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			
		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	1.57	1.33	6.54
		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 15.MIN.TIF	20	FORD-29648	FAIL	0.05			
	Week 48	SR-CR-16-003_GEL#19_DYS1_15.MIN.TIF	19	CHEVY-29648	PASS	0.12	0.12	0.01	1.09
		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	0.47	0.43	10.44
		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	0.09	0.07	4.50
		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	0.21	0.03	1.17
		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.
Source: P:\Sarepta\DMD\301\Programs\Analysis\Production\Listings\l_wb.sas, Date/time of run: 24JUN2016:18:59

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 methodoligical 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 estmates 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%¥	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%)

¥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[6] 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 ≥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

[6] End-of-Phase 2 meeting of March 13, 2013.

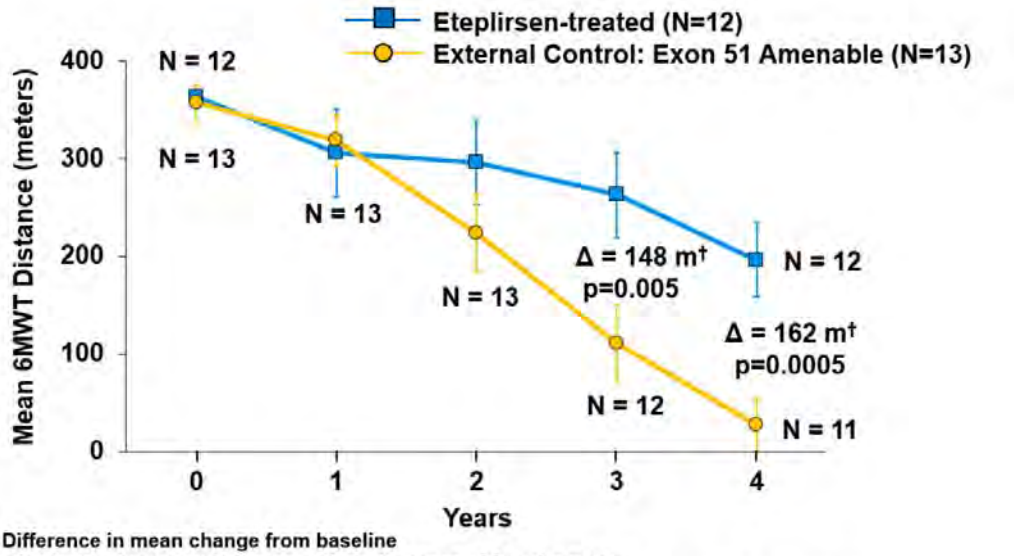
and Related Issues in Clinical Trials" – ICH E10 [2000]). FDA identified several issues related to the use of an external natural history for the applicant to address in the NDA. FDA asked the applicant to establish that treatment modalities, including the physical therapy programs and steroid regimens 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)



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)

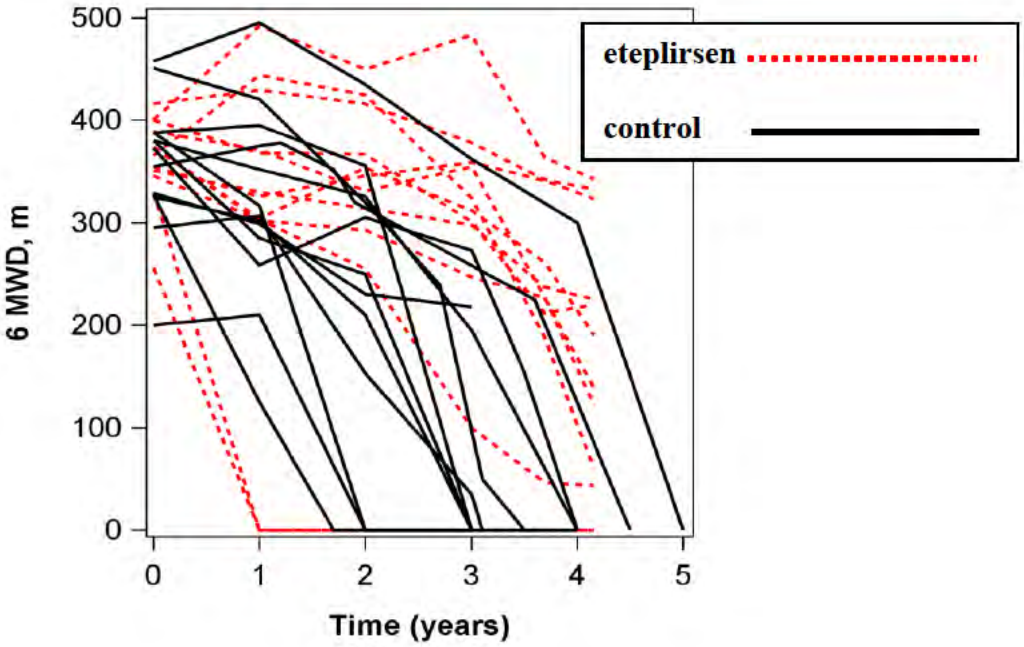
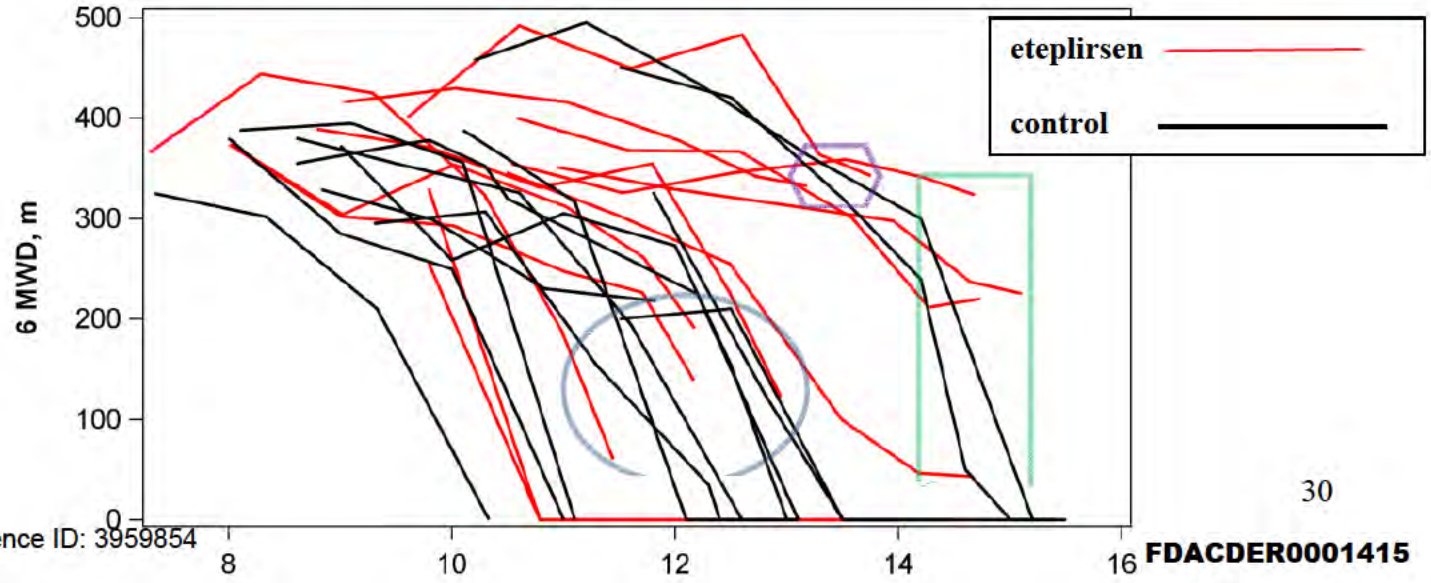


Figure 4: 6MWT distance vs. age in eteplirsen-treated patients in Study 201/202 and external control from the "Italian DMD Registry" and the "Leuven Neuromuscular Reference Center" registry (adapted from Dr. Farkas' memo)



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[7]. 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,

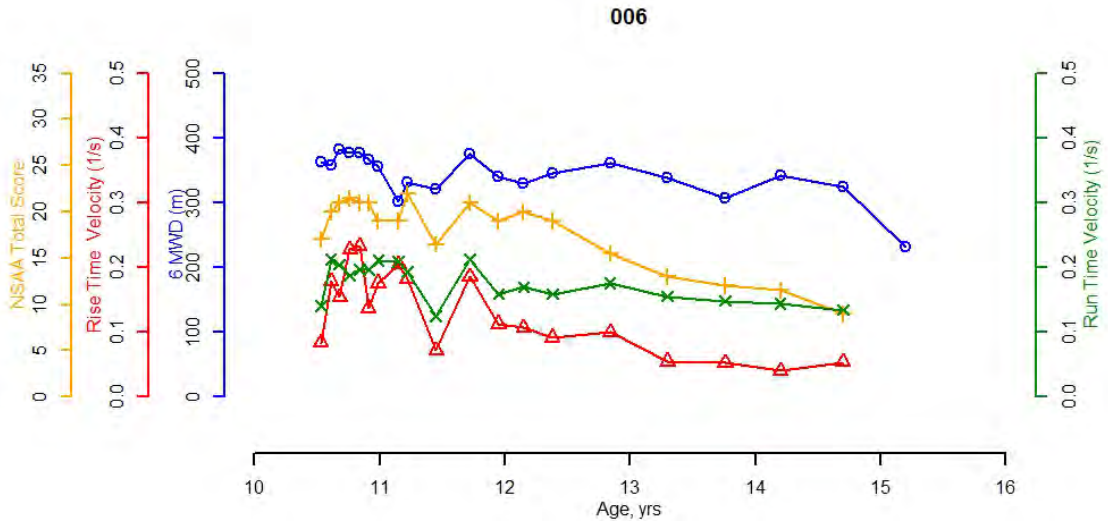
[7] Week 214 assessment, submitted to the NDA in December 2015, is the last one for which we have a complete set of clinical outcome measures. The applicant sent in May 2016 data from the Week 240 assessment limited to the 6MWT. These are presented in individual patient profiles, and have not been incorporated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

natural history data from the CINRG[8] 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[9] (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.

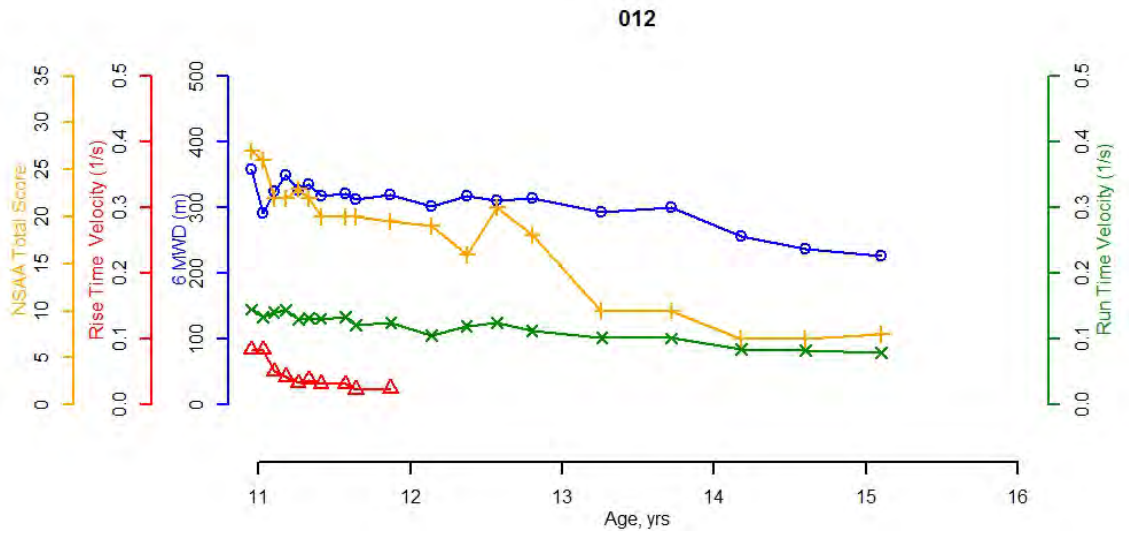
Figure 5: Clinical profile of Patient 006



[8] CINRG: Cooperative International Neuromuscular Research Group http://www.cinrgresearch.org/
[9] 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:

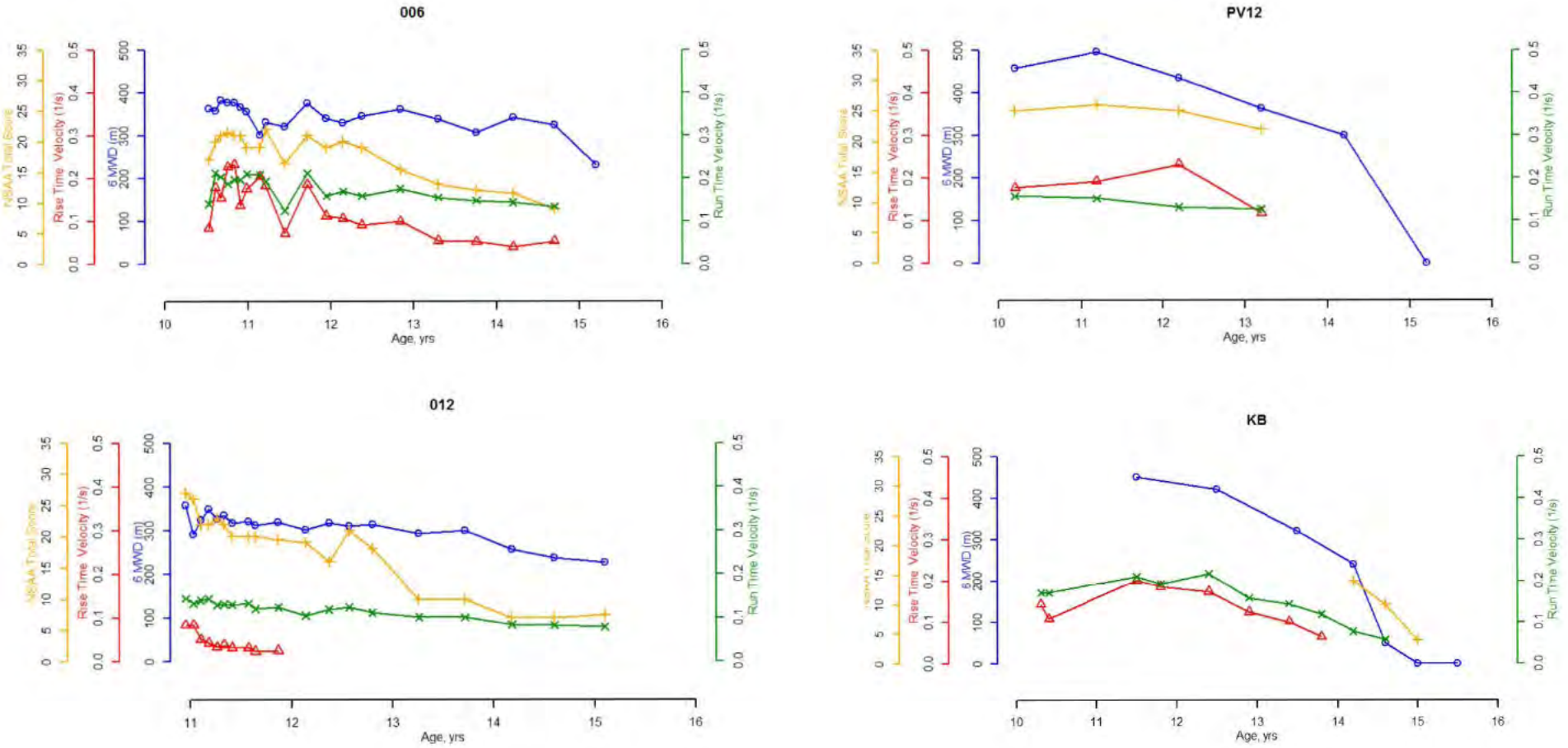
- At age 11 years, both patients had similar 6MWT distance, NSAA, rise time velocity and run time velocity.

- 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.

