1012\_V180\_BL1im3; Image for Normal Control 01006\_30TT\_48\_MD\_AL2im2.tif  
**Abbreviations** Reviewer A = Reviewer Aeffner, Reviewer C = Crabbs, Reviewer T = Thurman. Each “green dot (actually a “•3”) represents a fiber scored as being positively stained.

A final observation I made on the Percent Positive Fibers data was that Subjects 008, 013, and 015 had a notably different MANDYS106-ir fiber percentage relative to the normal controls when their original Baseline) tissues (noted in the figure as “Time of Staining Baseline Tissue – 4<sup>th</sup> bx”) were restained and analyzed as a bridge to the tissue stained as the original Baseline material (noted in the figure as “Time of Staining Baseline Tissue – 1<sup>st</sup> bx”) ( **Figure 18**).

**Figure 18 A Comparison Of The Percentage Of MANDYS106-IR Fibers From 3 Subjects Where The Tissue Was Stained At The Time Of Biopsy 1 Versus At The Time Of Biopsy 4**



Each error bar is constructed using 1 standard deviation from the mean.

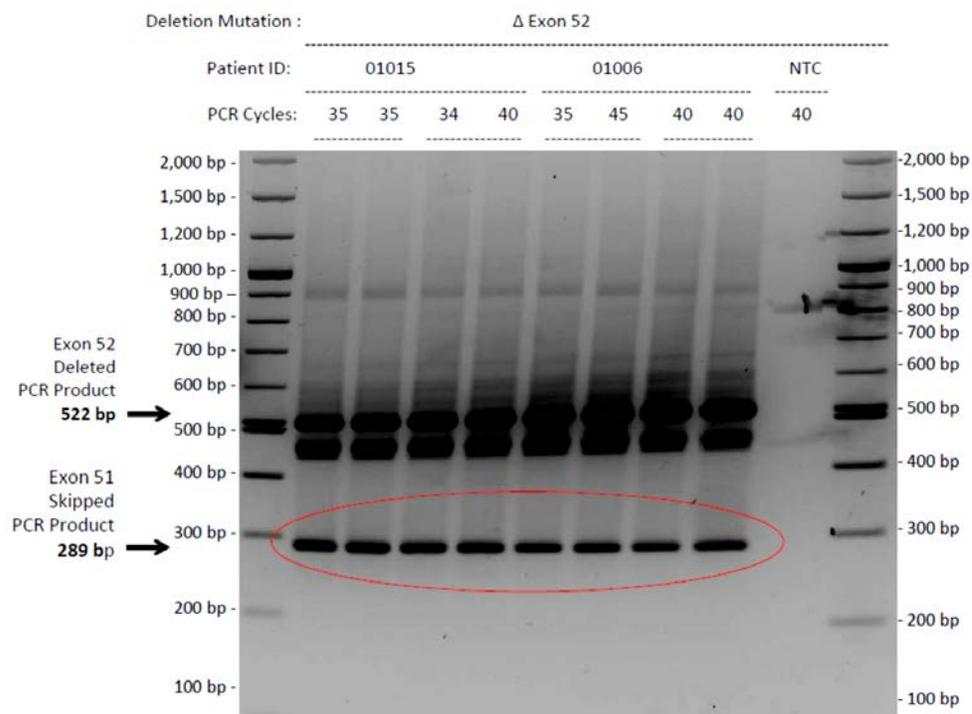
Source: Medical Reviewer's analysis of FIBERS2 dataset

The basis for the differences in the percent positive fibers from the time they were originally stained and the time of the 4th biopsy is not known; however, because they were stained with the same antibody and nearly the same procedure, one would expect the levels to be similar. One factor which is concerning to me is that the tissue for the fiber staining as well as the other biomarker assays had been in the freezer for about 3 years. Without a method to control for or evaluate the potential loss of immunoreactivity, I am concerned that the protein may have undergone changes which would result in a lesser level in the biomarker assays.

### Exon Skipping

The 180-week biopsies also showed the presence of exon 51-skipped band in each of the tested samples. The Figure below shows the skipped product in samples from patients 01015 and 01006 from the 4<sup>th</sup> biopsy.

**Figure 19 Exon Skipping in Subjects 006, 015 and a Normal Control from Biopsy 4 in Study 201 / 202**

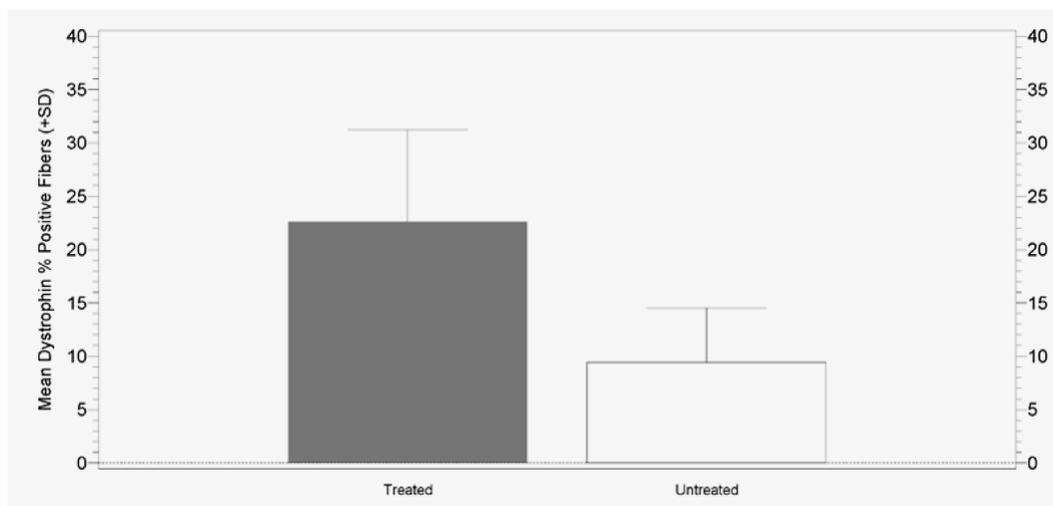


As with study 201/201, the applicant confirmed that the product was an exon 51-skipped product based on a sequencing result.

#### Immunofluorescence Intensity for Biopsy 4

For the fourth biopsy, the Applicant reported that the muscle biopsy from Week 180 displayed a statistically significant ( $p < 0.001$ ) increase in the relative [MANDYS106-IR] associated fluorescence intensity. The mean relative fluorescence value for treated patients was reported as 22.61 versus 9.41 for the untreated control samples, which were a population of 6 untreated DMD boys and Biopsy #1 tissue (baseline, untreated) from 3 of the original eteplirsen subjects (008, 013, 015) (**Figure 20**, note the Y-axis is mislabeled per the original figure below).

**Figure 20 MANDYS106-IR Intensity Relative to Normal Field Intensity as Measured by BIOQUANT at Week 180 (Treated versus Untreated Subjects)**



Source: 4658-us-202-sr-cr-15-007

I have the following concerns with the analysis of immunofluorescence intensity for the 4<sup>th</sup> biopsy:

- Use of Immunofluorescence Intensity as a Quantitative Technique

Immunofluorescence is not a quantitative technique in that the samples are not compared to a calibrated standard curve of a reference sample. It can be supportive to relative changes, e.g., an increase in positive fibers should correlate to an increase in fluorescence intensity; however, it gives no information as to the magnitude of change.

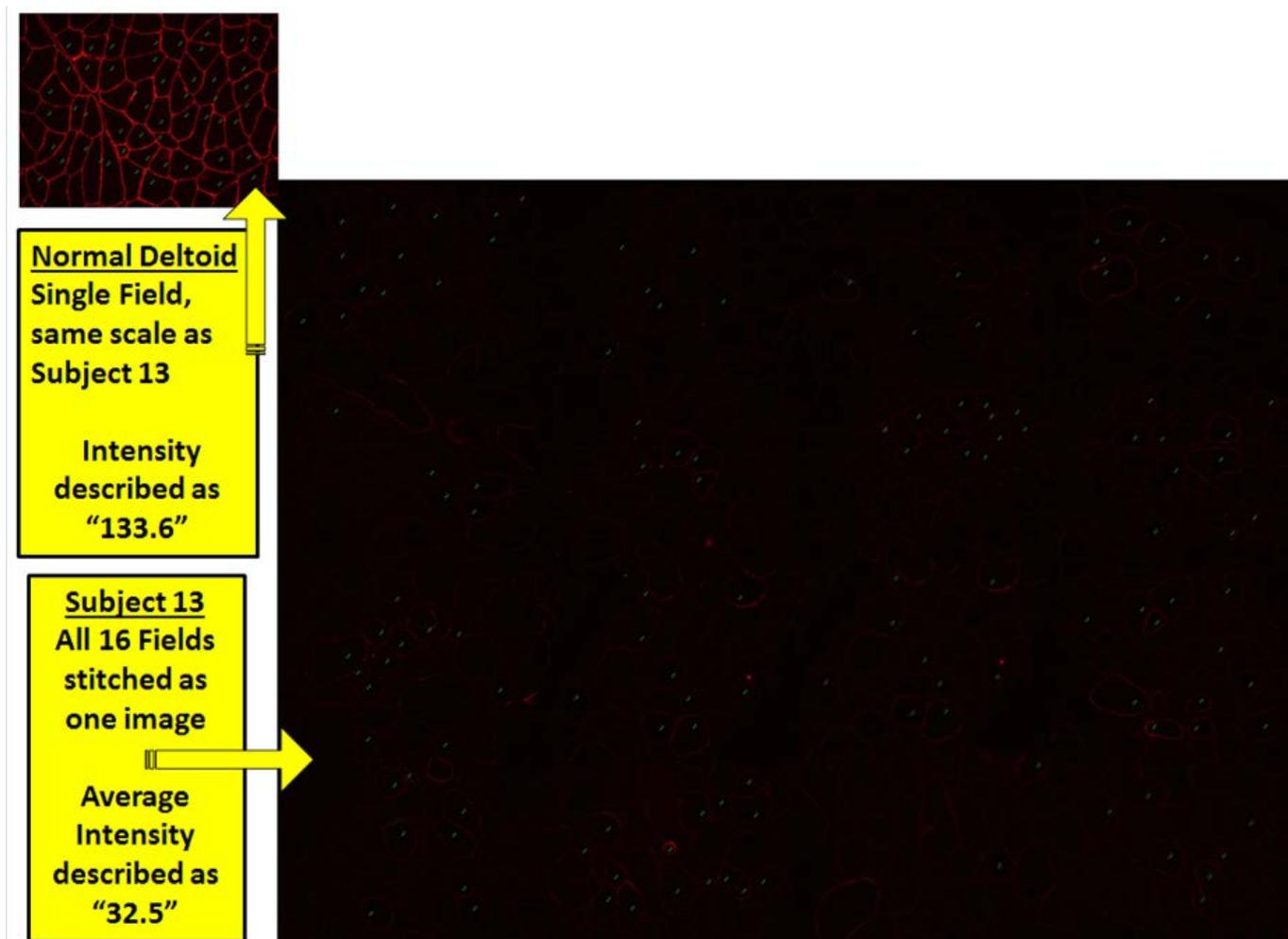
- Controls Used For Analysis Of The Fourth Biopsy

This has been discussed with the concerns about the fiber counts earlier in this section.

1. Discrepancy Between The Relative Reported Intensity Between Normal And Eteplirsen Stained Tissue

For example, in the average intensity for the fourth biopsy for subject 013 is described as “32.5” while the normal control is described as 133.6 (**Figure 21**). The Subject 013 sample does not appear even close in intensity to the normal subject. These are of course the numbers the instrument and data analysis software generated, which are difficult to visually assess with reproduced images; however, given the apparent disparity, I felt this was worth noting.

**Figure 21 BIOQUANT Intensity of Subject 013 and a Normal Control from the Fourth Biopsy**

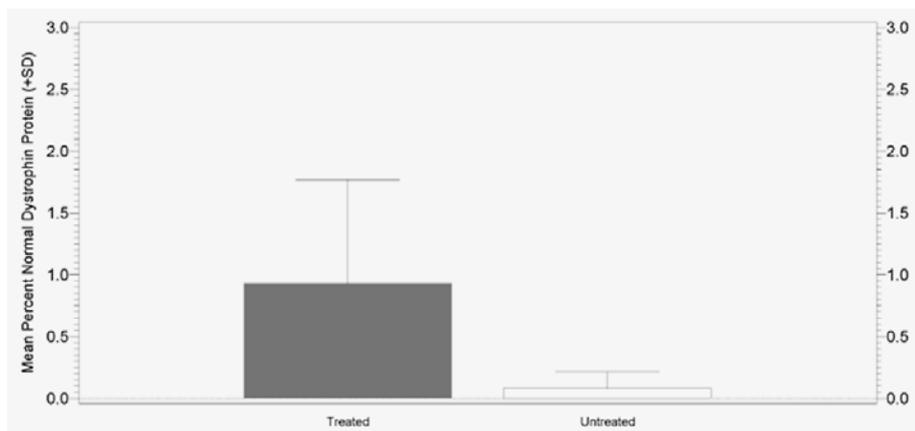


Source: Dataset from Study 202 images

### Western Blot Analysis

For the Fourth biopsy, the applicant reported that the group mean dystrophin protein level, expressed as percent of normal (non-DMD patients) dystrophin-protein levels was 0.92 % versus untreated patient levels of 0.08 % of normal tissue dystrophin-protein levels (**Figure 22**). According to the applicant the results indicated that weekly treatment with eteplirsen resulted in a statistically significant increase in dystrophin protein level ( $p < 0.007$ ) as measured in the Week 180 biopsy samples when contrasted with untreated DMD samples.

**Figure 22 Mean Percent of Normal of DYS1-Immunoreactive Protein as Assayed by Western Blot**



Source: 4658-us-202-sr-cr-15-004, Figure 1, p 13 of 101

**Reviewer’s Analyses and Comments**

**Table 12** contains the percent of normal for MANDYS –immunoreactivity for eteplirsen treated subjects as assessed by Western blot analysis. The numbers remain low despite 180 weeks of treatment. The clinical relevance of the small increase reported in some subjects is not clear since it does not correlate with clinical function (**Table 12** see also my analyses in **Section 7. Integrated Review of Effectiveness**).

**Table 12 Percentage of Normal DYS1-immunoreactivity the 4<sup>th</sup> biopsy.**

Treatment at Week 180	Subject	4 <sup>th</sup> Biopsy Gel #1	4 <sup>th</sup> Biopsy Gel #2	Average for 4 <sup>th</sup> Biopsy; BLOQ set to 0	Average for 4 <sup>th</sup> Biopsy Actual Values
30 mg/kg	002	BLOQ	0.28	0.14	0.15
	006	2.83	2.11	2.47	2.47
	007	BLOQ	BLOQ	0	0.16
	008	0.93	1.02	0.98	0.98
	009	0.58	0.46	0.52	0.52
	010	1.45	1.78	1.62	1.62
50 mg/kg	003	BLOQ	BLOQ	0	0.18
	004	1.22	0.66	0.96	0.96
	005 <sup>9</sup>	NA	NA	0	0
	012	0.75	BLOQ	0.38	0.5
	013	NA	1.15	1.15	1.15
	015	2.43	1.67	2.05	2.05

Source: nda206488\_0024\_m1\_us\_111-info-amend\_clinpharm-20151204.pdf

<sup>9</sup> No Western blot was available for the 4<sup>th</sup> biopsy of Subject 005

Western blots using DYS1 were also done for control subjects selected by the Applicant for the 4<sup>th</sup> biopsy (**Table 13**). The Applicant also provided the review team with the values of Western blots % MANDYS106-Immunoreactivity relative to normal controls that were below their limit of reliable quantitation, and had been assigned a value of zero instead of the actual value observed. As expected, using the actual value instead of zero increased the percent expression at baseline for this group and would have decreased the fold-increase of normal over control.

**Table 13 Untreated Controls Percent Dystrophin Results – A Comparison of Those That Included Levels below the Serial Dilution Curve and Those That Did Not For the Fourth Biopsy**

<b>Subject Number</b>	<b>Reported Value (per Protocol) – (Does not include levels below that of the serial dilution curve)</b>	<b>Average of Gel #1 and #2 (Includes levels below the serial dilution curve)</b>
01005	0	0.06
01013	0	0.14
01015	0	0.1
DMD1	0	0.13
DMD2	0	0.08
DMD3	0.37	0.37
DMD7	0.15	0.17
DMD8	0	0.20
DMD9	0.20	0.25

Source: Response-to 12nov15-clin-pharm-ir-re-wb-bloq-values

Reviewer CDB had several other concerns regarding the Western blots for the fourth biopsy data, as follows below. Reviewer AR concurred with the first and third concerns but reasoned that the applicant use of a highly sensitive Odyssey infrared detection system might allow for reasonable quantitation with bands of low intensity described in concerns 2 and 4 and that it may not be possible to gauge the accuracy of the densitometric quantitation with a visual examination of the pictures provided.

#### 1. Selection of Controls

Information on the performance characteristics of the Becker patients on standardized physical function tests (e.g., 6 Minute walk test, NSAA, Rise time), previous biopsy information, medical history, medications etc. was not provided in the NDA. Upon request of this information the Applicant informed the Review Team (responses-to-23oct15-clinical-and-bioassay-irs, 1.11.3 Clinical Information Amendment) that no data on physical function tests are available for these BMD control patients. It is therefore not possible to assess the relation of the percent of dystrophin expression by Western blot and the clinical benefit (i.e., physical functioning” of these levels. In the same Response to Information Request, the Applicant noted that (in contrast to the initial submission in the NDA), the mutation for the Becker subject #3 was unknown.

The untreated DMD controls used in the fourth biopsy analyses were not necessarily selected at random from a representative patient population. Tissue from patients from the ongoing eteplirsen Phase 3 confirmatory study 4658-301 (PROMOVI) were used.

**[AR]** The applicant compared dystrophin data from deltoid muscle from the 4<sup>th</sup> biopsy with baseline samples from bicep muscle for two patients (01013 and 01015). It is not clear to what extent the inherent variability in dystrophin expression between muscle groups may have contributed to the change in dystrophin reported for the 4<sup>th</sup> biopsy.

Immunofluorescence data from the mdx mouse model suggests that deltoid have 27% dystrophin-positive fibers compared to 45% in biceps and semitendinous muscle (Liang KW, Gene Therapy, 2004 and related findings by Lu QL et al, PNAS, 2005). The applicant used 8 DMD biceps muscle samples and deltoid muscle sample from 1 DMD patient as negative controls. The range of % healthy dystrophin for the bicep samples by western blotting was 0.08-0.37% compared to 0.12% for the deltoid sample from patient DMD1 (SR-CR-15-004 and Response to 23Oct15-Clinical Information Request, Table 9). Therefore, it is possible that some, but perhaps not all, of the change reported in the 4<sup>th</sup> biopsy samples from the 2 matched patient samples with biceps baseline data could be attributed to differences in dystrophin expression between different muscle groups. A systematic study on dystrophin would be needed to clearly account for inter-muscular differences in DMD patients.

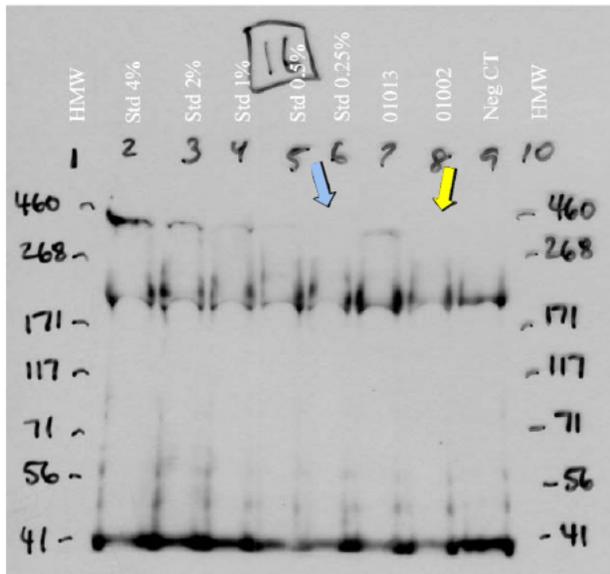
## 2. The quality of the standard dilution series affected their accurate quantitation

Data associated with an individual gel was considered acceptable if the standard curve R<sup>2</sup> value was  $\geq 0.90$ . Individual gels were graded as pass/fail based on this R<sup>2</sup> criteria. All five (5) data points of the standard curve must be incorporated into the R<sup>2</sup> evaluation. An individual gel that fails these acceptance criteria was repeated when necessary. In some cases the standard dilution series was imperceptible at the level of the band of the biopsy (**Figure 23**) and in others the quality of the bands was of such low quality that quantitation using that band does not seem credible (**Figure 24**).

In **Figure 23**, Subject 13 is reported as 1.15% and Subject 002 as 0028. The band for this subject and that of 0.25% in the gel do not seem perceptible. The actual selection of what the instrumentation will quantify is subjective and it does not seem that in the case of this gel, one can accurately discriminate a band for Subject 002 (yellow arrow) or the 0.25% band in the lane (blue arrow). In **Figure 24**, Subject 006 is reported as being 2.83 percent of normal (yellow arrow). The lane for the 2% serial dilution (blue arrow) does not appear usable as a reference.

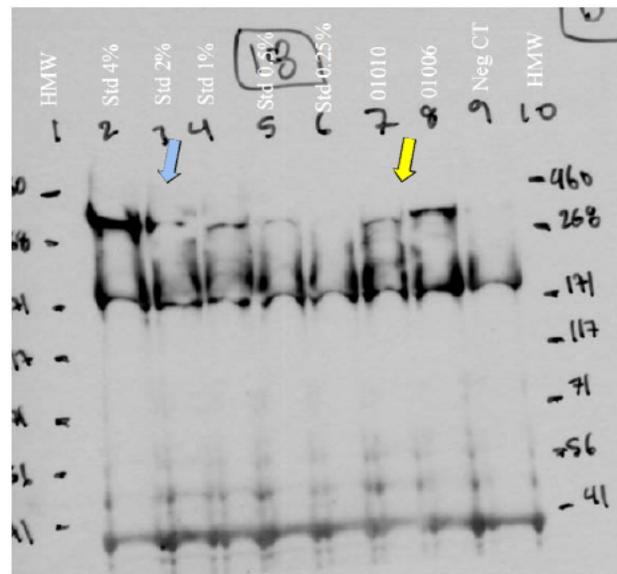
It is noteworthy that when levels below 0.25% were encountered, the applicant reported it as 0 (Below LOQ) and when levels above 4% were found, the samples were diluted to obtain quantitation within 0.25-4% and a dilution factor was then applied to the result. This would bias the results so that more control subjects would seem to have no immunoreactive band in the Western blot analyses. I would acknowledge it is methodologically more sound to dilute the samples that are too concentrated than to interpret levels below the limit of quantification; however, the rejection of so many gels seems to be biased against the controls.

Figure 23 Western blot for Subject 01013 and 01002, 4<sup>th</sup> biopsy



Source: 4658-us-sr-cr-15-004.pdf, p 96 of 101;  
data from p. 81 of the CSR.

Figure 24 Western blot for Subject 01010 and 01006, 4<sup>th</sup> biopsy



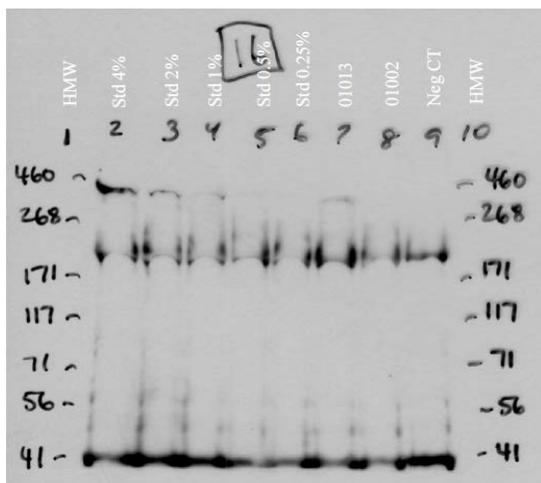
Source: 4658-us-sr-cr-15-004.pdf, p 93 of 100;  
data from p. 80 of the CSR.

3. The Applicant used a different antibody for the fourth biopsy Western Blots. This inhibited our ability to make comparisons to all of the subjects' pretreatment baseline from the previous studies. The Applicant included the pretreatment tissue from 3 subjects as a bridge between the different biopsy results however these data were not informative because it is not clear that these subjects represent a random representation of the entire original 12 subjects.

One of the 3 subjects with baseline DYS1 data did not have a Week 180 result

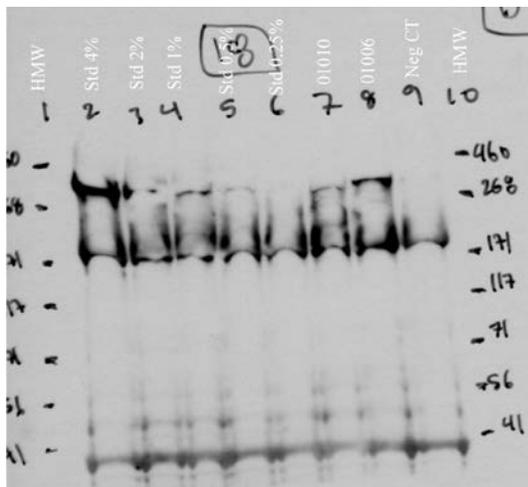
4. Certain DYS1-IR bands do not seem to correspond to the levels reported

**Figure 25 Subject 2 reported as 0.28%**



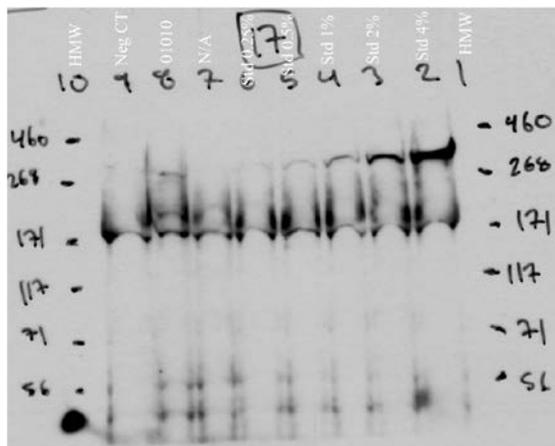
Source: 4658-us-sr-cr-15-004.pdf, p.88 of 101;  
 Lane 7 – Subject 002 sample, Lane 6 – 0.25% standard

**Figure 26 Subject 10 reported as 1.78%**



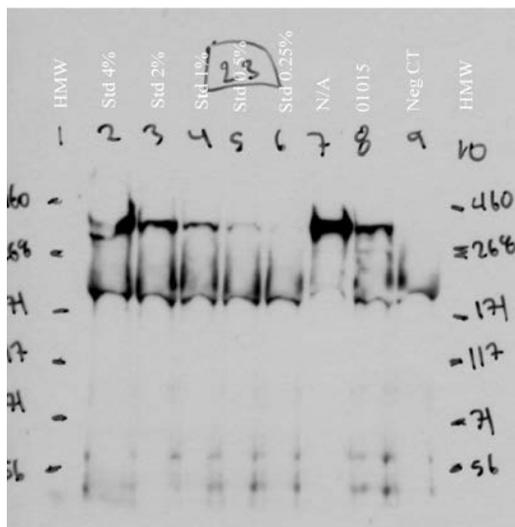
Source: 4658-us-sr-cr-15-004.pdf, p 93 of 101;  
 Lane 7 – Subject 101 sample, Lane 5 – 0.5% standard, Lane 4 – 1.0% standard, Lane 3 – 2% standard

**Figure 27 Subject 10 reported as 1.45%**



Source: 4658-us-sr-cr-15-004.pdf, p.92 of 101; Lane 8 – Subject 010 sample, Lane 5 – 0.5% standard, Lane 4 – 1.0% standard., Lane 3 – 2% standard

**Figure 28 Subject 15 reported as 2.43%**



Source: 4658-us-sr-cr-15-004.pdf, p. 101 of 101;  
 Lane 8 – Subject 015 sample, Lane 3 - 2.0% standard

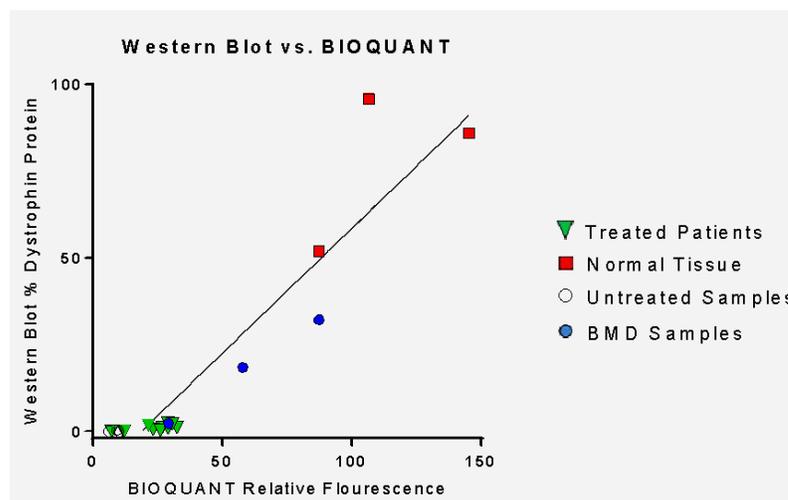
Such differences take on increased meaning given the few subjects in this “bridge” and in the original sample.

**[AR] Comments on the Applicant’s correlation of the Western Blot and Intensity Data**

The applicant has described (a) the correlation between dystrophin measured by western blotting and Bioquant fluorescence and (b) the proposed linear relationship between dystrophin amount and western blot band intensity. Both are reviewed below.

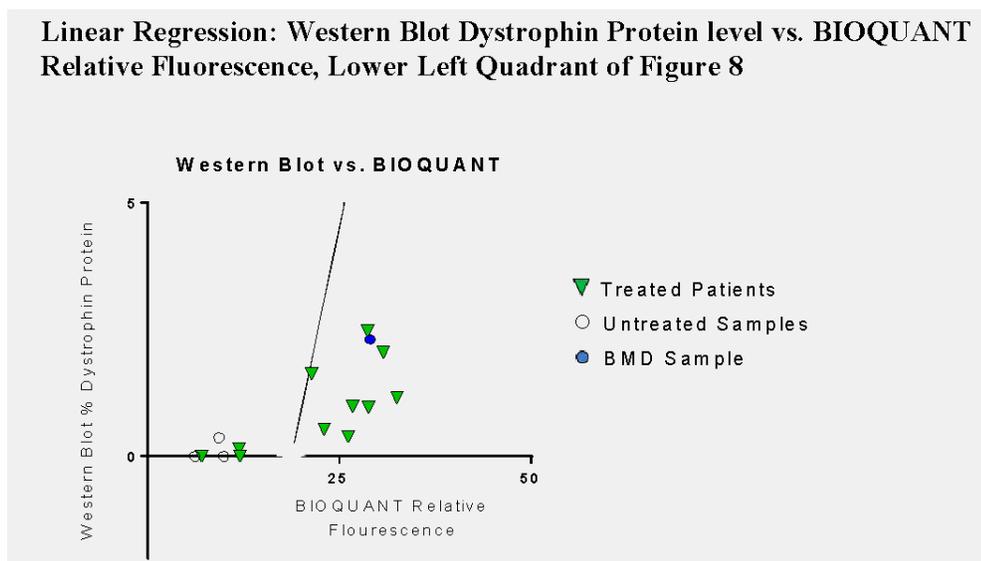
Based on their week-180 data with DMD, BMD, and healthy samples, the applicant claims that the r-square value of the western blotting and Bioquant data is 0.8741 (below from study report SR-CR-15-002). Much of the applicant’s data has very low dystrophin; hence they provided a second graph enlarging the lower left quadrant (**Figure 30** below). While the linear regression line shown on both graphs is the same, it does not appear that the dystrophin data points at levels below 1% on the western blot Y-axis support a linear relationship. It appears that the Bioquant quantitation tends to overestimate dystrophin levels because in instances where western blotting showed 0-0.25% dystrophin, the applicant shows 10-25% of dystrophin by Bioquant with the same biopsy samples. Hence, at less than 1% dystrophin levels, the two bioassays do not appear to correlate well with each other.

**Figure 29 Applicant’s Analysis of the Correlation between the Immunohistochemistry and Western Blot Intensity data by Subject**



Source: 4658-us-sr-cr-15-004

**Figure 30 Applicant's Analysis of the Correlation between the Immunohistochemistry and Western Blot Intensity data by Subject at the Lower End of The Intensity Scale**



Source: 4658-us-sr-cr-15-004

[AR] The applicant provided validation data with DMD, BMD, and healthy samples to support their proposed linear relationship between the dystrophin amount and western blot band intensity. I agree with the applicant's claim that it is not possible to have a single assay with a linear range of detection of 0.1 to 100% because there is currently no available reference standard (such as full-length or truncated recombinant human dystrophin) to allow direct measurements and because western blotting is not intended to be truly quantitative over a wide range of protein levels. The applicant's validation efforts were focused on low levels of dystrophin because they expected to have levels comparable to those found in BMD patients. Based on Anthony et al (Neurology, 2014), Brown et al (J Bioanal Biomed, 2012), and van den Bergen (J Neurol Neurosurg, 2014), the applicant focused on establishing conditions for linear measurements at levels of dystrophin <5%.

[AR] In validation report SR-15-023, the applicant describes their findings for testing (a) spike/recovery, (b) precision, (c) intermediate precision, (d) linearity, and (e) LOD/LOQ using predefined acceptance criteria and BMD, DMD, and healthy samples. A working range of 0.25% to 4% was established by the applicant, where 0.25% was their lower limit of quantitation (LLOQ) and 4% was their upper LOQ. When levels below 0.25% were encountered, the applicant reported it as 0 (Below LOQ) and when levels above 4% were found, the samples were diluted to obtain quantitation within 0.25-4% and a dilution factor was then applied to the result. A serial dilution was included on each gel with test samples and the applicant claims that an r-square of >0.9 was calculated on each set of serial dilution used for extrapolating patient sample data. Overall, the linearity of the Western blot assay between 0.25 to 4% appears to be reasonably qualified by the use of a serial dilution on each gel. However, the correlation between western blotting and Bioquant dystrophin levels does not appear linear at levels of dystrophin below 1% in the western blot method. There also seemed to be a large number of gels with levels below the level of quantification, both for baseline/untreated and treated samples (Table 12).

**Clinical Function Data, Study 201 Placebo-Controlled Trial**

This section describes the results from principle clinical assessments, the 6MWT, NSAA Total Score, Rise Time, 10-Meter Run in the placebo-controlled 201 Study. The 202 historically controlled study results are presented following this Section.

6-Minute Walk Test

On those visits where 2 tests were performed, the Applicant used the greatest 6MWT distance for the principal analysis. The Applicant performed an ANCOVA of ranked data to compare the 2 eteplirsen treatment groups to placebo because the assumptions of normality were violated. This analysis showed no significant differences between the treatment groups (**Table 14**).