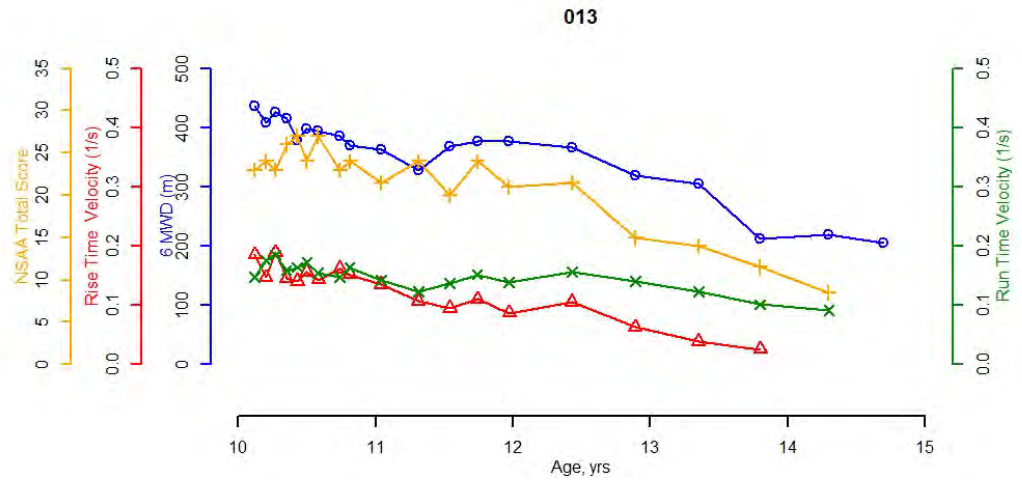
A similar observation can be made in a comparison between Patient 006 and Patient KB (Figure 7). Both patients had similar run time, rise time, and 6MWT around age 11. By age 14, they had similar 6MWT, NSAA, rise time and run time, indicating a similar disease course over a 3-year period of time. At 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



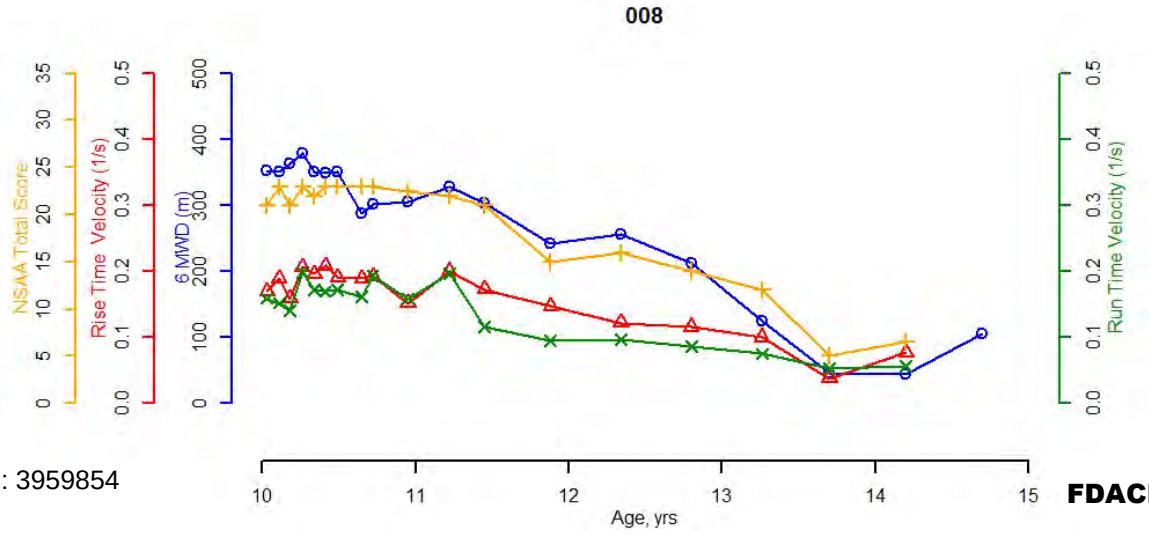
Patient 013 (Figure 8), who received placebo during Study 201, and was later switched to eteplirsen 50mg/kg, had the third highest 6MWT distance after age 14 years. Patient 013 is also showing a marked decline on most outcome measures, including rise time velocity from age 10.5, and a decline in NSAA 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.

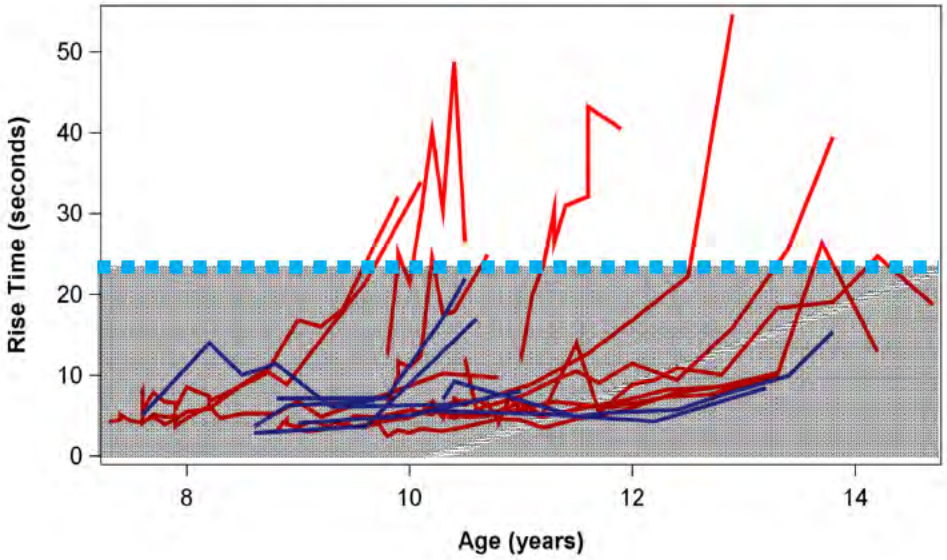
Figure 9: Clinical profile of Patient 008



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

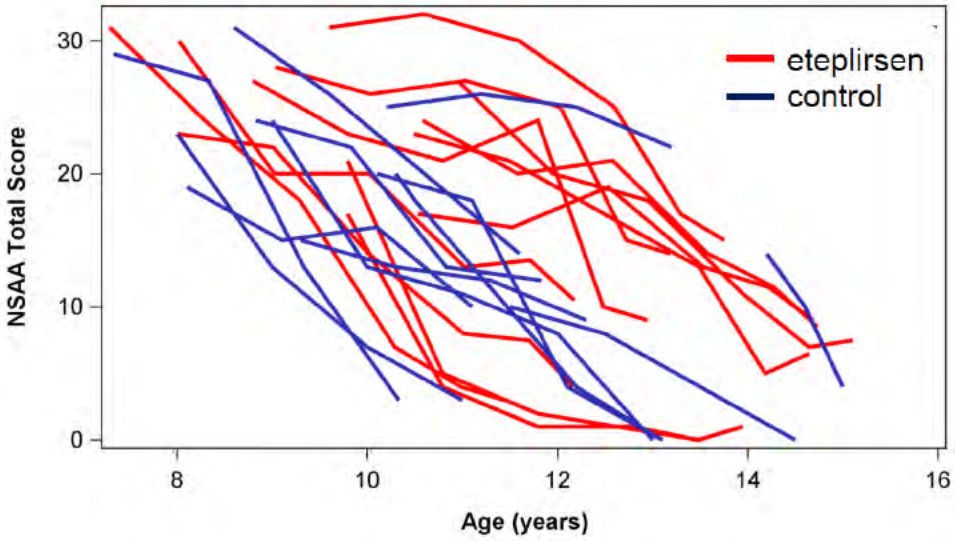
good work!," The Manual for Study 202 also stated that if the patient fell or could not rise from the floor, the test was over and time and distance should be recorded. On the other hand, the protocols for the historical control studies were very scant (see Appendix 2: Protocol of the Leuven Neuromuscular 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

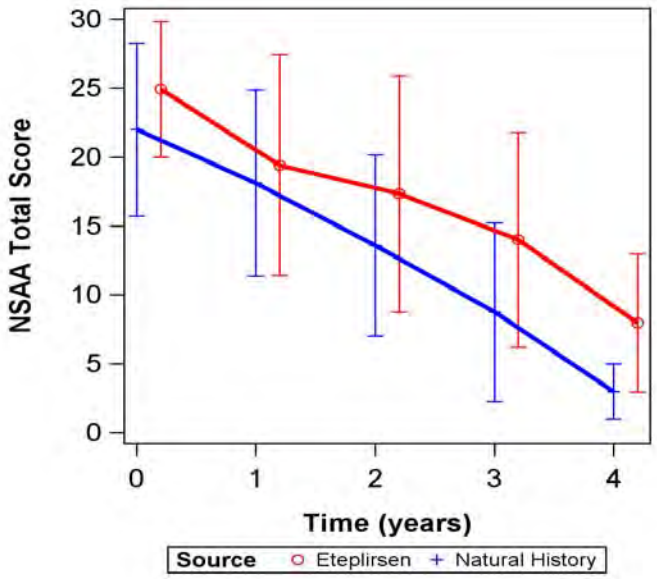
running. The NSAA is a comprehensive outcome measure, and arguably more fully reflects the functional abilities of DMD patients than the 6MWT. The NSAA remains, however, dependent on subject effort, and is not immune to possible bias. All eteplirsen-treated patients showed progressive declines in NSAA 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.

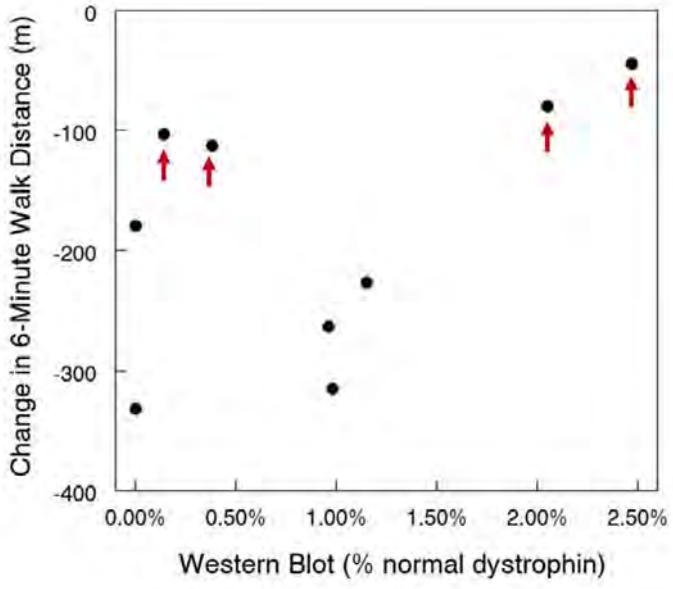
Figure 12: Mean NSAA scores over time



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, <u>in favor of placebo</u>, 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 it 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[10] 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

[10] Cooperative International Neuromuscular Research Group http://www.cinrgresearch.org/

have had an impact on study outcomes, as if 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.[11] 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.

[11] 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[12] 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[13] 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

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

[13] 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.

[14] 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.

[15] 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.

[16] 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% ± 0.84% of normal (mean ± 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

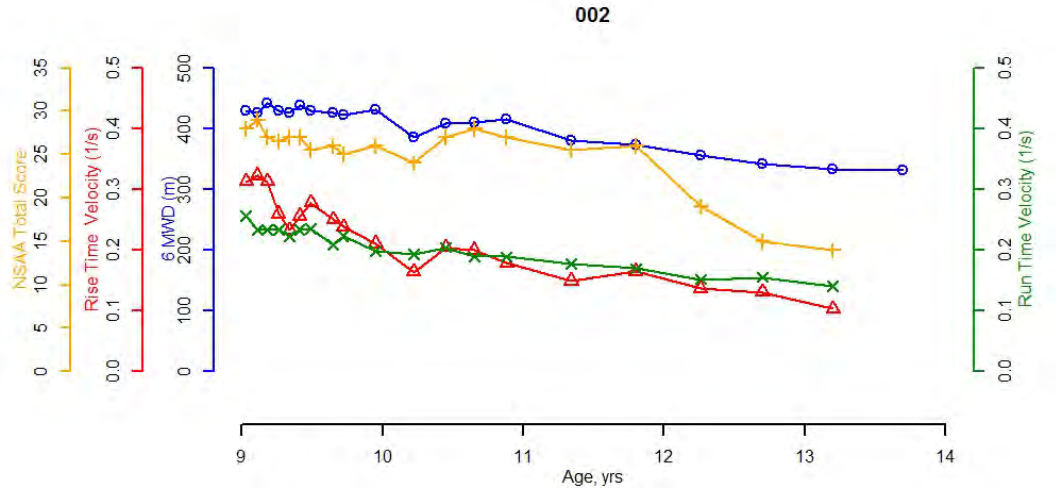
A Postmarketing Risk Evaluation and Mitigation Strategy is not needed for this product.

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.

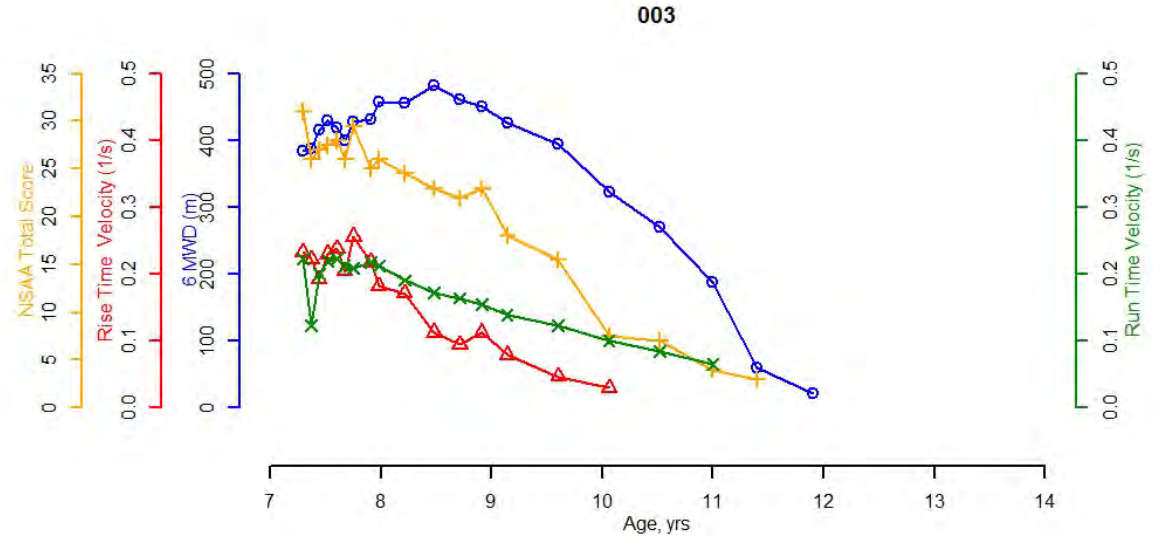
Appendix 1: Patient profiles

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.

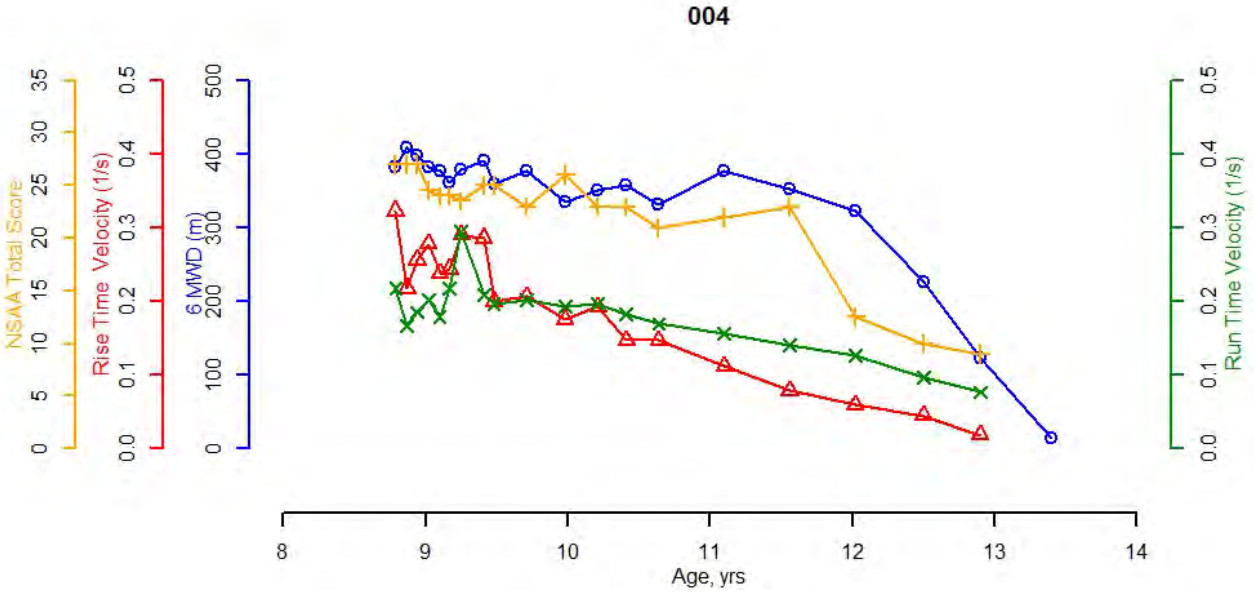
Patient 002 (eteplirsen 30 mg/kg) had a relatively mild course. Patient 002 has 0.14% dystrophin at Week 180, indication that eteplirsen is not likely to have contributed to the course of the disease in this patient.



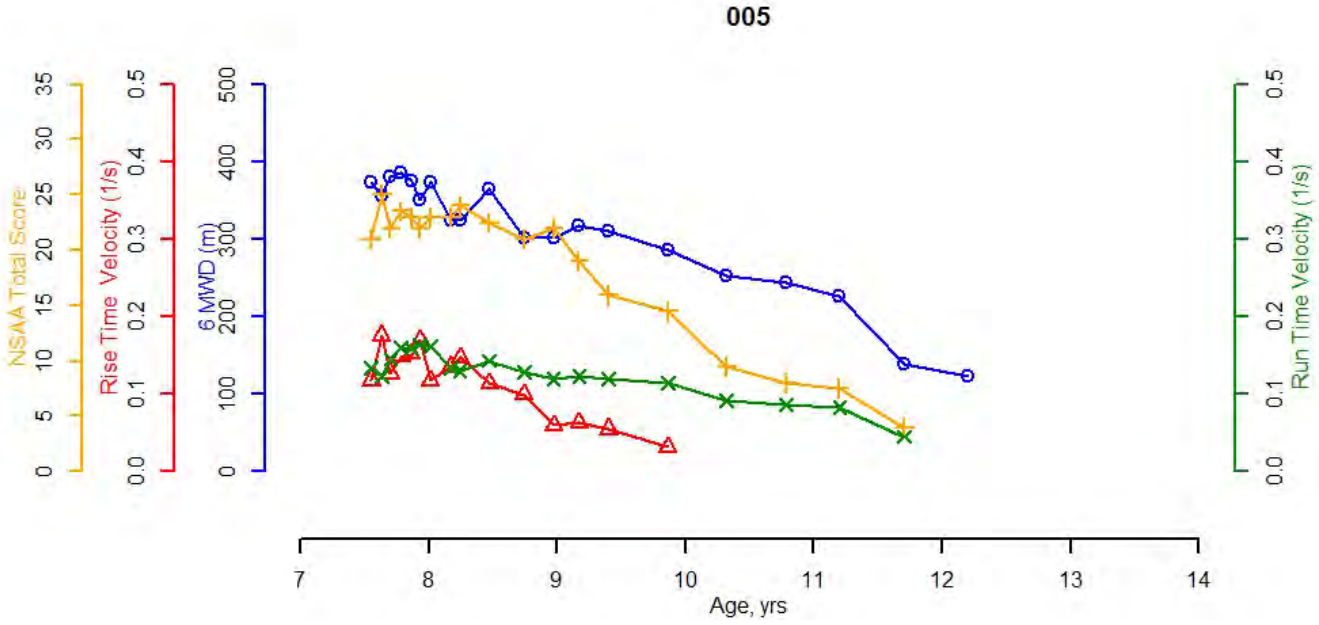
Patient 003 (eteplirsen 50 mg/kg) had a rapid decline in all outcome scales. Patient 003 had 0% dystrophin at Week 180.



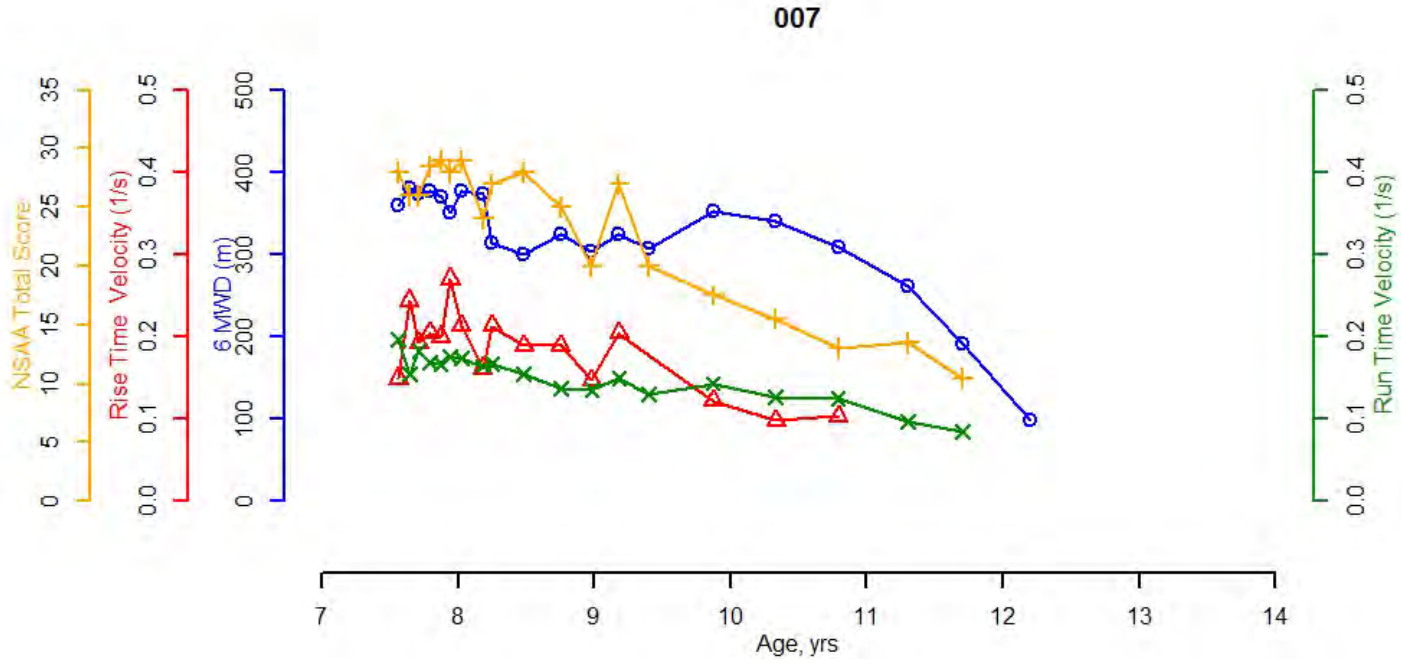
Patient 004 (eteplirsen 50 mg/kg) had relative stability up to age 11 and a half, and then rapidly declined in all outcome scales. Patient 004 had 0.96% dystrophin at Week 180.



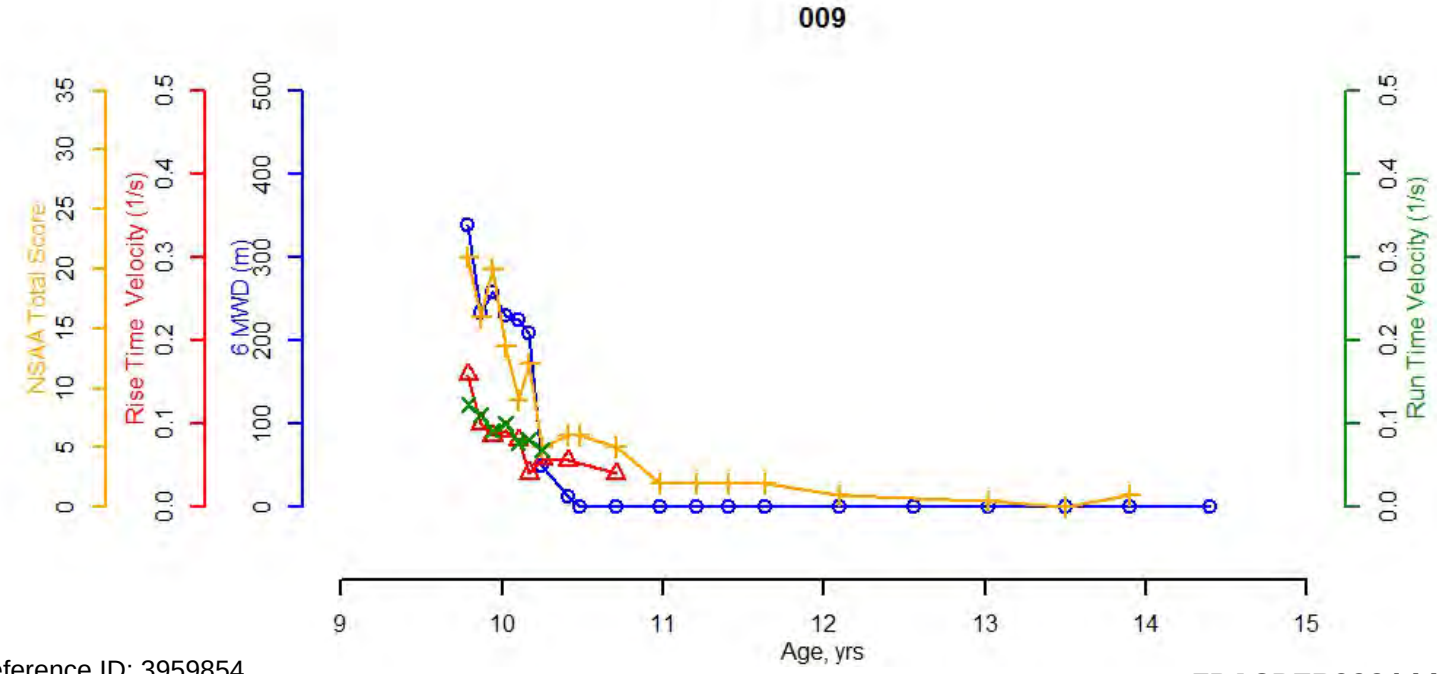
Patient 005 (eteplirsen 50 mg/kg preceded by placebo in Study 201) had a rapid decline in all outcome scales. Patient 005 lost the ability to rise at age 10 years. Patient 005 had no biopsy at Week 180.



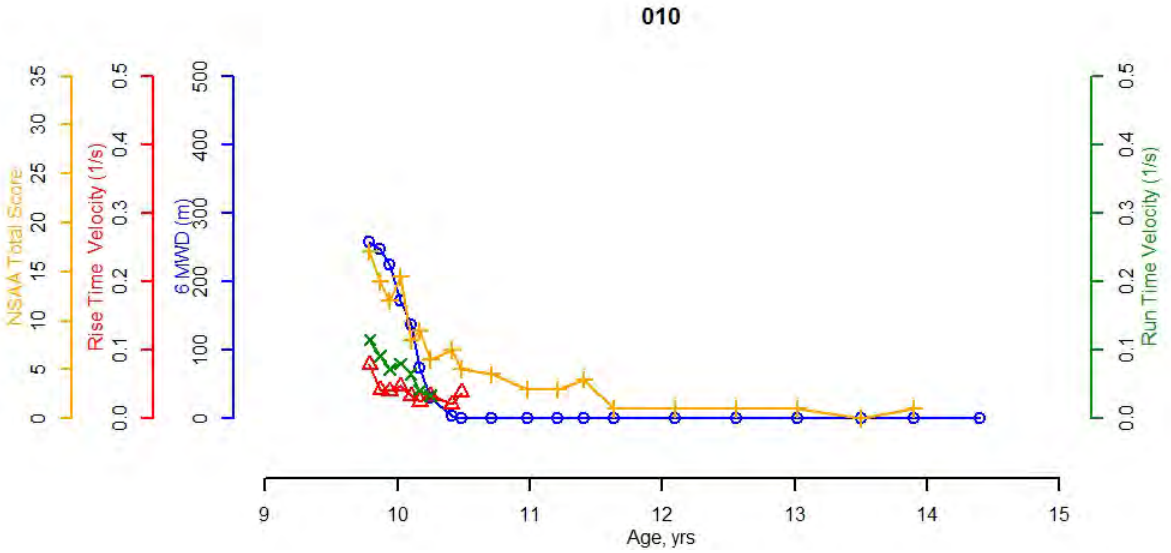
Patient 007 (eteplirsen 30 mg/kg preceded by placebo in Study 201) was relatively stable up to age 11 years, then had a steady decline in all outcome scales. Patient 007 had 0% dystrophin at Week 180.



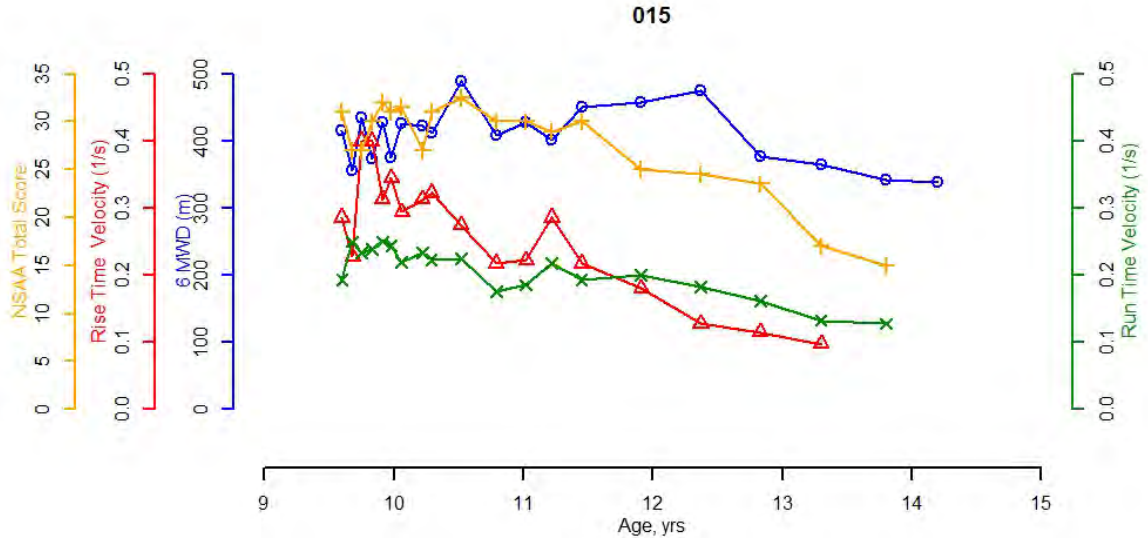
Patient 009 (eteplirsen 30 mg/kg) had a rapid decline in all scales from age 9.5 years. Patient 009 had 0.52% dystrophin at Week 180.



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 datacut 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" colom 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 fysio, 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 physioterapist 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 physioterapist/neurologist from each partecipating 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 subgroupsThese 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.