**Table 14 Analysis of Change from Baseline for 6 Minute Walk Test (Study 201/202 ITT and mITT Populations)**

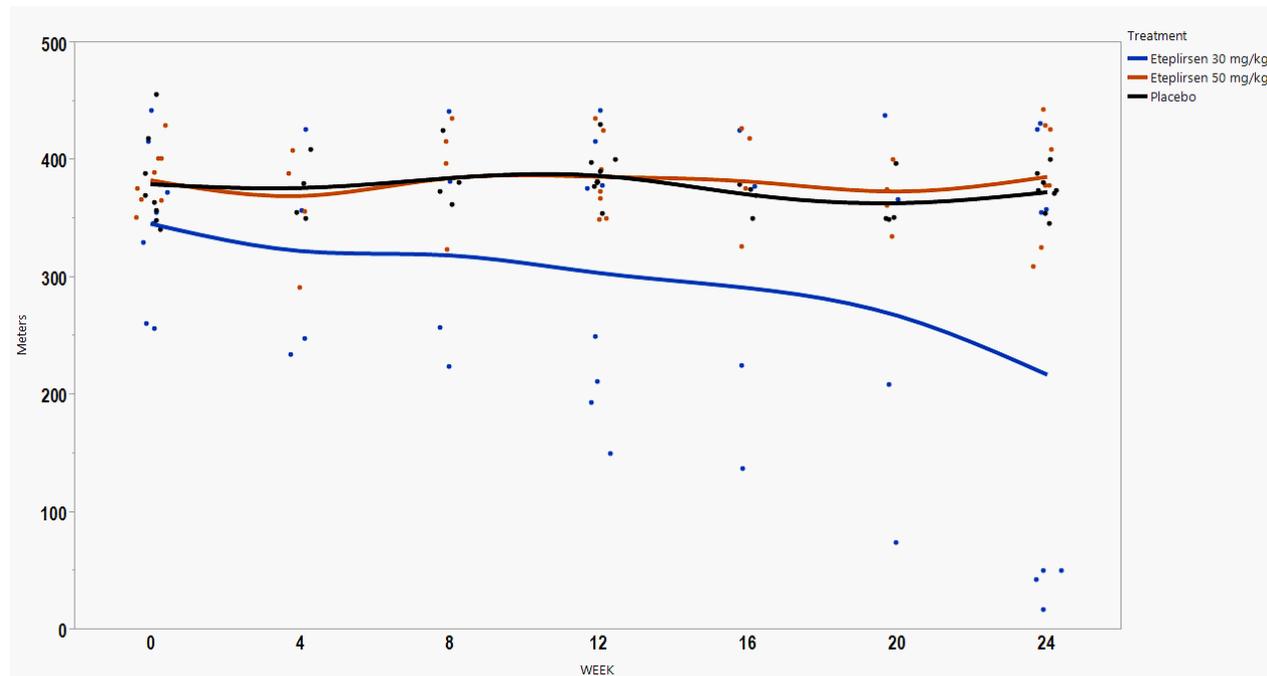
Treatment	Model Adjusted Change from Baseline	P Value for treatment vs PBO	Estimated Treatment Effect	95% CI
Analysis of Change from Baseline for 6 Minute Walk Test Using Ranked Data				
Pbo	6.4			
30 mg	4.3	.425	-2.2	( -8.2, 3.9)
50 mg	8.8	.378	2.3	( -3.5, 8.2)
Pbo vs. All AVI-4658	6.4 / 6.6	0.939	0.2	(-5.3, 5.7)

Source: 4658-us-201-tables and figures, Table 14.2.5.1 p. 607 of 2239 and Table A.14.2.5.1, p. 2057 of 2239

***Reviewer’s Analyses and Comments***

My own analysis concurred with the Applicants finding that there was no statistical difference between the Eteplirsen and placebo groups in the first 24 week, placebo controlled portion of the 201/202 Study.

**Figure 31 Six Minute Walk Test Performance by Treatment and Visit in the Placebo-Controlled Portion of Study 201/202 (ITT Population)**

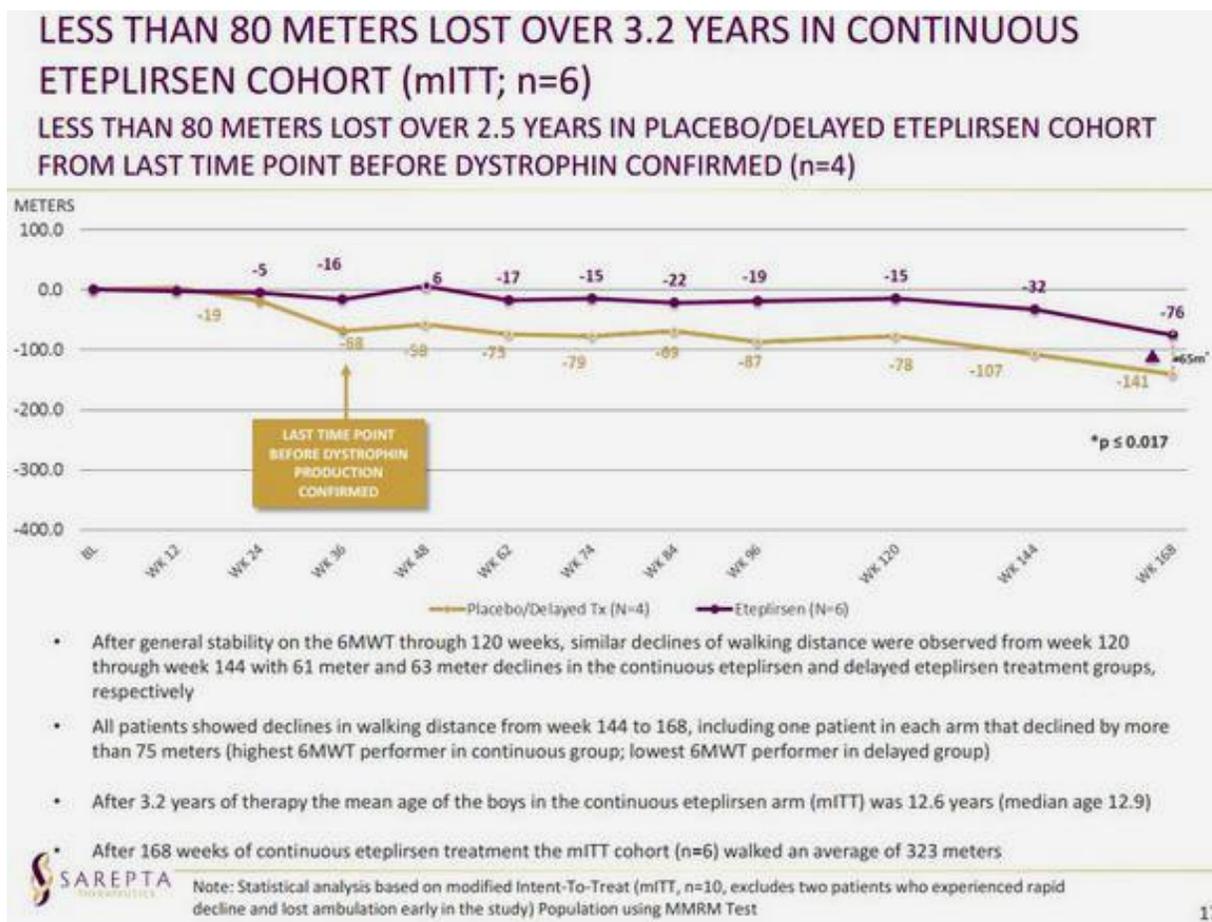


Source: Medical Reviewer Analysis of ADSMW.XPT

The Applicant has proposed removing Subjects 009 and 010 from the full analysis because of their decline in performance. This is violates the principles of the Intent to Treat. Authors in the literature who advocate using a “modified” Intent to Treat Population, note that “...excluding patients after randomisation may introduce non-comparability of characteristics across treatment groups and consequently lead to bias.” [Abraha and Montedori 2010; see also Sainani 2010]. It is noteworthy that Sarepta has made public claims on their website that when placebo subjects transitioned to eteplirsen they seemed to recover function from week 36 (**Figure 32**)<sup>10</sup>.

<sup>10</sup> <http://investorrelations.sarepta.com/phoenix.zhtml?c=64231&p=irol-newsArticle&ID=2006709>

**Figure 32 Figure Publically Released by Sarepta Erroneously Inferring Clinically Significant Treatment Effect Switching From Placebo to Eteplirsen by Week 36<sup>11</sup>**

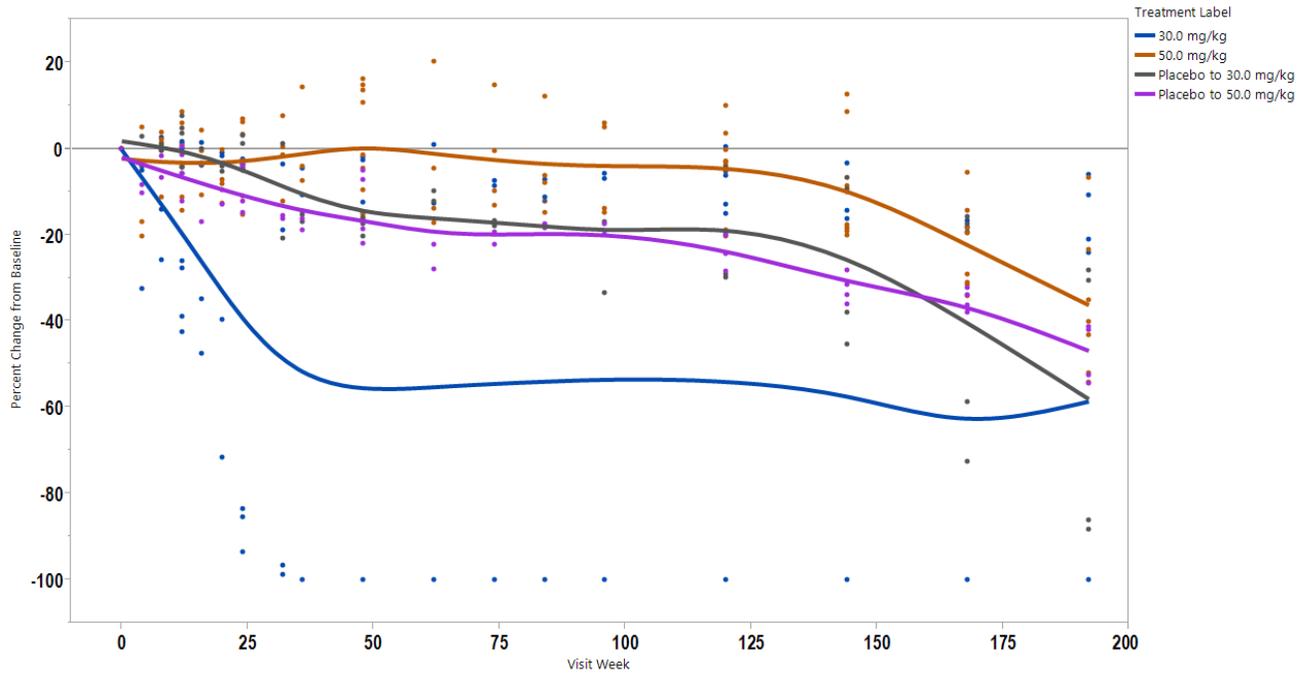


In this analysis, Sarepta has combined the patients actively treated in 2 dose groups from the beginning into a single group, which is different from the prespecified analysis, and then omitted the two subjects who declined in performance. They have also combined the two placebo sequence groups, although these subjects are treated by two different doses after Week 24.

For my own analysis of this issue, I plotted the performance on the Six Minute Walk Test by Treatment Sequence using an ITT population. As may be seen in **Figure 33**, the patients transitioned from placebo to drug decline without stabilization during this period.

<sup>11</sup> FORM 8-K January 9, 2015, Sarepta Therapeutics, Inc. p 14

**Figure 33 Percent Change from Baseline on the Six Minute Walk Test in Study 201/202 by Treatment Sequence and Visit (ITT Population)**



Source: Medical Reviewer's analysis of the t-smv-csv.txt dataset

In summary, the placebo controlled portion of study 201 does not show a clinical benefit for eteplirsen in the 6MWT.

### NSAA Total Score

The Applicant performed an MMRM analysis of the full analysis population that revealed a statistically significant difference between the placebo and 30 mg/kg/wk groups in favor of *placebo* at Week 24. The Applicant used the best score on visits where two tests were performed.

**Table 15 Summary and Change from Baseline in NSAA Total Scores (Full Analysis and mITT Populations)**

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT) <sup>a</sup> (N = 2)	50 mg/kg/wk (N = 4)
<b>Baseline<sup>b</sup></b>				
Mean	23.3	20.8	22.5	29.0
Median	22.0	19.0	22.5	29.0
SD (SE)	3.30 (1.65)	5.19 (2.59)	7.78 (5.50)	2.31 (1.15)
Min, Max	21, 28	17, 28	17, 28	27, 31
<b>Week 24<sup>c</sup></b>				
Mean	26.5	14.8	23.5	26.8
Median	26.5	13.5	23.5	27.0
SD (SE)	4.04 (2.02)	10.53 (5.27)	4.95 (3.50)	5.12 (2.56)
Min, Max	23, 30	5, 27	20, 27	21, 32
<b>Change at Week 24</b>				
Mean	3.3	-6.0	1.0	-2.3
Median	2.0	-5.5	1.0	-2.0
SD (SE)	2.50 (1.25)	8.60 (4.30)	2.83 (2.00)	2.99 (1.49)
Min, Max	2, 7	-16, 3	-1, 3	-6, 1

Source – 4658-us-201-body, Table 11-7, p. 69 of 107

<sup>a</sup> mITT excludes patients 009 and 010; <sup>b</sup> Baseline is the last non-missing value before first dose; <sup>c</sup> Week 24 is the best score achieved on days 1 and 2 of that visit.

**Abbreviations:** max = maximum; min = minimum; mITT = modified intent to treat population; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SE = standard error.

### Reviewer's Analyses and Comments

In my review of the NSAA, I noted two values for the 12 and 24 week visits in the datasets submitted by the applicants. Rather than use the maximum value, I used the average NSAA Score.

There was a potentially meaningful difference in the Baseline scores between the study arms. (Table 16).

**Table 16 Comparison of the Baseline in the NSAA Total Score by Treatment during the Placebo Controlled Portion of Study 201 / 202**

Level	- Level	Difference	Lower CL	Upper CL	p-Value
Eteplirsen 50 mg/kg	Eteplirsen 30 mg/kg	8.25	2.2	14.32	0.01*
Eteplirsen 50 mg/kg	Placebo	5.75	-0.32	11.82	0.06
Placebo	Eteplirsen 30 mg/kg	2.50	-3.57	8.57	0.38

Source: Medical Reviewer analysis of the ADEFF2.XPT dataset

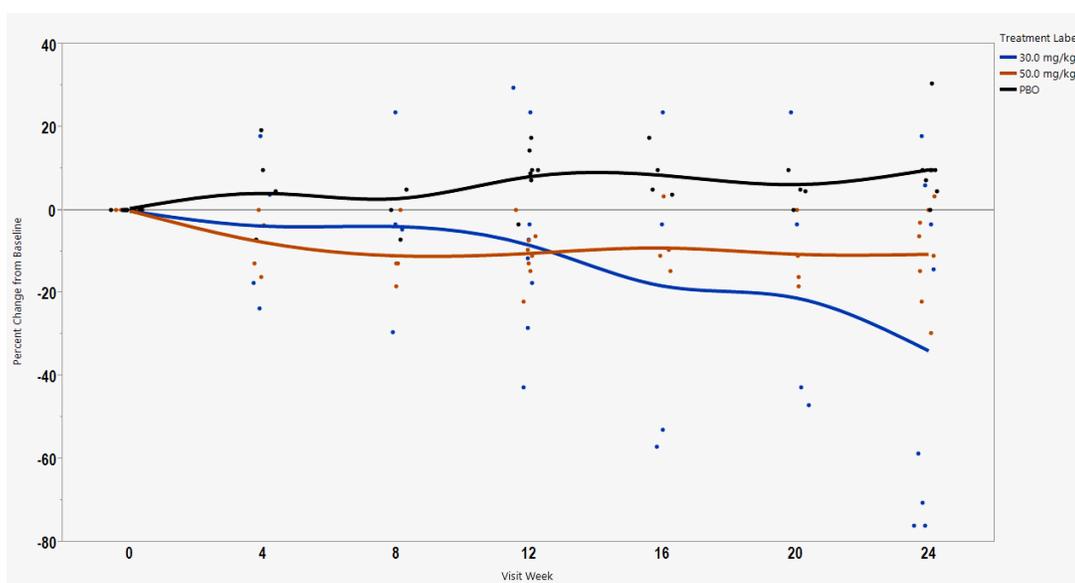
With respect to the treatment effects demonstrated in the placebo controlled portion of Study 201 / 202, there was a significant difference between the percent change from baseline for the contrast between placebo and 30 mg/kg group at 24 weeks in favor of *placebo* ( Table 17 and Figure 34).

**Table 17 Comparison of the Percent Change from Baseline in the NSAA Total Score by Treatment during the Placebo Controlled Portion (to Week 24) of Study 201 / 202 (ITT Population)**

Level	- Level	Difference	Lower CL	Upper CL	p-Value
Placebo	Eteplirsen 30 mg/kg	34.60	14.3930	54.80	0.002*
	Eteplirsen 50 mg/kg	10.61149	-9.59	30.82	0.30

Source: Medical Reviewer analysis of the ADEFF2.XPT dataset

**Figure 34 Percent Change from Baseline in the NSAA Total Score by Visit and Treatment (ITT Population)**



Source: Medical Reviewer analysis of the ADEFF2.XPT dataset

In summary, the placebo controlled portion of study 201 does not show a clinical benefit for eteplirsen in the NSA Total Score.

Rise time

The Applicant modified their planned analysis for Rise Time as their “...intent for this plan was to use the patient’s best score as a reflection of best effort made.<sup>12</sup>” According to the 201/202 study report, no statistically significant differences between the treatment groups were detected (**Table 18**).

<sup>12</sup> 4658-us-201-body.pdf, p 55 of 107

**Table 18 Summary and Change from Baseline in Rise Time (Full Analysis and mITT Populations)**

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT) <sup>a</sup> (N = 2)	50 mg/kg/wk (N = 4)
<b>Baseline<sup>b</sup></b>				
Mean	6.63	8.55	7.60	5.73
Median	6.30	9.15	7.60	3.90
SD (SE)	1.360(0.680)	4.576(2.288)	6.223(4.400)	4.213(2.106)
Min, Max	5.4, 8.5	3.2, 12.7	3.2, 12.0	3.1, 12.0
<b>Week 24<sup>c</sup></b>				
Mean	5.93	14.25	4.50	10.28
Median	5.45	11.55	4.50	3.50
SD (SE)	1.632(0.816)	12.479(6.239)	1.556(1.100)	13.822(6.911)
Min, Max	4.6, 8.2	3.4, 30.5	3.4, 5.6	3.1, 31.0
<b>Change at Week 24</b>				
Mean	-0.70	5.70	-3.10	4.55
Median	-0.65	5.70	-3.10	-0.20
SD (SE)	1.140(0.570)	10.852(5.426)	4.667(3.300)	9.635(4.818)
Min, Max	-2.1, 0.6	-6.4, 17.8	-6.4, 0.2	-0.4, 19.0

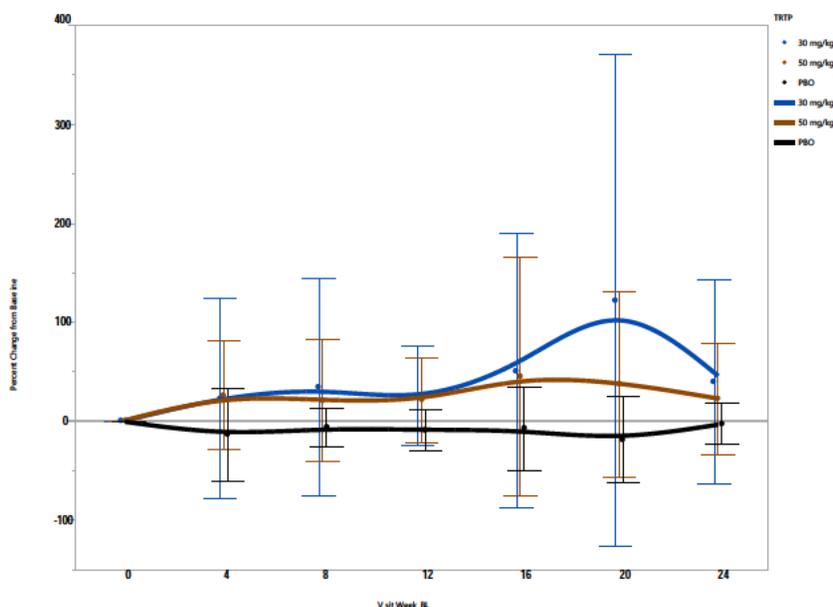
Source: Table 14.2.2.1, Table A.14.2.2.1  
<sup>a</sup> mITT excludes patients 009 and 010.  
<sup>b</sup> Baseline is the last non-missing value before first dose.  
<sup>c</sup> Week 24 is the best time achieved on days 1 and 2 of that visit.  
 Abbreviations: max = maximum; min = minimum; mITT = modified intent to treat population; SD = standard deviation; SE = standard error.

Source: Table 11-0, 4658-us-201-body.pdf, p. 71 of 107

**Reviewer’s Analyses and Comments**

The Rise Time is an important secondary outcome. My own analysis was based on the average rise time. I also found no differences between treatment groups for this test (**Figure 35**). Numerically, the 30 mg/kg group and the 50 mg/kg group did worse (i.e., rise time increased more) than placebo.

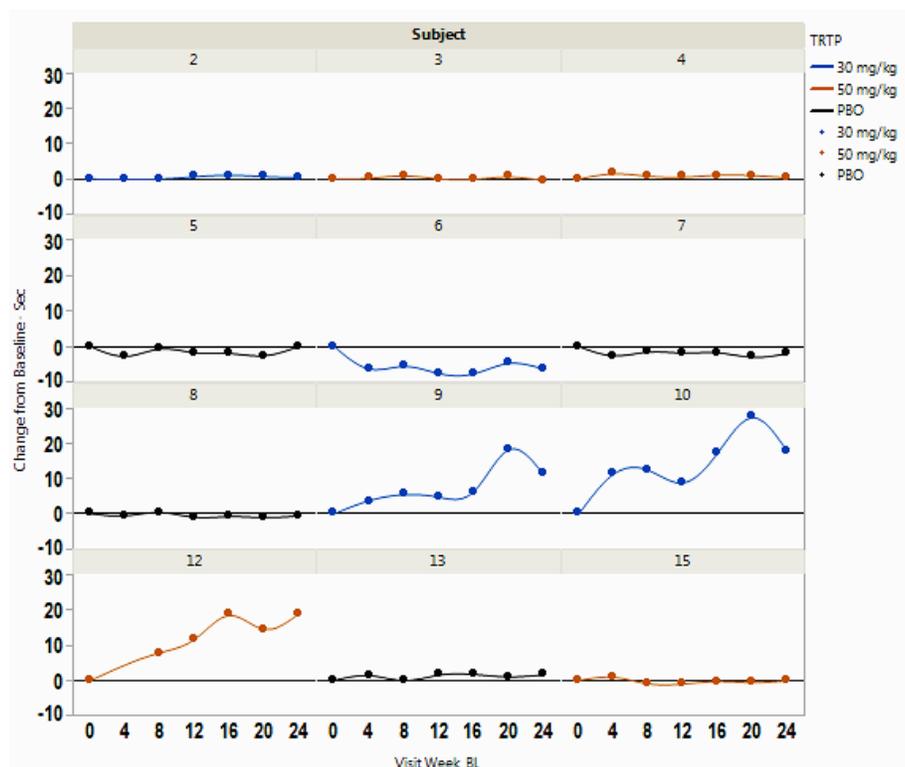
**Figure 35 Percent Change from Baseline in Rise Time (95% CI) by Visit and Treatment during the Placebo Controlled Portion of the 201/202 Trial (ITT Population)**



Source: Medical Reviewer analysis of t-nstar-csv.txt dataset  
 Each error bar is constructed using a 95% confidence interval of the mean.

Evaluation of the Rise Time data at the subject level demonstrates Subjects 009 and 010 on 30 mg/kg and Subject 012 on 50 mg/kg had marked increase in Rise time while on active treatment (**Figure 36**) during this portion of the study.

**Figure 36 Change in Rise Time (Seconds) By Visit and Subject during the Placebo Controlled Portion of the 201/202 Trial (ITT Population)**



Source: Medical Reviewer analysis of t-nstar-csv.txt dataset  
The black reference line marks no change

In summary, the placebo controlled portion of study 201 does not show a clinical benefit for eteplirsen in the Rise Time.

### Timed 10-meter run

The Applicant modified their analysis to only assess the best (lowest) time on the 10-Meter run, stating that it should reflect "... the best effort made<sup>13</sup>" (**Table 19**). They noted that the placebo group generally performed better than the 30 mg/kg group and that using an analysis appropriate for non-normal data (these data are not normal) favored the placebo group over the 50-mg/kg group at Week 4 (4.7 sec vs. 9.98 sec,  $P = 0.04$ ) but comparisons other timepoints were not significantly different (**Figure 37**).

<sup>13</sup> 4658-us-201-body, P. 55 of 107

**Table 19 Summary and Change from Baseline in 10-Meter Run (Full Analysis and mITT Populations)**

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT) <sup>a</sup> (N = 2)	50 mg/kg/wk (N = 4)
<b>Baseline<sup>b</sup></b>				
Mean	6.43	6.95	5.50	5.30
Median	6.55	7.60	5.50	4.90
SD (SE)	1.011(0.506)	2.138(1.069)	2.263(1.600)	1.111(0.555)
Min, Max	5.1, 7.5	3.9, 8.7	3.9, 7.1	4.5, 6.9
<b>Week 24<sup>c</sup></b>				
Mean	5.78	13.10	4.35	4.78
Median	5.70	9.40	4.35	4.30
SD (SE)	0.150(0.075)	11.836(5.918)	0.212(0.150)	1.839(0.920)
Min, Max	5.7, 6.0	4.2, 29.4	4.2, 4.5	3.1, 7.4
<b>Change at Week 24</b>				
Mean	-0.65	6.15	-1.15	-0.53
Median	-0.70	3.25	-1.15	-0.55
SD (SE)	0.985(0.492)	10.368(5.184)	2.051(1.450)	0.866(0.433)
Min, Max	-1.8, 0.6	-2.6, 20.7	-2.6, 0.3	-1.5, 0.5

Source: Table 14.2.2.1, Table A.14.2.2.1

<sup>a</sup> mITT excludes patients 009 and 010.

<sup>b</sup> Baseline is the last non-missing value before first dose.

<sup>c</sup> Week 24 is the best time achieved on days 1 and 2 of that visit.

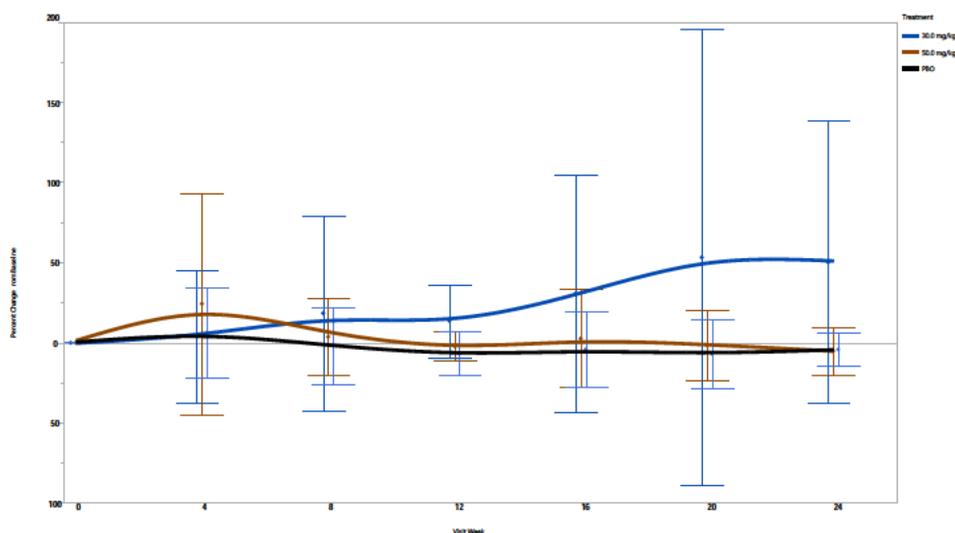
**Abbreviations:** max = maximum; min = minimum; mITT = modified intent to treat population; SD = standard deviation; SE = standard error.

Source: Table 11-8, 4658-us-201-body.pdf, p. 70 of 107

**Reviewer’s Analysis and Comments**

My own graphic analysis concurred with the Applicant’s (Figure 37) that there was no difference between treatment groups at Week 24.

**Figure 37 Percent Change from Baseline in the 10-Meter Run Time during the Placebo-Controlled Portion of the Study 201 / 202 (ITT Population)**



Source: Medical Reviewer analysis of t-nstar-csv.txt dataset  
 Each error bar is constructed using 1 standard deviation from the mean.

In summary, the placebo controlled portion of study 201 does not show a clinical benefit for eteplirsen in the 10-Meter Run.

### Timed 4 Step Test

In the placebo controlled portion of the study, the Applicant's analysis revealed statistically significant differences between the placebo and 30 mg/kg/wk eteplirsen groups in favor of *placebo* at Weeks 8, 16, and 20 (**Table 20**).

**Table 20 Timed Four Step Test in the Placebo-Controlled Portion of Study 201/202 (ITT and mITT population)**

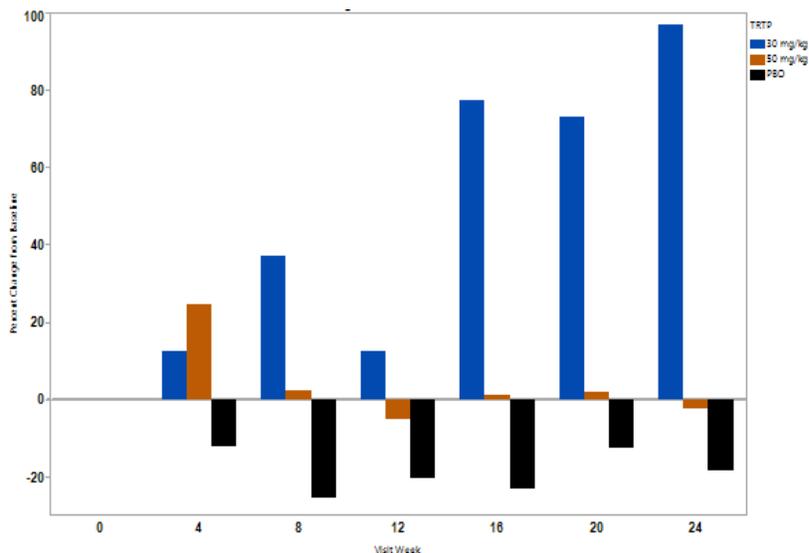
Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT) <sup>a</sup> (N = 2)	50 mg/kg/wk (N = 4)
<b>Baseline<sup>b</sup></b>				
Mean	5.30	4.88	3.75	3.50
Median	4.35	4.80	3.75	3.35
SD (SE)	1.934(0.967)	1.355(0.677)	0.354(0.250)	1.074(0.537)
Min, Max	4.3, 8.2	3.5, 6.4	3.5, 4.0	2.4, 4.9
<b>Week 24<sup>c</sup></b>				
Mean	4.08	14.73	3.70	3.35
Median	4.15	10.15	3.70	3.15
SD (SE)	0.685(0.342)	15.069(7.535)	0.990(0.700)	1.240(0.620)
Min, Max	3.3, 4.7	3.0, 35.6	3.0, 4.4	2.1, 5.0
<b>Change at Week 24</b>				
Mean	-1.22	9.85	-0.05	-0.15
Median	-0.80	5.35	-0.05	-0.05
SD (SE)	1.597(0.798)	13.797(6.898)	0.636(0.450)	1.115(0.558)
Min, Max	-3.5, 0.2	-0.5, 29.2	-0.5, 0.4	-1.6, 1.1

Source: [Table 14.2.6.1, Table A.14.2.6.1](#)  
<sup>a</sup> mITT excludes patients 009 and 010.  
<sup>b</sup> Baseline is the last non-missing value before first dose.  
<sup>c</sup> Week 24 is the best time achieved on days 1 and 2 of that visit.  
**Abbreviations:** max = maximum; min = minimum; mITT = modified intent to treat population; SD = standard deviation; SE = standard error.

### ***Reviewer's Analyses and Comments***

In my own analysis of the Four Step data, I noted that from the data from the ITT population, the placebo subjects appeared to have performed numerically better than those in the 30 or 50 mg/kg group (**Figure 38**).

**Figure 38 Median Percent Change from Baseline in Four Step Test by Treatment and Visit (ITT Population)**



Source: Medical Officer's review of t-fst-csv.txt

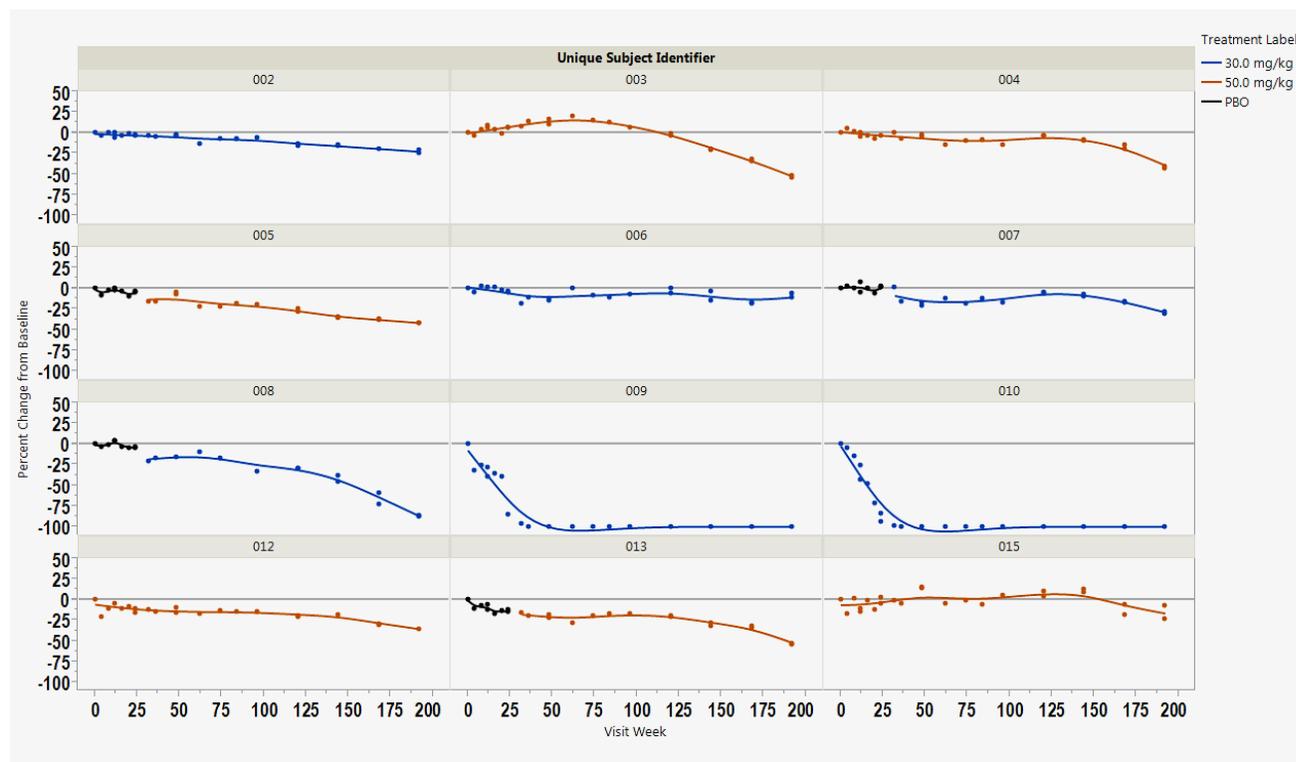
The Applicant has made claims that subjects originally randomized to placebo were notably stabilized after switching to eteplirsen by the 36 week visit (**Figure 32**). However, **Figure 57** (in long-term data description of next section) suggests that there is no clear improvement after subjects switch to the active treatment..