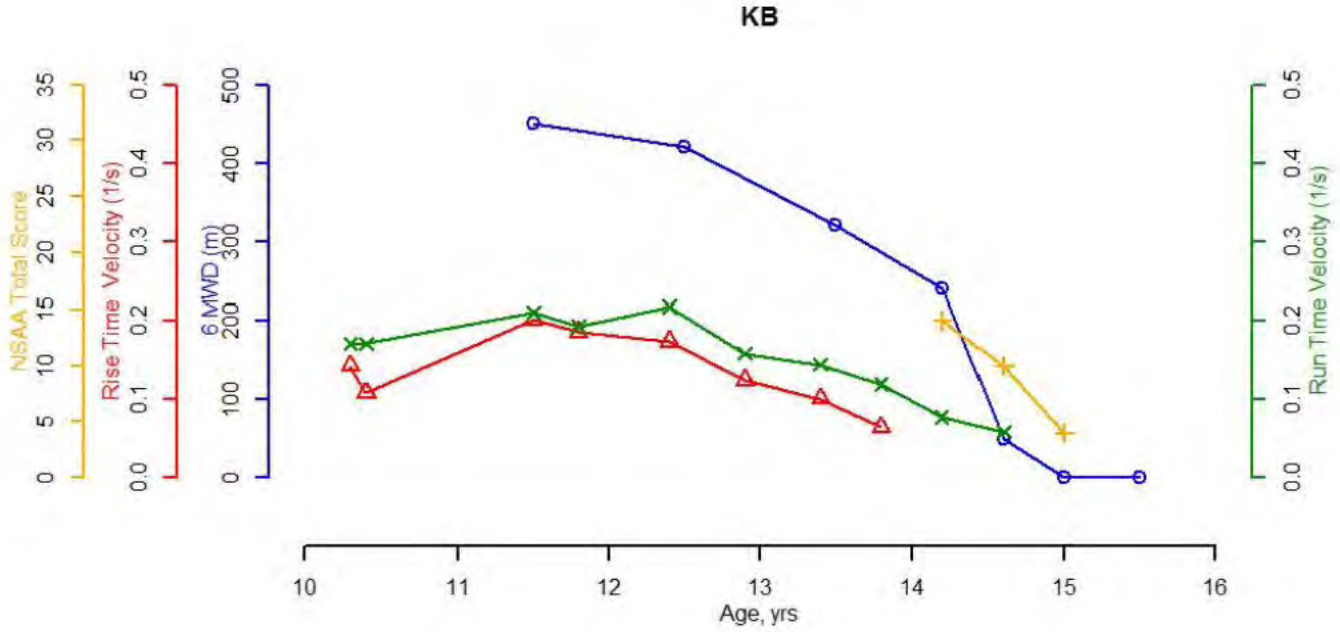
The patient CRF will be filled in by a part–time fellow in each centre. The CRF of each patient will be maintained on site under the responsibility of the P.I. in each institution. The CRF data of all centres will be then collected and entered in a dedicated database by the dedicated research fellow 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.

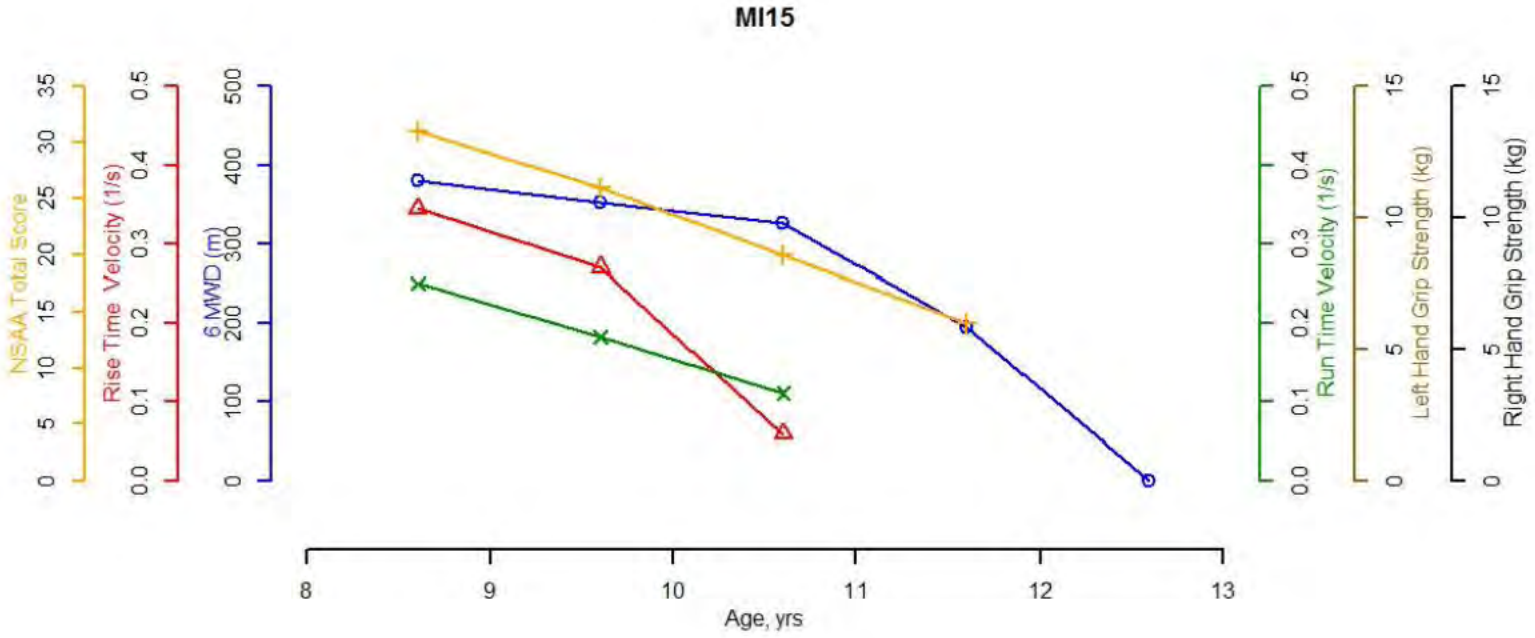
Maintenance of the study database will be the responsibility of the coordinator unit. The data will be stored and analyzed in accordance with Italian legislation. Only aggregate results will be disseminated.

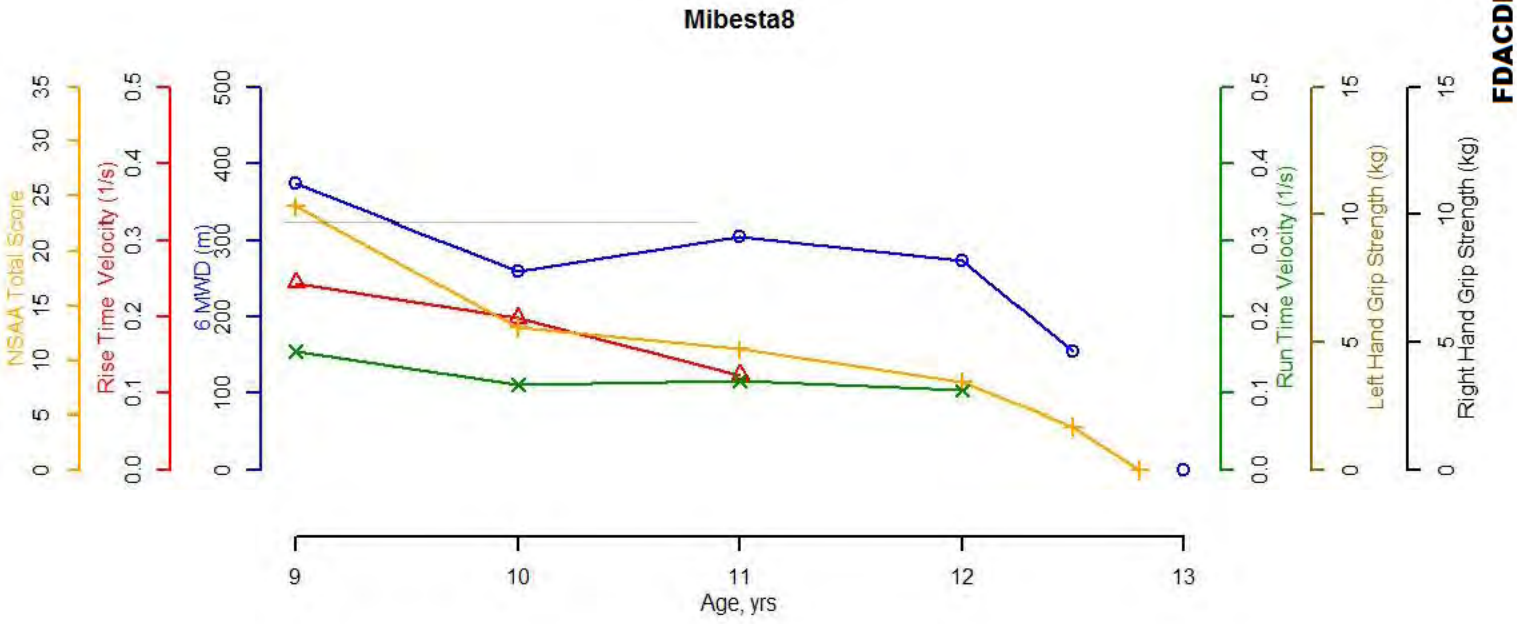
PLAN FOR STATISTICAL ANALYSIS:The distribution of each of the outcome variables (6MWT, NSSA, timed items) will be assessed. Descriptive analysis will be carried out by computing means and medians of continuous variables (as appropriate according to the type of distribution, i.e. whether approximately 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.

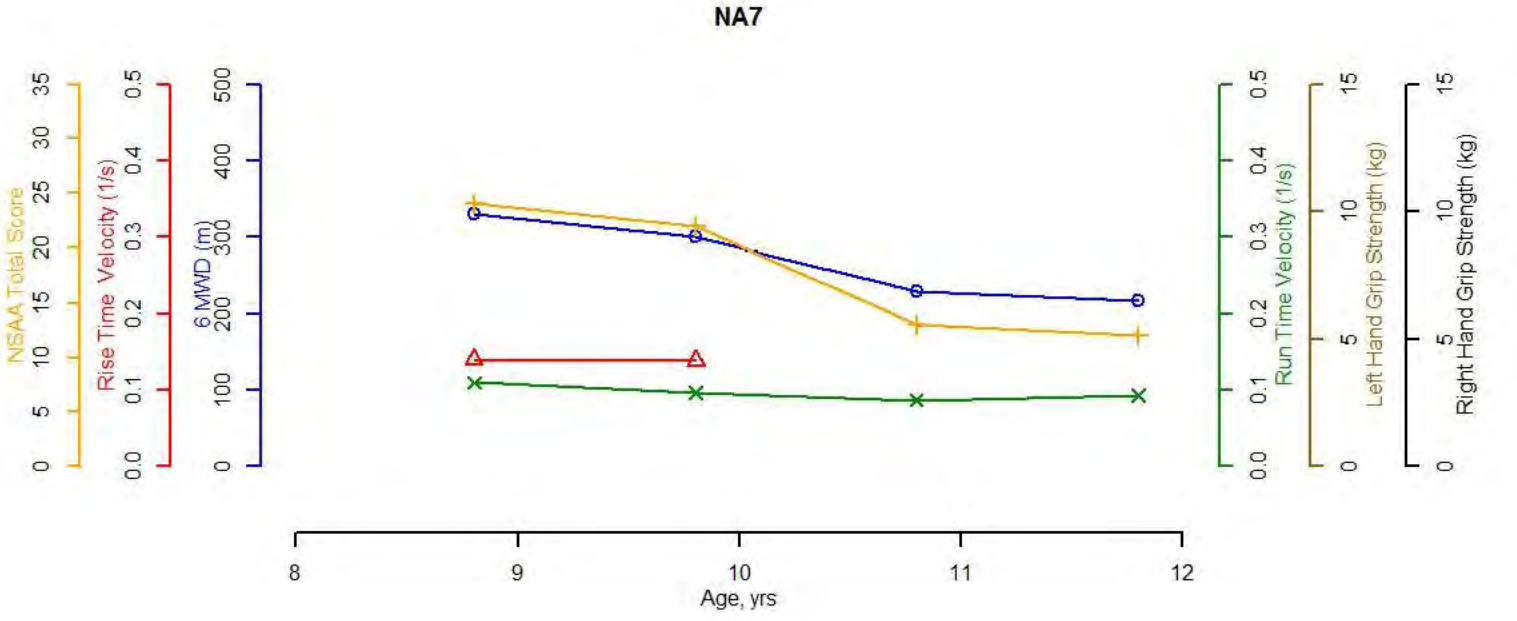
Verified: Date: 24 NOV 2015
Name: Prof E. Mercuri
Signature:

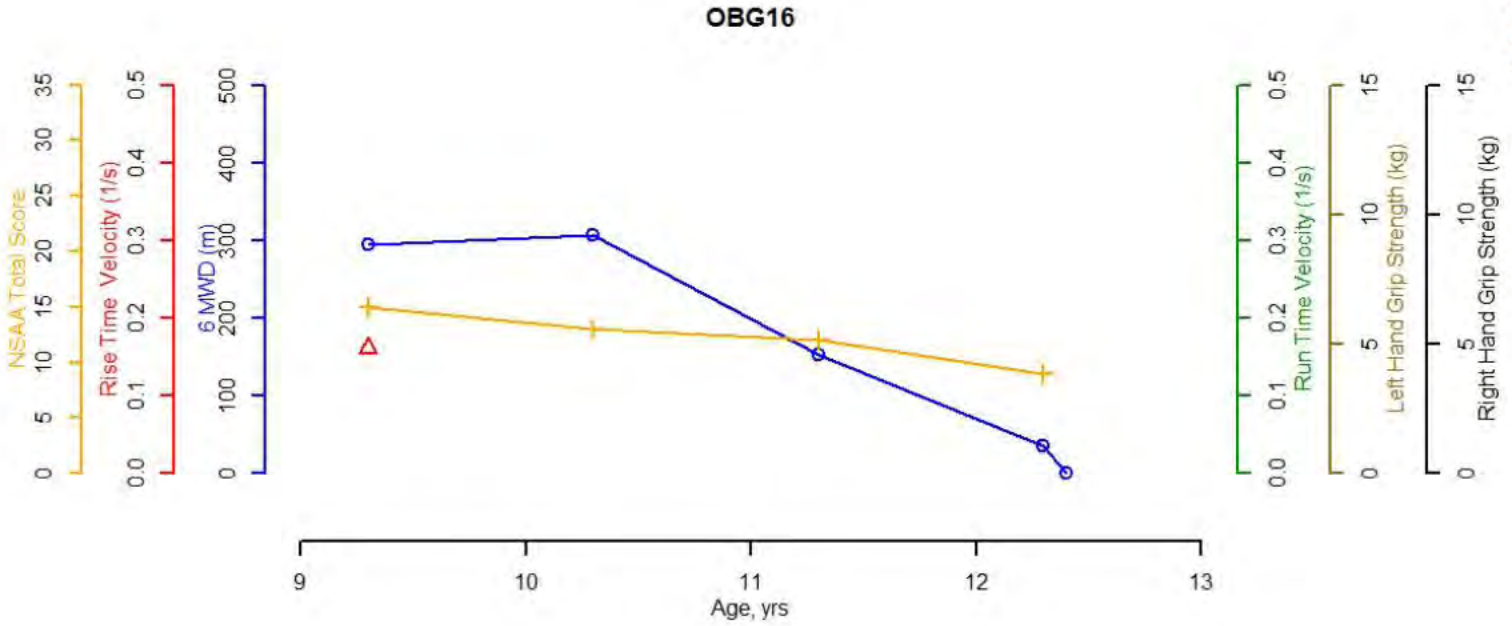
Appendix 4: Patient profiles from the Belgium and Italian Registries