### Long-Term (Open Label and Natural History–Contrasted) Data

#### 6 MWT

Several subjects in addition to 009 and 010 had notable declines in 6MWT (**Figure 39**).

**Figure 39 Percent Change from Baseline on the Six Minute Walk Test in Study 201/202 by Treatment Sequence, Subject and Visit Week (Intent to Treat Population)**



Source: Medical Officer's review of 6MWTDER.XPT dataset

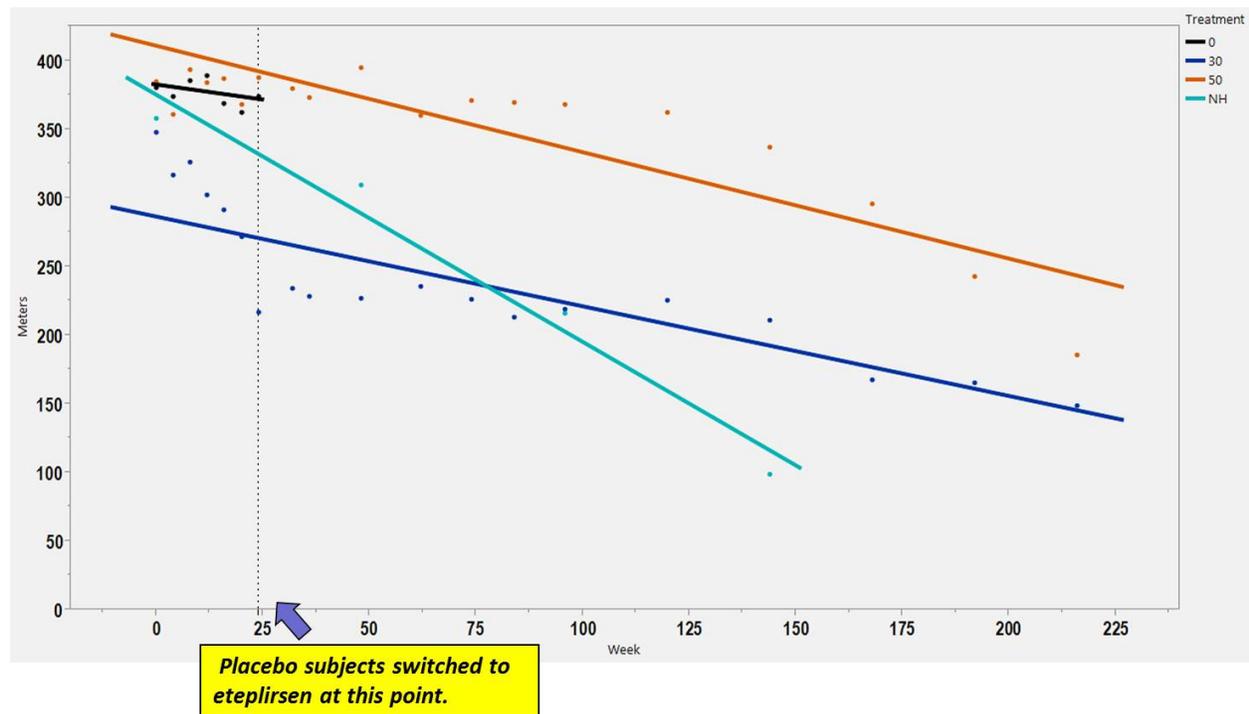
### ***Reviewer's Comments***

The Applicant desires to evaluate the treatment effect of eteplirsen by contrasting it to subjects in two natural history datasets. The subjects were selected based on (A) Age > 7 y (B) Genotype (Exon 51 skipping) (C) Steroid Use (D) 'sufficient' longitudinal 6MWD data. The subjects were not matched based on these criteria but rather, these were general criteria used to filter their natural history cohort.

In my own analysis, I first compared the Natural History subjects to the other treatment sequences in terms of baseline demographics (see **Figures 3-8**). This analysis suggested that the natural history subjects were not well matched with the eteplirsen and natural history cohorts, especially with respect to the steroid regimen, proportion of subjects with baseline 6MWT below 350 meters, and in their baseline NSAA score.

I then graphically looked at their performance versus all of the treatment sequences on the 6MWT to see if the natural history subjects performed in a manner similar to the subjects. The Natural History subjects declined more rapidly from the start of the documented observation period, whereas the placebo subjects have a roughly similar performance to the eteplirsen subjects with respect to the slope of their decline (**Figure 40**). This suggests to me that the natural history subjects were not well matched and that being part of a controlled trial was a bigger factor in performance than the treatment group to which one was assigned.

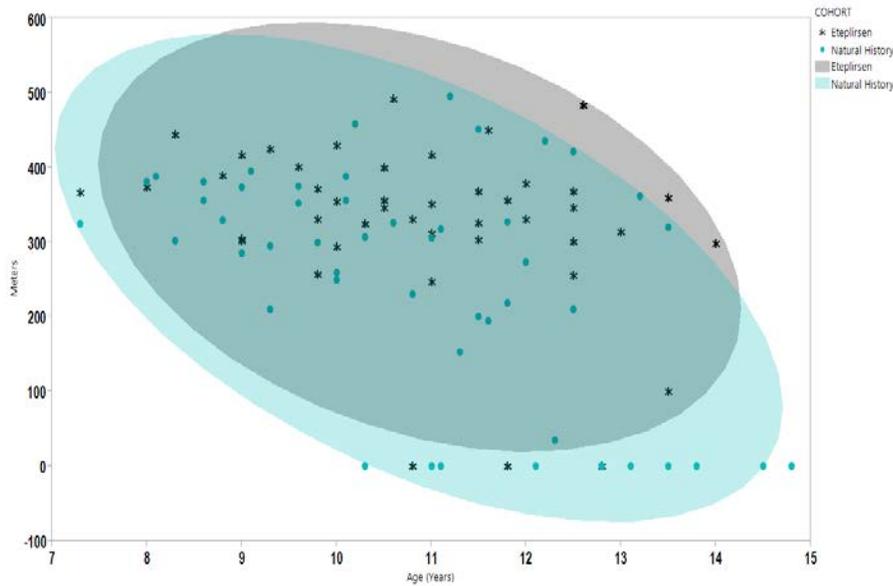
**Figure 40 Performance on the Six Minute Walk by Treatment Group and Week (ITT Population)**



Source: Medical Officer's review of 6MWTDER.XPT dataset

A scatterplot of the 6MWT for the Eteplirsen versus Natural History cohorts by Age was generated. I performed this analysis because I believe that age was a more relevant benchmark than study visit. It is related to disease progression whereas the timing of the clinic visit is a coincidence of when the subject was brought into the trial. For example, if someone is brought in at a younger age, they will more likely have sustained function compared to an older person at the same visit. While this association between age and disease progression is not absolute, it is at least as if not more sensible than looking for an association between visit week and disease progression. Density ellipses were generated at the 95% levels for each cohort. This display suggests that the eteplirsen and natural history cohorts when normalized for age had similar performances on the 6MWT.

**Figure 41 Scatterplot of 6MWT versus Age for the Eteplirsen and Natural History Cohort**

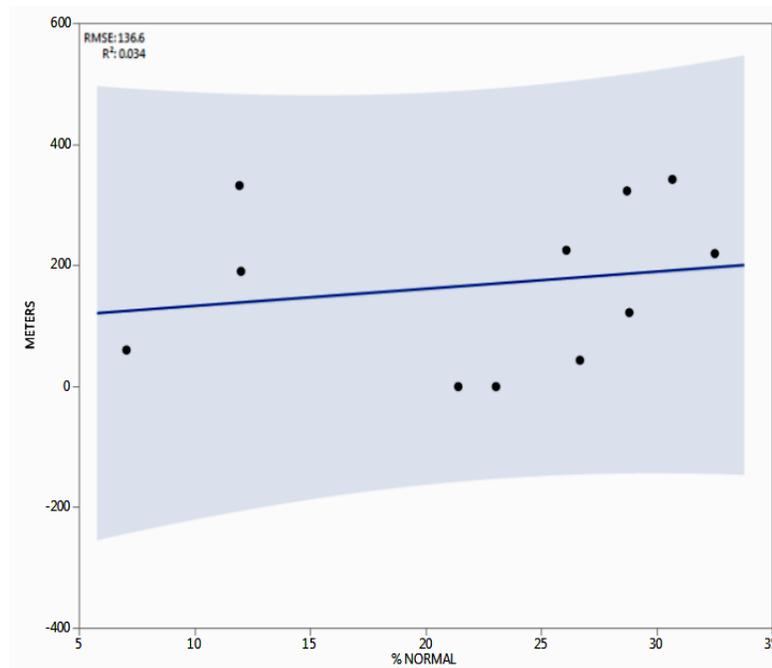


Source: Medical Reviewer Analysis of SWTDER dataset; the ellipses represent 95% normal density

### Correlations of 6MWT and Dystrophin Data

Graphical and correlational analysis of the 6MWT and Week 180 Dystrophin metrics suggests no predictive relationship between the two variables.

**Figure 42 6MWT Distance vs BQ % Normal for eteplirsen treated Subjects at 4 years**



Shaded area represents 95% prediction band

Figure 43 6MWT Distance vs PPF % Normal for eteplirsen treated Subjects at 4 years

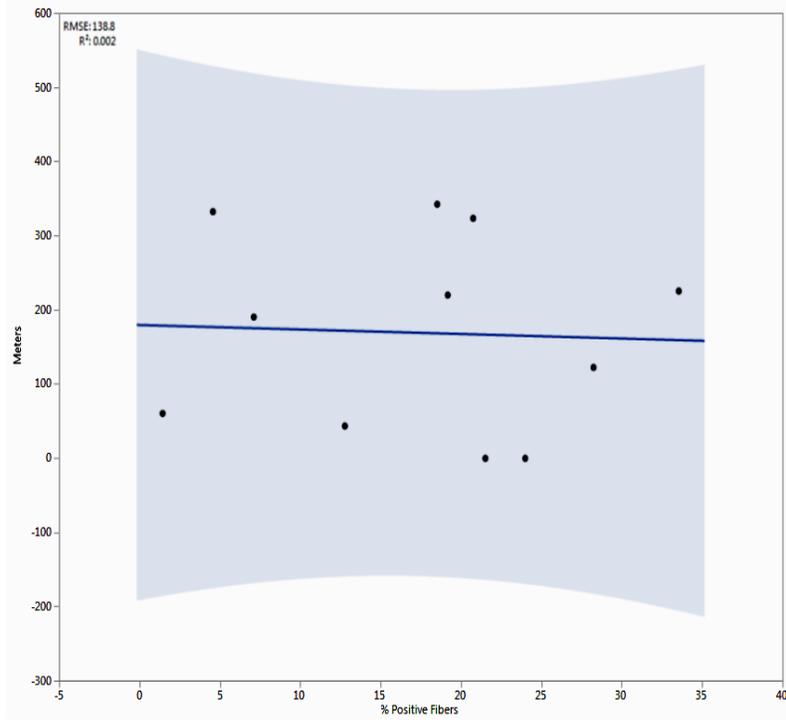
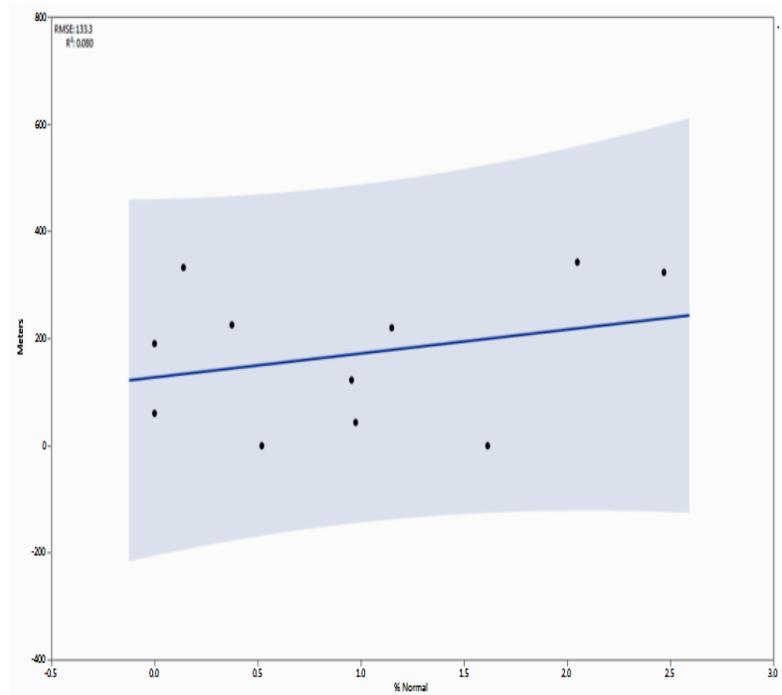


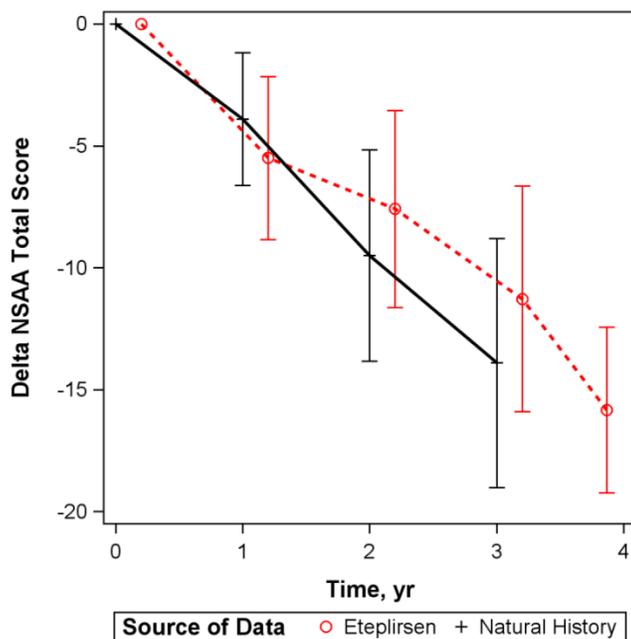
Figure 44 6MWT Distance vs WB % Normal for eteplirsen treated Subjects at 4 years



NSAA Total Score

Performance of the combined eteplirsen and natural history cohorts is depicted below (**Figure 45**).

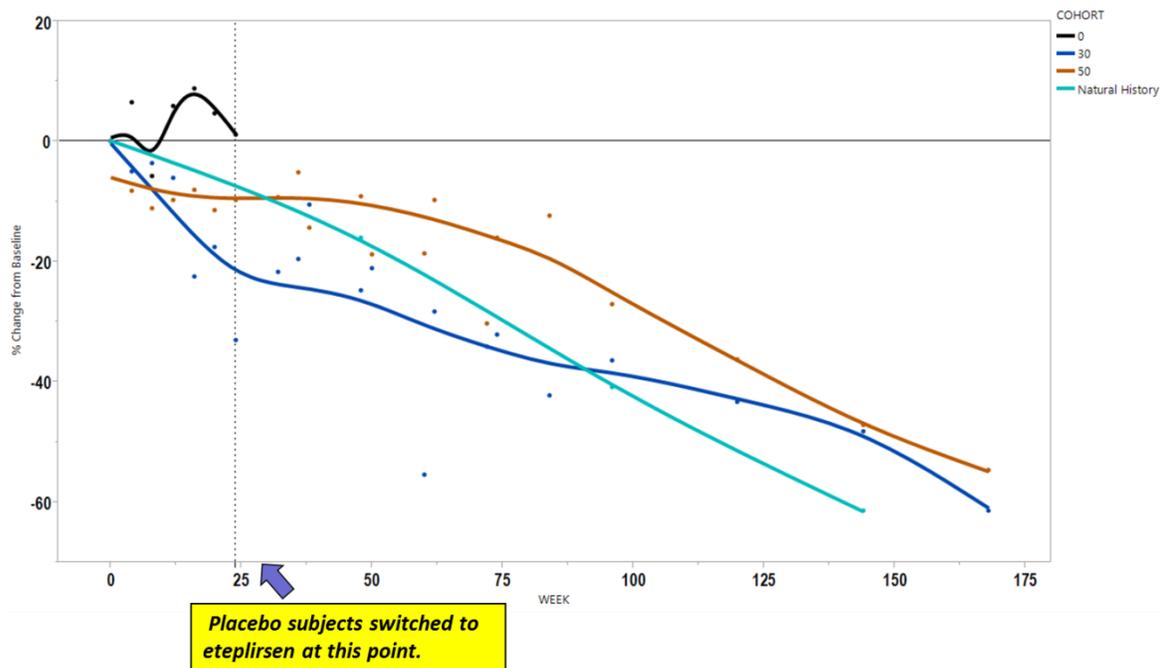
**Figure 45 Change in the NSAA Total Score (95%CI) by Treatment Cohort**



As an initial step in my evaluation of the natural history data related to NSAA performance, I evaluated the baseline data of subjects in this comparison. The Eteplirsen-treated subjects had a mean baseline of 25 and the Natural History cohort, 22, the difference of which was statistically significant ( $P = 0.01$ ). In combination with other numerical differences in baseline characterization, the difference in the eteplirsen and natural history cohort's baseline demographics represent a meaningful difference to me.

**Figure 46** demonstrates the change over time by treatment until Week 192 of the NSAA total score by Week and Original Treatment Group. It is apparent from the trajectories in the first 25 weeks that the natural history cohort performs inferiorly to the placebo subjects from the 201 trial.

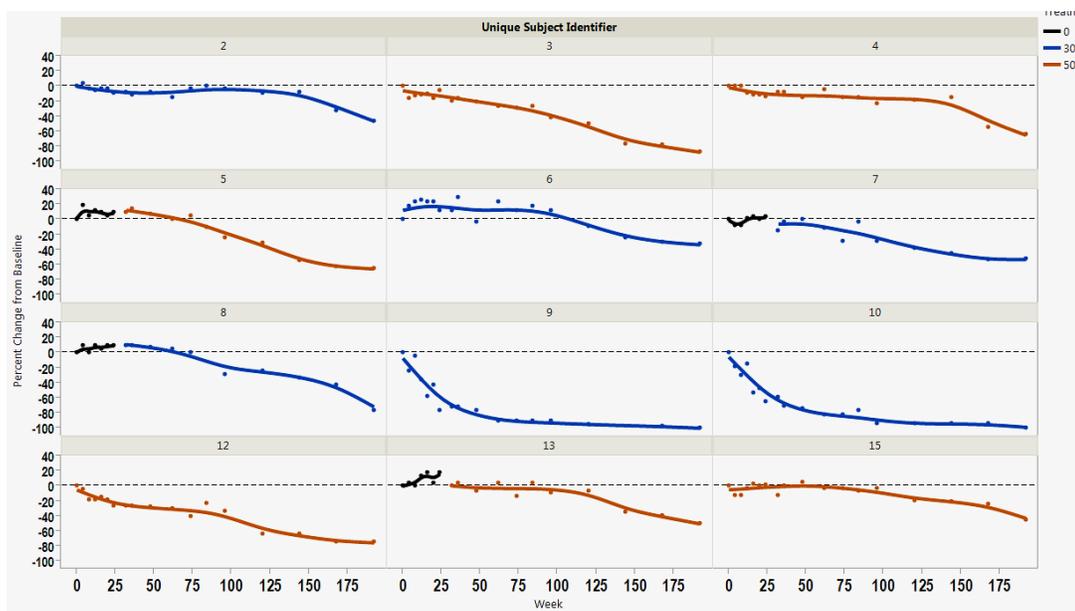
**Figure 46 Percent Change in NSAA Score by Week and Treatment in Study 201 / 202 (ISS Safety Population)**



Source: Medical Reviewer analysis of the t-nstar-csv.txt dataset

Figure 47 shows the change by subject.

**Figure 47 Percent Change in NSAA Total Score by Subject, Treatment and Visit Week in Study 201 / 202 (ITT Population)**

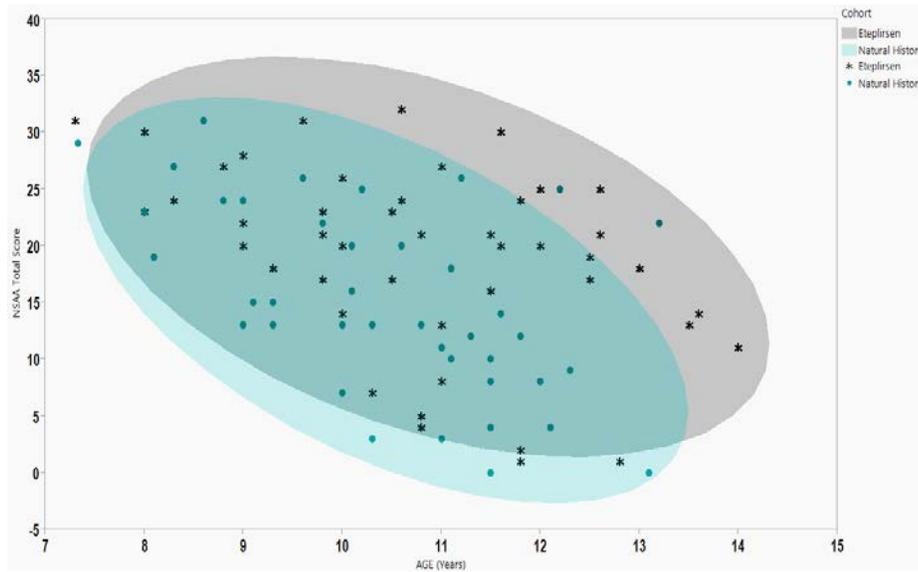


Source: Medical Reviewer analysis of the t-nstar-csv.txt dataset

Clinical Review Christopher Breder, MD PhD  
NDA 206488 (Eteplirsen)

A scatterplot of the NSAA for the Eteplirsen versus Natural History cohorts by Age was generated (Figure 48). This display suggests that the eteplirsen and natural history cohorts when normalized for age had similar performances on the NSAA.

**Figure 48 Scatterplot of Total NSAA Score Performance versus Age for the Eteplirsen and Natural History Cohort**



Source: Medical Reviewer analysis of the NSAADER dataset  
Ellipses represent 95% normal density

### Correlations of NSAA Total Score and Dystrophin Data

Graphical and correlational analysis of the NSAA total score and Week 180 Dystrophin metrics suggests no predictive relationship between the two variables.

Figure 49 Total NSAA Score vs BQ % Normal

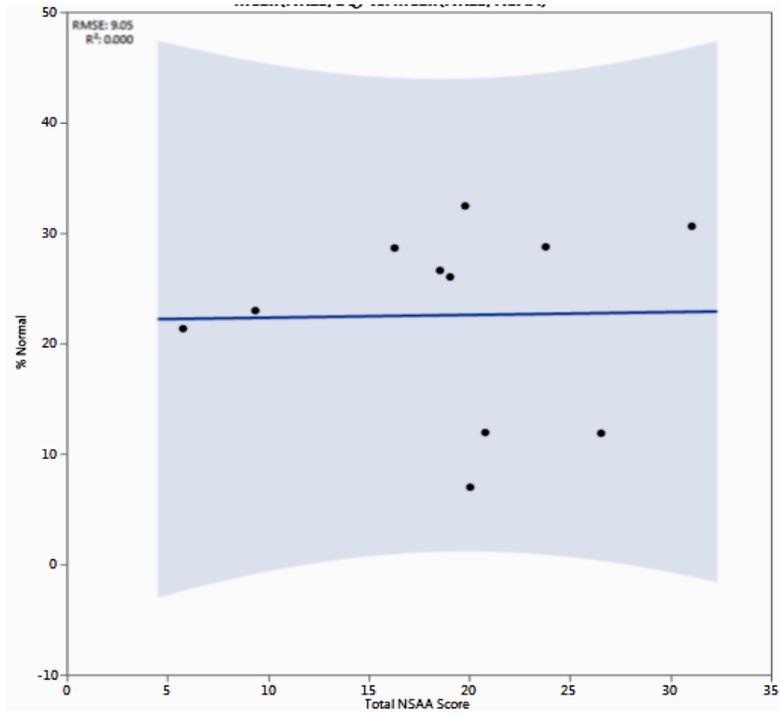
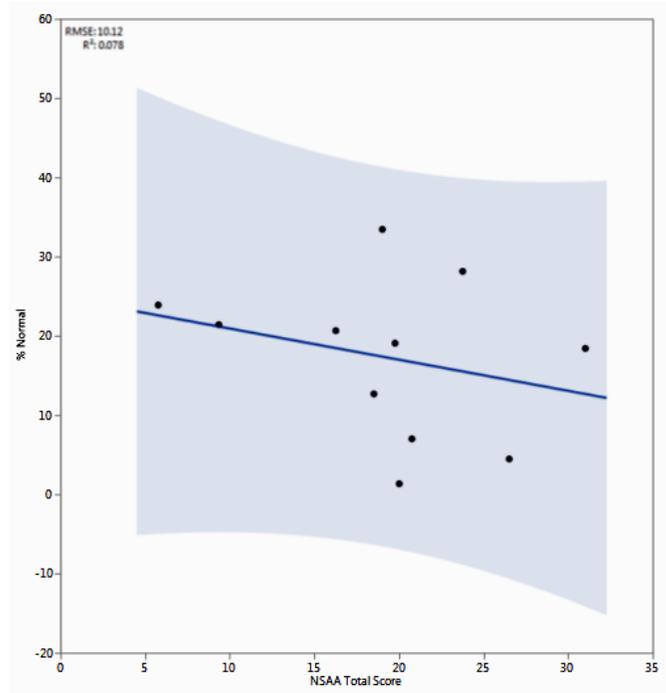
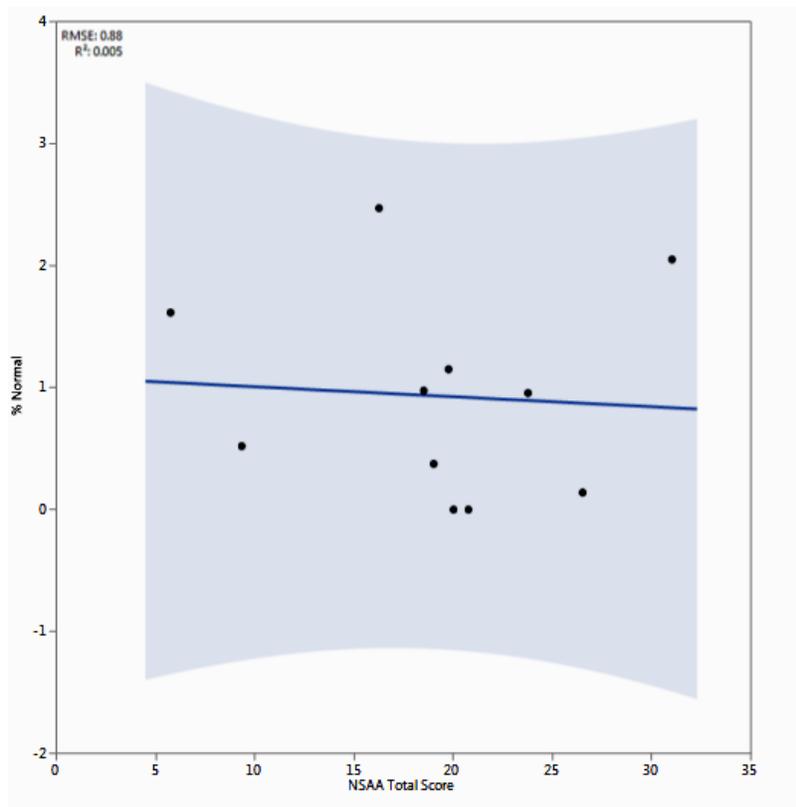


Figure 50 Total NSAA Score vs PPF % Normal



**Figure 51 Total NSAA Score vs WB % Normal**



Rise Time

A sizable proportion of subjects did not have rise time data from all visits, with some dropping out early. For this reason, viewing long term data (from baseline to Week 192) as the percent change from baseline by treatment, which would ordinarily be desirable as a method to normalize baseline performance, was not meaningful. I evaluated the data descriptively and graphically by subject to describe when they dropped out ( **Table 21**) and to visualize the time course of their performance (**Figure 52**). Subjects 009 and 010 (30 mg/kg) 003, 005, and 012 (50 mg/kg) dropped from performing the rise time before the end of testing.

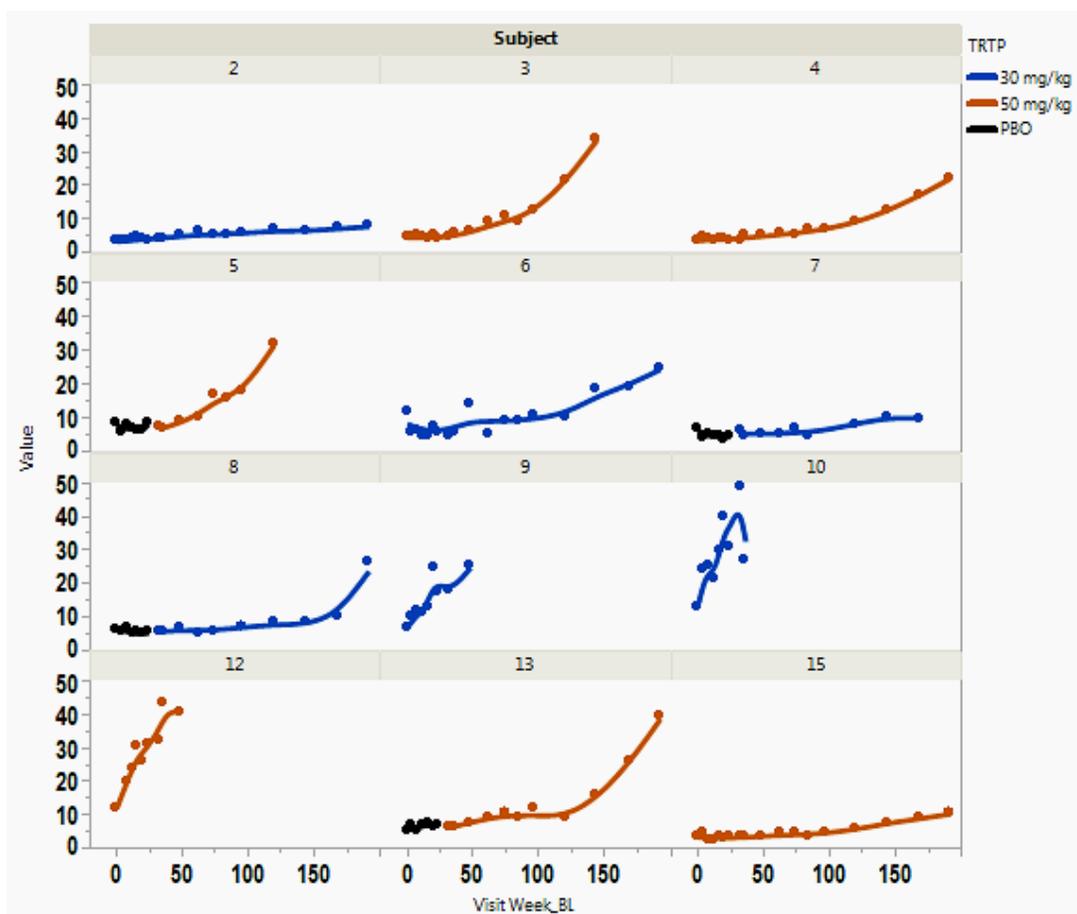
**Table 21 Subjects with ‘Missing’ Rise Time data in the 201/202 study (ITT Population)**

Subject	Original Treatment	Last Week with Rise Time
003	50 mg/kg	144
005	PBO – 50 mg/kg	120
009	30 mg/kg	48

Subject	Original Treatment	Last Week with Rise Time
010	30 mg/kg	36
012	50 mg/kg	48

Source: Medical Reviewer analysis of t-nstar-csv.txt dataset

Figure 52 Rise Time by Week by Subject from Baseline to Week 192 (ITT Population)



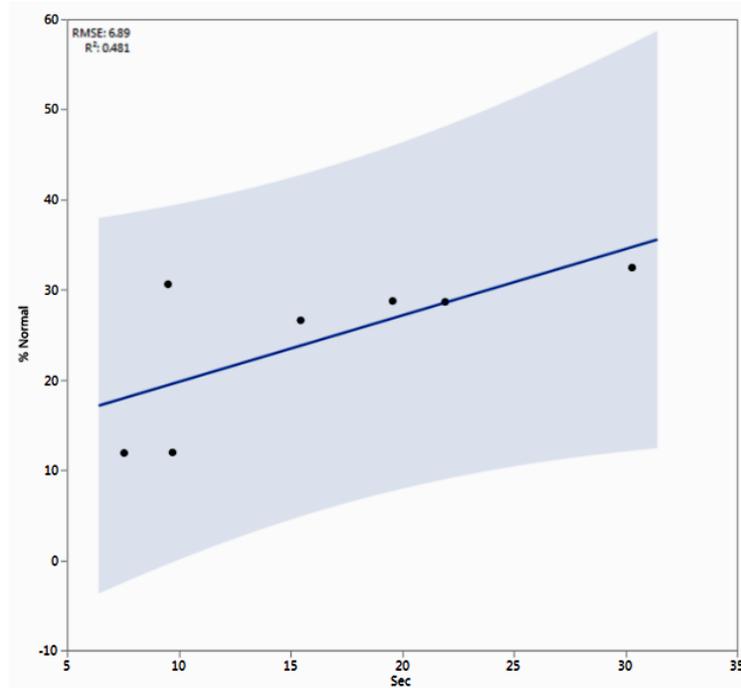
Source: Medical Reviewer analysis of t-nstar-csv.txt dataset

Evaluation of this data suggest that the rise time performance of subjects 003, 004, 005, 012, and 013 (50 mg/kg) and 006, 008, 009, 010, 012, and 013 (30 mg/kg) deteriorated. I believe this is meaningful because the performance of rise time is a less prone to bias than the 6MWT.

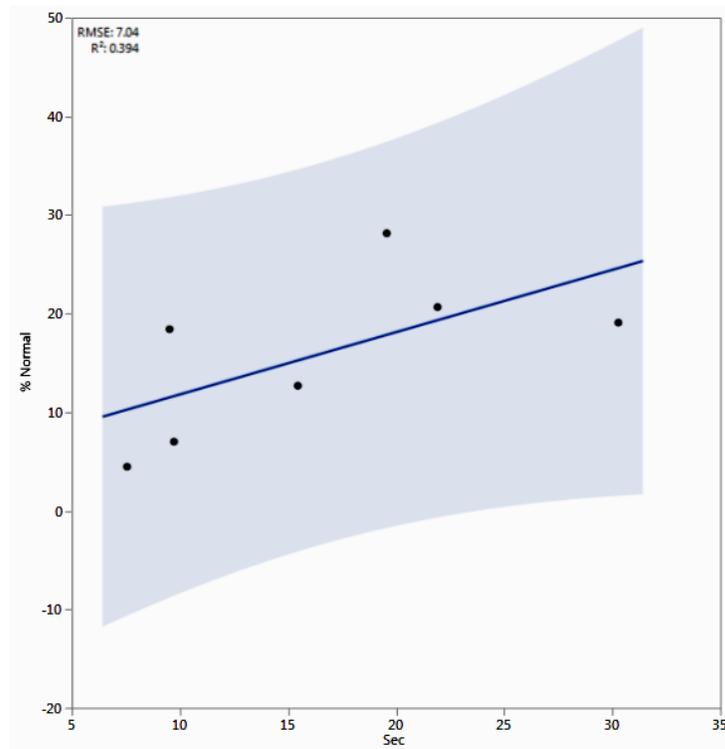
Correlations of Rise Time and Dystrophin Data

In general these graphs demonstrate a positive correlation between an increase in dystrophin metrics and the rise time.

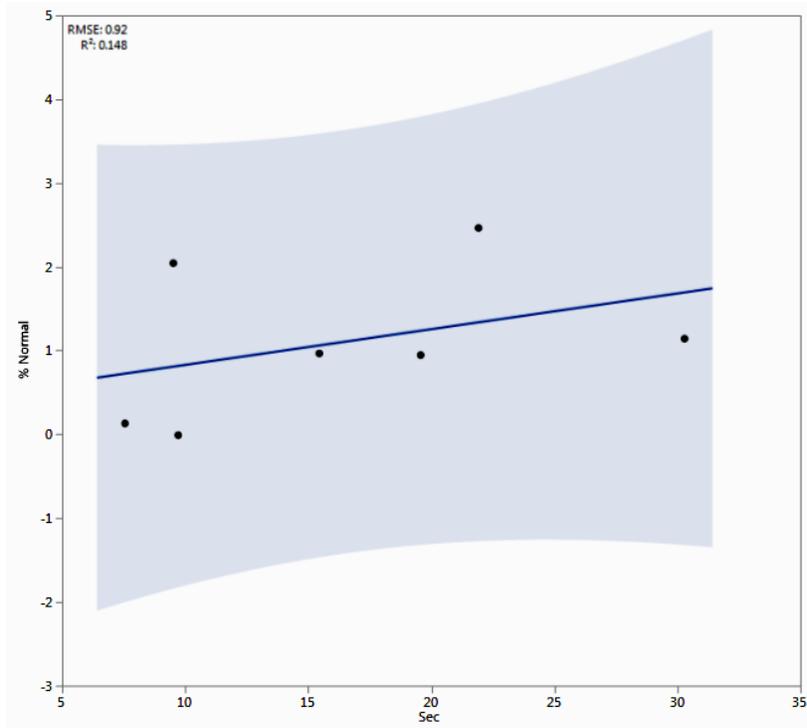
**Figure 53 Rise Time Sec vs BQ % Normal for eteplirsen treated Subjects at 4 years**



**Figure 54 Rise Time Sec vs PPF % Normal for eteplirsen treated Subjects at 4 years**



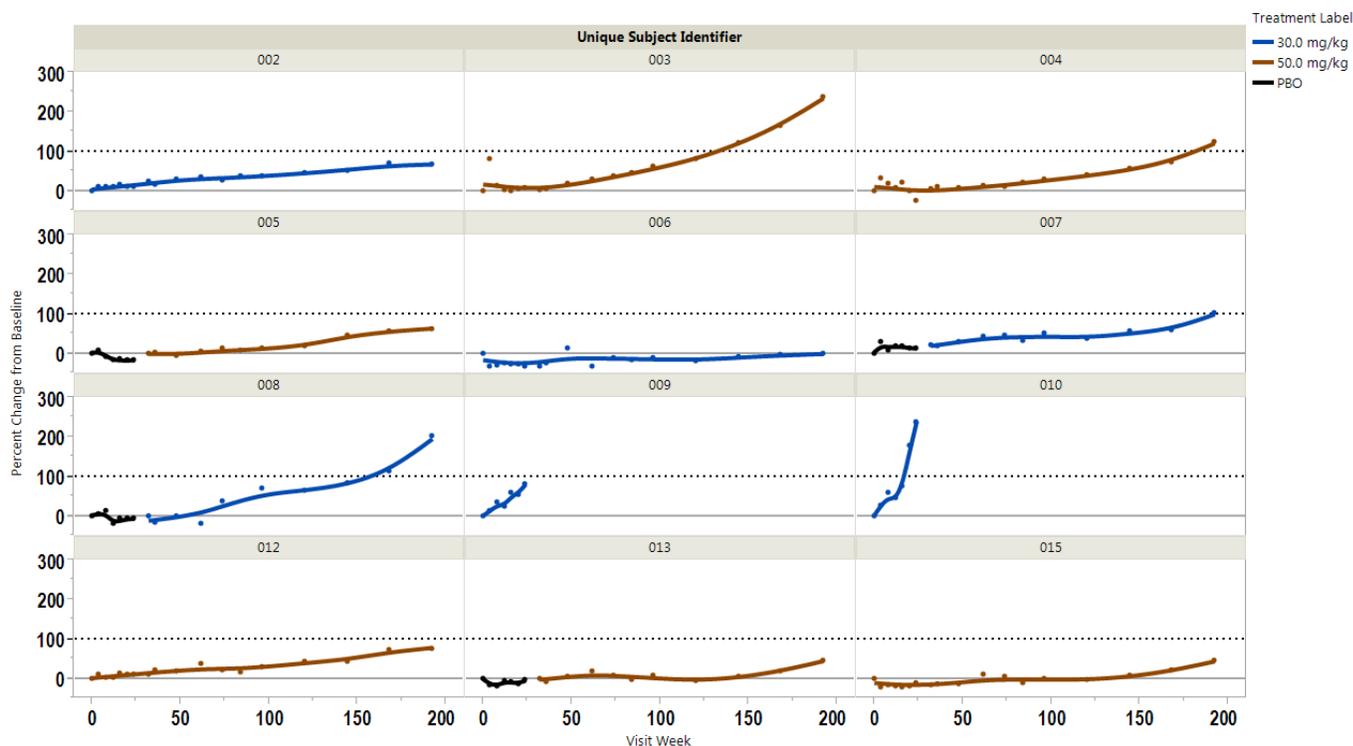
**Figure 55 Rise Time Sec vs WB % Normal for eteplirsen treated Subjects at 4 years**



### 10-Meter Run

As with the rise time data, several subjects (009 and 010 from the 30 mg/kg group) were missing data on the 10-Meter Run out to Week 196, so reporting of the percent change by treatment sequence was not feasible. Instead, I have reported this data by Subject, indicating their treatment before and after the Week 24 switch for those originally randomized to placebo. In addition to the subjects who did not do the 10-Meter run through to Week 196, these data demonstrate a deterioration (> 100%) in performance for Subjects 007 and 008 from the 30 mg/kg treatment group and Subjects 003 and 004 from the 50 mg/kg treatment group (**Figure 56**).

**Figure 56 Percent Change from baseline to Week 196 for the 10-Meter Run by Subjects and Treatment (ITT Population)**



Source: Medical Reviewer analysis of t-nstar-csv.txt dataset

#### Four Step Test

The Four Step test was performed to Week 192. **Table 22** lists the subjects who had continuous missing data.

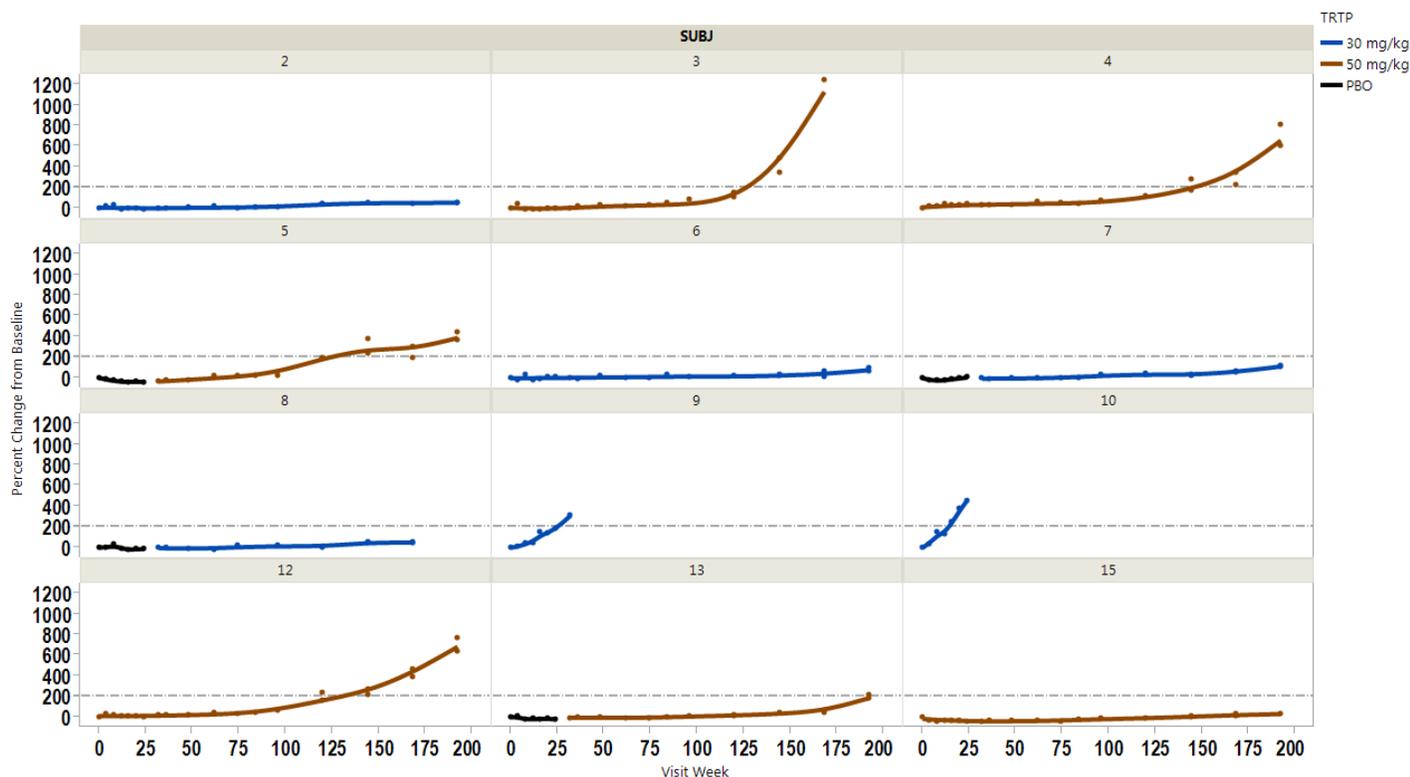
**Table 22 Subjects with Missing Four Step test data in the 201/202 study**

Subject	Treatment Sequence	Last Week with Four Step Test data
3	50 mg/kg	168
8	PBO – 30 mg/kg	168
9	30 mg/kg	32
10	30 mg/kg	24

Source: Medical Officer's review of t-fst-csv.txt

Figure 57 demonstrates a marked deterioration ( $\geq 200\%$ ) in Rise Time performance in subjects 003, 004, 005, 009, 010, 012, and 013.

**Figure 57 Percent Change from Baseline in the Four Step Test by Subject and Treatment from Baseline to Week 192 by Subject, Visit and Treatment**



Source: Medical Officer's review of t-fst-csv.txt; the dashed reference line is at 200% increase in Rise Time

- Additional Exploratory Efficacy Endpoints
  - Pulmonary function testing (PFT) measurements (forced vital capacity [FVC], percent predicted FVC, forced expiratory volume in 1 second [FEV1], FEV1%, FEV1/FVC ratio, maximum inspiratory pressure [MIP], and maximum expiratory pressure [MEP]).

The Applicant noted that no significant differences between the treatment groups on any PFT parameter were observed for the full analysis population at any time point regardless of the statistical analysis used.

**Reviewer's Analysis and Comments**

I performed my own analysis of the PFT results from the controlled portion of the study. I agree with the applicant that there were no significant statistical results when analyzing the data from the placebo-controlled portion of the study. While none of the analyses revealed a significant change for change from baseline, there were significant baseline imbalances between treatment groups in the analysis of FEV1%, FVC% and MEP%.

The Applicant has commented on their PFT results in the Integrated Summary of Efficacy:

*Over the course of 36 months of treatment, mean percent predicted MIP improved by 1.0% (from 91.7% at baseline to 92.7% at Month 36), while mean percent predicted MEP declined by 4.4% (80.7% to 76.3%) and mean percent predicted FVC declined by 7.5% (97.7% to 90.2%). In comparison, pulmonary function data from recent natural history studies in patients with DMD suggest that percent predicted MIP and MEP decline at a rate of 4% per year, while FVC declines at a rate of 5% per year (Khirani 2014; Mayer, 2015). Thus, over a period of 36 months, patients not receiving eteplirsen might be expected to show declines in MEP and MIP of 11.5% and declines of FVC in 14.3%<sup>14</sup>*

Comparison to the populations in Khirani et al., and Mayer et al. is not appropriate since the populations in those studies differed considerably from the Eteplirsen-treated subjects.

Table 23 lists the major differences from the information provided in the publications.

**Table 23 Differences between the Eteplirsen-treated Subjects and Referenced Comparator Populations for Pulmonary Function Test Data**

	<b>Eteplirsen-treated Subjects (Study 201 / 202)</b>	<b>Khirani et al.</b>	<b>Mayer et al.</b>
<b>Age</b>	7 to 11 years old, median age = 9.7	...age range 8–19 years old	...between 5.0 and 24.1 years (median 10.3 years)
<b>Steroid Regime n</b>	92% (11/12) on continuous steroids	45.0% were being treated with glucocorticoids... During the course of this study, the treating physician sometimes reduced subjects' steroid dosages in an effort to temper side effects	According to our regional guidelines, all patients over the age of 10 years received prophylactic cardiac treatment with ACE-inhibitors, while none received corticosteroids....At the time of their first visit, 27 subjects (45.0%) were being treated with glucocorticoids (age: median 8.9 years, range: 5.1–16.4 years), of which 16 (59.3%) were using prednisone / prednisolone and 11 (40.7%) were taking deflazacort.

<sup>14</sup> Source: summary-clin-efficacy, p. 57 of 85

	<b>Eteplirsen-treated Subjects (Study 201 / 202)</b>	<b>Khirani et al.</b>	<b>Mayer et al.</b>
<b>Other Key Factors</b>	<ul style="list-style-type: none"> <li>• None had scoliosis surgery prior to the study</li> <li>• All subjects ambulatory at the start of the study; subjects 009 and 010 could not complete the 10M run by week 192</li> </ul>	<p><i>... Of the 48 remaining patients [screened,] 25 had spinal surgery to correct scoliosis</i></p>	<p><i>63.3% were ambulatory at their first visit and 4 subjects (mean age: 12.2 years) became non-ambulatory (couldn't walk 10 M) during follow-up visits</i></p>

- Changes from Baseline in CD3, CD4, and CD8 lymphocyte counts in muscle biopsy tissue at Week 12 for groups 1 and 3a and at Week 24 for groups 2 and 3b.

There were no statistically significant differences between the treatment groups in the change from baseline in CD3, CD4, or CD8 levels. There were also no reported statistically significant differences between the treatment groups in the change from baseline in MHC1 or MHC2 levels.

- Upper Extremity Function

The subject did testing of grip strength. Plots of these data are included in **Appendix 2. Patient Profiles**. Upper extremity is difficult to interpret in this age of DMD boys in open label, under powered, and nonrandomized studies because upper extremity strength peaks at an age higher than the lower extremities so it is difficult to know where each subject is in their development in this respect. The figure below depicts the changes in grip strength in normal and DMD boys by age.

**Figure 58 Grip Strength in DMD and Healthy Controls<sup>15</sup>**



**6.2. AVI-4658-28 Dose-Ranging Study of AVI-4658 to Induce Dystrophin Expression in Selected Duchenne Muscular Dystrophy (DMD) Patients (“Study 28”)**

**6.2.1. Study Design**

**Overview and Objective**

Primary objective – To assess the safety of escalating doses of eteplirsen when administered by 12 weekly doses in boys with DMD.

Secondary objectives were to:

- Evaluate the pharmacokinetics (PK) of eteplirsen in patients, and
- Evaluate the efficacy of eteplirsen over 12 weeks of dosing.

**Trial Design**

**Medical Reviewer’s Comment**

*Considering the blinding (open label), brief duration (12 weeks) and that doses were below the desired labeled dose, this study is inadequately designed to provide substantial evidence for approval.*

- Basic study design

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<sup>15</sup> [http://www.nmd-journal.com/article/S0960-8966\(07\)00761-4](http://www.nmd-journal.com/article/S0960-8966(07)00761-4)

This was an open-label, multiple-dose, dose-ranging study. Patients were sequentially allocated to 1 of 6 dose cohorts (of 2 to 4 patients per cohort) to receive eteplirsen administered intravenously (IV) once a week for 12 weeks. Weekly doses ranged from 0.5 to 20.0 mg/kg.

Initially, 1 patient was dosed in each cohort. Cohort expansion occurred after the first patient had been treated for 3 weeks and that patient's safety data had been examined by the safety review committee. Patients resided at the clinic for 24 hours following study treatment administration at Weeks 1, 6, and 12 and for 4 hours after study treatment administration at all other study weeks, provided there were no safety concerns. A follow-up visit for muscle biopsy and safety assessment was conducted at Week 14. Subsequent follow-up was to occur at monthly intervals for 12 weeks following the Week 14 visit (i.e., through Week 26).

- Population
  - Key Inclusion / Exclusion Criteria

#### Inclusion

1. Had an out of frame deletion(s) that could be corrected by skipping exon 51 [45-50; 47-50; 48-50; 49-50; 50; 52; 52-63], based on DNA sequencing data.
2. Male, between the ages of 5 and 15 years.
3. Had a muscle biopsy analysis showing <5% revertant fibers present.
4. DNA sequencing of exon 51 confirmed that no DNA polymorphisms occurred that could have compromised PMO duplex formation or there was confirmation of *in vitro* dystrophin production after eteplirsen exposure to fibroblast or myoblast in vitro cultures.
5. Had sufficiently preserved right and left biceps muscles or alternative arm muscle group.
6. Able to walk independently for at least 25 meters.
7. Had a forced vital capacity (FVC)  $\geq$ 50% of predicted and did not require ventilator support or supplemental oxygen.
8. Received the standard of care for DMD as recommended by the DMD care recommendations from the North Star UK and Translational Research in Europe – Assessment and Treatment of Neuromuscular Diseases (TREAT-NMD).

#### Exclusion

1. A DNA polymorphism within exon 51 that may have compromised PMO duplex formation.
2. Known antibodies to dystrophin.
3. Lacked intact right and left biceps muscles or alternative arm muscle group.
4. A calculated creatinine clearance <70% of predicted normal for age based on the Cockcroft and Gault Formula.
5. A left ventricular ejection fraction (EF) of <35% and/or fractional shortening (FS) <25% based on ECHO during Screening.
6. A history of respiratory insufficiency as defined by a need for ventilator support and/or supplemental oxygen.
7. A severe cognitive dysfunction rendering the potential patient unable to understand and comply with the study protocol.

8. Any known immune deficiency or autoimmune disease.
  9. A known bleeding disorder or receipt of chronic anticoagulant treatment within 3 months of study entry.
  10. Receipt of pharmacologic treatment, apart from corticosteroids, that might have affected muscle strength or function within 8 weeks of study entry (viz., growth hormone and/or anabolic steroids).
  11. Surgery within 3 months of study entry or planned for anytime during the duration of the study.
- Study Treatments
    - Dose Selection

According to the Applicant, dose levels of 0.5 to 4.0 mg/kg/wk were initially selected based on animal data that suggested a Human Equivalent Dose of 4.0 mg/kg in the mdx mouse model led to up-regulation of dystrophin production. However, efficacy, which was measured by up-regulation of dystrophin expression at Week 14, might require higher doses in humans than that predicted and extrapolated from the mouse model. Therefore, assuming satisfactory safety at the original 4 dose levels (each of which were assessed by an independent DSMB prior to dose escalation decisions), 2 higher dose cohorts of 10.0 and 20.0 mg/kg/wk were added by protocol amendment.

- Assignment to Treatment

A total of 19 patients were enrolled and treated across the following 6 dose groups: 0.5 mg/kg/wk (n=4), 1.0 mg/kg/wk (n=2), 2.0 mg/kg/wk (n=2), 4.0 mg/kg/wk (n=3), 10.0 mg/kg/wk (n=4), and 20.0 mg/kg/wk (n=4).

**Table 24 Schedule of Key Events**

Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Week	-12	-1	1	2	3	4	5	6	7	8	9	10	11	12	14	18	22	ET/ 26
<b>Parameter</b>																		
Genetic Analysis	X <sup>18</sup>																	
Study Drug Administration			X	X	X	X	X	X	X	X	X	X	X	X				
Magnetic Resonance Imaging (MRI)	X <sup>9</sup>																	
<b>Safety Assessments</b>																		
Laboratory Assessments	X	X	X		X			X						X <sup>7</sup>	X			X
AE and SAE Assessment <sup>17</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Electrocardiogram (ECG)		X <sup>3</sup>	X <sup>4</sup>					X <sup>4</sup>						X <sup>4</sup>				X
Echocardiography (ECHO)		X <sup>3</sup>												X				X <sup>8</sup>
<b>Pharmacodynamic Assessments</b>																		
Pulmonary Function Tests (PFTs)		X <sup>3</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Muscle Function Assessments <sup>12</sup>		X	X					X						X		X	X	X
Muscle Biopsy		X <sup>10</sup>													X <sup>15</sup>			
<i>In Vitro</i> Dystrophin Assessment		X																
SAM Download <sup>5</sup>		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<b>Pharmacokinetic Assessments</b>																		
PK Sampling (blood/plasma and urine)			X					X						X				

Key Footnotes for (3) Screening ECG, ECHO, and PFTs were performed within 30 days prior to the first study drug administration. (4) ECG was performed within 8 hours following study drug administration and was interpreted by medically qualified personnel prior to discharge from the study site. (5) SAM was to be worn for 7 days during Baseline (note that up to 10 days prior to study drug start was allowed for obtaining the Baseline); 7 days, once a month during the treatment period (Weeks 1-12); and 7 days once every month during the follow-up period (Weeks 14-26). (8) Final ECHO must have been performed any time between Week 22 and Week 26 (or any time before the Early Termination Visit for patients who discontinued prematurely) such that the results were available for the Investigator, or designee, to review during the Week 26 visit or Early Termination Visit. (9). MRI (without contrast) of the muscle proposed for biopsy at Screening was to be taken at Investigator's discretion. (10) Required if a suitable historical biopsy sample, as determined by the Investigator, had not been obtained within 24 months before the first study drug administration. (12) Quantitative Muscle Testing (QMT), North Star Ambulatory Assessment (NSAA), and Six-minute walk test (6MWT). (15) The muscle biopsy was obtained from the contralateral bicep of the Screening muscle biopsy (or alternative). (17) AE/SAEs were reviewed before and after all study drug administrations. (18) If this assessment had not been performed prior to signing consent, previously performed genetic testing may have been used to qualify the patient for this study; Abbreviations, AE = adverse event; HR = heart rate; PK = pharmacokinetic; SAE = serious adverse event; SAM = StepWatch Activity Monitor

○ Concomitant Medications

Permitted therapies

1. Oral steroids such as, but not limited to, prednisolone, prednisone, and deflazacort, before enrollment and for the duration of the study. Other concomitant medications may have also been taken (e.g., bisphosphonates) but every attempt should have been made to keep dosing constant during the Screening period and throughout the study duration (i.e., through Week 26).
2. Oral angiotensin-converting enzyme (ACE) inhibitors, such as but not limited to perindopril or lisinopril, before enrollment and for the duration of the study. Dosage should have been kept constant if at all possible.
3. Oral  $\beta$ -blockers, such as but not limited to carvedilol or atenolol, before enrollment and for the duration of the study, at a constant dosage if at all possible.
4. Angiotensin receptor blockers, such as but not limited to losartan, irbesartan, valsartan, and candesartan, at constant dosage.
5. Oral laxatives, such as but not limited to lactulose, Senokot, or Movicol, before enrollment and for the duration of the study.
6. Vitamin D and calcium supplements if clinically indicated before enrollment and for the duration of the trial.

Prohibited Therapies (not permitted before and/or during the trial) included:

1. Initial prescription of intranasal and/or inhaled and topical steroids for a condition other than muscular dystrophy in the week before enrollment or during the study.
2. Investigational therapy or participation in any other clinical trial (involving receipt of an investigational drug) for 4 weeks prior to study treatment administration.
3. Prior exposure to eteplirsen.
4. All other prescribed medications with the potential to affect muscle mass, strength and/or function, such as (but not limited to) growth hormone, were not to be taken within 8 weeks of study entry.
5. Use of immunosuppressants during the Screening period or while on study (through Week 26).

## Study Endpoints

No primary efficacy endpoint was defined. However, the primary dystrophin expression analysis was the percentage of dystrophin-positive fibers as measured in the muscle biopsy tissue using immunohistochemistry (IHC) at Week 14 compared to Baseline.

### Dystrophin-related endpoints

- Percentage of dystrophin-positive fibers

For IHC detection of dystrophin, biopsy sections were incubated (1 hr) with MANDYS106, washed and subsequently incubated (30 min) with an appropriate biotinylated secondary antibody. Prior to mounting, sections were washed and labeled by incubation (15 min) with streptavidin conjugated to Alexa 594. The detection threshold was adjusted for each patient so that only the revertant fibers were detected in the pre-treatment sample, and 2 independent investigators counted the number of

dystrophin-positive fibers as a percentage of the total number of fibers in a given field Normal, healthy muscle tissue would be expected to have 100% dystrophin-positive fibers.

Additional biopsy-related efficacy endpoints included:

- Number and proportion of patients achieving a  $\geq 10\%$  level of internally shortened dystrophin production (measured as a percentage of dystrophin-positive fibers) at Week 14 compared to Baseline
- Dystrophin intensity (as assessed by IHC) at Week 14 compared to Baseline

To correct each measurement for background dystrophin intensity, the minimum intensity level (representative of the cytoplasm or background intensity) was subtracted from the maximum intensity level (from the sarcolemma) for each region where intensity values were measured. Actual fluorescence intensity units were also converted to a percentage of normal by setting a normal control (normal healthy tissue) to 100 for dystrophin.

- Dystrophin protein level (as assessed by Western blot) at Week 14 compared to Baseline

A biopsy from the quadriceps femoris of a normal healthy adult female was used as the control. Band intensity was measured using software from Image J, and quantification was based on relative density values (area and percentage of the bands). In order to report results as a percentage of control (normal muscle tissue), the relative density values for all samples (Dys in DMD and control samples) and their loading protein ( $\alpha$ -actinin) bands were calculated. Then the values for the Dys and  $\alpha$ -actinin bands were divided by the control value. Finally, the sample relative density for each lane was divided by the loading protein relative density for the same lane, and the results are presented as a percentage of normal control.

- Exon skipping (as assessed by reverse transcription polymerase chain reaction [RT-PCR]) at Week 14 compared to Baseline

The extent of exon skipping observed in the muscle biopsies was classified into 3 categories, referred to as Skip (1), Skip (2), and Skip (3).

- Skip (1) samples showed variable skipping of exon 51 under enhanced conditions (35/40 cycles of nested RT-PCR).
- Skip (2) samples showed variable skipping of exon 51 under standard conditions (30/35 cycles of nested RT-PCR) but consistent skipping under enhanced conditions.
- Skip (3) samples exhibited robust skipping of exon 51 under standard conditions.
- Dystrophin Detection in Peripheral Lymphocytes

Dystrophin detection in the mRNA of peripheral lymphocytes was conducted only for patients treated at the 10.0 and 20.0 mg/kg/wk dose levels to assess skipped or unskipped mRNA products.

Functional endpoints included:

- 6MWT

Patients are asked to walk a 25-meter course for 6 minutes and the distance walked is recorded. This study used a modified version of the American Thoracic Society (ATS) guidelines for the test (ATS 2002), which included the addition of a rest period prior to testing, scripted encouragement from the testing staff at regular intervals, and use of a “safety chaser” to walk along behind the participant during testing.

- QMT

Muscle groups were tested with the patient in either the sitting or supine position, as shown below:

- Tested in sitting position:
  - Knee extensors, right and left
  - Knee flexors, right and left
- Tested in supine position:
  - Elbow flexors, right and left
  - Elbow extensors, right and left
  - Grip strength, right and left

The placement of the myometer for each assessment was standardized. Each measurement was performed 3 times.

- North Star Ambulatory Assessment (NSAA)

Patients were asked to perform 17 different functional activities, including a 10 m walk/run, rising from a sit to stand, standing on 1 leg, climbing stairs, descending stairs, rising from lying to sitting, rising from the floor, lifting the head, standing on heels, and jumping. Patients were graded as follows: 2 = normal, no obvious modification of activity; 1 = modified method but achieves goal independent of physical assistance from another; and 0 = unable to achieve goal independently.

- StepWatch Activity Monitor (SAM)

The device was worn during the waking hours for 7 consecutive days during Baseline (up to 10 days before study treatment administration started), and for 7 consecutive days once every month during the treatment period (starting after study treatment administration), and the follow-up period.

## **Statistical Analysis Plan**

### Analysis Populations

Three study populations were defined for analysis:

**Safety Population:** Included all patients who were enrolled in the study and received at least 1 dose of study treatment.

**Per Protocol Population:** Included all patients who received all 12 doses of study treatment.

**PK Evaluable Population:** Included all patients who provided at least 1 PK sample. The reportable PK population included those patients with at least C<sub>max</sub>, T<sub>max</sub>, and AUC<sub>0-24</sub> computed from 1 or more of the 3 sampling days (1st, 6th, 12th dose [Weeks 1, 6, and 12]).

All demographic, baseline, and safety analyses were conducted on the Safety Population. PK data were evaluated for the PK population. Exploratory efficacy data, when summarized, were evaluated for the Per Protocol Population and also for the Analyzable Safety Population, which included patients with pre- and post-treatment biopsies.

Analysis of Percent Positive fibers

The percentage of dystrophin-positive fibers (assessed by IHC) at Baseline and after 12 weekly doses of eteplirsen (Week 14) were summarized with descriptive statistics by dose group, and data are presented as actual value and change from Baseline.

### Sample Size

No formal sample size calculations were performed

### **Safety Assessments**

Safety assessments included:

- Physical Exams,
- vital signs and tests
  - heart rate (HR)
  - oxygen saturation (SaO<sub>2</sub>)
  - ECGs
  - ECHO (EF and/or fractional shortening [FS])
- safety laboratory tests
  - hematology and coagulation
  - clinical chemistry
  - urinalysis
  - anti-dystrophin antibodies
  - immune cell infiltration (presence of CD3, CD4, and CD8 cells in biopsied muscle)
- PFTs
  - FVC and percent predicted FVC
  - forced expiratory volume in one second (FEV<sub>1</sub>)
  - Percent predicted FEV<sub>1</sub> (FEV<sub>1</sub>%)
  - FEV<sub>1</sub>/FVC

Tolerability was assessed by passive reporting, (i.e., from the patient and/or parent[s] or legal guardian[s]) and elicitation of adverse events (AEs) by the study staff. Adverse events were coded using Medical Dictionary for Regulatory Activities (MedDRA) (Version 12.0)

### **Protocol Amendments**

Changes implemented by Protocol Amendment 1.0, dated 18 March 2009, included:

- Reduced the duration of the follow-up period from 40 to 14 weeks

- Reduced the number of patients from 4 to 2 for dose cohorts 2, 3, and 4 to allow faster determination of an effective dose for further studies.
- Modified the following inclusion criteria:
  - Added deletions “52-63” to the list of acceptable out of frame deletions that could be corrected by exon 51 skipping
  - Modified the criterion to walk independently to include “for at least 25 meters”
  - Modified the standard of care for DMD to be that recommended by the “North Star UK and Translational Research in Europe – Assessment and Treatment of Neuromuscular Diseases (TREAT-NMD)”
- Modified the following exclusion criteria:
  - Antibodies to dystrophin were specified to be “known” antibodies to dystrophin
  - Fractional shortening was changed from <30% to <25% to be excluded
  - Immune deficiency or autoimmune disease was specified to be any “known” immune deficiency or autoimmune disease
  - Receipt of “creatine protein supplementation” within 8 weeks of study entry was removed

## Analyses

Of note, the laboratory performing the Western blot analyses used multiple samples from the same patients to re-analyze the results. Initially, the Western blot analyses reported the results from one sample per patient and any post-treatment increases in dystrophin protein level were reported as an ‘X’-fold increase from baseline. Subsequently, while preparing the *Lancet* publication, the laboratory repeated several Western blots to achieve publication standard results and also to test different pieces of muscle within a patient. These results were reported as the *maximum amount of dystrophin per patient* and were expressed as a percentage of normal.

StepWatch data were not to be analyzed.