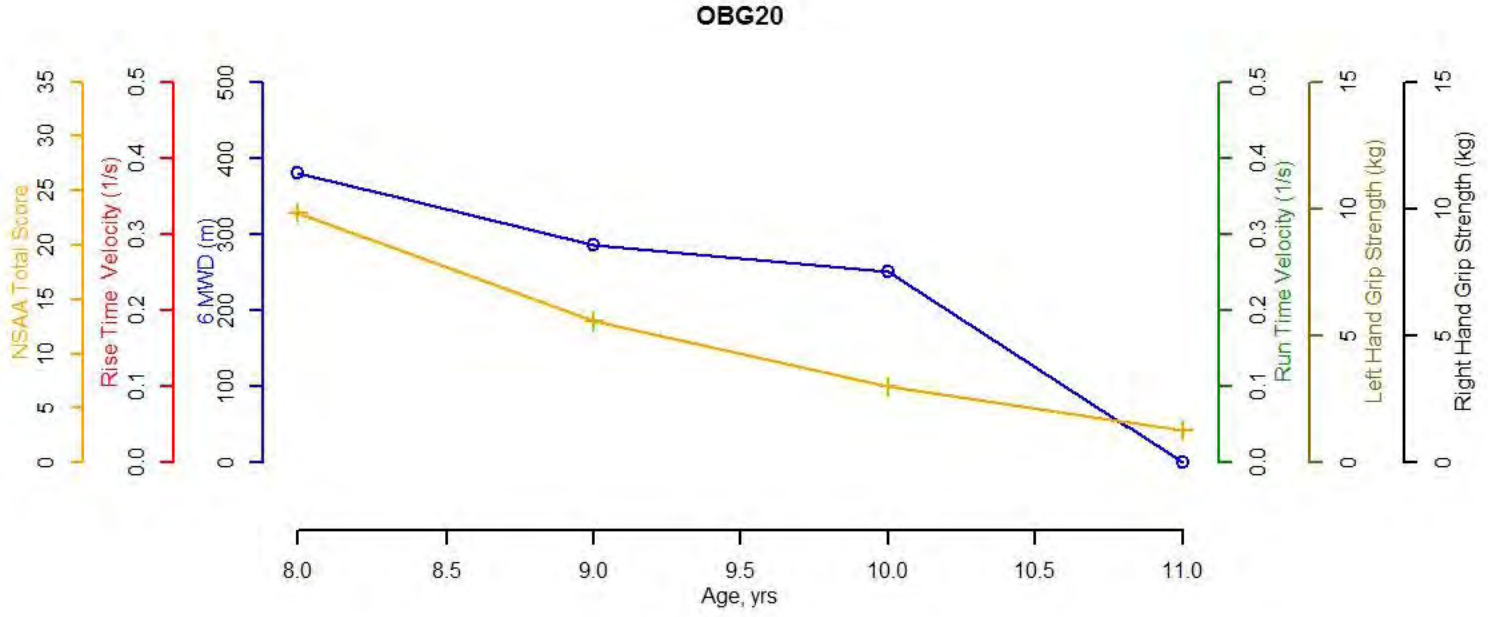


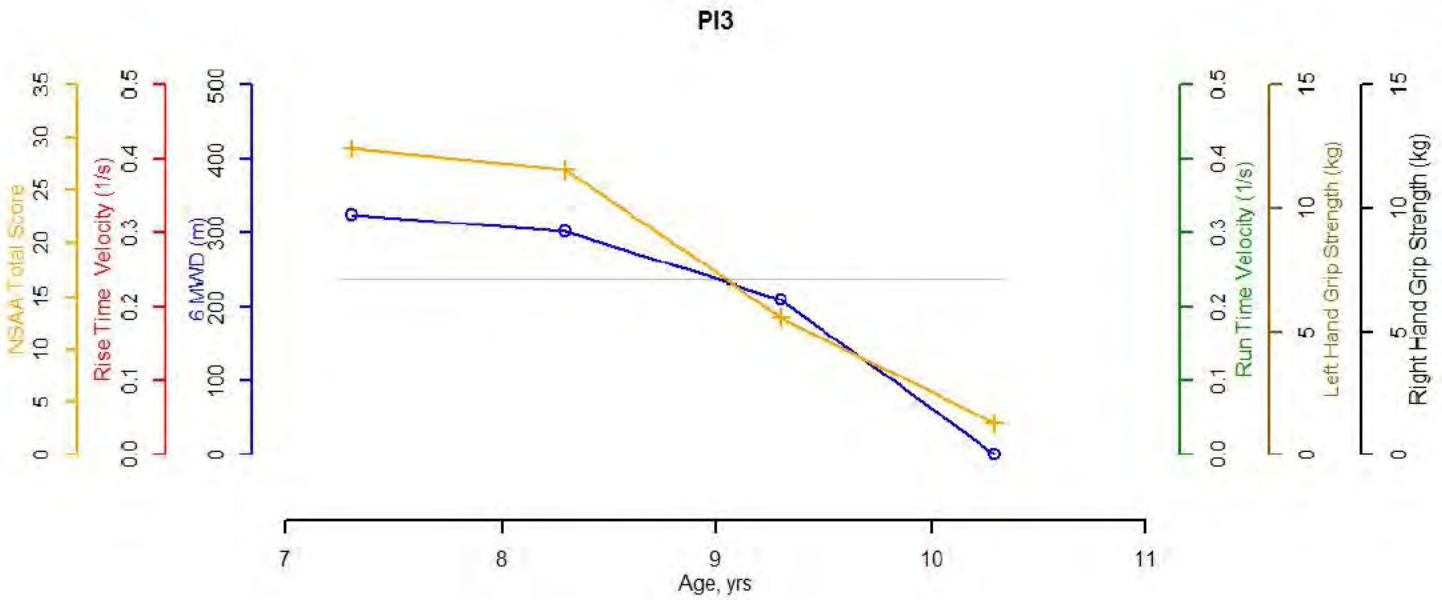


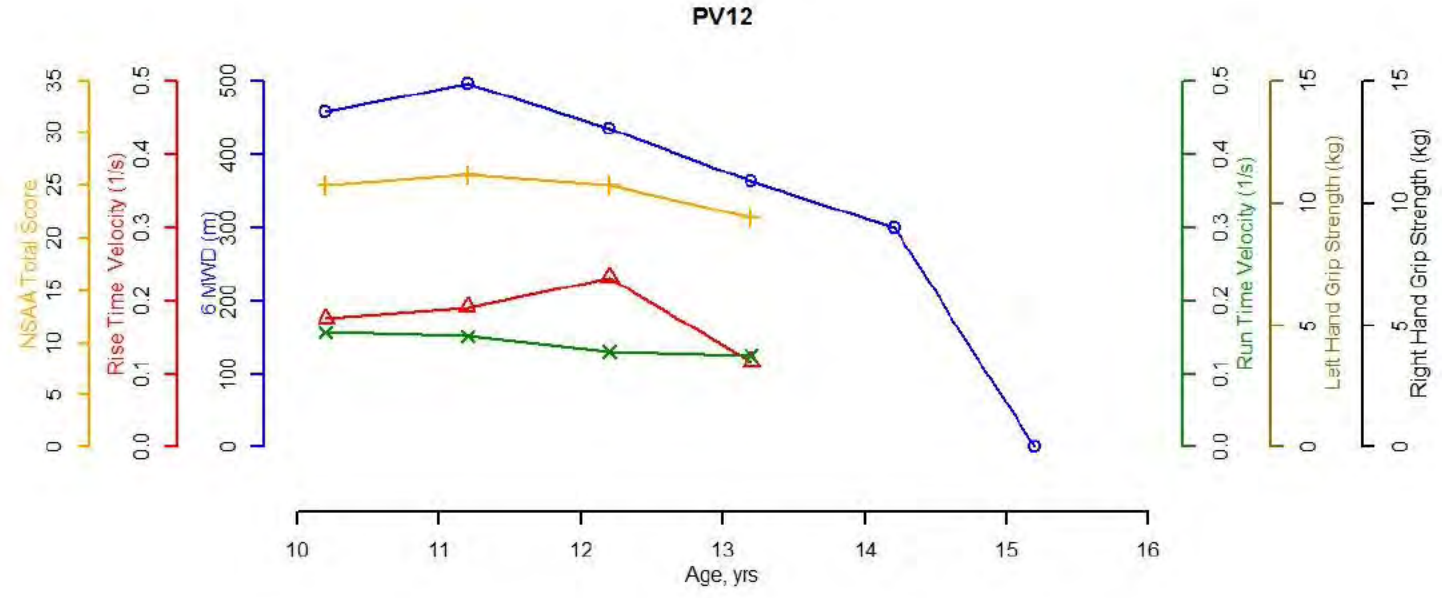

Mibesta8
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs


NA7
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs






PI3
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs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NSAA Total Score
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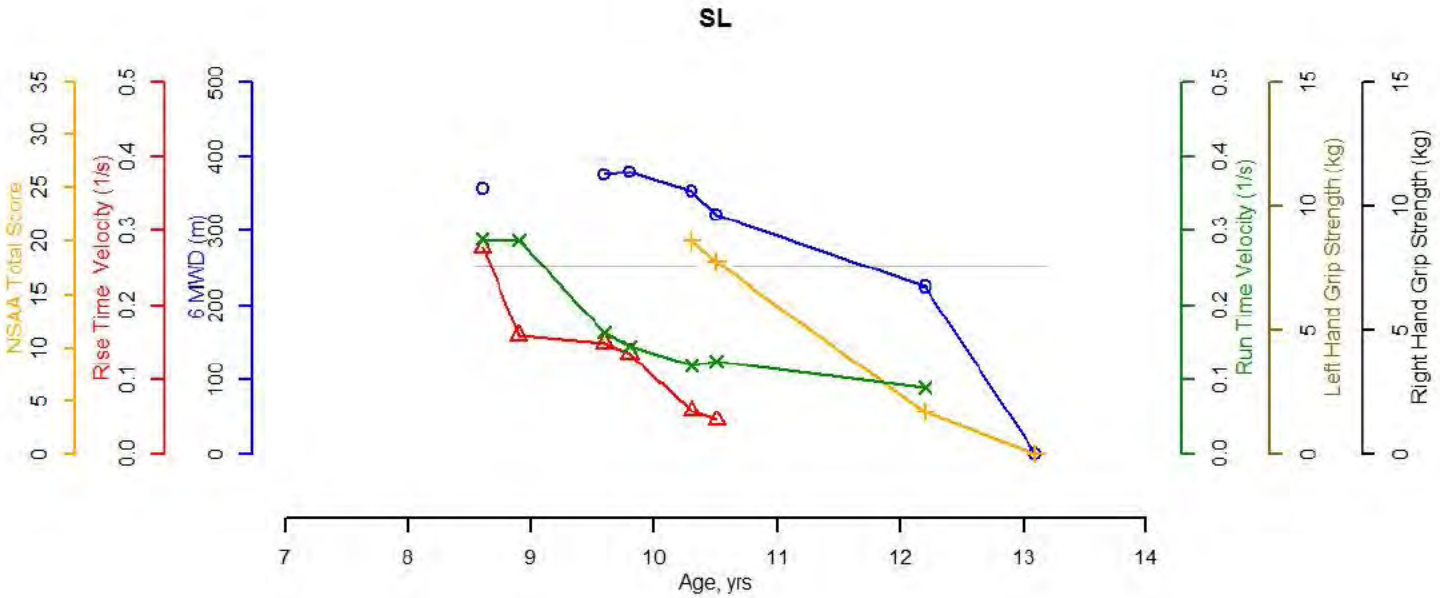
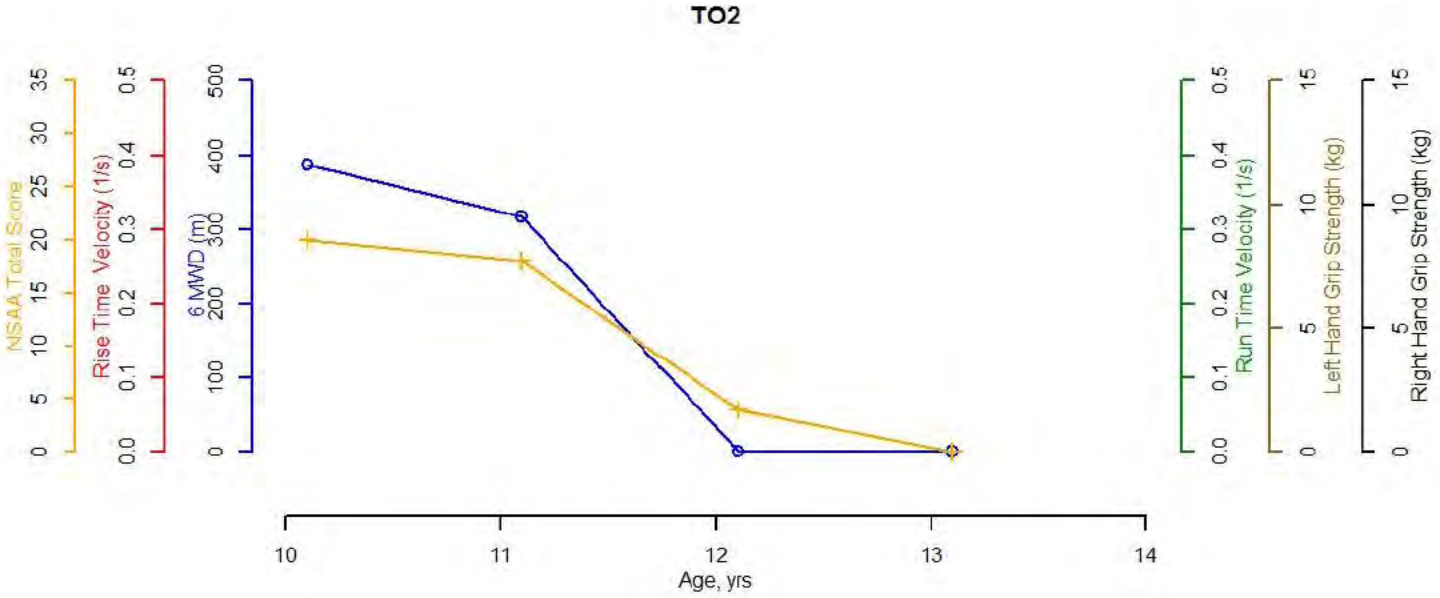
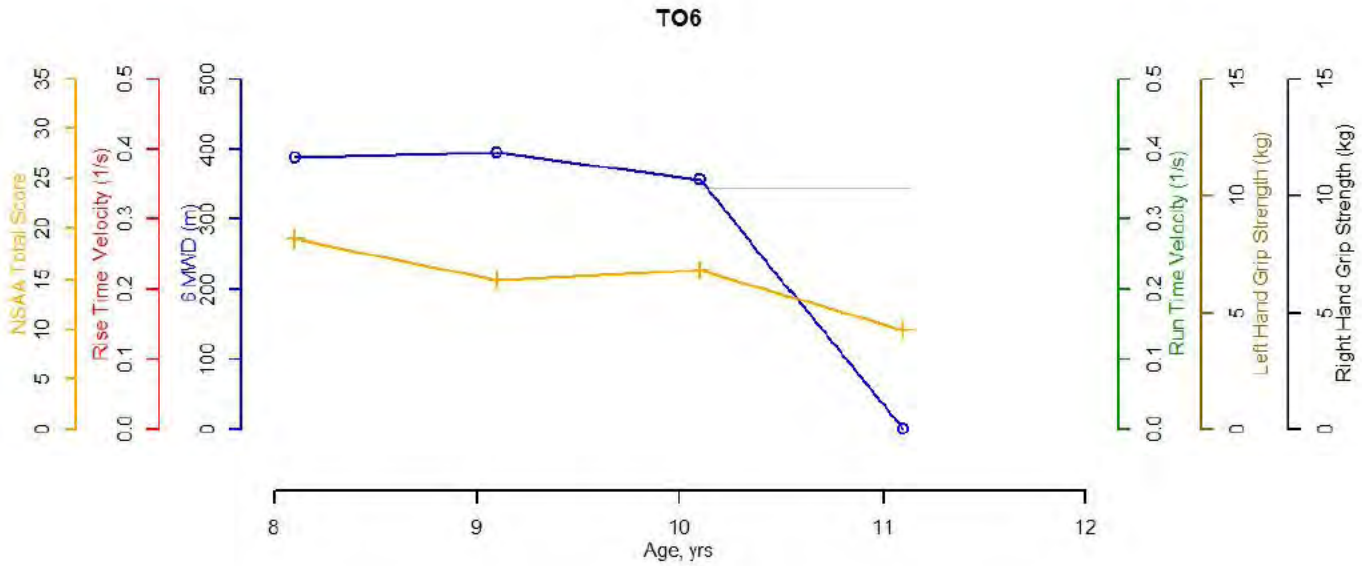
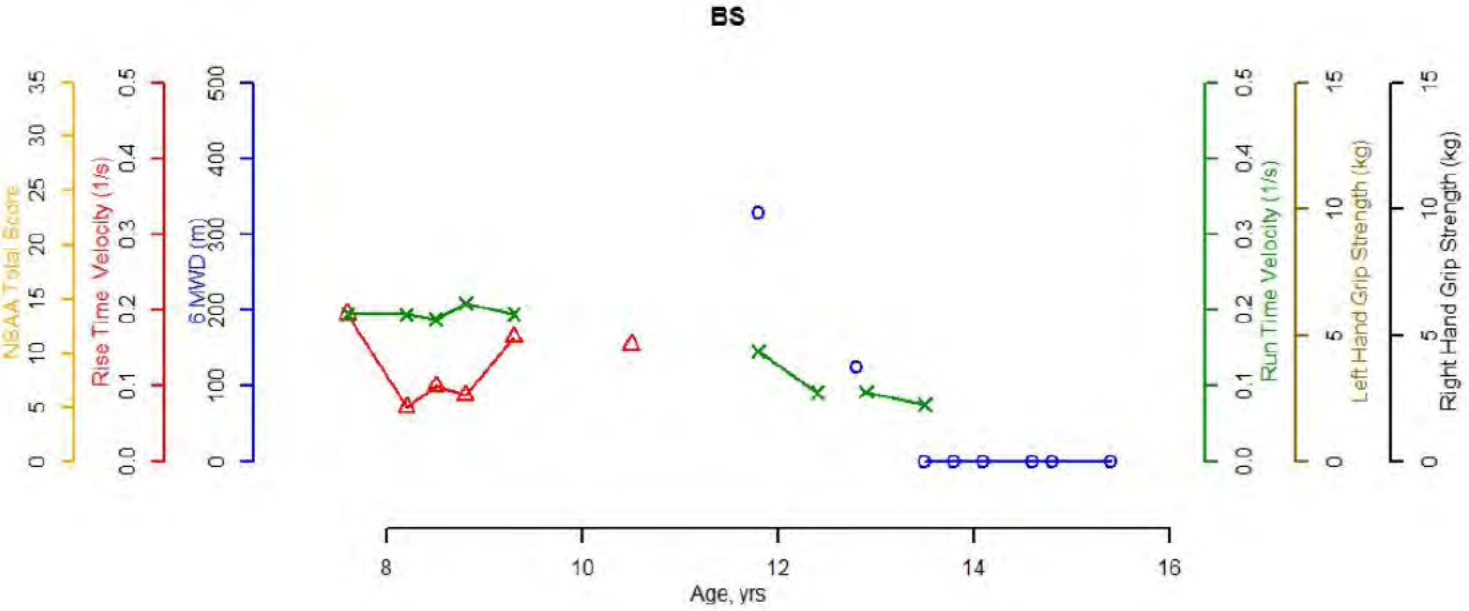
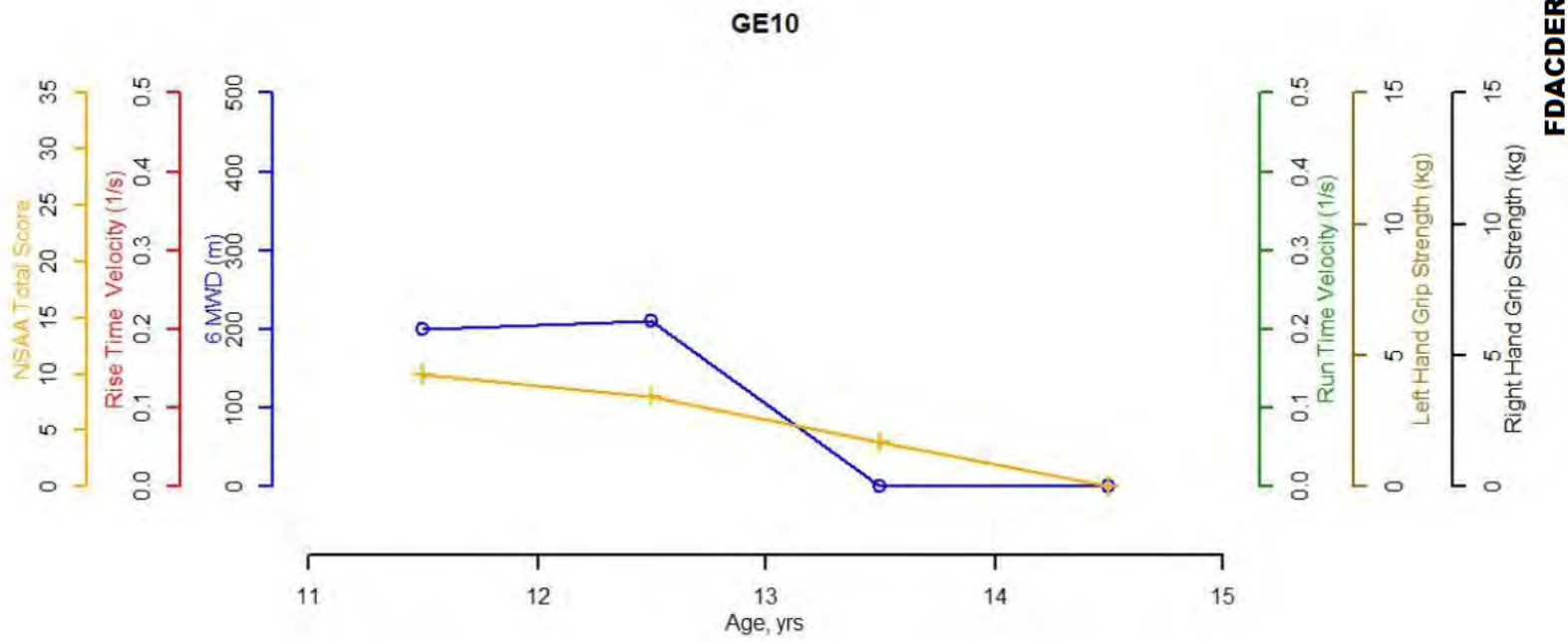
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs


SL
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs


TO2
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs






GE10
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs

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) [redacted] 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) 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
Nationwide Children's Hospital		
(b) (4)	Laboratory Director	Protocol and final report review
	Laboratory Coordinator	Tissue allocation
	Histology technician	Assigned blinding codes to samples
Sarepta Therapeutics, Inc.		
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

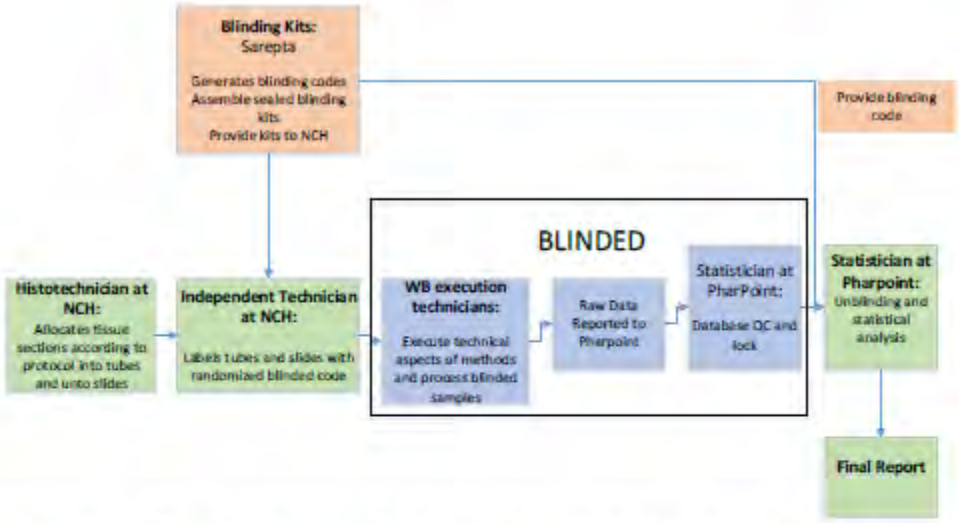


Figure 1: Flowchart Depicting Key Stages of Sample Blinding, Analysis and Unblinding

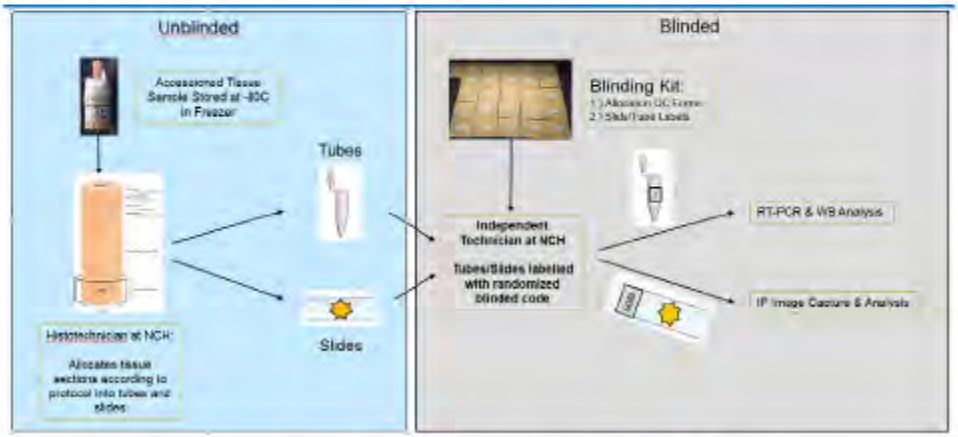


Figure 2: Blinding for Tissue Allocation for Western Blot Analysis

From: Rao, Ashutosh
To: Woodcock, Janet
Subject: RE: so are my changes OK? tx jw
Date: Wednesday, July 13, 2016 6:07:39 PM

Fantastic! Please enjoy your dinner outing!

From: Woodcock, Janet
Sent: Wednesday, July 13, 2016 6:06 PM
To: Rao, Ashutosh
Subject: RE: so are my changes OK? tx jw

Thank you!! We are done! jw

From: Rao, Ashutosh
Sent: Wednesday, July 13, 2016 6:03 PM
To: Woodcock, Janet
Subject: RE: so are my changes OK? tx jw

I made some suggested edits to the items through page 7 (only question# 1).

From: Woodcock, Janet
Sent: Wednesday, July 13, 2016 6:00 PM
To: Rao, Ashutosh
Subject: so are my changes OK? tx jw

From: Rao, Ashutosh
To: Woodcock, Janet
Subject: RE: Only one more question
Date: Wednesday, July 13, 2016 5:43:29 PM
Attachments: image003.png

They did re-read from the first 3 biopsies (and from an older study 28) because we asked them to. The re-read was done with the same images and not fresh tissue sections/fresh slides. The table below shows the re-read summary.

Table 7: Summary of Raw, Change, and Percent Change from Baseline in Dystrophin Positive Fibers by Treatment and Study Week – MANDYS106, Combined Studies 4658-us-201/202

Cohort (n)	Baseline	Week 12 Raw Data (Change from BL)[P] % Change from BL	Week 24 Raw Data (Change from BL)[P] % Change from BL	Week 48 Raw Data (Change from BL)[P] % Change from BL
All Eteplirsen (8)	14.27	-	-	24.06 (9.80)[0.004] 70.28
Eteplirsen 30mg/kg (4)	13.63	-	27.33 (13.70)[0.007] 137.02	23.23 (9.60)[NS] 59.75
Eteplirsen 50mg/kg (4)	14.91	16.74 (1.83)[NS] 24.20	-	24.90 (9.99)[0.026] 80.80
Placebo/Delayed Etep (4)	10.50	-	-	9.69 (-0.81)[NS] 9.11
Placebo to 30mg/kg (2)	10.42	-	10.02 (-0.39)[NS] -7.86	10.42 (-1.02)[NS] -12.36
Placebo to 50mg/kg (2)	10.58	8.52 (-2.06)[NS] -12.14	-	10.58 (-0.60)[NS] 30.58

Note: BL=Baseline; p=p-value (based on 1-sample t-test); NS=Not Significant.
Source: Tables 1.1.1 and 1.1.2 and Listing 1.4.

From: Woodcock, Janet
Sent: Wednesday, July 13, 2016 5:30 PM
To: Rao, Ashutosh
Subject: Only one more question

John Jenkins says in his note to me that there was a re-read of the first 3 biopsies and that this was considered satisfactory. My understanding is that you can't re-do reads of these tissue sections and that what was done is what I described in the memo. Can I give John an unambiguous answer that they did not re-read the slides of the first 3 bx? I know they made new slides of 3 baseline samples, that is different. jw

From:	Unger, Ellis
To:	Woodcock, Janet
Cc:	Jenkins, John K; Dunn, Billy; Bastings, Eric; Temple, Robert; Choy, Fannie (Yuet); Farkas, Ronald; Lowy, Naomi
Subject:	RE: Proposed PMR/PMC for Sarepta
Date:	Wednesday, July 13, 2016 5:03:41 PM

OK, thanks. We'll go with these.

From: Woodcock, Janet
Sent: Wednesday, July 13, 2016 4:33 PM
To: Unger, Ellis
Cc: Jenkins, John K; Dunn, Billy; Bastings, Eric; Temple, Robert; Choy, Fannie (Yuet); Farkas, Ronald; Lowy, Naomi
Subject: RE: Proposed PMR/PMC for Sarepta

These look good to me. thanks. jw

From: Unger, Ellis
Sent: Wednesday, July 13, 2016 3:28 PM
To: Woodcock, Janet
Cc: Jenkins, John K; Dunn, Billy; Bastings, Eric; Temple, Robert; Choy, Fannie (Yuet); Farkas, Ronald; Lowy, Naomi
Subject: Proposed PMR/PMC for Sarepta

Hi Janet,

Please review and comment on our proposed PMR and PMC for eteplirsen:

PMR:

In order to verify the clinical benefit of eteplirsen, conduct a 2-year randomized, double-blind, controlled trial of eteplirsen in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping. Patients should be randomized to the approved dosage of eteplirsen (30 mg/kg weekly) or to a dosage that provides significantly higher exposure, e.g., 30 mg/kg daily. The primary endpoint will be the North Star Ambulatory Assessment.

Draft Protocol Submission:	MM/YY
Final Protocol Submission:	MM/YY
Trial Completion:	MM/YY
Final Report Submission:	MM/YY

PMC:

Conduct a 2-year controlled trial in patients who have a confirmed mutation of the DMD gene that is amenable to exon 45 or 53 skipping with a phosphorodiamidate morpholino oligomer (PMO) designed to bind to a regulatory site governing splicing of the corresponding exon. The study should include at least two well-separated doses of each PMO, with the high dose designed to provide the greatest dystrophin response possible, based upon preliminary dose-finding, with an expectation of acceptable tolerability. The primary objective of this study will be to evaluate the effect of the two PMO doses (combined-active group) compared to control on the North Star Ambulatory Assessment.

The secondary objective will be to evaluate dystrophin levels as percent of normal by Western blot, with tissue to be obtained by needle biopsy. A double-blind, placebo-controlled trial design should be used, if feasible, as this would be most informative. If it is not feasible to include a placebo group, an untreated concurrent control group may be considered, with appropriate care to reduce bias in outcome assessments given the lack of randomization and blinding. You should allow sufficient time for the Agency to review, provide feedback, and come to concurrence on the protocol prior to initiation of the study.

Draft Protocol Submission: MM/YY
Final Protocol Submission: MM/YY
Trial Completion: MM/YY
Final Report Submission: MM/YY

From: Rao, Ashutosh
To: Woodcock, Janet
Subject: RE: I'm around till 2 if you have learned anything, at the same number. jw
Date: Wednesday, July 13, 2016 3:28:13 PM
Attachments: image001.png

From the data (tabulated below), it appears that there is a difference for the two matched samples regardless.

	Old baseline	New baseline	Week 180
"005"	8.9	11.6	no biopsy
"015"	9.9	7.4	30.7
"013"	9.3	16.3	32.5

From: Woodcock, Janet
Sent: Wednesday, July 13, 2016 3:24 PM
To: Rao, Ashutosh
Subject: RE: I'm around till 2 if you have learned anything, at the same number. jw

OK but there is a diffence between the baseline and 180 week readings regardless of which baseline you use, right? jw

From: Rao, Ashutosh
Sent: Wednesday, July 13, 2016 3:02 PM
To: Woodcock, Janet
Subject: RE: I'm around till 2 if you have learned anything, at the same number. jw

Some suggested edits are attached.

With regards to differences in protocol for Bioquant analysis – I did find the original study 201/202 protocol and there are no significant differences with the one used for the week-180 samples. The MuscleMap algorithm is used only for percent positive fiber counting and not during Bioquant intensity measurement. After our visit to Columbus, we didn't have major issues with the Bioquant method itself and most of our advice was focused on the WB and percent positive fiber scoring during discussions about the week-180 sample testing.

I also do not see a significant difference in the baseline values for the old and new Bioquant values (tables below) that requires an explanation, please let me know I am missing anything.

Thanks
Ash

From: Woodcock, Janet
Sent: Wednesday, July 13, 2016 2:07 PM
To: Rao, Ashutosh
Subject: RE: I'm around till 2 if you have learned anything, at the same number. jw

Can you look at what I have written about the percent positive fibers in my memo? In the section on production of dystrophin protein. Part I need you to fill in. jw

From: Rao, Ashutosh
Sent: Wednesday, July 13, 2016 1:38 PM
To: Woodcock, Janet
Subject: RE: I'm around till 2 if you have learned anything, at the same number. jw

The old BIOQUANT intensity protocol under IND is no longer visible in Global Submit, I've asked Fannie to find it and email it to me asap.