### 6.2.2. Study Results

**Table 25 Patient Disposition in Study AVI-4658-28 (Safety Population)**

Disposition	N (%) at the Eteplirsen dose (mg/kg)						
	0.5	1.0	2.0	4.0	10.0	20.0	Total
Enrolled	4	2	2	3	4	4	19
Treated	4 (100)	2 (100)	2 (100)	3 (100)	4 (100)	4 (100)	19 (100)
Completed	4 (100)	2 (100)	2 (100)	2 (66.7)	4 (100)	4 (100)	18 (94.7)
Withdrew <sup>a</sup>	0	0	0	1 (33.3)	0	0	1 (5.3)
Reasons for Withdrawal							
Adverse Event	0	0	0	1	0	0	1 (5.3)
Voluntary Withdrawal	0	0	0	0	0	0	0
Sponsor Discretion	0	0	0	0	0	0	0

	N (%) at the Eteplirsen dose (mg/kg)						
Lost to Follow-up	0	0	0	0	0	0	0

Source AVI-4658-28 CSR, Table 10-1, p. 56 of 105

**Table 26 Number of Patients per Analysis Data Set in Study 28 (Safety Population)**

	0.5 (n=4)	1.0 (n=2)	2.0 (n=2)	4.0 (n=3)	10.0 (n=4)	20.0 (n=4)	Total (n=19)
<b>Analysis Data Set</b>	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>				
Safety Population	4 (100)	2 (100)	2 (100)	3 (100)	4 (100)	4 (100)	19 (100)
Per Protocol Populatio	3 (75.0)	2 (100)	2 (100)	1 (33.3)	4 (100)	3 (75.0)	15 (78.9)
PK Population (plasm	2 (50.0)	2 (100)	2 (100)	3 (100)	4 (100)	4 (100)	17 (89.5)
PK Population (urine)	4 (100)	2 (100)	2 (100)	3 (100)	4 (100)	4 (100)	19 (100)

Source:

4 subjects completed less than 12 doses: 1.104, (0.05 mg/kg), 1.108 (4 mg/kg), 2.202 4.0 mg/kg (d/c after 7 doses (cardiomyopathy), 2.207 (20 mg/kg)

17 subjects had baseline and post baseline muscle biopsies (used in Cirak et al (Lancet, 2011); 2 subjects did not have post treatment biopsies, Subjects 2.202 and 1.104 (refused)

### Table of Demographic Characteristics

**Table 27 Demographic Characteristics of Subjects in AVI-4658-28 by Dosing Cohort (Safety Population)**

	Dose level (mg/kg/wk) / N						
Parameter	0.5 / 4	1.0 / 2	2.0 / 2	4 / 3	10 / 4	20 / 4	Total /
Age (mean yo)	8.3	6	11	9.7	8.8	8.8	8.7
Weight (kg)	33.3	23.7	42.6	40.1	34.6	33	34.5
Height (cm)	127.3	110.7	126.9	126.9	123.7	126.5	124.5
Age at Dx (mean yo)	3.8	3	4.5	5	2	3.3	3.5
Age in study (mean	8.3	6	11	9.7	8.8	8.8	8.7

	Dose level (mg/kg/wk) / N						
<b>Duration of dz (mean years)</b>	4.5	3	6.5	4.7	6.8	5.5*	5.3

Source: AVI-4658-28 CSR, Table 11-2, p. 59 of 105; Age demographics calculated from data in Listing 16.2.4.2 by Medical Reviewer; \* Duration of disease approximated for 2 subjects in 20 mg group because of partial missing dates of diagnosis

## Efficacy Results - Primary Endpoint

### Dystrophin Positive Fibers

The Applicant reported that across the 17 evaluable patients in the Analyzable Safety Population, the mean percentage of dystrophin-positive fibers increased by 6.5% of normal relative to baseline [range: -4, 52] at Week 14 with the greatest increase observed in the 20.0 mg/kg/wk dose group (15.3% [range: 2, 52]) (Table 28).

**Table 28 Percent MANDYS106-immunoreactive fibers in Study 28 (Safety Population)**

Timepoint Statistic	eteplirsen dose (mg/kg/wk)						Total (n=17)
	0.5 (n=3)	1.0 (n=2)	2.0 (n=2)	4.0 (n=2)	10 (n=4)	20 (n=4)	
Screening/Baseline Actual Value							
Mean (SD)	1.7 (1.15)	2.5 (3.54)	1.0 (0.00)	3.0 (2.83)	1.5 (1.29)	3.5 (1.00)	2.2 (1.68)
Median	1	3	1	3	2	3	2
Min. Max	1, 3	0, 5	1, 1	1, 5	0, 3	3, 5	0, 5
Week 14 Actual Value							
Mean (SD)	2.7 (3.79)	0.5 (0.71)	13.0 (11.31)	2.5 (2.12)	8.5 (4.36)	18.8 (24.20)	8.8 (13.08)
Median	1	1	13	3	7	8	6
Min. Max	0, 7	0, 1	5, 21	1, 4	6, 15	5, 55	0, 55
Week 14, Change from Baseline							
Mean (SD)	1.0 (4.58)	-2.0 (2.83)	12.0 (11.31)	-0.5 (0.71)	7.0 (4.97)	15.3 (24.54)	6.5 (13.13)
Median	0	-2	12	-1	6	4	3
Min. Max	-3, 6	-4, 0	4, 20	-1, 0	3, 14	2, 52	-4, 52

Source: Table 14.2.1A; max = maximum; min = minimum; SD = standard deviation

Source: 4658-28-body CSR, p.61 of 105

### Mandys106-Immunoreactive Fluorescence Intensity as a Percentage of Normal

The Applicant reported a mean change from baseline in dystrophin intensity level (IHC) at Week 14 was 3.6% of normal in the Analyzable Safety Population

**Table 29 Mandys106-Immunoreactive Fluorescence Intensity as a Percentage of Normal (Analyzable Safety Population)**

Timepoint Statistic	eteplirsen dose (mg/kg/wk)						Total (n=17)
	0.5 (n=3)	1.0 (n=2)	2.0 (n=2)	4.0 (n=2)	10 (n=4)	20 (n=4)	
Screening/Baseline Actual Value							
Mean (SD)	5.0 (0.00)	6.0 (2.83)	6.0 (1.41)	8.5 (0.71)	9.8 (0.96)	9.8 (0.96)	7.9 (2.29)
Median	5	6	6	9	10	10	9
Min, Max	5, 5	4, 8	5, 7	8, 9	9, 11	9, 11	4, 11
Week 14 Actual Value							
Mean (SD)	6.0 (1.73)	5.0 (1.41)	12.0 (9.90)	10.5 (0.71)	16.8 (7.41)	13.8 (3.77)	11.5 (6.24)
Median	5	5	12	11	15	13	10
Min, Max	5, 8	4, 6	5, 19	10, 11	10, 27	10, 19	4, 27
Week 14, Change from Baseline							
Mean (SD)	1.0 (1.73)	-1.0 (1.41)	6.0 (11.31)	2.0 (1.41)	7.0 (8.21)	4.0 (4.08)	3.6 (5.68)
Median	0	-1	6	2	6	3	2
Min, Max	0, 3	-2, 0	-2, 14	1, 3	-1, 18	1, 10	-2, 18

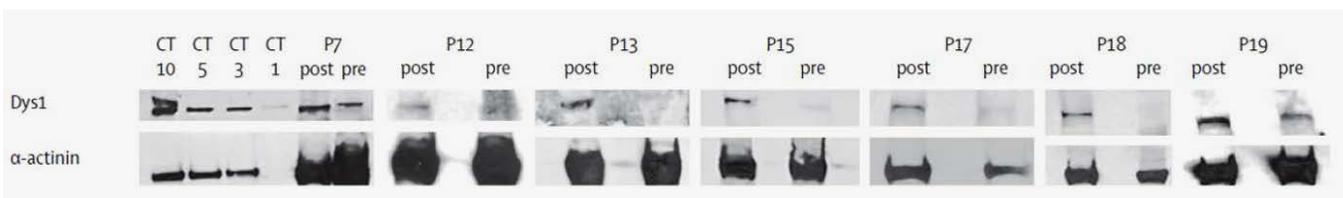
Source: Table 14.2.1A

max = maximum; min = minimum; SD = standard deviation

### DYS1-immunoreactivity assessed by Western Blot

The initial Western Blot analysis reported results from one sample per patient with post-treatment increases in “dystrophin protein level” reported as an ‘X’-fold increase from baseline. Subsequent analyses were performed using multiple pieces of muscle per patient; these results were reported as the *maximum amount of dystrophin per patient* and were expressed as a percentage of normal. The dystrophin bands (pasted below) appear to be reasonably well resolved but not likely to be quantifiable in a linear range because of the large differences in the loading concentrations and saturated bands for alpha-actinin.

**Figure 59 Examples of Western blots from Study 28**



Source:

**[AR]** No assay validation data and information on variability, linearity, or limits of detection were provided for the methods used in study 28. The applicant stated that they considered the methods to be exploratory at the time.

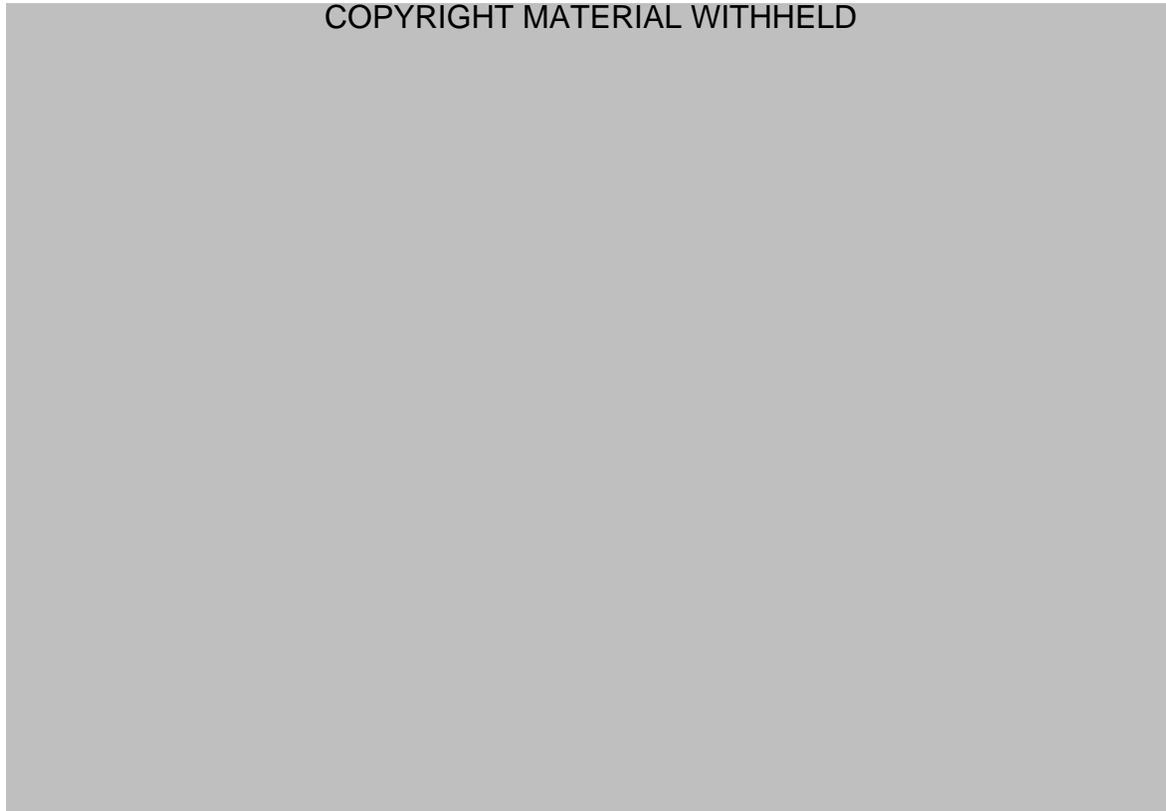
**Table 30 Patient Level Biomarker Data for Study 28 (Safety Population)**

Dose Level (mg/kg/wk)	Eteplirsen dose (mg/kg)																		
	0.05				1.0		2.0		4.0			10				20			
Subject	1101	1102	1103	1104	1105	1106	1107	2201	1108	2202	2206	1109	1110	1203	2204	1111	1112	2205	2207
<b><i>Percent "Positive" Fibers</i></b>																			
Pre-TX	1	3	1	ND	0	5	1	1	5	----	1	3	2	0	1	3	3	3	5
Post-TX (%)	1	0	7	NA	0	1	5	21	4	NA	1	6	6	7	15	5	8	55	7
<b><i>Mean Fluorescence /Fiber (% Normal)</i></b>																			
Pre-TX	5	5	5	ND	4	8	7	5	9	-----	8	9	11	10	9	11	9	9	10
Post-TX (%)	8	5	5	NA	4	6	5	19	10	NA	11	17	10	13	27	13	10	19	13
<b><i>Western Blot Analysis with DYS-1 Antibody (percentage of normal controls)</i></b>																			
Pre-TX	ND	ND	ND	-----	ND	1.8	ND	1.3	ND	-----	1.1	ND	ND	ND	1	1	1	1	1
Post-TX (fold)	ND	ND	ND	-----	ND	1.1	ND	4.3	ND	-----	1.24	ND	ND	ND	5	ND	5	10	1.5

Source: extracted from 4658-28-body, pp. 63-4 of 105

In Study 28, the clinical investigator also investigated the co-localization with dystrophin-associated glycoprotein complex proteins to the sarcolemma. The figure below (from [Cirak *et al.* 2011]) shows the colocalization of dystrophin in two patients, 18 and 19 with alpha-sarcoglycan and neuronal nitric oxide synthase (nNOS).

**Figure 60 Colocalization of Dystrophin-immunoreactivity with  $\alpha$ -sarcoglycan and Neuronal NOS immunoreactivity in muscle from patients from Study 28.**



Source: Cirak et al., 2011

***Reviewer's Analysis and Comments***

The biomarker percent positive fibers and intensity data from Study 28 has similar issues as Study 201 / 202 and had even shorter duration of treatment and was in doses well below that proposed for labeling.

It is important to perform the colocalization tests to further support the anatomical and “functional localization” of the MANDYS106-immunoreactivity. However in the case of the figures which have been produced from this study, it seems that, for example, the cluster of fibers from Subject 18 in **Figure 60** are likely revertant fibers. It may not be surprising that they have these dystrophin-associated molecules co-expressed with dystrophin. It is not clear whether this is in fact an effect of the drug therapy, since this was not systematically investigated.

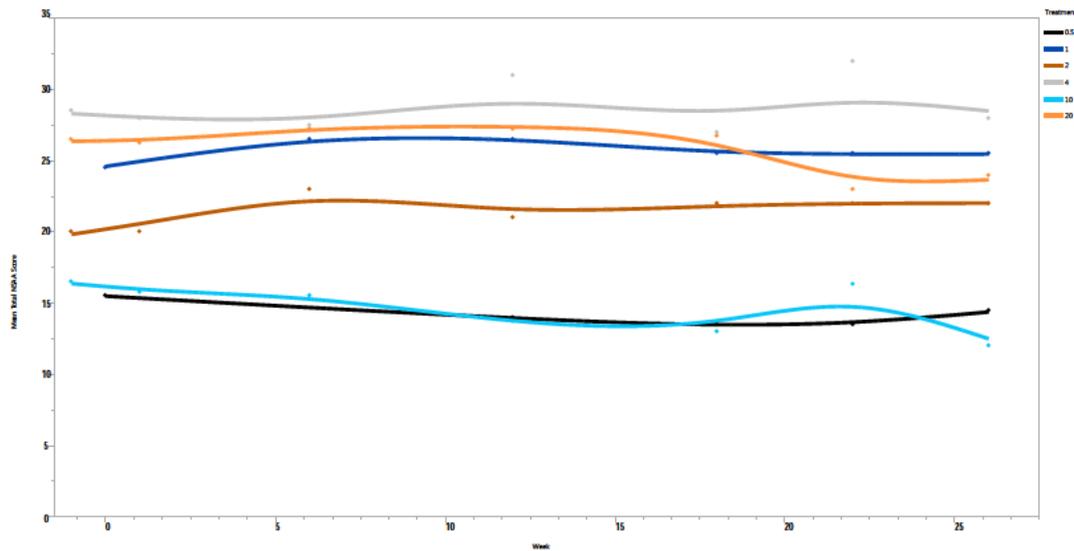
### Clinical Tests of Function

The Applicant did not analyze and report the results of the 6MWT and NSAA Total Score, so I have analyzed them from the datasets submitted with the NDA.

#### Six-Minute Walk Test (6MWT)

Subjects 104 and 108 were missing considerable data for the 6MWT so were not included in the analysis of mean results per treatment and visit (**Figure 61**).

**Figure 61 Mean Six Minute Walk Test by Dose Cohort and Week in Study 28 (Per Protocol Population)**

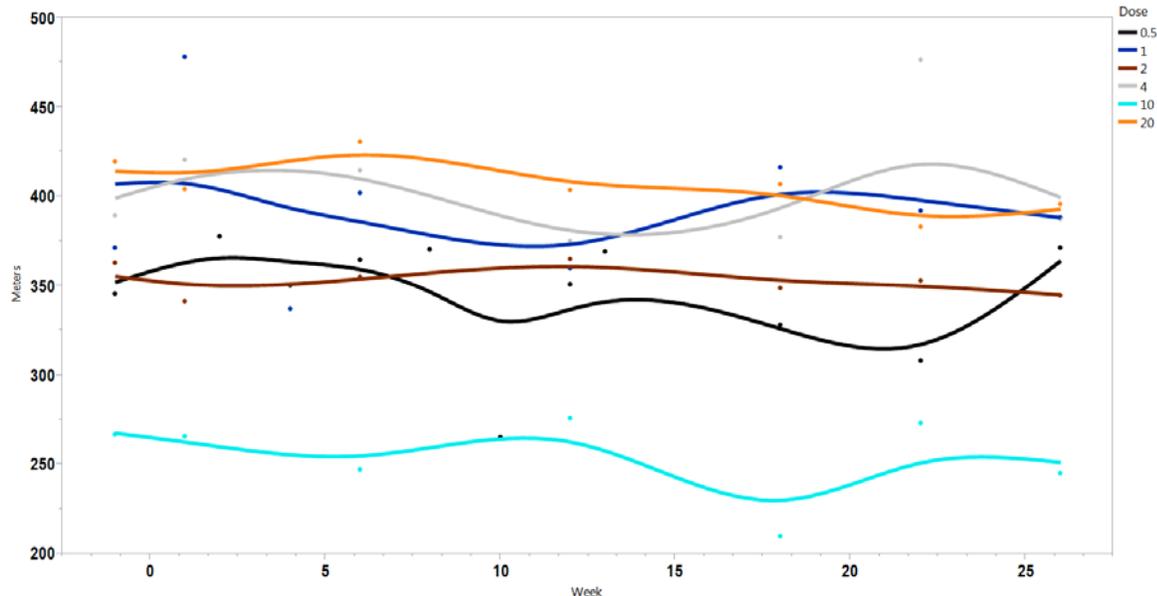


Source: Medical Reviewer's analysis of the Study 28 WA dataset

#### NSAA

Subjects 101, 104 and 108 were considerable missing data and so they were omitted from the analysis of mean results by visit and treatment (**Figure 62**).

**Figure 62 Mean NSAA Total Score by Dose Cohort and Week in Study 28 (Per Protocol Population)**



Source: Medical Reviewer's analysis of the Study 28 AA dataset

### ***Reviewer's Analysis and Comments***

The clinical function tests in Study 28 do not support a clinical benefit of eteplirsen treatment.

***The following studies were reviewed for safety but were not considered evaluable for the labeled indication.***

An abbreviated summary of each is provided. Safety of each study is reviewed in **Sections 8.1.3.**

### **6.3. CRO490: Restoring Dystrophin Expression in Duchenne Muscular Dystrophy: A Phase I/II Clinical Trial Using Avi-4658 Study Design ("Study 33")**

#### **Overview and Objective**

The objectives of this study were to determine the safety and tolerability of AVI-4658 when administered as intramuscular injections that comprised a single dose and to determine the ability of AVI-4658 to restore dystrophin protein production by skipping exon 51. This was a single-blind, placebo-controlled, study with planned treatments of 0.09 mg or 0.9 mg injected IM in a foot muscle and placebo injected in the contralateral foot.

**Table 31 Treatment groups in the AVI-4658-33 Study (Safety Population)**

Group	AVI-4658 <sup>a</sup>					Placebo <sup>a</sup>				
	Dose per Injection (mg)	Injections		Total		Dose per Injection (mg)	Injections		Total	
		No.	Volume (µL)	Volume (µL)	Dose (mg)		No.	Volume (µL)	Volume (µL)	Dose (mg)
1	0.01	9	100	900	0.09	0	9	100	900	0
2	0.10 <sup>b</sup>	9	100	900	0.90	0	9	100	900	0
	0.225 <sup>c</sup>	4	225	900	0.90	0	4	225	900	0

<sup>a</sup> Subjects received intramuscular injections of AVI-4658 in the extensor digitorum brevis (EDB) muscle in one foot and intramuscular injections of placebo in the EDB muscle in the other foot.

<sup>b</sup> The first 3 subjects in Group 2 received 9 intramuscular injections to deliver the 0.9 mg dose; the last 2 subjects in Group 2 received 4 intramuscular injections to deliver the dose.

Source Table 1 p. 15 of 443

The subjects in Group 1 (0.09 mg AVI-4658 dose group) were enrolled in the study under Version 2.1 of the protocol, and the subjects in Group 2 (0.9 mg AVI-4658 dose group) were enrolled in the study under Version 2.2 of the protocol. Version 2.2 of the protocol differed from Version 2.1 of the protocol as follows:

- The number of dose groups was reduced from 3 dose groups (0.09, 0.27, and 0.9 mg of AVI-4658) to 2 dose groups (0.09 and 0.9 mg of AVI-4658).
- The number of planned subjects was changed from up to 9 subjects (3 subjects in each of the 3 originally planned AVI-4658 dose groups) to up to 7 subjects (2 subjects in Group 1 and 5 subjects in Group 2).
- The minimum age requirement for study participation was changed from  $\geq 12$  years to  $\geq 10$  years.
- The requirement for subjects to be nonambulatory or unable to stand independently (inclusion criterion 4) was deleted.

#### Pharmacodynamic Endpoints

- Exon Skipping
- Fiber counts
- Colocalization studies with  $\alpha$ -sarcoglycan and  $\beta$ -dystroglycan

#### Safety Assessments

- Adverse Events, including injection site reactions as an event of special interest
- laboratory studies
- vital signs
- physical examinations
- electrocardiograms

#### **6.4. Study 4658-301 – An Open-Label, Multi-Center, 48-Week Study with a Concurrent Untreated Control Arm to Evaluate the Efficacy and Safety of Eteplirsen in Duchenne Muscular Dystrophy (“Study 301”)**

Study 301 is an open-label study of eteplirsen safety and efficacy in patients with DMD. Approximately 80 male ambulatory patients (able to walk >300 meters on 6MWT) between the ages of 7 to 16 years who have a confirmed diagnosis of DMD amenable to exon 51 skipping are being enrolled. Patients assigned to eteplirsen treatment will receive eteplirsen 30 mg/kg IV weekly for 48 weeks and will be compared with an untreated control group (i.e., patients who are non-amenable to exon 51 skipping). The primary endpoint is the change in walking ability as measured by the 6MWT over 48 weeks. Pulmonary function, dystrophin expression, and other clinical measures of efficacy and safety will also be assessed. As of 17 April 2015, 25 patients have been dosed with eteplirsen in this study.

#### **6.5. Study 4658-203 – An Open-Label, Multi-Center Study to Evaluate Safety, Efficacy and Tolerability of Eteplirsen in Early Stage Duchenne Muscular Dystrophy (“Study 203”)**

Study 203 is an open-label study designed to evaluate the safety, efficacy and tolerability of eteplirsen in patients with early stage DMD. Approximately 40 male ambulatory patients between the ages of 4 and 6 years, inclusive, who have a confirmed diagnosis of DMD amenable to exon 51 skipping will be enrolled. Patients will receive eteplirsen 30 mg/kg/ IV weekly for 96 weeks. Dystrophin expression, MRI of muscle tissue, and functional efficacy using the North Star Ambulatory Assessment (NSAA), as well as other functional clinical measures, will be assessed. No patients have been dosed with eteplirsen in this study as of 17 April 2015.

#### **6.6. Study 4658-204 – An Open-Label, Multi-Center Study to Evaluate the Safety and Tolerability of Eteplirsen in Patients with Advanced Stage Duchenne Muscular Dystrophy (“Study 204”)**

Study 204 is an open-label study designed to evaluate the safety and tolerability of eteplirsen in patients with advanced stage DMD. Approximately 20 male ambulatory impaired or non-ambulatory patients between the ages of 7 and 21 years, inclusive, who have a confirmed diagnosis of DMD amenable to exon 51 skipping are being enrolled. Patients will receive eteplirsen 30 mg/kg IV weekly for 96 weeks. Pulmonary function, as well as other functional clinical measures will be assessed. As of 17 April 2015, 9 patients have been dosed with eteplirsen in this study.

## **7 Integrated Review of Effectiveness**

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### **7.1. Assessment of Efficacy across Trials**

#### **7.1.1. Primary Endpoints**

In this section, the overall perspectives of the drug effect, as it relates to the biomarker, will be discussed. This includes results related to Exon Skipping, Western blot analyses, Counts of immunoreactive muscle fibers, and immunofluorescence intensity. Each reviewer's comments are independently expressed.

**[AR] OBP Reviewer Dr. Ashutosh Rao's overall comments on the methodologies for the early biopsies and 4<sup>th</sup> biopsy from study 201/202:**

The applicant's early biopsy methods used for study 201/202 were exploratory in nature and not validated prior to use. The RT-PCR method was qualitative but reasonably well-performed to be able to predict the presence of an exon 51-skipped mRNA. Their western blotting was being optimized with multiple antibodies and several of their blots had a saturated healthy control that precluded meaningful quantitation. The applicant's immunofluorescence methods were also being optimized during studies 201/202. Between the measurements of positive fibers and fluorescence intensity, the measurement of intensity is likely to be more objective because it was relative to a healthy sample slide and did not include a subjective assessment by an analyst but rather by the Bioquant software. However, neither method appeared capable of reliably differentiating between newly expressed dystrophin and revertant dystrophin. Additionally, comparisons between baseline/weeks 12 or 24 and the week 48 samples are confounded by the use of different muscle types for the week 48 (deltoid) and the other biopsies (biceps). As discussed earlier some, but perhaps not all, of the changes reported in the dystrophin levels could be attributed to the differences in dystrophin expression and rates of degeneration in different DMD muscle groups. Other technical issues with the early biopsies are discussed within the review in Section 6.

More standardized procedures and positive/intermediate/negative controls were validated based on multiple discussions with FDA and prior to the 4<sup>th</sup> biopsy testing. Hence, the data using the 4<sup>th</sup> biopsy is likely to represent more robust measurement of dystrophin. Some, but not all, treated patient-matched samples had a baseline comparator sample. The applicant worked around this confounding factor by generating a set of "reference" control samples that consisted of pooled samples from either healthy, DMD, or Becker patients. However, there are some concerns about the choice of control samples, including their genotype, muscle of origin, and variability. Each of the treated samples was compared to the same set of controls. Within the three methods, namely RT-PCR/immunofluorescence/western blotting, the western blotting method is more likely to be quantitative because of the use of a serial dilution of healthy control and due to the inclusion of a negative and intermediate control sample on the same gel each time. The RT-PCR measurement is capable of being a reliable indicator that exon skipping occurred in these patients post-treatment. The immunofluorescence method, with independent reassessments, could serve as supportive data for the total dystrophin protein levels and localization. At this point it is not clear if the immunofluorescence method overestimates the true amount of newly formed dystrophin protein or if the western blotting method underestimates the true amount because no purified protein reference standard is available to make these types of clear assessments. The relative extent to which the antibodies recognize native or truncated protein is also not clear. The co-localization of dystrophin with nNOS and sarcoglycans can serve as additional supportive measurements to suggest that the dystrophin being expressed is "functional" within cells because it localizes to the sarcolemmal membrane and associates with its known functional partners as part of the dystrophin associated protein complex.

Overall, keeping in mind the concerns with the control samples, the applicant's methods with their fourth biopsy at week 180 were reasonably well-performed that they should be able to reliably estimate the relative levels and localization of dystrophin in muscle fibers. The expression of exon-skipped mRNA levels and co-localization to other dystrophin-associated proteins can also provide supportive data for the pharmacodynamic effect of this exon-skipping therapeutic.

Christopher D. Breder, MD PhD, Medical Reviewer Division of Neurology Products.

My comments relate not only to the primary endpoint but also extend to other biomarker data, such as exon skipping, fiber counts, immunofluorescence intensity, western blot analysis, as well as the clinical function tests including the 6MWT, NSAA, Rise time and 10-Meter run, I will combine these summary statements.

I agree with my colleague, Dr. Rao that the first 3 biopsies are not informative for all of the reasons summarized in my review. However, from my perspective, I think the fourth biopsy showed that there was some form of dystrophin present but it is not clear if the amount actually represents

- some effect of the drug
- variation in the tissue collection
- choice of controls, or
- the natural variation of these parameters in the disease

The exact amount seems to be slightly less than 1% of normal.

Similarly, the tests of clinical function in the placebo controlled portion are uniformly negative. Studies using natural history controls are not adequately done since these subjects were picked after the clinical course of the eteplirsen treated subjects was largely established and because they do not seem well matched. I do not discount that there is a difference between the eteplirsen and natural history cohorts in the 6MWT; however, it is not at all clear that this is drug-related.

Considering how small the database was known to be before submission, the Division commented that a single study needed to demonstrate particularly strong evidence of clinically meaningful benefit. Since the Sponsor wished to use a natural history cohort, the Division communicated that effect was to be of a magnitude so it was clear that it was not due to variation in the disease. I have not found the evidence in this NDA to satisfy either request.

## **8 Review of Safety**

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### **8.1. Safety Review Approach**

The Safety Review was performed on all data up through the 120 Day Safety Update (cutoff: August 12, 2015), which included data from a total of 129 patients, including 15 untreated patients and 114 patients who received eteplirsen. As of the D120 data cutoff, a total of 82 patients have been treated in clinical studies with the proposed treatment regimen (30 mg/kg administered once weekly by IV infusion) and an additional 6 patients have received a higher dose (50 mg/kg once weekly by IV

infusion); all other eteplirsen-treated patients received dose(s) <30 mg/kg.

This 120-Day Safety Update provides updated safety data for the 46 patients reported in the original NDA who were treated at the proposed eteplirsen dose or higher; this includes 12 patients treated with 30 mg/kg or higher once weekly for approximately 4 years in Studies 201/202 and 34 patients treated with 30 mg/kg once weekly for up to 9 months in Studies 204 (n=9) and 301 (n=25). Additionally, this D120 Update also provides safety data from 57 new patients, including 42 who received eteplirsen at the proposed dose regimen for up to 4 months in Studies 203 (n=4), 204 (n=15), or 301 (n=23) and 15 untreated patients in Study 301.

## 8.2. Review of the Safety Database

### 8.2.1. Overall Exposure

There were a relatively small number of subjects exposed to the intended labeled dose (30 mg/kg/week) and those doses which would yield useful safety information (e.g., 20 and 50 mg/kg/week) (**Table 32**).

### 8.2.2. Adequacy of the safety database:

In general, the *quality* of the safety database was adequate for review. However, the number of subjects is not adequate for an assessment of safety in this application. The duration of treatment of placebo comparators should also be longer to allow comparisons to eteplirsen treatments that were extended. For example, comparing adverse events that occurred in Eteplirsen treatments out to 196 weeks to placebo subjects with 24 week exposures is not optimal.

## 8.3. Adequacy of Applicant's Clinical Safety Assessments

### 8.3.1. Categorization of Adverse Events

Adverse Events were for each study coded in MedDRA versions appropriate to the timing of the finalization of the study reports. The Adverse Event dataset from the Integrated Summary of Safety was coded in MedDRA version 14.1.

## 8.1. Safety Results

### 8.1.1. Death

No deaths have been reported in the eteplirsen application, through the 120-Day cutoff.

**Table 32 Extent of Exposure to Study Drug: Integrated Analyses (Safety Population)**

	Placebo (N=4)	Untreated (N=15)	Eteplirsen							All IV (N=107)	All Eteplirsen (N=114)
			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)			
<b>Days on Study Drug<sup>a</sup></b>											
n	4	15	7	11	4	4	82	6	107	114	
Mean	162.3	67.9	1.0	74.4	78.0	78.0	213.7	1394.8	255.4	239.8	
SD	1.26	53.50	0.00	10.59	1.63	0.00	342.29	87.03	412.93	404.62	
Median	162.0	66.0	1.0	78.0	78.0	78.0	126.5	1449.5	97.0	89.5	
Min, Max	161, 164	1, 170	1, 1	43, 79	76, 80	78, 78	1, 1451	1282, 1453	1, 1453	1, 1453	
<b>Number of Infusions</b>											
n	4	0	7	11	4	4	82	6	107	114	
Mean	24.0		1.0	11.3	12.0	11.8	31.1	197.8	37.0	34.8	
SD	0.00		0.00	1.56	0.00	0.50	48.23	14.12	58.26	57.09	
Median	24.0		1.0	12.0	12.0	12.0	19.0	205.5	15.0	14.0	
Min, Max	24, 24		1, 1	7, 12	12, 12	11, 12	1, 208	176, 208	1, 208	1, 208	
<b>Number of Weeks Category</b>											
<13 Weeks	0	10 (66.7%)	7 (100%)	11 (100%)	4 (100%)	4 (100%)	27 (32.9%)	0	46 (43.0%)	53 (46.5%)	
≥13 Weeks to <24 Weeks	0	3 (20.0%)	0	0	0	0	25 (30.5%)	0	25 (23.4%)	25 (21.9%)	
≥24 Weeks	4 (100%)	2 (13.3%)	0	0	0	0	30 (36.6%)	6 (100%)	36 (33.6%)	36 (31.6%)	
<b>Patient Years at Actual Dose<sup>b</sup></b>											
n	4	15	NA	NA	NA	NA	82	6	88	88	
Mean	0.459	0.183					0.559	3.804	0.817	0.817	
SD	0.0016	0.1465					0.9275	0.2715	1.2106	1.2106	
Median	0.459	0.178					0.365	3.952	0.385	0.385	
Min	0.46	0.00					0.02	3.38	0.02	0.02	
Max	0.46	0.46					4.00	4.00	4.00	4.00	
Sum	1.834	2.746					49.096	22.827	71.923	71.923	

<sup>a</sup> For the untreated patient population, Days on Study Drug equals post-baseline Days on Study.

<sup>b</sup> Placebo patient years are calculated as the number of days on placebo divided by 365.25. Untreated patient years are calculated as the number of days from the Week 1 visit to the last visit date divided by 365.25.

Source: Module 5.3.5.3 Table ISS.3.1.1

Source: NDA 206488 S0018, Summary of Clinical Safety, Table 11, p 44 of 184

### 8.1.2. Serious Adverse Events

Four subjects with Serious Adverse Events (SAEs) were reported in the original NDA submission (**Table 33**). Two additional subjects had non-fatal serious SAEs in the period between the NDA submission and the 120-Day cutoff (Study 4658-203, Subject 202.202, PT term, *Oxygen saturation decreased*; Study 4658-301-A1, Subject 216.003, PT term *Lymphadenitis viral*). These boys were not in the active treatment group at the time of these SAEs.

There does not appear to be a causal relationship between treatment and these SAEs although a contribution cannot be ruled out

#### 8.1.1. Dropouts and/or Discontinuations Due to Adverse Effects

One subject (1/119 eteplirsen treated (0.9%), 1/11 @ 4 mg/kg IV (9.1%)), **Patient 28-02-202** from Study 28, discontinued treatment in the development program due to Cardiomyopathy. He was a 10-year-old boy being treated with 4 mg/kg/week. A retrospective review of the echocardiograms for this patient showed that the patient had pre-existing cardiomyopathy. The patient discontinued study treatment after receiving 7 once weekly IV infusions of eteplirsen at 4 mg/kg, but remained in the study for safety follow-up. His outcome at the time of the 120-Day safety update was listed as not recovered.

### 8.1.2. Significant Adverse Events

A total of 9 AEs occurring in 6 patients, were assessed as severe by the Investigator (**Table 34**). Two events met the criteria for seriousness. All of the events were judged by the investigator to be “Not Related” except the case of cardiomyopathy in Subject 28-02-202 which was judged to be “Possibly Related.”

#### *Reviewer’s Analyses and Comment’s*

Overall the incidence of severe AEs is low and not concentrated in one type of event. As with most of the safety analyses in this application, an accurate perspective on significant AEs is difficult with such a small safety database, many of whom were treated with doses not intended for labeling.

**Table 33 Summary of Nonfatal Serious Adverse Events Reported in the Original NDA Submission (ISS Safety Population)**

Patient	Dose	Preferred term	Description	Severity	Prior dose	Date Onset / Resolved
33-01-006	eteplirsen 0.9 mg) Single dose	<i>Wound infection</i>	Suspected bilateral local infection at bx site was reported as an A (onset date of (b) (6)). Hospitalized (b) (6) with a diagnosis of 'superficial late bilateral wound infection on the site of the EDB muscle biopsies'. He received 6 doses of IV flucloxacillin on (b) (6)	Mod	14 Oct 2008	(b) (6)
28-01-107	12 once weekly doses eteplirsen 2.0 mg/kg IV started on 02 July 2009.	<i>Vomiting</i>	Vomiting (post-operative nausea and vomiting)	Mod	17 Sep 2009	29 Sep 2009 / 30 Sep 2009
28-01-108	11 doses of once weekly eteplirsen 4.0 mg/kg IV started on 23 July 2009.	<i>Ankle fracture</i>	Fall on 10 November 2009; On (b) (6), seen in the hospital Emergency Room where an X-ray confirmed that he had suffered a closed stable medial malleolus fracture of his left ankle	Mod	08 Oct 2009	(b) (6)
201/202-01-009	once weekly 30 mg/kg IV,	<i>Femur fracture</i>	closed stable femoral fracture s/p falling out of wheelchair in vehicle incident	Sev	17 Apr 2013	22 Apr 2013 / 18 Jun 2013

Source: Integrated Summary of Safety, Section 2.1.6, pp. 59-60

**Table 34 Cases of Severe Adverse Events (Safety Population)**

Subject ID	Study	Dose (mg/kg)	Study Day	Event Duration (Days)	Preferred Term	Serious	Outcome	Action
203-202-201	203	30	10	17	incision site haemorrhage	N	Resolved	Dose not changed
201/201-01-005	201	50	900	18	haemorrhoids	N	Resolved	Dose not changed
			885	3	back pain	N	Resolved	Dose not changed
301-216-003	301	Un-treated	NA	1	lymphadenitis viral	Y	Resolved	Not applicable
28-02-202	28	4	46	UNK	cardiomyopathy with left ventricular dysfunction	N	Not resolved	Withdrawn
201/202-01-006	201	30	101	4	nasal congestion	N	Recovered	No change
201/202-01-009	201	30	144	8	bone pain	N	Recovered	No change
			144	8	loss of balance	N	Recovered	No change
			608	57	Fracture of right distal femur	N	Recovered	Dose not changed, medication, non-drug therapy

Source: Summary of Clinical Safety (SCS) and 120-Day Safety Update of the SCS  
 Abbreviations – UNK, unknown

### 8.1.3. Treatment Emergent Adverse Events and Adverse Reactions

I evaluated the events in the 201 / 202 Study comparing the placebo versus actively treated subjects, since this was the best controlled adverse event daatabase. I also summarized the safety from the smaller studies and those not placebo controlled. I looked for disproportionate amounts of AEs in the smaller studies (28, 33, 301, 203, 204) that would otherwise be obscured when pooled with the entire safety population of the application.

Because the number of subjects and number of adverse events was small in the placebo controlled portion of the study, I looked at the incidence of all events that satisfied ALL of the following criteria:

- The number of AEs in the Eteplirsen 30 mg/kg OR the 50 mg/kg group is greater than 1
- The number of AEs in the Eteplirsen 30 mg/kg or the 50 mg/kg group is greater than the number in the placebo group

The analysis AE dataset (ADAE) contained 478 events. I evaluated the coding of this dataset and proposed changing the preferred terms for 25 events based on the verbatim terms:

<b><u>Original term</u></b>	<b><u>New Preferred Term</u></b>
▪ <i>Cataract Subcapsular</i>	<i>Cataract</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Abdominal Pain Upper</i>	<i>Abdominal Pain</i>
▪ <i>Thrombosis In Device</i>	<i>Device Occlusion</i>
▪ <i>Thrombosis In Device</i>	<i>Device Occlusion</i>
▪ <i>Thrombosis In Device</i>	<i>Device Occlusion</i>
▪ <i>Non-Cardiac Chest Pain</i>	<i>Chest Pain</i>
▪ <i>Rhinitis</i>	<i>Nasopharyngitis</i>
▪ <i>Rhinitis</i>	<i>Nasopharyngitis</i>
▪ <i>Rhinitis</i>	<i>Nasopharyngitis</i>
▪ <i>Respiratory Disorder</i>	<i>Upper Respiratory Infection</i>
▪ <i>Viral Upper Respiratory Tract Infection</i>	<i>Upper Respiratory Tract Infection</i>
▪ <i>Femur Fracture</i>	<i>Fracture</i>
▪ <i>Foot Fracture</i>	<i>Fracture</i>
▪ <i>Foot Fracture</i>	<i>Fracture</i>
▪ <i>Lower Limb Fracture</i>	<i>Fracture</i>
▪ <i>Radius Fracture</i>	<i>Fracture</i>
▪ <i>Post Procedural Haematoma</i>	<i>Haematoma</i>
▪ <i>Bone Pain</i>	<i>Pain In Extremity</i>

The analysis AE dataset of the 201 study contained 478 events in both study periods (Pre and post Week 24). When tabulating them by preferred term by treatment, there were 109 unique events. Of these 29 preferred terms satisfied the criteria outlined at the beginning of this section. Since there were only 4 subjects per dose group, I did not calculate the percent of the incidence.

**Table 35 Absolute counts of AE Preferred terms in the placebo controlled portion (Weeks 0 to 24) of Study 201/202 (Safety Population)**

<b>System Organ Class</b>	<b>Preferred term</b>	<b>N Total Events</b>	<b>N (30 mg/kg)</b>	<b>N (50 mg/kg)</b>	<b>N (Placebo)</b>
Gastrointestinal disorders	<i>Abdominal pain</i>	4	0	3	1
	<i>Diarrhoea</i>	2	1	1	0
	<i>Vomiting</i>	4	2	2	0
General disorders and administration site conditions	<i>Catheter site pain</i>	4	2	2	0
	<i>Device occlusion</i>	3	2	1	0
	<i>Infusion site extravasation</i>	3	1	2	0
	<i>Oedema peripheral</i>	2	2	0	0
Infections and infestations	<i>Upper respiratory tract infection</i>	4	1	3	0
Injury, poisoning and procedural complications	<i>Contusion</i>	3	2	1	0
	<i>Excoriation</i>	2	1	1	0
	<i>fracture</i>	2	1	1	0
	<i>Joint injury</i>	2	2	0	0
	<i>Muscle strain</i>	2	1	1	0
	<i>Procedural pain</i>	10	3	4	3
Investigations	<i>Activated partial thromboplastin time prolonged</i>	2	0	2	0
	<i>Blood creatine phosphokinase increased</i>	2	2	0	0
	<i>C-reactive protein increased</i>	2	0	2	0
Metabolism and nutrition disorders	<i>Obesity</i>	2	2	0	0
	<i>Vitamin D deficiency</i>	2	2	0	0
Musculoskeletal and connective tissue disorders	<i>Arthralgia</i>	3	1	2	0
	<i>Back pain</i>	6	3	1	2
Nervous system disorders	<i>Balance disorder</i>	4	2	2	0
	<i>Headache</i>	7	2	3	2
Renal and urinary disorders	<i>Proteinuria</i>	6	2	3	1

System Organ Class	Preferred term	N Total Events	N (30 mg/kg)	N (50 mg/kg)	N (Placebo)
Respiratory, thoracic and mediastinal disorders	<i>Nasal congestion</i>	5	3	1	1
	<i>Pharyngeal erythema</i>	2	1	1	0
	<i>Upper respiratory tract congestion</i>	2	2	0	0
Skin and subcutaneous tissue disorders	<i>Dermatitis contact</i>	3	2	1	0
	<i>Erythema</i>	2	1	1	0

Source: Medical Reviewer's analyses of the Study 201/202 ADAE dataset

I also evaluated this dataset by MedDRA High Level Term (HLT) versus treatment. Only two new HLTs arose from this analysis. The first was for *Urticarias*, HLT, which emanated from a case of *Hives to neck and right forearm*, AETERM and one case of *Cold-induced urticaria*, AETERM.

The following observations were made on the long term (Onset at > 24 week) adverse events:

- Infections arise with extended eteplirsen treatment, including an increase in respiratory infections
- There are different lab investigations declared as AEs in the different periods of the study. Initially, the incidence of aPTT, CK, and CRP are higher and in the second period, elevated glucose is the most prevalent event related to investigations. These are discussed more in **Section 8.1.4**.
- AEs related to neuromuscular symptoms are increased in the later part of the trial, and
- There are more hypersensitivity-related events in the later part of Study 201/202.

### AEs in the Smaller Studies

Small and uncontrolled studies in the ISS Adverse Event dataset from the 120-Day safety update was reviewed individually because of their unique doses, routes of administration, or population.

1. **AVI-4658-28** – A 2 site (UK) open label, multiple dose (qW x 12 Weeks), dose ranging study in 19 ambulatory males between 5 and 15 years old (Status: completed)

There were 150 adverse events, 120 were unique<sup>11</sup>. The most common preferred terms were *Headache*, *Upper respiratory tract infection* (N=8), *Back pain*, *Rhinitis* (N=7) *Abdominal pain*, and *Fall* (N=5). Preferred terms of *Abdominal pain*, *Nausea*, *Disease progression*, *Rhinitis*, *Upper respiratory tract infection*, *Fall*, *Lumbar vertebral fracture*, *Back pain*, and *headache* seemed to have an increase in incidence with dose.

There were 12 events with an intensity of moderate (ToxGrade of 2) or greater. One event of *Cardiomyopathy* (discussed in **Section 8.4.1**) was a severe (ToxGrade 3) event. Most of these events of moderate or greater intensity, except for CNS events, began after an extended time on drug. There were 12 events reported as not resolved at the time of the 120-Day Safety Update. The preferred terms in the Moderate-or-Greater and

Unresolved categories were reflective of the most common types experienced in the trials.

2. **AVI-4658-33** – A single site (UK), single blind, placebo-controlled study of 7 males, 10-17 years old DMD subjects treated with 0.09 (N=2) or 0.9 (N=5) mg IM in EDB muscle of one foot and placebo in the opposite foot (completed)

There were 16 adverse events, of which were 14 unique. Events of myoglobinuria were disproportionately higher in this study. Four subjects, 004, 006, 007, and 008, all from study 28 were the only individuals with an adverse event of Myoglobinuria. However, myoglobin was not assayed in the Study 33 urinalysis screen. There were 6 individuals in Study 28 who did have myoglobin in their urine when it was not present at baseline, which did not have an adverse event of myoglobinuria declared (see **Figure 74**). Most concerning is that myoglobin was not tested for in any study except for Study 28.

This study was also unique because the drug was dosed by IM injections in the muscles of feet rather than by the intravenous route. The Applicant presented data on different aspects of the local reaction to injection in an index score related to erythema, induration, pruritus, pain, nodules and cysts, ecchymosis, and reactive pain.

**Table 36 Cumulative Injection Site Reaction Score in Study 33 (Safety Population)**

AVI-4658 Dose Group	Study Day	Grade [Cumulative Injection Site Reaction Score] <sup>a</sup>					ND/NR
		0 [0]	1 [1 to 2]	2 [3 to 5]	3 [6 to 14]	4 [>14]	
0.09 mg (N=2)	1	--	--	2 (100%)	--	--	--
	2	--	1 (50%)	--	1 (50%)	--	--
	3	1 (50%)	--	1 (50%)	--	--	--
	14-28 <sup>b</sup>	1 (50%)	--	--	--	--	1 (50%)
	120	2 (100%)	--	--	--	--	--
0.9 mg (N=5)	1	--	4 (80%)	1 (20%)	--	--	--
	2	3 (60%)	2 (40%)	--	--	--	--
	3	2 (40%)	1 (20%)	1 (20%)	--	--	1 (20%)
	14-28 <sup>b</sup>	5 (100%)	--	--	--	--	--
	120	4 (80%)	1 (20%)	--	--	--	--

ND/NR = not done or not reported

<sup>a</sup> Sum of the grades for erythema, induration, pruritus, pain, nodules and cysts, ecchymosis, and reactive pain (see **Supplement E** in protocol in Appendix 13.1.1).

<sup>b</sup> Day on which posttreatment biopsies of extensor digitorum brevis (EDB) muscle were done.

Source: **Table 12.3, Listing 13.2.30**

Source: CSR avi-4658-33, Table 1, p. 27 of 443

3. **4658-301** – An open-label, multi-center vs untreated control group (i.e., patients with DMD not amenable to exon 51 skipping) in approximately 80 patients amenable to exon 51 skipping and 80 untreated controls for up to 48 weeks of treatment treated with 30 mg/kg/wk IV infusions (Ongoing)

There were 207 adverse events in 48 subjects, with 102 preferred terms unique (counting once even if subject had more than 1 of a certain event)<sup>11</sup>. The most common preferred terms that occurred with an incidence greater than placebo were *Vomiting* (N=11), *Back pain* (N=8), *Excoriation* and *Headache* (N=7), *Pain in extremity* and *Nasopharyngitis* (N=6), *Cough* (N=5), and *Contusion* and *Fall* (N=4). There were 11 events in treated subjects that had a ToxGrade of 2; one ToxGrade 3 was in untreated subject. There are 18 events not resolved at the time of the 120-Day Safety Update. There were 64 events which required medication or other actions in response. The preferred terms in the Moderate-or-Greater, Unresolved, and Requiring Actions categories were generally reflective of the most common types experienced in the trials.

4. **4659-203** – An open-label, multicenter study of approximately 40 subjects (4 ongoing, 0 completed) subjects amenable to Exon 51 skipping ages 4-6 treated with 30 mg/kg/wk once weekly IV for up to 96 weeks or Untreated controls.

At this time the preferred terms and the Moderate-or-Greater, Unresolved, and Requiring Actions categories in the ongoing trial are generally reflective of the most common types experienced in all of the trials in this development program.

5. **4658-204** – An open-label, multicenter study of 30 mg/kg/wk for up to 96 weeks of treatment in approximately 20 non-ambulatory patients between 7-21 years of age, incapable of walking  $\geq 300$  meters on 6MWT (24 patients enrolled, study ongoing)

At the point of the 120-Day Safety Update, there are 105 events in n 89 subjects, 53 of them are unique terms. Eighteen events occurred in greater than 1 subject. There seems to have been a disproportionately high number of events with the preferred term of Rash. Events occurring with the highest incidence have been *Headache* (N=8), *Catheter site pain* and *Rash* (N=7), and *Vomiting* and *Cough* (N=6). Two events in one subject (*Fatigue* and *Vomiting*) were judged to by the Investigator be ToxGrade 2. Six events were unresolved at the time of the 120-Day Safety Update, including one of *pericardial fibrosis*. Several events occurred only once so far but bear mentioning and close monitoring during the trial: *Pericardial fibrosis*, *Wound dehiscence*, *Urine ketone body present*, *Aggression*, *Ecchymosis*, and *Pruritus*.

#### 8.1.4. Laboratory Findings

##### ***Medical Reviewer's Analyses and Comments***

Laboratories in the 201 Study were conducted at the National Children's Hospital for the first 28 weeks and by the CRO <sup>(b) (4)</sup> for the multi-site portion of the 201/202 study following week 28. During this second period, a multitude of normal ranges are present for each of several key analytes in the analysis laboratory dataset (ADLB) from this period. Some of these lab reference ranges vary to the extent that the low reference range from some subjects approaches the high limits of others (see my description of Creatinine). Because a description of the absolute values for the labs would not be informative, I performed my lab analysis by highlighting labs with abnormal values graphically and by describing multiples of the relevant abnormal ranges rather than the absolute values.

Notably missing from the laboratory assessments were Anti-dystrophin antibodies from all studies except Study 33 and urine myoglobin from all studies except Study 28.

- Electrolytes and Renal-Associated labs
  - **BUN** – Overall, abnormal BUN values appeared to have increased with dose (**Figure 63**). Subjects 002 (30 mg/kg group) and 015 (50 mg/kg group) had the highest multiples over an extended period of time. Subjects 003 and 012, both in the 50 mg/kg group) also had brief elevations of BUN. Subject 003 had the highest elevation at 2.3 times the upper limit of normal (**Table 37**).

**Figure 63 Multiples of the Abnormal HI Reference Limit for BUN Values Versus Time by Treatment (Days) (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT  
 Each dot represents a different lab value; green dots are normal and red abnormal.

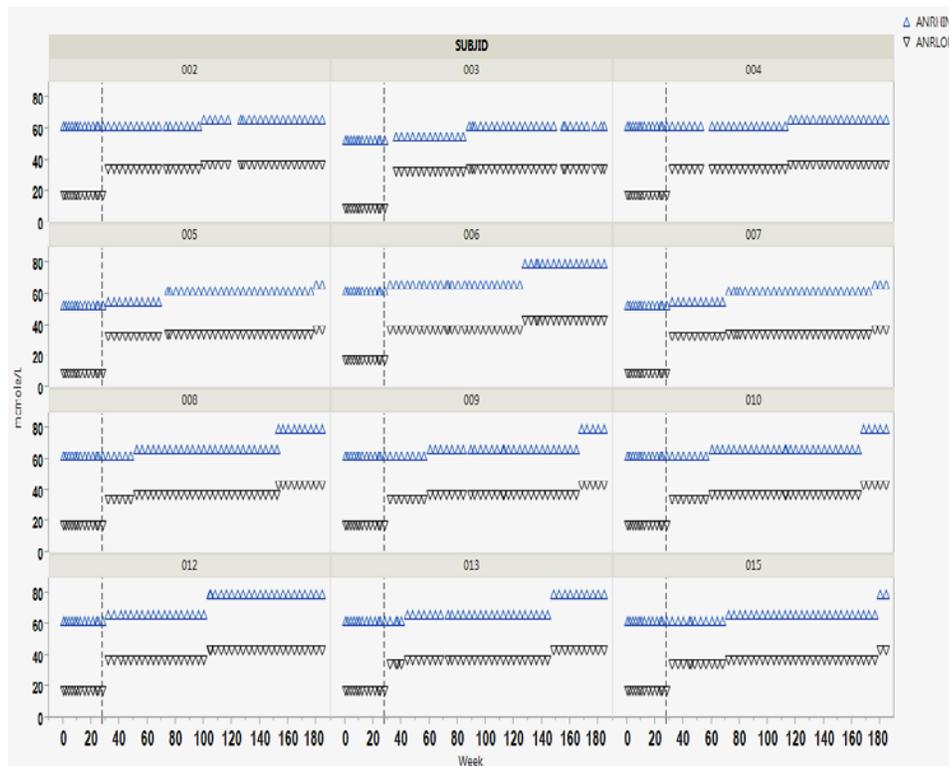
**Table 37 Mean Multiples of the Abnormal HI Limit in Subjects with Abnormal BUN (201/202 Safety Population)**

Treatment	Subject	N visits with abnormal values	Mean Value	Mean Multiples of Abnormal HI Limit
Eteplirsen 30 mg/kg	002	8	7.27	1.13
	007	2	6.78	1.06
Eteplirsen 50 mg/kg	003	2	10.7	1.67
	012	1	7.5	1.17
	015	21	7.53	1.17

Source: Medical reviewer analysis of ADLB.XPT

- **Calcium** – Three subjects had low Ca levels, one in the 30 mg/kg group and 2 in the 50 mg/kg/group. The lowest Ca level was 1.83 mmol/L (LO normal = 2 mmol/L)
- **Chloride (Cl)** – Most labs were normal with abnormal results found in all treatment groups with a maximum multiple of 1.02.
- **Creatinine (Cr)** – Creatinine is one of the labs that have normal multiple reference ranges, some of which were so different that the low values of one range approached the high limits of others (**Figure 64** and **Table 38**).

**Figure 64 Normal reference limits for Creatinine by Subject (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT

**Table 38 Number of Values of the Different Normal Reference Limits for Creatinine (201/202 Safety Population)**

Abnormal High Reference Limit	Abnormal Low Reference Limit	N Lab Values at each Level
53.04	8.84	42
54.808	32.708	33
61.88	17.68	126
61.88	34.476	151
66.3	37.128	223
79.56	43.316	69

Source: Medical reviewer analysis of ADLB.XPT

**Figure 65** demonstrates the appearance of the Creatinine lab values plotted as the log function to allow better visualization, since there is a floor-effect of abnormal low values between multiples of 1 and 0 versus 1 and no actual limit for HI values)

**Figure 65 Creatinine Log Scale Multiples of Abnormal LO (201/202 Safety Population)**

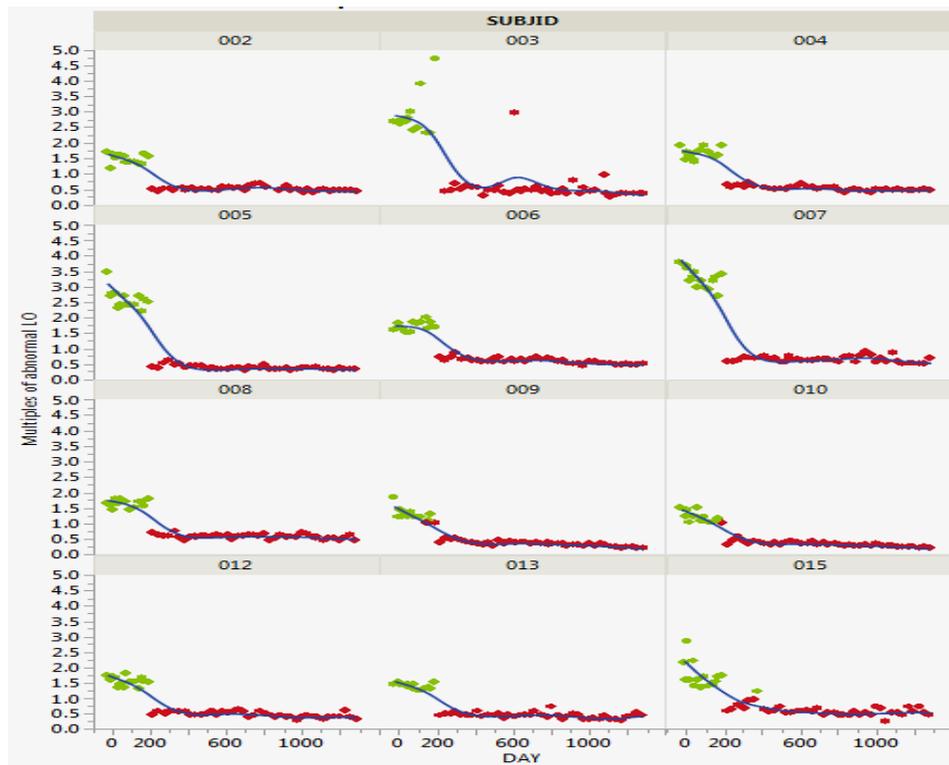


Source: Medical reviewer analysis of ADLB.XPT

Each dot represents a different lab value; green dots are normal and red abnormal.

As is evident from **Figure 66**, this is an issue with all subjects, not just a select few.

**Figure 66 Multiples of Abnormal LO Versus Day by Subject (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT

Each dot represents a different lab value; green dots are normal and red abnormal.

- **Potassium** – One subject (006) in the 30 mg/kg group had a value of 6.1 mmol/L (ULN 5.5) on day 499. Isolated values below the LLN were observed in all treatment groups.
- Liver -related labs
  - **ALT** – Every lab result for all treatments was abnormally HI in both periods of the study. There was no discernable difference between placebo and active arms during the double blind, placebo controlled portion of the 201 Study.
  - **AP** – This lab had no abnormally high results
  - **AST** - Every lab result for all treatments was abnormally HI in both periods of the study. There was no discernable difference between placebo and active arms during the double blind, placebo controlled portion of the 201 Study. Two subjects in the 50 mg/group had values at 15.4 (Subject 003 at Day 443) and 14.7 (Subject 15 at Day 889) times the high reference limit.
  - **GGT** – no high values
  - **Total bili** – There were two slightly high Total bilirubin values.
- Hematology
  - **Eosinophils** – A few subjects with abnormally high number of eosinophils were seen in all groups (**Table 39**). The highest was from Subject 007 who had a 3x increase over normal on day 891 (Week 128 visit).

**Table 39 Abnormal Eosinophils in the 201/202 study (201/202 Safety Population)**

Subject	Treatment	Multiples of abnormal HI	Value (10 <sup>9</sup> /L)	Change	Abnormal HI Limit	Study Day	VISIT
005	Placebo	1.07	0.62	0.521	0.58	8	Week 2
	50 mg/kg	2.73	1.91	1.811	0.7	274	Week 40
		1.09	0.76	0.661	0.7	721	Week 104
007	30 mg/kg	3.06	2.14	2.14	0.7	891	Week 128

Source: Medical reviewer analysis of ADLB.XPT

- **Hematocrit** – Slightly elevated values (to ~ 1.06 x ULN) were noted in all treatment groups in both periods.
- **Leukocytes** – All treatments had some slightly elevated (~1.5 – 1.6x ULN) with a few on treatment below the LLN (~ 0.9 x LLN)
- **Lymphocytes** – Several subjects in the active treatment groups had values below the LLN (~ 0.5 – 0.6x LLN). This lab is one where there were multiple reference ranges in both placebo-controlled and open-label parts of the study

**Table 40 Number of Values of the Different Normal Reference Limits for Lymphocytes (201/202 Safety Population)**

Abnormal LO Reference Limit	Abnormal HI Reference Limit	Number of values assessed based on t limit per Period	
		Period 1	Period 2
1.1	5.9	235	76
1.26	6.48	53	2
1.1	6.5	103	82
1.35	8.265	103	2

Source: Medical reviewer analysis of ADLB.XPT

- **Monocytes** –There was an increase in results below the LLN in the active treatment groups. One subject (007) had a value 0.05x the LLN at the week 36 visit. Most of the low values were .15x the LLN or greater. There were a few results above the ULN (~1.3-1.4x ULN) present in all treatment groups.

**Figure 67 Multiples of Abnormal LO Monocyte Values (Log Scale) Versus Time (Days) (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT

Each dot represents a different lab value; green dots are normal and red abnormal. Values above the abnormal HI limit omitted

- **Neutrophils** – The limits of normal shifted between 10.13 or 10.4 in the first 28 Weeks to 7.8/1.5 after Week 28, in the open label part of the study. Two subjects in the active treatment groups had abnormally low values, including a value of 0 neutrophils in Subject 009 at Week 28. One subject, 005, started out with abnormally low neutrophils but these levels elevated to normal during the study. There were a few values above the ULN (1.2-1.3x ULN) in the placebo group, however the active treatment groups (Subjects 006, 007 in the 30 mg/kg and 004, 013 in the 50 mg/kg group) had values up to 2.2x ULN that were sustained throughout both periods of the study (**Figure 68**).

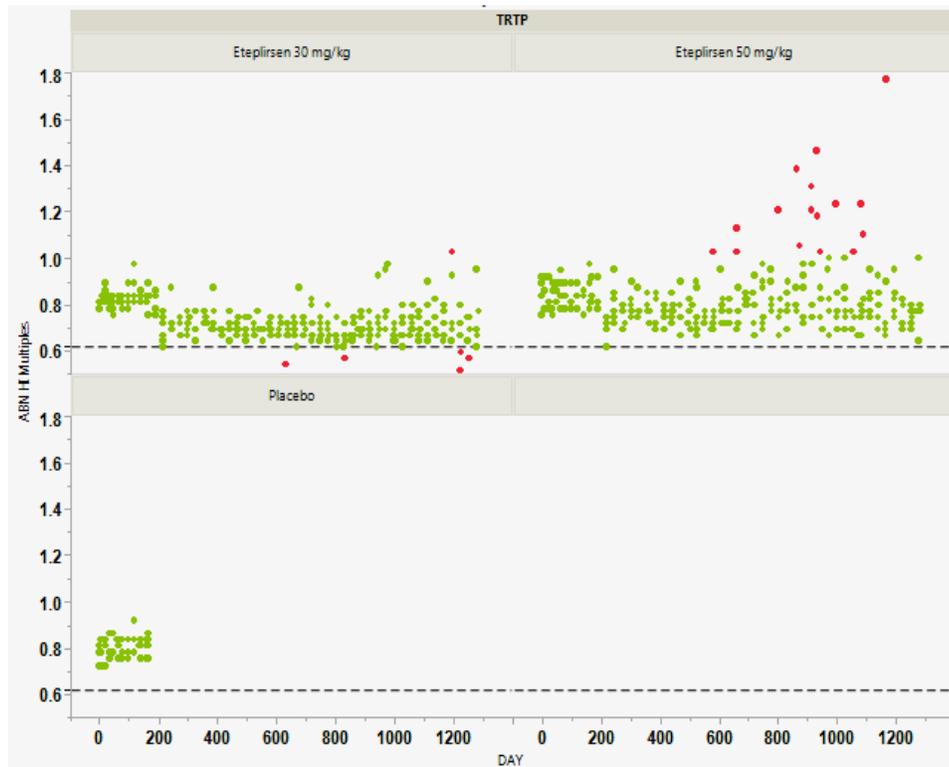
**Figure 68 Multiples of the Abnormal HI Reference Limit for Neutrophil Values Versus Time by Treatment (Days) (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT  
Each dot represents a different lab value; green dots are normal and red abnormal. Values below the abnormal LOW limit omitted

- **Platelets** – Several individuals in both the placebo and active treatment groups had slightly elevated values.
- **RBCs** – There were no abnormal RBC values
- Coagulation related labs
  - **aPTT** – One subject of six in the Period 2, 30 mg/kg and five of six in the Period 2, 50 mg/kg group had abnormally high aPTT values ( $\bar{x} \pm sd$ ;  $1.2 \pm 0.2$ ). Three of six subjects in the 30 mg/kg group had low aPTT values in Period two that ranged from 0.83-0.95x the abnormal LO reference limit.

**Figure 69 Multiples of the Upper Limit of Normal aPTT Value Versus Time (Days) (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT  
 Each dot represents a different lab value; green dots are normal and red abnormal.

**Table 41 Subjects with Abnormal aPTT Values (201/202 Safety Population)**

Treatment	Subject	Number of Abnormal Labs	Mean Value (sec)	Mean Multiples of the Abnormal HI Reference Limit
Eteplirsen 30 mg/kg	009	2	40	1.03
Eteplirsen 50 mg/kg	004	3	64	1.64
	005	6	43	1.10
	012	3	40	1.03
	013	4	46.5	1.19
	015	3	55	1.41

Source: Medical reviewer analysis of ADLB.XPT

- **Prothrombin Time** –The Prothrombin time was another lab that had several, highly variable reference limits

**Table 42 Number of Values of the Different Normal Reference Limits for Prothrombin Time (201/202 Safety Population)**

Abnormal LO Reference Limit	Abnormal HI Reference Limit	Number of values assessed based on this limit per Period	
		Period 1	Period 2
8.8	14.1	318	160
12.4	14.7	154	4

Source: Medical reviewer analysis of ADLB.XPT

Two Subjects, 002 and 008, on active therapy had abnormally high Prothrombin times (15.4 and 15.2 sec with a HI Limit of 14.1) on days 978 and 832, respectively. One subject (003) also had high values but he started abnormally high (15.2 with a HI Limit of 14.7).

- **Prothrombin Time INR** – The Prothrombin time INR was another lab that had several, highly variable reference limits

**Table 43 Number of Values of the Different Normal Reference Limits for Prothrombin Time INR (201/202 Safety Population)**

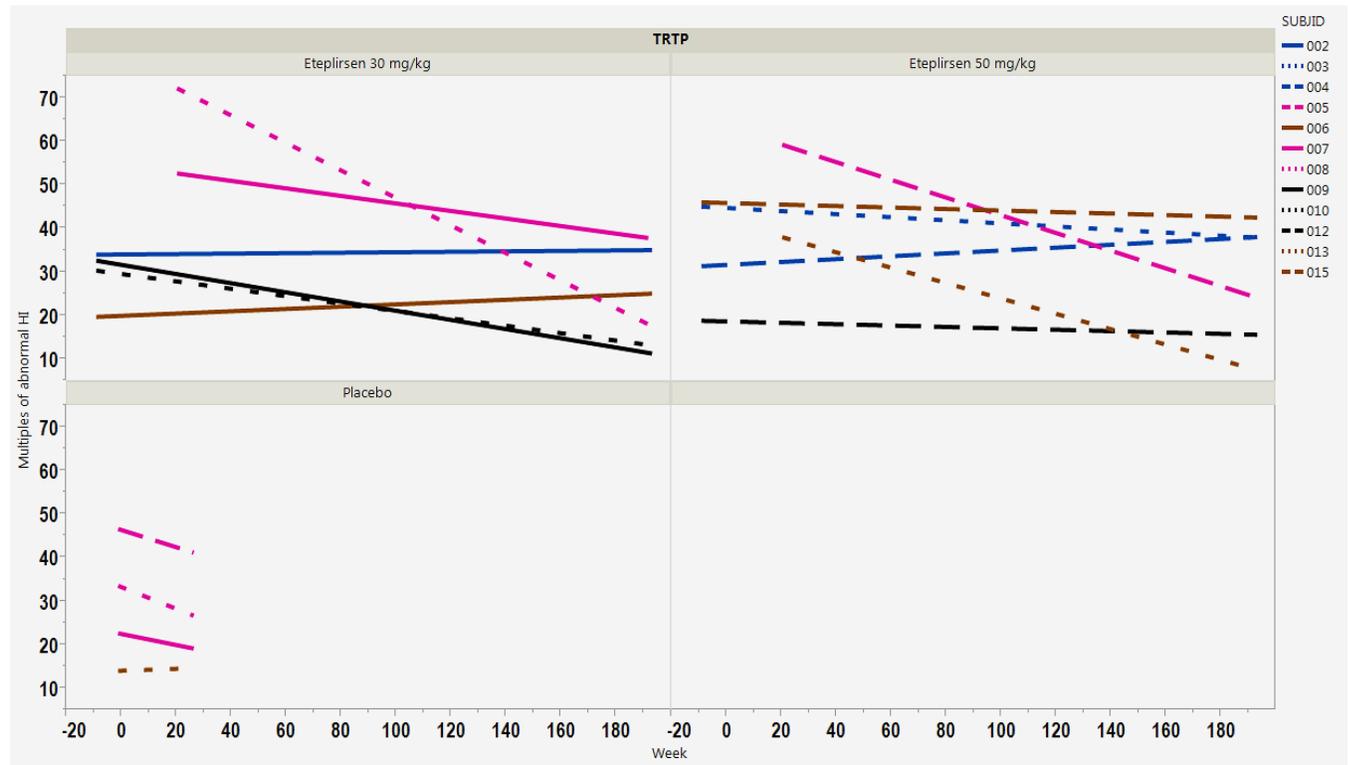
Abnormal LO Reference Limit	Abnormal HI Reference Limit	Number of values assessed based on this limit per Period	
		Period 1	Period 2
0	3	148	4
0.9	1.3	322	159

Source: Medical reviewer analysis of ADLB.XPT

Several subjects (002, 008, 003) had slightly elevated Prothrombin time INR values (1.4 with a HI Limit of 1.3) while on active treatment. One subject on 50 mg/kg had an isolated value that was very high (5.3, 4.1x the ULN) on Day 865, Visit Week 124.