I am not sure everyone is looking at the same set of numbers. Below, I have drawn arrows matching the baselines from the old intensity analysis (left table) and new one with the week 180 samples (right)

Subject	Treatment	Week			
		0	12	24	48
002	30 mg/kg	10.9		19.8	25.3
006	30 mg/kg	14.4		28.1	34.9
009	30 mg/kg	11.9		19.3	18.8
010	30 mg/kg	11.4		17.6	21.0
003	50 mg/kg	8.7	20.6		25.5
004	50 mg/kg	8.7	21.5		24.5
012	50 mg/kg	8.8	18.8		23.0
015	50 mg/kg	9.9 ←	~~17.0~~		~~34.8~~
007	P to 30 mg/kg	9.2		8.7	22.9
008	P to 30 mg/kg	9.0		~~9.9~~	29.0
005	P to 50 mg/kg	8.9 ←	9.0		22.1
013	P to 50 mg/kg	9.3 ←	9.1		19.7

Table 2: Week 180 Individual Treated Patients, BIOQUANT Fluorescence Intensity

Subject Number	Average
01002	11.9
01003	7.0
01004	28.1
01006	28.7
01007	12.0
01008	26.7
01009	23.0
01010	21.4
01012	26.1
01013	32.5
01015	30.7

Source: Listing A.1.1

Table 3: Individual Untreated DMD Control Samples, BIOQUANT Fluorescence Intensity

Subject Number	Average
01005	11.6
01013	16.3
01015	7.4
DMD1	9.9
DMD2	6.1
DMD5	9.2
DMD7	3.5
DMD8	17.6
DMD9	3.1

Source: Listing A.1.2

From: Unger, Ellis
To: Woodcock, Janet
Cc: Jenkins, John K; Dunn, Billy; Bastings, Eric
Subject: RE: MEMO
Date: Wednesday, July 13, 2016 3:19:12 PM

Janet,

I've canvassed the Division, and we have no additional comments.

Thanks for sharing,

Ellis

From: Woodcock, Janet
Sent: Monday, July 11, 2016 7:21 PM
To: Jenkins, John K; Unger, Ellis; Dunn, Billy; Bastings, Eric
Subject: MEMO

Here is a draft version of my decisional memo. I welcome comments on it. An appeal can't be done until I finalize this, I am told. Thanks. jw

From: Dunn, Billy
To: Woodcock, Janet
Subject: RE: Update on FDA Review of PMCs/PMRs?
Date: Wednesday, July 13, 2016 2:02:01 PM

Janet – Note that the PMR/PMC language has been undergoing extensive discussion above, but including, the Division. I am as eager to get this out as Sarepta is to receive it. My understanding is that it will be going out today and my intent in that regard is certainly clear to those over here.

Billy

From: Shamim Ruff [mailto:SRuff@Sarepta.com]
Sent: Wednesday, July 13, 2016 1:43 PM
To: Choy, Fannie (Yuet)
Cc: Woodcock, Janet; Throckmorton, Douglas C; Dunn, Billy; Ed Kaye
Subject: Update on FDA Review of PMCs/PMRs?

Dear Fannie,

Can you please confirm when we can expect to get feedback from FDA on the PMCs/PMRs? We don't understand why this is taking so long given that both the PROMOVI and ESSENCE studies were agreed with FDA in 2014 (FDA September 2014 Meeting Minutes). In addition, both studies were extended to 96 weeks based on subsequent FDA feedback.

The only outstanding item is the dose ranging study which we sent to FDA a week ago. If there are any issues or concerns, we would like to request an urgent tcon with the Division to agree a way forward.

Finally, we have conceded to **ALL** of FDA requests on the USPI so that should now be final.

We would appreciate receiving FDA feedback on the PMCs/PMRs as soon as possible- by end of day today. There is a great deal of anxiety from the patient community and we believe it is in the best interest of all of us concerned to complete the accelerated approval process by the end of this week.

We look forward to hearing from you as soon as possible.

Regards,
Shamim

Shamim Ruff
SVP Regulatory Affairs and Quality
p 617-274-4009 c (b) (6)
e sruff@sarepta.com

215 First Street, Cambridge, MA 02142

From: Choy, Fannie (Yuet) [mailto:Fannie.Choy@fda.hhs.gov]
Sent: Tuesday, July 12, 2016 6:33 PM
To: Shamim Ruff <SRuff@Sarepta.com>
Cc: Choy, Fannie (Yuet) <Fannie.Choy@fda.hhs.gov>
Subject: RE: FDAedits-COMPLETE_Round4_12July16-Eteplirsen PI_to Sarepta SRPT.docx

Dear Shamim,

I confirm receipt of email and the attached draft labeling. I will communicate revision, if any, after the team reviews your edits.

Regarding the proposed PMR/PMC, I have forwarded your request/email to the Division. I will share the information as soon as it's available.

Regards,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products

From: [redacted]
To: [redacted]
Subject: RE: I'm around till 2 if you have learned anything, at the same number. jw
Date: Wednesday, July 13, 2016 1:30:20 PM
Attachments: [redacted].jpg

The old BIOQUANT intensity protocol under IND is no longer visible in Global Submit. I've asked Fannie to find it and email it to me asap.

I am not sure everyone is looking at the same set of numbers. Below, I have drawn arrows matching the baselines from the old intensity analysis (left table) and new one with the week 180 samples (right).

Subject	Treatment	Week			
		0	12	24	48
002	30 mg/kg	10.9		19.8	25.3
006	30 mg/kg	14.4		28.1	34.9
009	30 mg/kg	11.9		19.3	18.8
010	30 mg/kg	11.4		17.6	21.0
003	50 mg/kg	8.7	20.6		25.5
004	50 mg/kg	8.7	21.5		24.5
012	50 mg/kg	8.8	18.8		23.0
015	50 mg/kg	9.9	17.0		34.8
007	P to 30 mg/kg	9.2		8.7	22.9
008	P to 30 mg/kg	9.0		9.9	29.0
005	P to 50 mg/kg	8.9	9.0		22.1
013	P to 50 mg/kg	9.3	9.1		19.7

Table 2: Week 180 Individual Treated Patients, BIOQUANT Fluorescence Intensity

Subject Number	Average
01002	11.9
01003	7.0
01004	28.8
01006	25.7
01007	12.0
01008	26.7
01009	23.0
01010	21.4
01012	26.1
01013	32.5
01015	30.7

Source: Listing A.11

Table 3: Individual Untreated DMD Control Samples, BIOQUANT Fluorescence Intensity

Subject Number	Average
01005	11.6
01013	16.3
01015	7.4
DMD1	9.5
DMD2	6.1
DMD3	9.2
DMD7	3.5
DMD8	17.6
DMD9	3.1

Source: Listing A.12

From: Unger, Ellis
To: Woodcock, Janet
Subject: FW: NSAA numbers
Date: Wednesday, July 13, 2016 11:18:59 AM
Attachments: RE Northstar for latest assessment of Study 202.msg

---

Here are the Week 240 data (drill down). I don't see that it ever came in through the gateway.

From: Farkas, Ronald
Sent: Wednesday, July 13, 2016 11:04 AM
To: Bhattaram, Atul; Unger, Ellis; Bastings, Eric; Breder, Christopher D
Cc: Dunn, Billy
Subject: RE: NSAA numbers

Here it is
Thanks
Ron

From: Bhattaram, Atul
Sent: Wednesday, July 13, 2016 10:35 AM
To: Unger, Ellis; Bastings, Eric; Farkas, Ronald; Breder, Christopher D
Cc: Dunn, Billy
Subject: RE: NSAA numbers

Hi Ellis

I got the datasets via email from Ron and Eric.

Atul

From: Unger, Ellis
Sent: Wednesday, July 13, 2016 10:28 AM
To: Bhattaram, Atul; Bastings, Eric; Farkas, Ronald; Breder, Christopher D
Cc: Dunn, Billy
Subject: RE: NSAA numbers

Greetings, Atul! One more question. Do you know where you got the Week 240 data? In other words, was this an email communication from Sarepta, or did it come in through the gateway? And if it came in through the gateway, I can probably find it if you know the approximate date.

Thanks for all,

Ellis

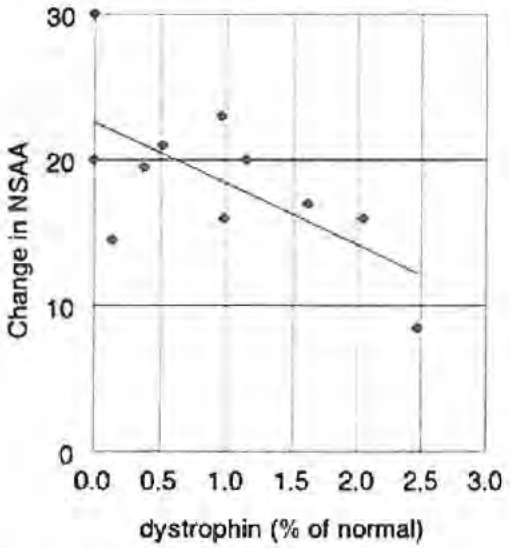
From: Bhattaram, Atul
Sent: Tuesday, July 12, 2016 12:19 AM
To: Unger, Ellis; Bastings, Eric; Farkas, Ronald; Breder, Christopher D
Cc: Dunn, Billy
Subject: RE: NSAA numbers

In the dataset that I sent before, there are blank cells in the column I (visit_number) after 168 weeks. The subsequent data points are for 192, 216 and 240 weeks. The reason behind is missing information at these visits for muscle strength measures which I integrated into this database. Let me know if it is not clear.

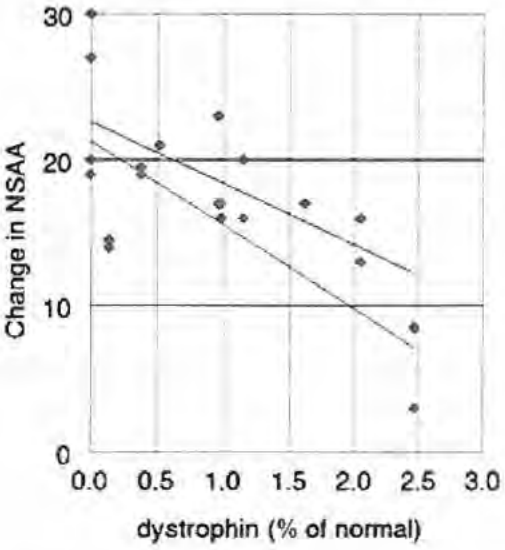
Atul

From: Unger, Ellis
To: Woodcock, Janet
Cc: Jenkins, John K; Dunn, Billy; Bastings, Eric
Subject: RE: MEMO
Date: Wednesday, July 13, 2016 12:57:30 AM

I just noticed that the y-axis scales for the blue and red plots were different. This was not intentional. Here's the red plot on the same 0 to 30 scale as the blue plot:



And here they are together:



From: Unger, Ellis
Sent: Wednesday, July 13, 2016 12:52 AM
To: Woodcock, Janet
Cc: Jenkins, John K; Dunn, Billy; Bastings, Eric
Subject: RE: MEMO

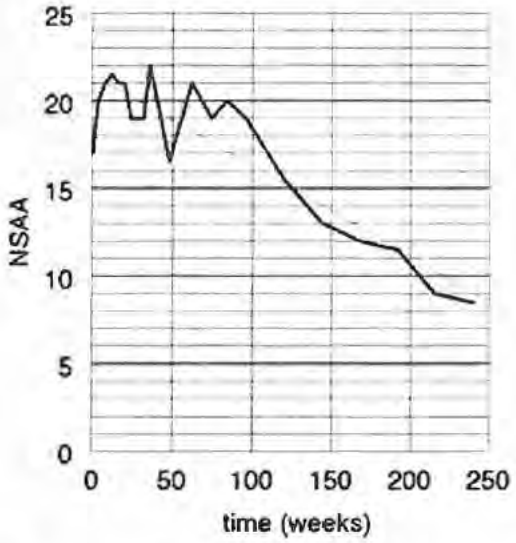
Janet,

I have some concerns with respect to Table 1 and Figure 2. You say that $R^2 = 0.8$. I've plotted the NSAA data in your Table 1, and I was able to reproduce your graph exactly. But I got R=0.8, which means that $R^2 = 0.64$. So I think that "$R^2 = 0.8$" is a typo. If R^2 were actually 0.8, then R would have been 0.9 (0.9 squared = 0.81).

More importantly, I cannot tell how you got the deltas for the NSAA for each patient in Table 1. If you want to include the table and figure as they are now in your draft, I think you might consider citing your source for the table (presumably a Sarepta table or a reviewer's table). If it is your own work, then you might want to explain how you calculated the numbers, i.e., identify the name and location of the original data file you used and explain the methods you used to calculate the deltas.

Looking deeper at your table, I was curious about the data from patient 006. This patient had the highest dystrophin value (2.47%) and an NSAA change of just 3 units, as noted in the table.

Here are the NSAA data on this boy:



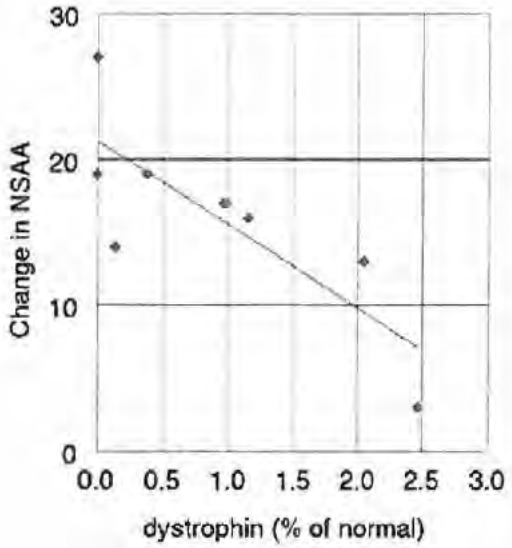
Looking at the data above, I have some difficulty characterizing his change as "3."

Instead of picking an arbitrary time point as the "final" point, I thought that the most reasonable way of doing this would be to include all of the data that have been collected through Week 240. Because all patients have remained on weekly treatment for 240 weeks, I see no reason to truncate the data at the time the biopsy was obtained. Thus, for each patient I calculated their delta as their Week 240 score minus their baseline score. EXCEPT - for the 4 patients who started on placebo for 24 weeks, I considered Week 24 to be their baseline.

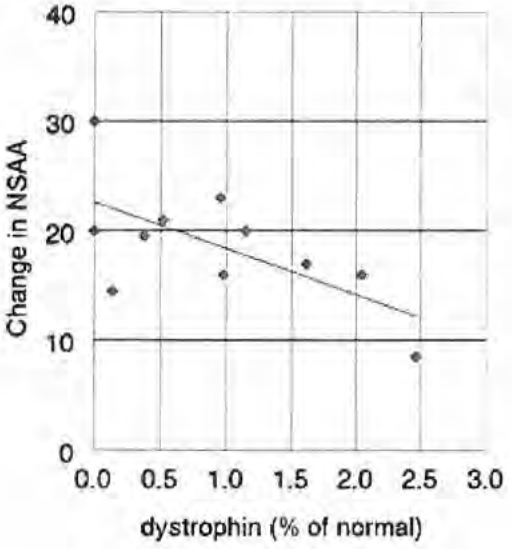
Also, I think we should use the data from all 11 patients with Week 180 biopsies, including the 2 who lost ambulation early, though I understand you might not agree.

Here is what I found...

First, below in blue is my version of your plot of 9 patients. My plot looks the same as yours, but I get R=0.80. (At the dystrophin value of 1%, there are two closely overlapping data points.)