- **Other Labs**
  - **Amylase** – No abnormally high results (though see results for Study 33 discussed in Section 8.1.3).
  - **Creatine Kinase** – CK references limits shifted from 430/37 in Period 1 to 204/24 in Period 2 so the Multiples of HI approach was used to evaluate these lab values. CK levels were elevated (up to 120x ULN) in all treatment groups and remained so for the duration of the trial. I note that the individual responses are quite varied (**Figure 70**); Subjects with the greatest decline in functional status, 009, 010, 008, and 012 had Creatine Kinase results that either declined or seemed to stabilize, while Subject 006, who seemed to have the greatest increase in dystrophin also seemed to have increasing Creatine Kinase levels.

**Figure 70 Multiples of Upper Limit of Normal for Creatine Kinase Range by Subject and Treatment over Time (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT

- **C-Reactive Protein** – CRP values were elevated in all groups in both periods of the trial. The highest multiple of the normal HI reference limit was subject 008 with an elevation to 7.49 during the Week 100 Visit while on 30 mg/kg. Levels in this subject were not chronically elevated but seemed to intermittently spike during the trial.
- **Cystatin** – Only one value was elevated in these data to a multiple of 1.06 times the upper limit of normal (Subject 007 while on 30 mg/kg during the Week 44 visit).
- **Glucose** – Subjects in active treatment groups had an increase in labs greater than the ULN. A few abnormally low values were seen in all treatment groups.

**Figure 71 Multiples of the Upper Limit of Normal Glucose Value versus Time (Days) (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT

Each dot represents a different lab value; green dots are normal and red abnormal

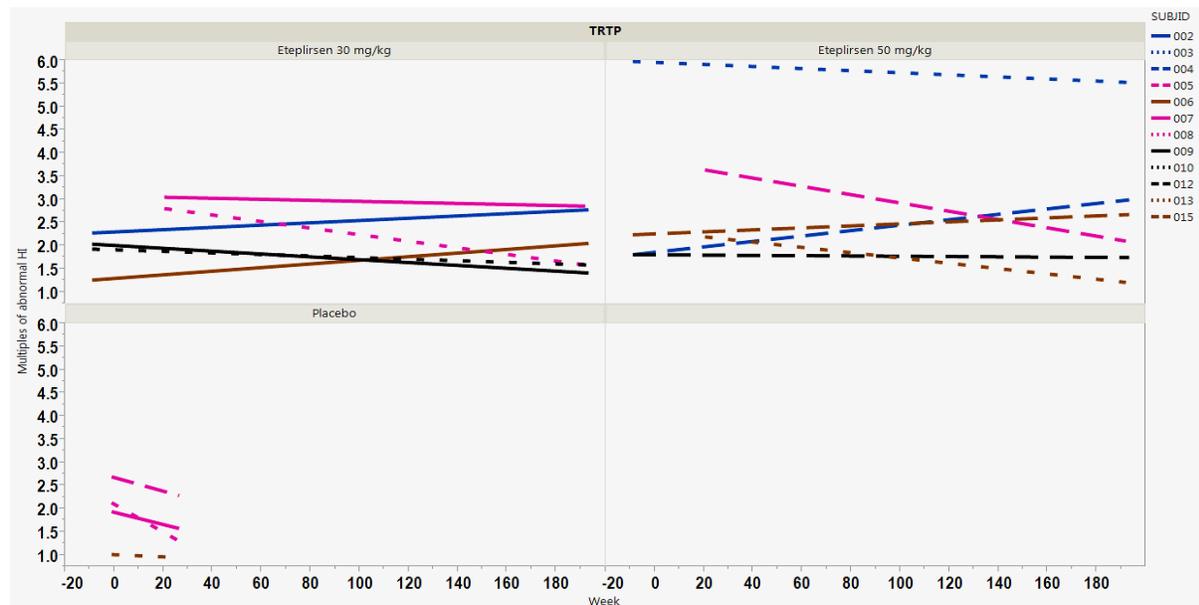
- **LDH** – The reference range for the first 28 weeks was 1250 / 400 and then it shifted to 250 / 100 after Week 28 (**Figure 72**). In the 50 mg/kg group, Subject 003 had elevations in LDH up to 7-8x ULN for several visits. As with the Creatine Kinase values, Subjects with the greatest decline in functional status, 009, 010, 008, and 012 had Creatine Kinase results that either declined or seemed to stabilize, while Subject 006, who seemed to have the greatest increase in dystrophin also seemed to have increasing Creatine Kinase levels (**Figure 73**).

**Figure 72 Multiples of the High Reference Range for Lactate Dehydrogenase Range by Subject and Visit (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT  
Each dot represents a different lab value; green dots are normal and red abnormal

**Figure 73 Multiples of Upper Limit of Normal for Lactate Dehydrogenase Range by Subject and Treatment over Time (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT

▪ **Urine Myoglobin**

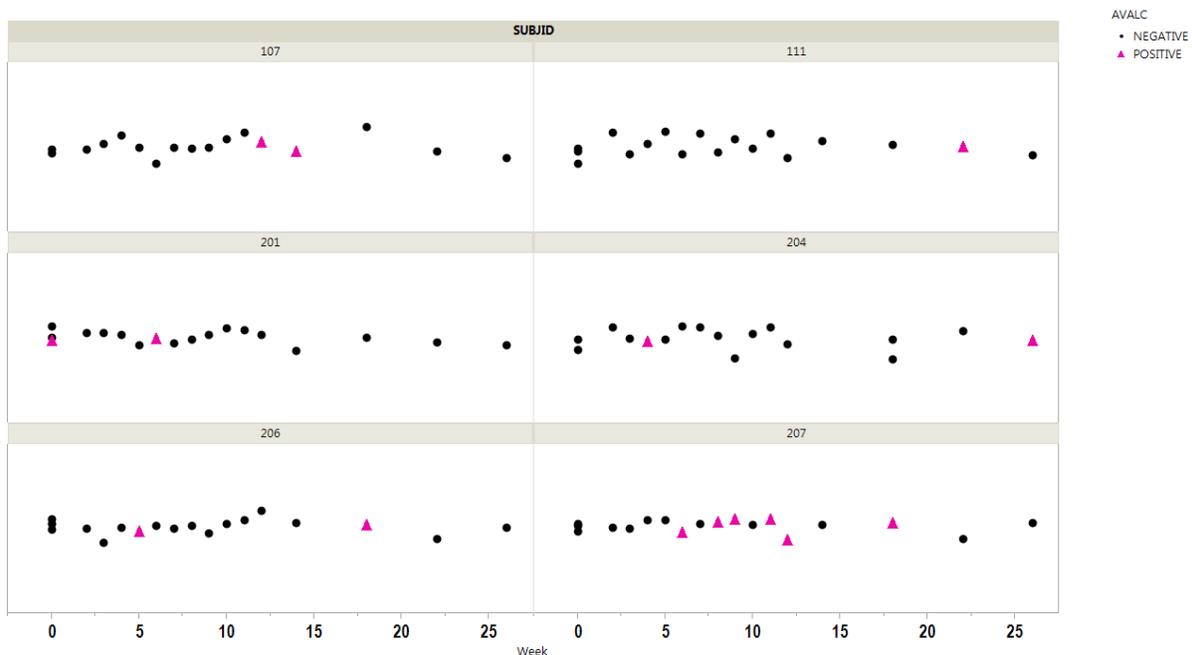
The event of being “positive” for urine myoglobin occurred in 6 subjects in Study 28 who were negative at baseline (Table 44 and Figure 74). Notably, this lab was not performed in other studies.

**Table 44 Subjects with Positive Myoglobin in the Urinalysis from Study 28 (ISS safety Population)**

Subject	Age	Treatment
107	9	Eteplirsen 2 mg/kg
111	9	Eteplirsen 20 mg/kg
201	13	Eteplirsen 2 mg/kg
204	10	Eteplirsen 10 mg/kg
206	9	Eteplirsen 4 mg/kg
207	9	Eteplirsen 20 mg/kg

Source: Medical reviewer analysis of ADLB.XPT from the 120 Day Safety Update

**Figure 74 Events of Myoglobiuria by Visit (ISS Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT from the 120 Day Safety Update

- Urine pH** – The reference range for the first 28 weeks was 8 / 4.5 and then it shifted to 7.5 / 5 after Week 28. There seemed to be an increase in urinary pH and an increase in abnormally values above the ULN in subjects on active treatment. Graphical inspection of the data suggests that there may have been technique differences between the National Children’s and (b) (4) sites that resulted in higher values in the latter site.

**Figure 75 Multiples of the high reference range for Urinary pH by Treatment and Visit (201/202 Safety Population)**



Source: Medical reviewer analysis of ADLB.XPT  
 Each dot represents a different lab value; green dots are normal and red abnormal

### 8.1.5. Vital Signs

Vital signs were from the 201 / 202 study were assessed since there was a placebo control for at least part of the study. Other studies were evaluated for results that seemed clinically significant.

**Diastolic Blood Pressure** – I used the diastolic blood pressure limits listed by the Applicant of 90 (ULN) and 40 (LLN). The ULN is higher than several authoritative sources [NHLBI 2004; American Heart Association 2012] to demonstrate some of the most abnormal values. Several subjects in all of the treatment groups had diastolic blood pressures that were slightly lower than normal [range 37-39].

**Table 45 Abnormally High Diastolic Pressure Readings from Study 201/202 (Safety Population)**

Treatment	Subject	Diastolic BP mM/Hg	Baseline BP mM/Hg	Study Day	VISIT
Eteplirsen 30 mg/kg	006	98	74	167	Week 24.5
		95		225	Week 5
	010	94	54	533	Week 49
		93		666	Week 68

Treatment	Subject	Diastolic BP mM/Hg	Baseline BP mM/Hg	Study Day	VISIT
		92		750	Week 80
Eteplirsen 50 mg/kg	003	92	64	284	Week 13
		93		289	Week 14
		94		843	Week 93
		91		148	Visit 23
	012	93	42	610	Week 60
		013	92	53	1082

Source: Medical Reviewer analysis of ISS ADVS dataset

**Systolic Blood Pressure** – Several Subjects in both active and placebo controlled groups had elevations in their systolic blood pressure. The distribution and magnitude seemed balanced considering the cumulative time of exposure of the different groups.

**Heart rate** – There did not seem to be a difference in the number of abnormal heart rates when the ULN was 110 as suggested by the Applicant as the ULN; almost every subject on all treatments had values above this limit. However those subjects with the highest heart rates in active treatment groups

**Table 46 Subjects with Heart Rates Greater than 130 from Study 201/202 ( Safety Population)**

Treatment	Subject	Study Day	Baseline Heart Rate (Beats / Minute)	Heart Rate (Beats / Minute)
Eteplirsen 30 mg/kg	006	43	111	137
		204		134
		232		131
	007	450	98	131
		793		131
		1129		132
	008	517	114	152
		566		132
		615		134
		846		139
009	666	88	133	
Eteplirsen 50 mg/kg	003	120	94	135
	004	50	80	131
		1285		131
	013	1009	102	137
		1082		132
		1092		132
		1225		132
	015	598	93	142
		1071		131
Placebo	008	120	114	131

Source: Medical Reviewer analysis of ISS ADVS dataset

**Respiratory rate** – There were a few minor reductions in respiratory rate in the active treatment groups but these did not appear clinically significant.

**Weight** – When matched by age, the weights of all treatment sequences progressed at the same rate.

### 8.1.1. Echocardiograms and Electrocardiograms

**Echocardiograms** – There were no discernable changes in the % Ejection Fraction and of the % fractional shortening by treatment or sequence.

**Electrocardiograms** – Electrocardiograms (ECGs) in this population were evaluated by this reviewer using age appropriate limits as suggested by [Rijnbeek *et al.* 2001]♦.

**Heart Rate** – Several subjects on active treatment had increased heart rates as was noted in the previous section on Vital Signs.

**PR interval** – No subjects had PR interval measurements outside of the 98% CI of 105 -174 msec♦.

**QRS Interval** – Subjects 003 and 012 had values above the 98% interval (103, 67) but had baseline values in the high normal range as well.

**Figure 76 QRS Interval by Visit Week (Study 201 / 202 Safety Population)**



Source: Medical Reviewer analysis of Study 201 / 202 ISS ADEG dataset

**QT interval** – I analyzed the data for measurements that went outside of the 98% CI of 373 – 440 for boys of 8-12 years of age♦. Almost every boy (except Subject 15 on Eteplirsen 50 mg/kg) had at least one measurement above 440 msec but none remained elevated.

**ECG Interpretation** – **Table 47** demonstrates the principal EKG interpretation changes observed in Study 201/202.

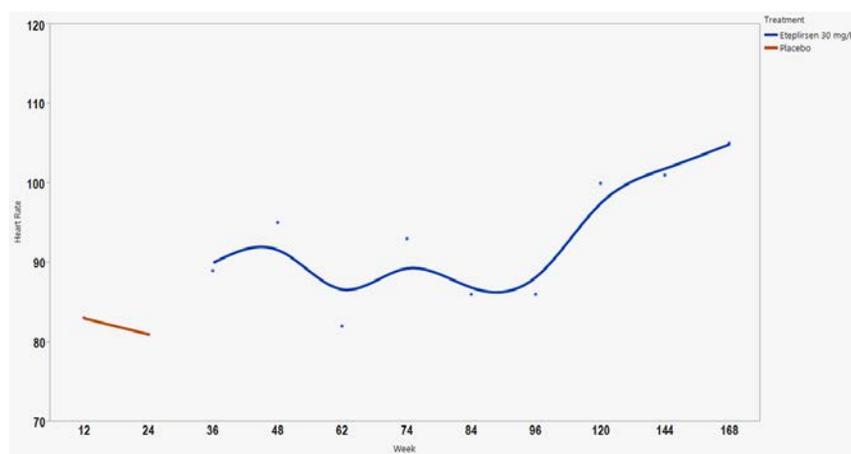
**Table 47 Changes from Normal EKG Interpretation to Ventricular Hypertrophy in Study 201 / 202**

Interpretation		# ECG Interpretations by Dose				Subjects (Treatment(s) during ECG)
Baseline	on Treatment	Total	30 mg/kg	50 mg/kg	Placebo	
(N)	(BVH)	2	2	0	0	<b>007</b> (30 mg/kg)
(N)	(LVH)	6	2	2	2	<b>005</b> (Placebo + 50 mg/kg) <b>007</b> (30 mg/kg)
(N)	(RVH)	8	5	2	1	<b>006</b> (30 mg/kg); <b>007</b> PBO+30 mg/kg; <b>012</b> (50 mg/kg)
(N)	SHORT PR QINF/LAT	1	0	0	1	<b>005</b> (Placebo)
(RVH)	(BVH)	9	0	9	0	<b>003</b> (50 mg/kg)

Source: Medical Reviewer analysis of Study 201 / 202 ISS ADEG dataset

When Subject 007's ECG findings were inspected in isolation, only the heart rate stood out beyond the *Interpretation* findings.

**Figure 77 Subject 007 Heart Rate by Visit and Treatment (Week 0 – 168 of Study 201 / 202)**



Source: Medical Reviewer analysis of Study 201 / 202 ISS ADEG dataset

### 8.1.2. Immunogenicity

Anti-dystrophin labs were tested in Study 33. Of 7 subjects, all were negative in this single dose study. Anti-dystrophin labs were not found for other studies.

## 8.2. Safety in the Postmarket Setting

### 8.2.1. Integrated Assessment of Safety

The principal finding of the safety review is that there were insufficient subjects exposed to adequately characterize the safety profile of eteplirsen. Another concern is the quality of the labs considering the frequent shift in normal ranges even with the same Subject for a given lab.

There are safety signals for the following issues:

- **Potential for bleeding-related events**
  - In the *Haemorrhages (SMQ)*, there was a dose response for most identified events
  - Adverse events for eteplirsen treated subjects for *Infusion site haematoma, epistaxis, and ecchymosis* were elevated above 5% and greater than placebo
  - Several subjects on active treatment had abnormally elevated PT, INR and/or aPTT
- **Accident and injury-related events**
  - There seemed to be a dose response for events of Fracture, Contusion, Excoriation, and Injury<sup>16</sup>
  - Events of *Contusion* and *Excoriation* were 15% greater than placebo
- **Infections**
  - Events of Upper respiratory infection, rhinorrhea and Nasopharyngitis substantially elevated
  - - *Several subjects in the active treatment groups had values below the LLN (~ 0.5 – 0.6x LLN).*
- **Renal disorders**
  - The BUN was elevated particularly in the 50 mg/kg treatment group
- **Cardiovascular signals**
  - Increased diastolic pressure,
  - Increased heart rate
  - Increased proportions of subjects who transitioned from a normal EKG to having some form of ventricular hypertrophy as an abnormal finding.

Urine myoglobin and Anti-Dystrophin antibodies were not routinely collected.

Several of these events are also consistent with disease progression in DMD. The possibility that these signals appear disproportionately higher in the actively treated subjects may be related to the small sample size of actively treated subjects and the inadequate size and exposure duration of the comparator database.

Duchene Muscular Dystrophy is serious and fatal disease and that, in this context, these issues would be concerning but that labeling and routine monitoring could be a sufficient method for postmarketing safety surveillance.

## 9 Advisory Committee Meeting and Other External Consultations

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A meeting of the Peripheral and Central Nervous System Drug Products Advisory Committee was held on April 25, 2016. The principle questions dealt with whether sufficient evidence had been presented for accelerated approval or a full approval.

The vote on the Accelerated Approval question was 7-6, not in favor. Concerns by those who voted

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<sup>16</sup> Preferred terms containing *fracture* and preferred terms containing *injury* were combined

No included that

- It is was not clear what threshold was necessary for a clinical benefit
- Whether the biopsies themselves yielded generalizable data because of the patchiness of tissue types in the extremities, especially with advanced disease.

The vote on the Full Approval question was 7 (no) – 3 (yes) – 3 (abstain). Concerns from those who voted No included the trial design and conduct. There was a concern that the subjects may have showed effects in domains that were not measured.

## **10 Labeling Recommendations**

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### **10.1. Prescribing Information**

Labeling recommendations are not given at this time since the regulatory recommendation is for a Complete Response.

## **11 Risk Evaluation and Mitigation Strategies (REMS)**

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### **11.1. Recommendations on REMS**

In light of the paucity of safety information submitted in this application, it is not possible to know if a REMS would be necessary and exactly what should be monitored. At this point in time, if eteplirsen were to be approved, the following risk management approaches are recommended:

- A patient registry as a post-marketing requirement will help to evaluate the main safety risks (as noted above) of eteplirsen in the postmarketing setting. An issue is that the premarket safety database was not adequate to ensure the type and magnitude of these risks is well defined.
- Future clinical trials should be adequately designed to include necessary assessments and to provide controls to allow for interpretation of long-term safety data.
- Labeling should be clear about uncertainties and deficiencies of the eteplirsen clinical program.

## **12 Postmarketing Requirements and Commitments**

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Postmarketing requirements and commitments are not given at this time since the regulatory recommendation is for a Complete Response. If there is a decision for Approval, I would recommend a Post-Marketing Commitment to first do a dose ranging study to determine the Maximal Tolerated Dose.

## 13 Appendices

### Appendix 1. Submissions from the Applicant

**Table 48 Applicant Submissions to the NDA following the Original NDA**

Date of Submission	Sequence Number	Content per the Applicant
20160108	0029	<ul style="list-style-type: none"> <li>• An updated dataset for the 10 external control patients amenable to exon 51 skipping from the Italian DMD Telethon database which contains patient-level data (baseline through Year 4) on the 6MWT.</li> <li>• An updated dataset for the 3 external control patients amenable to exon 51 skipping from the Leuven Neuromuscular Reference Center database which contains patient-level data (baseline through Year 4) on the 6MWT.</li> </ul>
20151217	0028	<ul style="list-style-type: none"> <li>• An updated dataset for the 3 external control patients amenable to exon 51 skipping from the Leuven Neuromuscular Reference Center database which contains patient-level data (baseline through Year 3) on: <ul style="list-style-type: none"> <li>○ - Height and weight</li> <li>○ - Rise time (labeled as Gowers in xlsx)</li> </ul> </li> </ul>
20151214	0025	<ul style="list-style-type: none"> <li>• Results for select Week 216 (4.5 year) functional assessments in study 201/202 are presented in tabular format</li> </ul>
20151210	0024	<ul style="list-style-type: none"> <li>• Responses to information requests <ul style="list-style-type: none"> <li>○ Study 201/202 Week 180 western blot study and validation reports: Controls, RSD acceptance criteria, Gel images Quantitation</li> <li>○ Start and resolution dates of 9 severe adverse events in Summary of Clinical Safety</li> <li>○ Analysis of DMD control sample BLOQ values in Study 201/202 Week 180 western blot report</li> <li>○ Study 28 western blot pre-treatment values</li> <li>○ Prior dose dates for ankle fracture and femur fracture SAEs; Study 33 myoglobinuria criteria</li> </ul> </li> <li>• Anti-dystrophin antibody and urine myoglobin assessments</li> <li>• Study 28 annotated western blot images; PCR product sequencing</li> <li>• Study 33 myoglobinuria criteria; Study 28 anti-dystrophin antibody dataset</li> <li>• Analysis of treated sample BLOQ values in Study 201/202 Week 180 western blot report</li> </ul>

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Date of Submission	Sequence Number	Content per the Applicant
20151207	0023	<ul style="list-style-type: none"> <li>• A recently obtained dataset containing patient-level pulmonary function test data (FVC and FVC percent predicted) from DMD patients reported in the published literature (Mayer 2015) and used for comparison to eteplirsen-treated patients in Sarepta report SR-CR-010</li> <li>• Sarepta report SR-CR-010, entitled Pulmonary Function Measurements in Eteplirsen-Treated Patients Over 168 Weeks: Comparison to External Control Data and Scientific Literature</li> <li>• An updated dataset for the 10 external control patients amenable to exon 51 skipping participating in the Italian DMD Telethon registry which contains patient-level data (baseline through Year 3) on: <ul style="list-style-type: none"> <li>○ height and weight</li> <li>○ - supportive care (physical therapy, orthoses, corrective surgery)</li> <li>○ - Rise Time (labeled as Gowers maneuver in xlsx)</li> <li>○ - 10-meter run/walk</li> </ul> </li> <li>• A newly obtained dataset which contains patient-level supportive care data for the 12 patients participating in Study 4658-us-202</li> </ul>
20151102	0021	<ul style="list-style-type: none"> <li>• newly obtained datasets for subjects in the Italian DMD Registry (i.e. Professor Eugenio Mercuri's registry): <ul style="list-style-type: none"> <li>○ Baseline rise time for all subjects</li> <li>○ Additional steroid treatment information for 6 subjects with genotypes amenable to exon skipping therapy</li> <li>○ Prior steroid treatment duration and baseline height for 10 subjects with genotypes amenable to exon 51 skipping</li> </ul> </li> </ul>
20151026	0019	<ul style="list-style-type: none"> <li>• Response to clinical Information Requests <ul style="list-style-type: none"> <li>○ upper and lower limits of quantitation</li> <li>○ orthoses or other devices</li> </ul> </li> </ul>
20151023	0018	<ul style="list-style-type: none"> <li>• 120 Day Module 2.7.4 —Summary of Clinical Safety</li> <li>• Datasets, Data Documentation, Programs, and Tables Figures Listings for: <ul style="list-style-type: none"> <li>○ Study 4658-us-202</li> <li>○ Study 4658-203</li> <li>○ Study 4658-204</li> <li>○ Study 4658-us-301</li> <li>○ Integrated Summary of Safety</li> </ul> </li> </ul>
20151013	0016	<ul style="list-style-type: none"> <li>• Response to an information request sent by the Division on 08 October 2015, regarding the Western blot methodology used in study 4658-us-201/202 at Weeks 12, 24, and 48.</li> </ul>
20151001	0013	<ul style="list-style-type: none"> <li>• Marked images from immunohistochemistry</li> </ul>

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Date of Submission	Sequence Number	Content per the Applicant
20150916	0011	<ul style="list-style-type: none"> <li>• Response to Clinical Information request containing:               <ul style="list-style-type: none"> <li>○ Week 180 4<sup>th</sup> Biopsy datasets</li> </ul> </li> </ul>
20150828	0009	<ul style="list-style-type: none"> <li>• Datasets for the fourth biopsy (Tabulation and Analysis) are provided in this amendment.</li> <li>• SAS codes and programs</li> <li>• Clarification in labset variables</li> <li>• Biomarker information from requested by Dr. Rao</li> </ul>
20150820	0008	<ul style="list-style-type: none"> <li>• Response to 20150806 Information Request               <ul style="list-style-type: none"> <li>○ Revised define files</li> <li>○ Initial clarification of ‘supportive care’ in the 201/202 study</li> <li>○ Promise for marked images</li> <li>○ Multiple revised clinical data tables</li> <li>○ PK datasets</li> <li>○ Information of images used in publications</li> </ul> </li> </ul>
20150730	0004	<ul style="list-style-type: none"> <li>• Reports on the 4<sup>th</sup> biopsy assays               <ul style="list-style-type: none"> <li>○ Western blot assessment of dystrophin protein levels</li> <li>○ RT-PCR assessment of DMD patient mRNA</li> <li>○ BIOQUANT® assessment of dystrophin signal intensity</li> <li>○ Scoring of immunofluorescence images for the presence of dystrophin positive muscle fibers</li> </ul> </li> </ul>
20150724	0003	<ul style="list-style-type: none"> <li>• Week 192 (4 year) functional assessments in study 201 /202</li> </ul>
20150626	0001	<ul style="list-style-type: none"> <li>• Final submission of Rolling NDA               <ul style="list-style-type: none"> <li>○ the complete clinical content contained in Modules 2 and 5</li> </ul> </li> </ul>
20150520	0000	<ul style="list-style-type: none"> <li>• Nonclinical and chemistry, manufacturing and controls content submission for original NDA</li> </ul>

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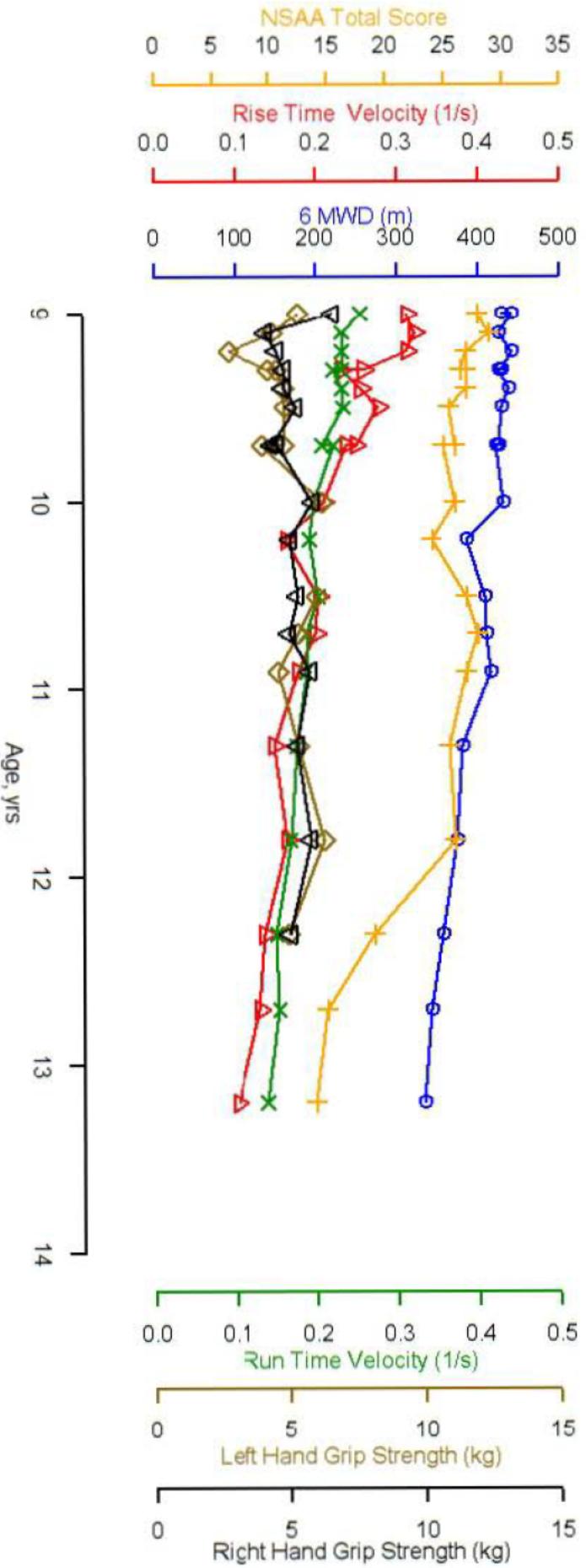
NDA 206488 (Eteplirsen)

13.1. **Appendix 2. Patient Profiles**

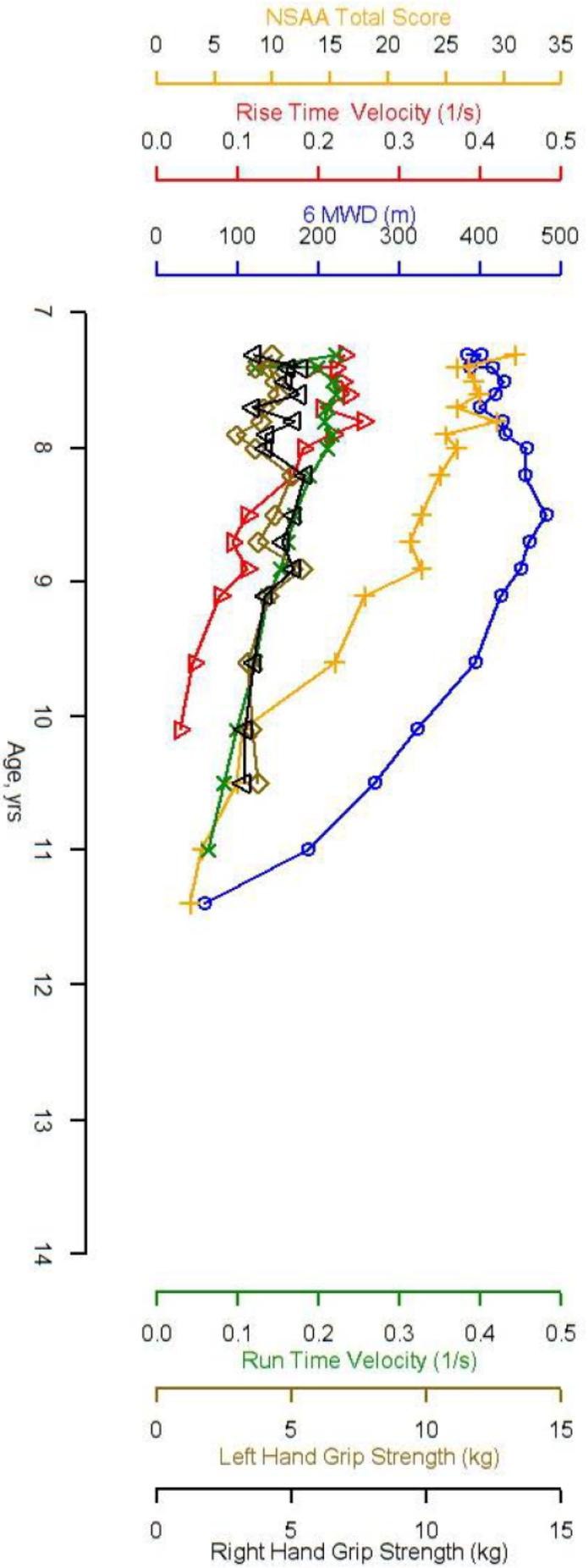
Patient profiles containing results from each of the Eteplirsen subjects in studies 201 / 202 are included in this section.

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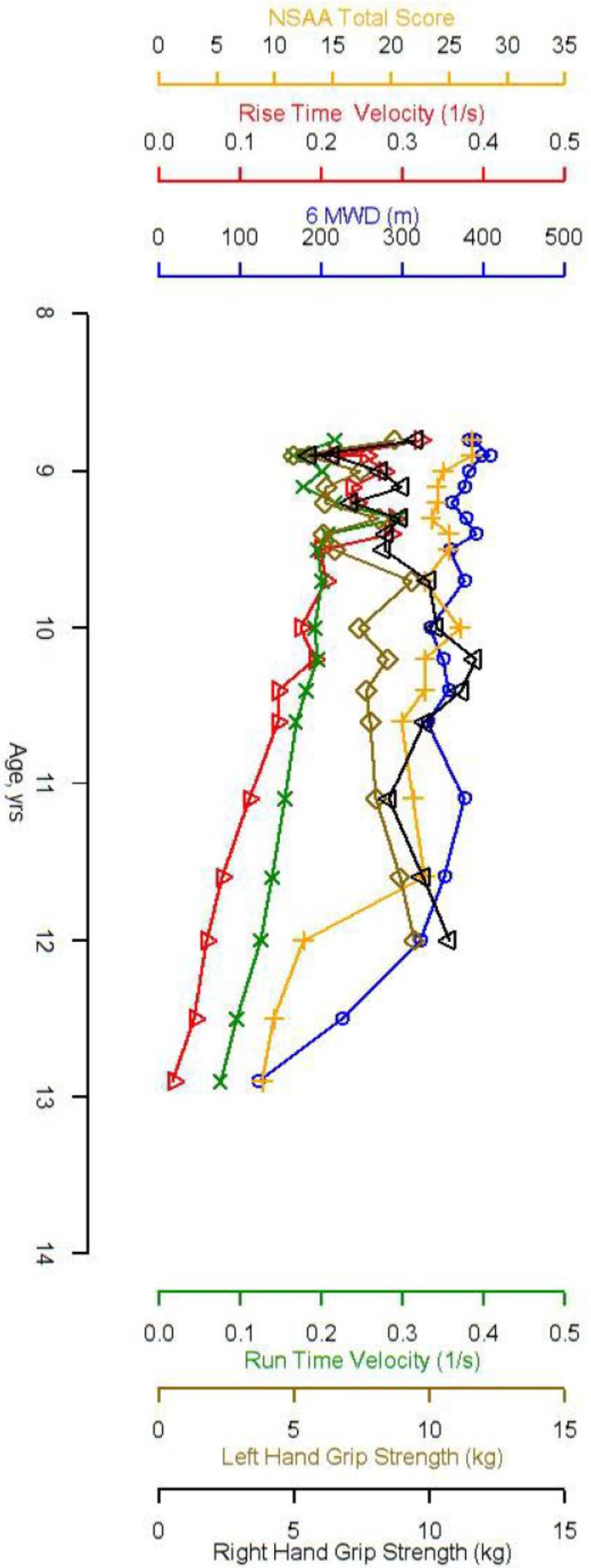


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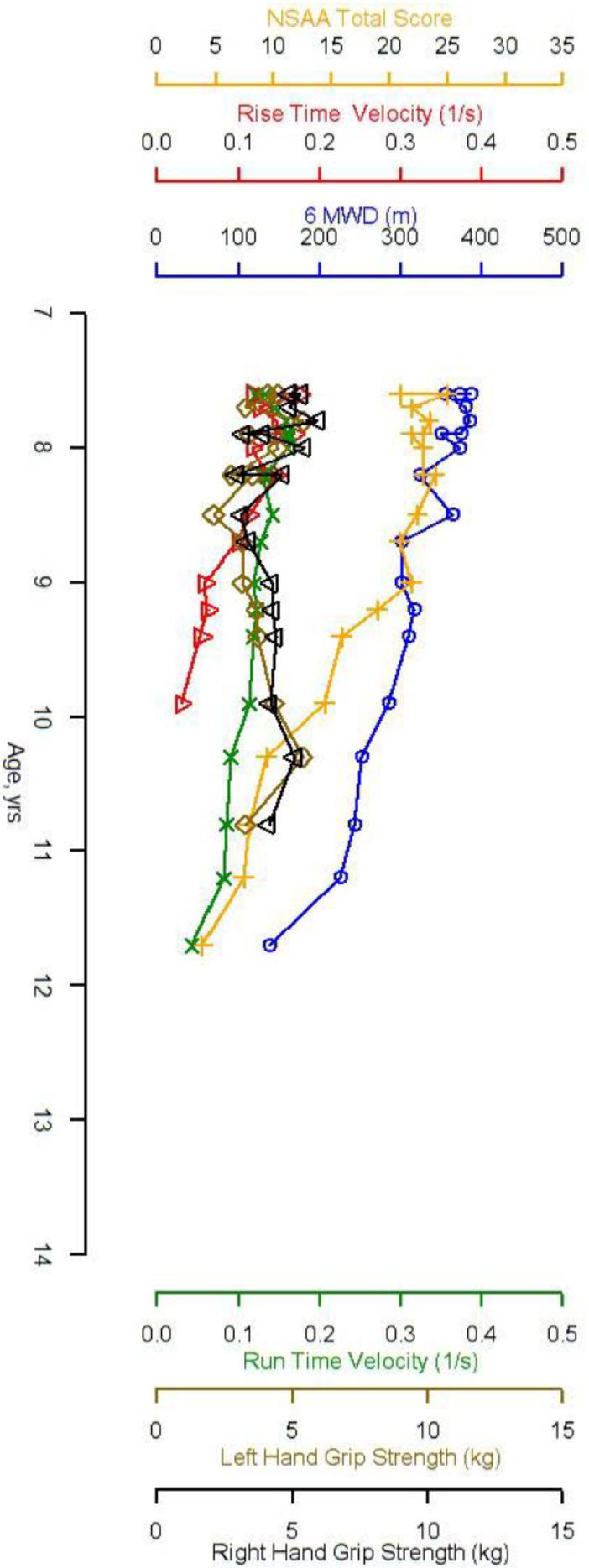


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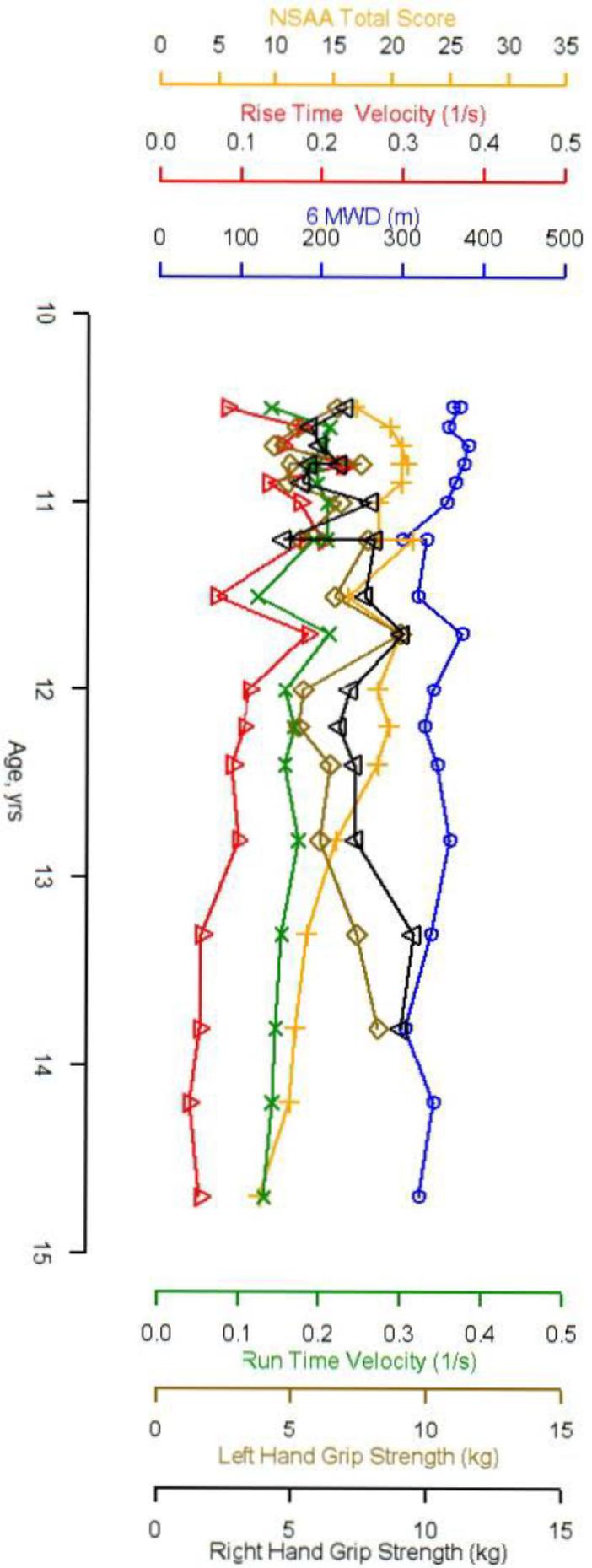


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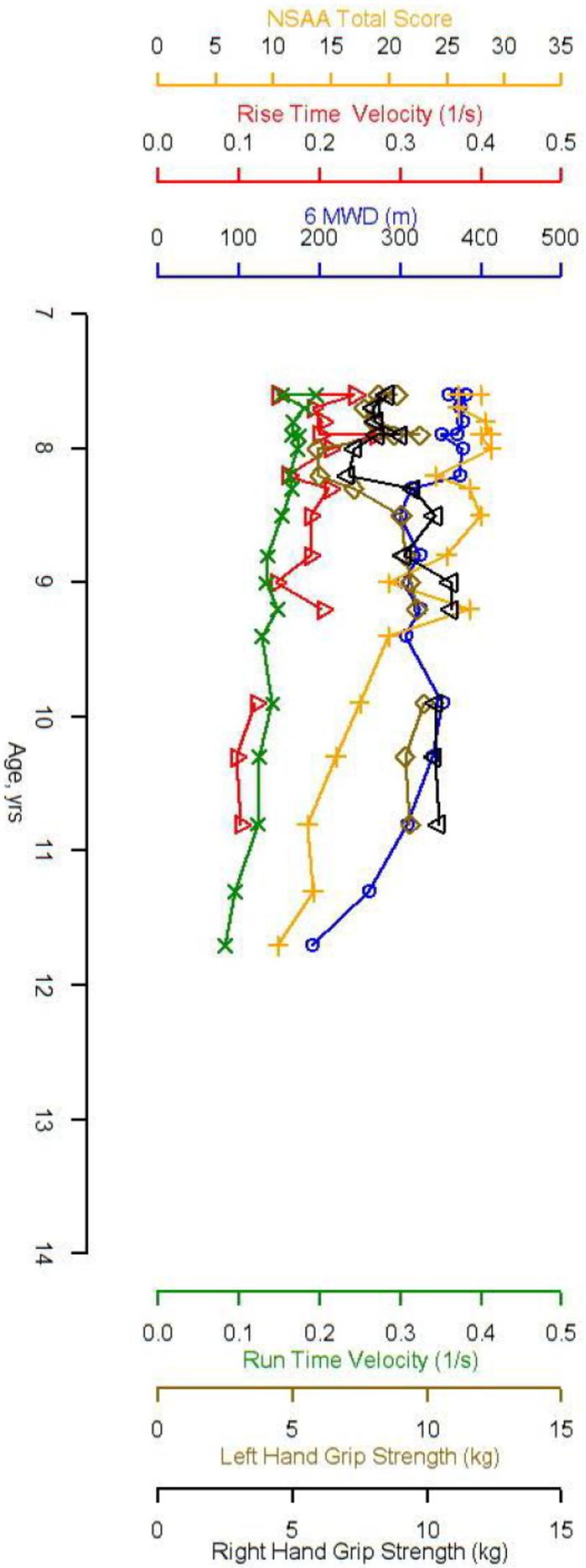
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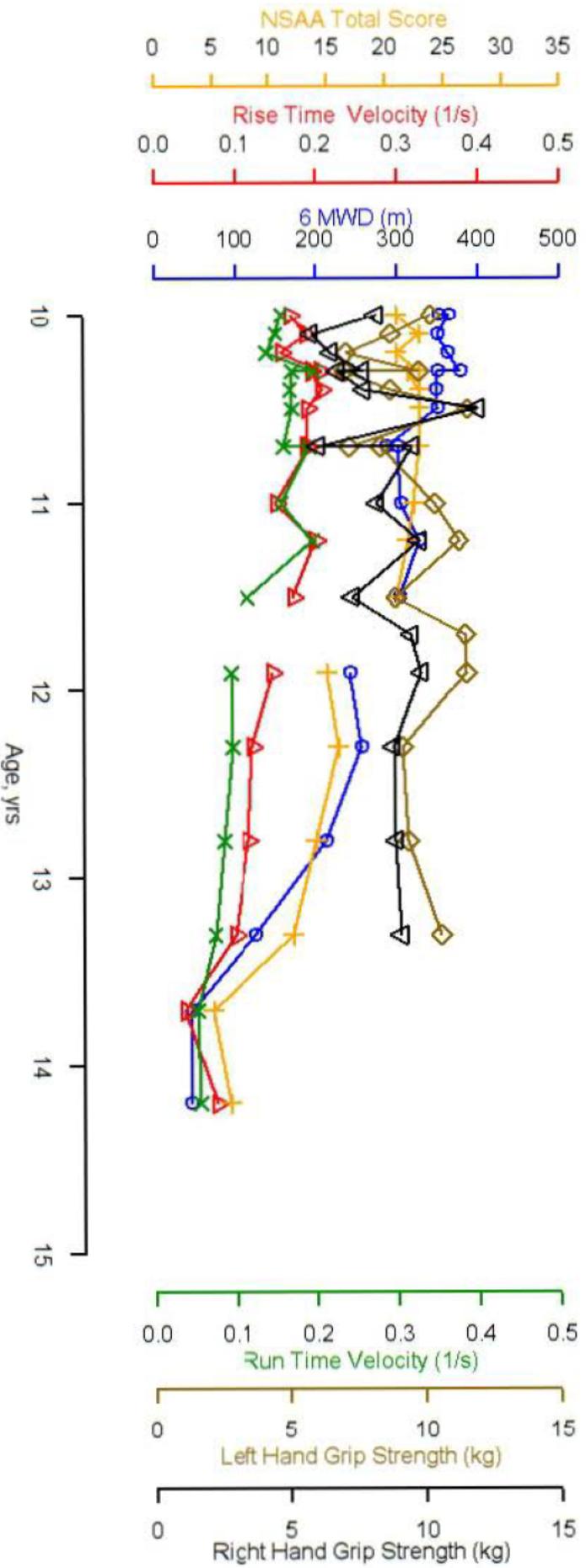
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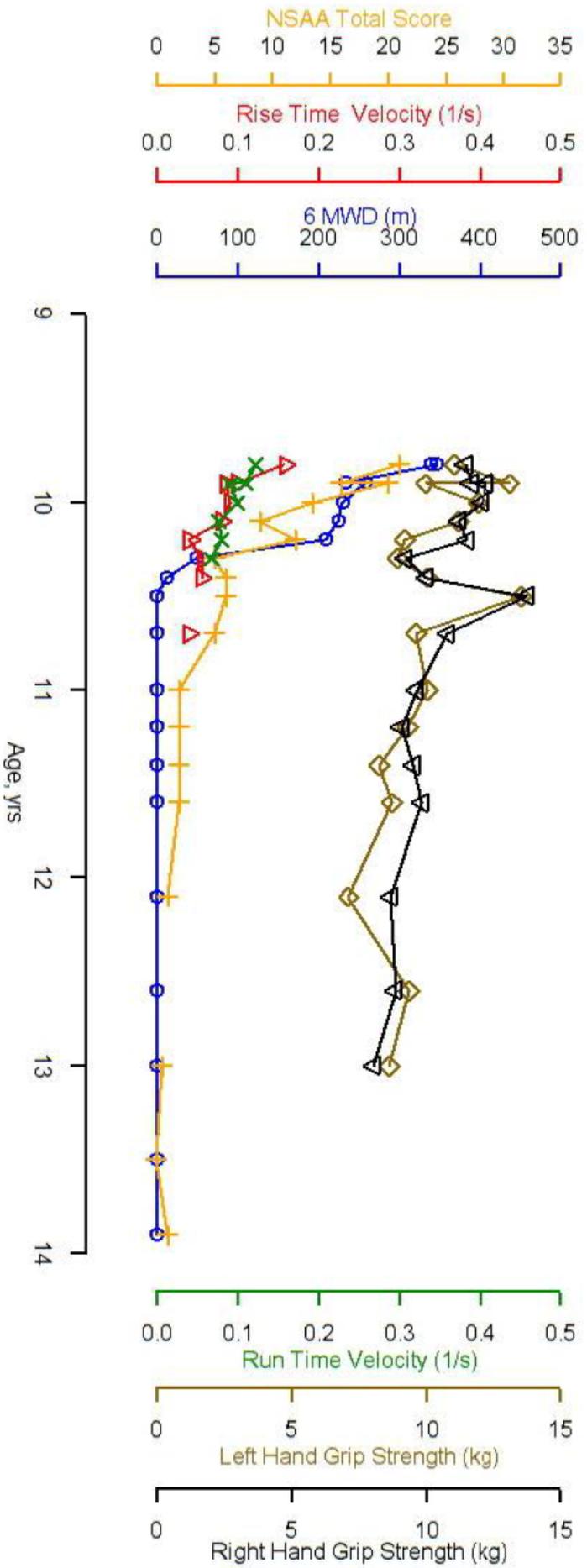
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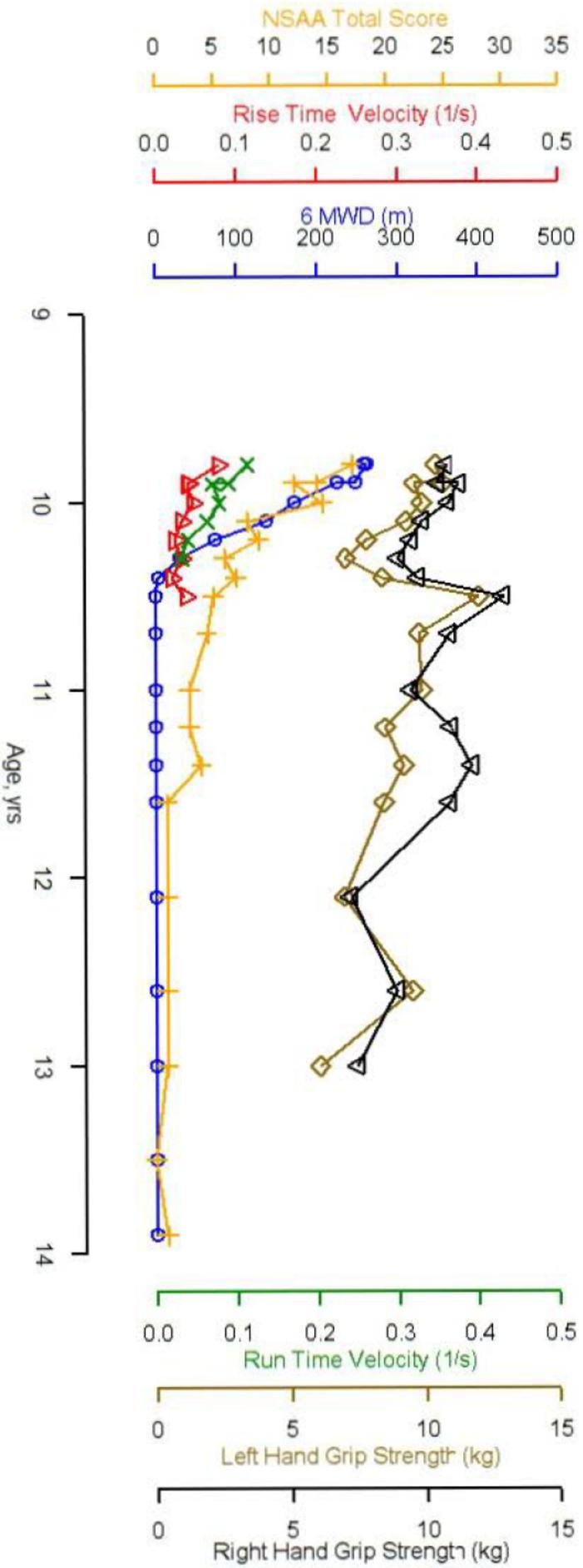
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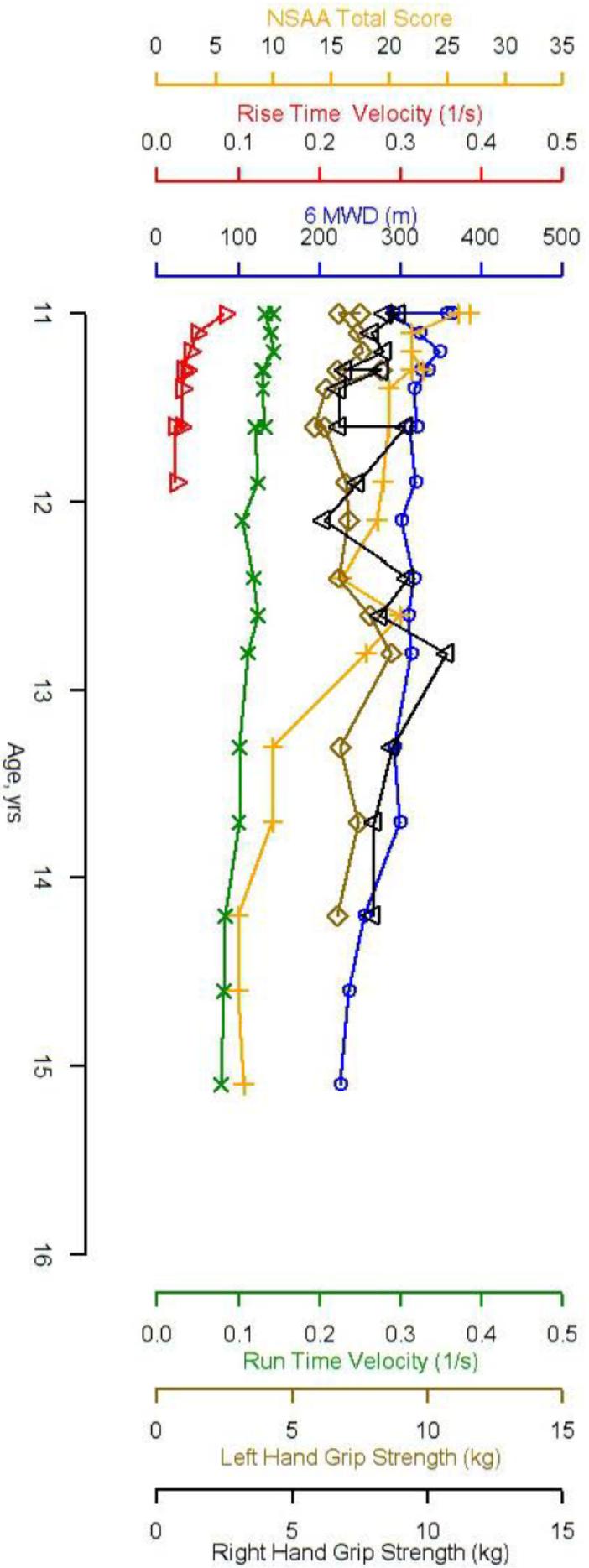
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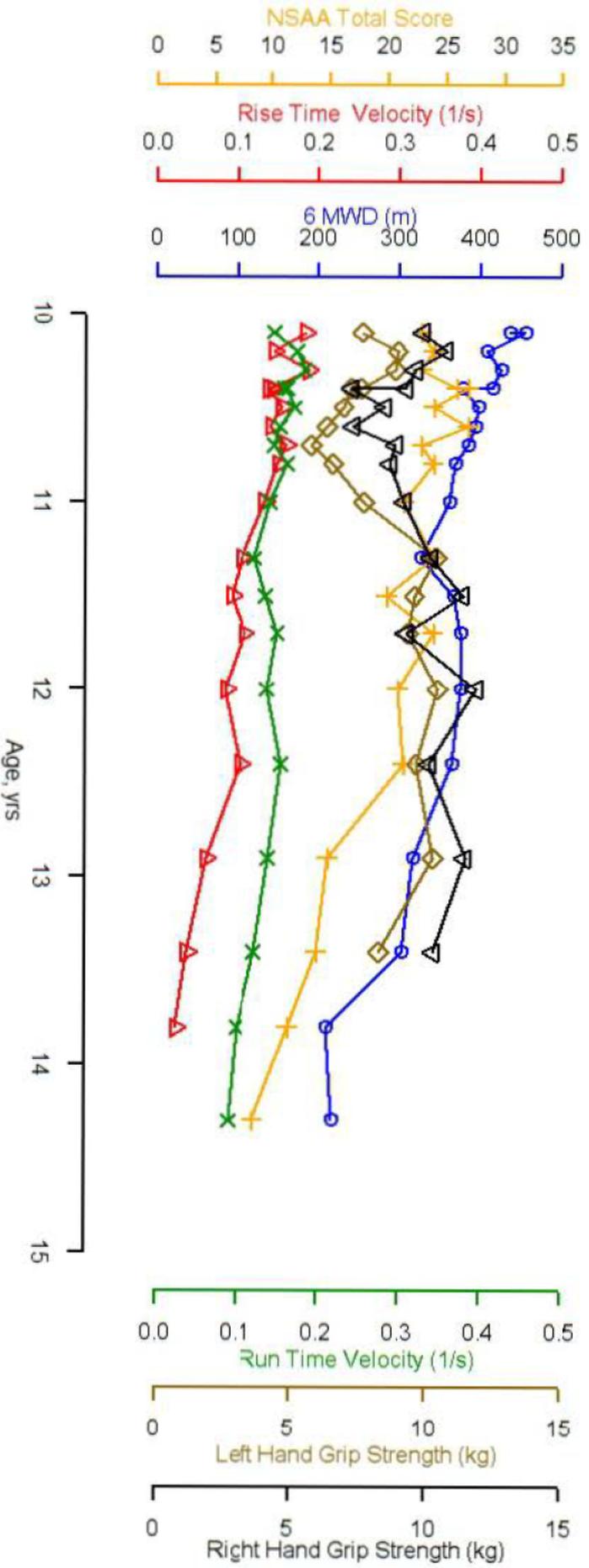
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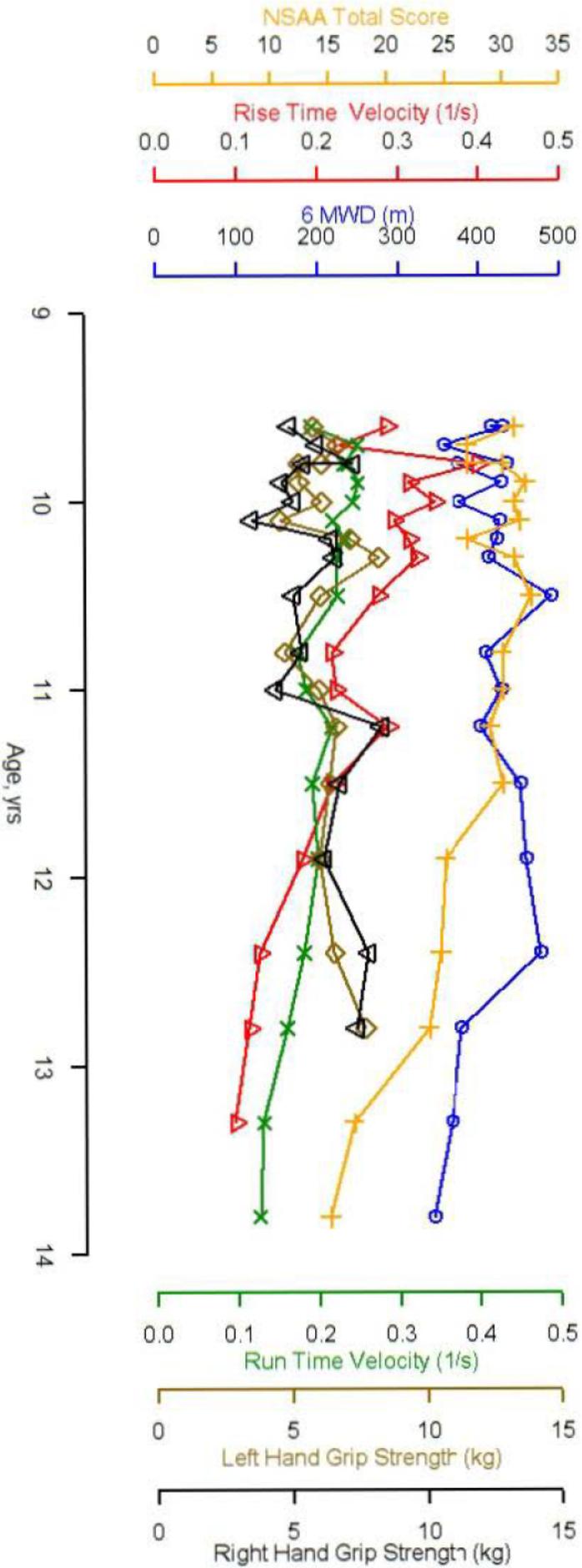


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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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CHRISTOPHER D BREDER  
05/09/2016

V ASHUTOSH RAO  
05/09/2016

RONALD H FARKAS  
05/09/2016

NDA/BLA Number: 206488

Applicant: Sarepta

Stamp Date: 6/26/15

Drug Name: Eteplirsen

NDA/BLA Type: 505(b)(1)

On initial overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	NA	Comment
<b>FORMAT/ORGANIZATION/LEGIBILITY</b>					
1.	Identify the general format that has been used for this application, e.g. electronic CTD.	X			
2.	On its face, is the clinical section organized in a manner to allow substantive review to begin?	X			
3.	Is the clinical section indexed (using a table of contents) and paginated in a manner to allow substantive review to begin?	X			
4.	For an electronic submission, is it possible to navigate the application in order to allow a substantive review to begin (e.g., are the bookmarks adequate)?		X		See note that follows table
5.	Are all documents submitted in English or are English translations provided when necessary?	X			
6.	Is the clinical section legible so that substantive review can begin?	X			
<b>LABELING</b>					
7.	Has the applicant submitted the design of the development package and draft labeling in electronic format consistent with current regulation, divisional, and Center policies?	X			
<b>SUMMARIES</b>					
8.	Has the applicant submitted all the required discipline summaries (i.e., Module 2 summaries)?	X			
9.	Has the applicant submitted the integrated summary of safety (ISS)?	X			
10.	Has the applicant submitted the integrated summary of efficacy (ISE)?	X			
11.	Has the applicant submitted a benefit-risk analysis for the product?	X			
12.	Indicate if the Application is a 505(b)(1) or a 505(b)(2). If Application is a 505(b)(2) and if appropriate, what is the reference drug?	X			
<b>DOSE</b>					
13.	If needed, has the applicant made an appropriate attempt to determine the correct dosage and schedule for this product (i.e., appropriately designed dose-ranging studies)? Study Numbers 4658-us-201 and AVI-4658-28:	X			
<b>EFFICACY</b>					
14.	Do there appear to be the requisite number of adequate and well-controlled studies in the application?  Study Numbers 4658-us-201/202 Study Numbers 4658-us-201 and AVI-4658-28, AVI-4658-33	X			

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>NA</b>	<b>Comment</b>
15.	Do all pivotal efficacy studies appear to be adequate and well-controlled within current divisional policies (or to the extent agreed to previously with the applicant by the Division) for approvability of this product based on proposed draft labeling?	X			The Division was aware of the duration and nature of controls in these studies.
16.	Do the endpoints in the pivotal studies conform to previous Agency commitments/agreements? Indicate if there were not previous Agency agreements regarding primary/secondary endpoints.	X			
17.	Has the application submitted a rationale for assuming the applicability of foreign data to U.S. population/practice of medicine in the submission?			X	
<b>SAFETY</b>					
18.	Has the applicant presented the safety data in a manner consistent with Center guidelines and/or in a manner previously requested by the Division?	X			
19.	Has the applicant submitted adequate information to assess the arrhythmogenic potential of the product (e.g., QT interval studies, if needed)?	X			
20.	Has the applicant presented a safety assessment based on all current worldwide knowledge regarding this product?	X			
21.	For chronically administered drugs, have an adequate number of patients (based on ICH guidelines for exposure <sup>1</sup> ) been exposed at the dose (or dose range) believed to be efficacious?	X			
22.	For drugs not chronically administered (intermittent or short course), have the requisite number of patients been exposed as requested by the Division?			X	
23.	Has the applicant submitted the coding dictionary <sup>2</sup> used for mapping investigator verbatim terms to preferred terms?		X		The Clinical Reviewer will derive this from the AE datasets
24.	Has the applicant adequately evaluated the safety issues that are known to occur with the drugs in the class to which the new drug belongs?	X			
25.	Have narrative summaries been submitted for all deaths and adverse dropouts (and serious adverse events if requested by the Division)?	X			
<b>OTHER STUDIES</b>					

<sup>1</sup> For chronically administered drugs, the ICH guidelines recommend 1500 patients overall, 300-600 patients for six months, and 100 patients for one year. These exposures MUST occur at the dose or dose range believed to be efficacious.

<sup>2</sup> The "coding dictionary" consists of a list of all investigator verbatim terms and the preferred terms to which they were mapped. It is most helpful if this comes in as a SAS transport file so that it can be sorted as needed; however, if it is submitted as a PDF document, it should be submitted in both directions (verbatim -> preferred and preferred -> verbatim).

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>NA</b>	<b>Comment</b>
26.	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?		X		The Division has requested certain natural history data (e.g., rise time and standards of care” that the Applicant has stated they could not obtain.  The Applicant has not supplied the overlay images indicating what muscle fibers were considered dystrophin + by the individuals counting these fibers. See note that follows table for discussion of this issue.
27.	For Rx-to-OTC switch and direct-to-OTC applications, are the necessary consumer behavioral studies included (e.g., label comprehension, self selection and/or actual use)?			X	
<b>PEDIATRIC USE</b>					
28.	Has the applicant submitted the pediatric assessment, or provided documentation for a waiver and/or deferral?				
<b>ABUSE LIABILITY</b>					
29.	If relevant, has the applicant submitted information to assess the abuse liability of the product?			X	
<b>FOREIGN STUDIES</b>					
30.	Has the applicant submitted a rationale for assuming the applicability of foreign data in the submission to the U.S. population?			X	
<b>DATASETS</b>					
31.	Has the applicant submitted datasets in a format to allow reasonable review of the patient data?		X		See note that follows table
32.	Has the applicant submitted datasets in the format agreed to previously by the Division?		X		See note that follows table
33.	Are all datasets for pivotal efficacy studies available and complete for all indications requested?		X		See note that follows table
34.	Are all datasets to support the critical safety analyses available and complete?		X		See note that follows table
35.	For the major derived or composite endpoints, are all of the raw data needed to derive these endpoints included?			X	
<b>CASE REPORT FORMS</b>					
36.	Has the applicant submitted all required Case Report Forms in a legible format (deaths, serious adverse events, and adverse dropouts)?	X			
37.	Has the applicant submitted all additional Case Report Forms (beyond deaths, serious adverse events, and adverse drop-outs) as previously requested by the Division?	X			
<b>FINANCIAL DISCLOSURE</b>					

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>NA</b>	<b>Comment</b>
38.	Has the applicant submitted the required Financial Disclosure information?	X			1 investigator in w 300 shares of Applicant stock (>\$12K)
<b>GOOD CLINICAL PRACTICE</b>					
39.	Is there a statement of Good Clinical Practice; that all clinical studies were conducted under the supervision of an IRB and with adequate informed consent procedures?	X			

**IS THE CLINICAL SECTION OF THE APPLICATION FILEABLE? \_\_\_ Yes \_\_\_**

If the Application is not fileable from the clinical perspective, state the reasons and provide comments to be sent to the Applicant.

The Division met with the Applicant and discussed deficient Define files and hyperlinking. The Applicant has promised to replace or repair this material in a timely manner.

The Applicant has not supplied the overlay images indicating what muscle fibers were considered dystrophin + by the individuals counting these fibers. The Applicant has promised to furnish this material in a timely manner.

See items submitted by L Kelley (8/12/15) and Y. Choy (8/6/15) to the NDA for more detail on these matters.

Reviewing Medical Officer

Date

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Clinical Team Leader

Date

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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CHRISTOPHER D BREDER  
08/25/2015

RONALD H FARKAS  
08/25/2015