Second, below, my plot of all 11 patients, where I calculate delta as Week 240 minus baseline (for patients who started on placebo, it's Week 240 minus Week 24):

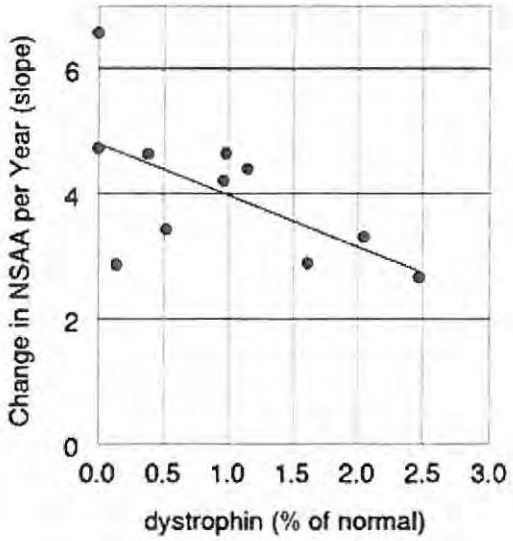


Using all of the data through Week 240, the slope is less steep and the correlation is worse. And knowing that you might not agree with inclusion of the 2 boys who became unable to ambulate, I will tell you that if I include all 11 boys, R=0.65. If I leave out the 2 non-ambulatory boys, R=0.64.

So it makes no difference whether they are included or not. And the slope is essentially the same.

An alternate analysis I've been interested in is one that uses ALL of the aforementioned data for each patient. Rather than using just the first and last data points for each patient, we can calculate a slope for each patient using linear regression. For example, consider patient 006 graphed above. The red line is the linear regression. His slope is -0.051 units per week, or (multiply by 52) -2.7 units per year.

Below is a plot of the slope for each boy vs. dystrophin at Week 180 by Western blot. The slope for each boy is expressed as the change in NSAA per 52 weeks. For this plot, R=0.60, which is quite similar to the correlation of simple pre-treatment minus post-treatment (above).



In short, when you consider all of the NSAA data through Week 240, you get essentially the same plot whether you simply subtract pre- from post (red plot), or calculate a slope for each patient (black plot). And in either case, R is in the 0.6-range.

You can feel free to use some or all of these plots. Obviously, you can use your own table/plot, but if you do, I suggest you explain how you got your numbers, as I noted above.

Ellis

From: Jenkins, John K
Sent: Tuesday, July 12, 2016 5:25 PM
To: Woodcock, Janet; Unger, Ellis; Dunn, Billy; Bastings, Eric
Cc: Jenkins, John K
Subject: RE: MEMO

Janet

As we discussed in the hallway, I had some comments on your memo:

1. You said you did not consider data from the first three biopsies, but you do not say why. It was my understanding that the re-read of the first three biopsies for IHC was considered valid and the review team saw no change from baseline from the first biopsy to week 180. This is an important part of their perspective on the amount of dystrophin produced and its significance. They noted that the baseline ICH results from the patients in Study 201 were very different from the "new" baseline data that were conducted in parallel to the week 180 biopsy and that these differences were not explained. So, I think you need to explain why you are not considering the IHC data from the first three biopsies in your review, and instead focusing on the comparison of the "new" controls versus the week 180 IHC results.
2. I am not familiar with the references you added to the memo and cannot comment on your summary of the findings of each paper. Perhaps the review team know those references and can provide their take on the findings. Even taking your summaries at face value as accurate, I did not find the summary of the references to be particularly helpful in determining whether the dystrophin findings for eteplirsen are "reasonably likely" to predict clinical benefit. As you noted, there are issues related both to the quantity of dystrophin produced as well as its quality (functionality) that are hard to tease apart in the small sample of boys treated. It is possible that the "responders" are making functional protein, it is also possible they are making non-functional protein, and I don't think we have the data to sort this out. It still seems to be a judgment on the totality of evidence where reasonable people may disagree.
3. I find the correlation graphs at the end to be problematic since they graph a delta for a clinical endpoint on the y-axis against an absolute endpoint value for dystrophin on the x-axis. While one might assume the baseline dystrophin levels were low and therefore using the absolute endpoint value is a reasonable estimate of the effect of the drug on dystrophin (e.g., a surrogate for the delta); we don't know that and therefore it is hard to know whether the graphics are isolating a drug effect or capturing a prognostic difference that may have been present at baseline. So, I don't find them as convincing as you suggest, but again, that may be a judgment.
4. You focus on the "responders" in the population to support your argument, but we don't know how to identify the responders and therefore will have to approve based on the sponsor's grouping of mutations that are "amenable to exon 51 skipping," which likely include mutations that are not responsive to eteplirsen. This is a frustrating intersection of "targeted" therapies and approval based on a group mean based on characteristics chosen by the sponsor that we cannot validate. This is very analogous to the CF cases that we have been discussing and I think the review teams in both cases are struggling with these issues. It feels like we have to "unlearn" what we think we know from targeting and regress to approving an un-validated grouping of mutations. I know we do this all the time for non-targeted therapies, but it is still discomforting to face this for targeted therapies. While the drug at the proposed dose has not been shown to be toxic, it will likely require a central line for chronic administration, which is not benign, and families/society will be burdened with a very expensive drug that may only be working in a small subset of the indicated population. This is an issue we will have to grapple with for many "targeted" therapies for

rare diseases.

As we discussed, I am asking the review team members to provide any feedback they have on your memo by COB tomorrow so you can finalize the memo. That step is necessary for the appeal process to be formally triggered and I think we all agree we need to move quickly to resolve this case on way or the other.

John

From: Woodcock, Janet
Sent: Monday, July 11, 2016 7:21 PM
To: Jenkins, John K; Unger, Ellis; Dunn, Billy; Bastings, Eric
Subject: MEMO

Here is a draft version of my decisional memo. I welcome comments on it. An appeal can't be done until I finalize this, I am told. Thanks. jw

From: Jenkins, John K
To: Woodcock, Janet; Unger, Ellis; Dunn, Billy; Bastings, Eric
Cc: Jenkins, John K
Subject: RE: MEMO
Date: Tuesday, July 12, 2016 5:25:01 PM

Janet

As we discussed in the hallway, I had some comments on your memo:

1. You said you did not consider data from the first three biopsies, but you do not say why. It was my understanding that the re-read of the first three biopsies for IHC was considered valid and the review team saw no change from baseline from the first biopsy to week 180. This is an important part of their perspective on the amount of dystrophin produced and its significance. They noted that the baseline ICH results from the patients in Study 201 were very different from the "new" baseline data that were conducted in parallel to the week 180 biopsy and that these differences were not explained. So, I think you need to explain why you are not considering the IHC data from the first three biopsies in your review, and instead focusing on the comparison of the "new" controls versus the week 180 IHC results.
2. I am not familiar with the references you added to the memo and cannot comment on your summary of the findings of each paper. Perhaps the review team know those references and can provide their take on the findings. Even taking your summaries at face value as accurate, I did not find the summary of the references to be particularly helpful in determining whether the dystrophin findings for eteplirsen are "reasonably likely" to predict clinical benefit. As you noted, there are issues related both to the quantity of dystrophin produced as well as its quality (functionality) that are hard to tease apart in the small sample of boys treated. It is possible that the "responders" are making functional protein, it is also possible they are making non-functional protein, and I don't think we have the data to sort this out. It still seems to be a judgment on the totality of evidence where reasonable people may disagree.
3. I find the correlation graphs at the end to be problematic since they graph a delta for a clinical endpoint on the y-axis against an absolute endpoint value for dystrophin on the x-axis. While one might assume the baseline dystrophin levels were low and therefore using the absolute endpoint value is a reasonable estimate of the effect of the drug on dystrophin (e.g., a surrogate for the delta); we don't know that and therefore it is hard to know whether the graphics are isolating a drug effect or capturing a prognostic difference that may have been present at baseline. So, I don't find them as convincing as you suggest, but again, that may be a judgment.
4. You focus on the "responders" in the population to support your argument, but we don't know how to identify the responders and therefore will have to approve based on the sponsor's grouping of mutations that are "amenable to exon 51 skipping," which likely include mutations that are not responsive to eteplirsen. This is a frustrating intersection of "targeted" therapies and approval based on a group mean based on characteristics chosen by the sponsor that we cannot validate. This is very analogous to the CF cases that we have been discussing and I think the review teams in both cases are struggling with these issues. It feels like we have to "unlearn" what we think we know from targeting and regress to

approving an un-validated grouping of mutations. I know we do this all the time for non-targeted therapies, but it is still discomforting to face this for targeted therapies. While the drug at the proposed dose has not been shown to be toxic, it will likely require a central line for chronic administration, which is not benign, and families/society will be burdened with a very expensive drug that may only be working in a small subset of the indicated population. This is an issue we will have to grapple with for many "targeted" therapies for rare diseases.

As we discussed, I am asking the review team members to provide any feedback they have on your memo by COB tomorrow so you can finalize the memo. That step is necessary for the appeal process to be formally triggered and I think we all agree we need to move quickly to resolve this case on way or the other.

John

From: Woodcock, Janet
Sent: Monday, July 11, 2016 7:21 PM
To: Jenkins, John K; Unger, Ellis; Dunn, Billy; Bastings, Eric
Subject: MEMO

Here is a draft version of my decisional memo. I welcome comments on it. An appeal can't be done until I finalize this, I am told. Thanks. jw

From: Rao, Ashutosh
To: Woodcock, Janet
Subject: RE: did you get my memo? jw
Date: Tuesday, July 12, 2016 2:19:47 PM

Hi Janet,

I did not catch anything technically wrong with the dystrophin method-related write-ups in my reading.

The points related to clinical efficacy and relevance are outside my expertise so I couldn't comment on them. From a non-clinician perspective, I would have liked to see a qualifier with this sentence "These patients also fared the best clinically" on page 13 to clarify if you meant 6MWT/NSAA/both.

I sensed that you wanted to keep the memo at a high-level and not get into very technical details. The following clarifying points might be worth keeping in mind or including if you think it would help. Most of these (except #4 below) are covered in the primary/secondary memos so I don't think you necessarily need them.

(1) the Sanger sequencing confirmed that the correctly skipped mRNA sequence was observed in the post-treated samples,
(2) a reference to spontaneous dystrophin from revertant fibers is missing and could be one cause for the variability in previous findings but previously published studies doesn't really suggest a consensus on the extent to which these contribute to the pathology or tx response. Having matched baseline samples from the same muscle in current and future studies could reduce some of the variability from any unusually high revertant fibers in some individuals,
(3) one of the challenges towards interpretation that isn't mentioned is the stability of the very large protein after de novo expression. Either due to the inflammatory environment or inherent structural instability in the novel/truncated form of the protein, the protein is likely is not very stable in vivo. At least two lines of work support this hypothesis – Ervasti's group has been unable to purify recombinant protein because it misfolds and degrades upon cloning and expression in traditional expression systems and Aartsma-Rus's group has shown that the skipped mRNA is inherently unstable because of its large size and this instability of transcripts with specific mutations is a critical determinant of protein levels (Spitali P et al, FASEB J, 2013).
(4) dystrophin is an extremely low abundance protein accounting for only 0.002% of the total striated muscle protein (Hoffman EP et al, Cell, 1987) so while it may be tempting to think of dystrophin as an "actin" or "myosin"-like structural/cytoskeletal protein, it isn't comparably abundant but apparently very critical in muscle function at very low levels relative to other proteins that are part of the machinery that drives muscle fiber function.

Hope this is helpful.

Ash

From: Woodcock, Janet

Sent: Tuesday, July 12, 2016 1:28 PM
To: Rao, Ashutosh
Subject: RE: did you get my memo? jw

Technically, did you find anything wrong? I incorporated most of your suggestions, and am looking at a bit more of the literature. jw

From: Rao, Ashutosh
Sent: Tuesday, July 12, 2016 10:30 AM
To: Woodcock, Janet
Subject: RE: did you get my memo? jw

Hi Janet,

I thought the memo reads very well. Some comments and suggested edits are attached. Please let me know if there are questions.

Thanks
Ash

From: Woodcock, Janet
Sent: Tuesday, July 12, 2016 9:32 AM
To: Rao, Ashutosh
Subject: did you get my memo? jw

Center Director Decisional Memo DRAFT

NDA#	206488
Drug Name:	EXONDYS 51™ (eteplirsen)
Indication:	Duchenne Muscular Dystrophy (DMD)
Sponsor:	Sarepta
Author:	Janet Woodcock, M.D. Director, Center for Drug Evaluation and Research, FDA

(b) (5)

16 pages of draft language have been withheld as b(5) immediately following this page

From: Throckmorton, Douglas C
To: Woodcock, Janet
Subject: FW: FDA Information: re: NDA 206488 / eteplirsen USPI
Date: Thursday, July 07, 2016 10:06:56 PM
Attachments: FDAedits-COMPLETE withNewTable-Round2_Sarepta Response 07 July 16 Redline Ver 2.docx

---

He's persistant. Anything I can do please let me know. Doug

Douglas C. Throckmorton MD
Deputy Director for Regulatory Programs
CDER, FDA
301-796-5400

From: Shamim Ruff [mailto:SRuff@Sarepta.com]
Sent: Thursday, July 07, 2016 2:32 PM
To: Choy, Fannie (Yuet)
Cc: Woodcock, Janet; Throckmorton, Douglas C; Shamim Ruff
Subject: RE: FDA Information: re: NDA 206488 / eteplirsen USPI

Dear Fannie,

Please find attached our reviewed version of the USPI. We have pretty much accepted all your requests and have only made minor tweaks to a few sentences.

As per my email yesterday, we really want to wrap up this filing by the end of day tomorrow; we are being contacted by the patients on daily basis on the status of this NDA.

Thank you for your help.

Regards,
Shamim

Shamim Ruff
SVP Regulatory Affairs and Quality
p 617-274-4009 c (b) (6)
e sruff@sarepta.com

SAREPTA

215 First Street, Cambridge, MA 02142

From: Choy, Fannie (Yuet) [mailto:Fannie.Choy@fda.hhs.gov]
Sent: Wednesday, July 06, 2016 8:26 PM
To: Shamim Ruff <SRuff@Sarepta.com>
Cc: Choy, Fannie (Yuet) <Fannie.Choy@fda.hhs.gov>
Subject: FDA Information: re: NDA 206488 / eteplirsen

Importance: High

Dear Shamim,

Attached please find the latest FDA working version of the draft labeling for NDA 206488 for eteplirsen. The base document is the firm's version dated June 16, 2016. Please provide any edits as tracked changes using our proposed text as the base.

-

-

Kindly confirm receipt of email.

-

Regards,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products
ODE I/OND/CDER
Food and Drug Administration
10903 New Hampshire Avenue, WO22 Rm. 4215
Silver Spring, MD 20993-0002
301-796-2899 phone
fannie.choy@fda.hhs.gov

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11 pages of draft labeling have been withheld as b(5) immediately following this page

From: Rao, Ashutosh
To: Woodcock, Janet
Subject: Fw: some additional explanation of dystrophin levels in exon 44 patients
Date: Thursday, July 07, 2016 2:43:38 PM
Attachments: image005.png
image006.png

Hi Janet,

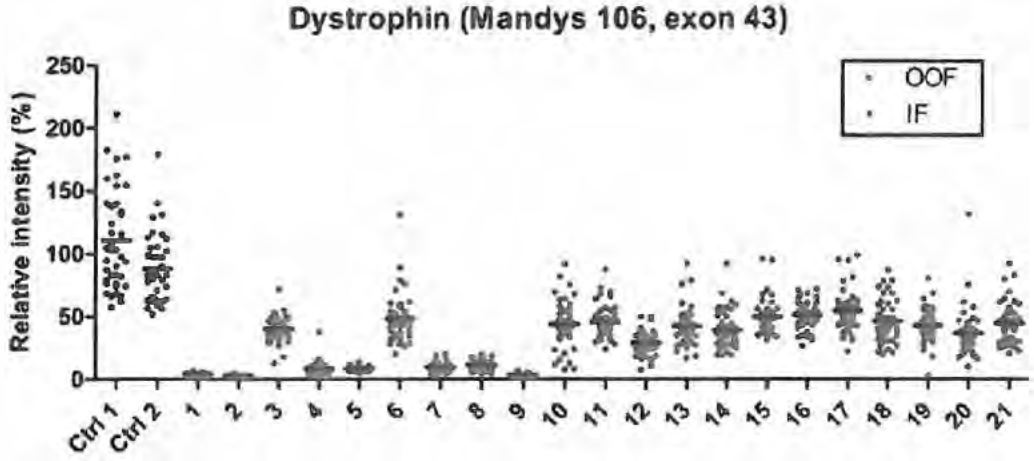
The reference is Anthony K, Neurology, et al 2014. See related email from Ron below.

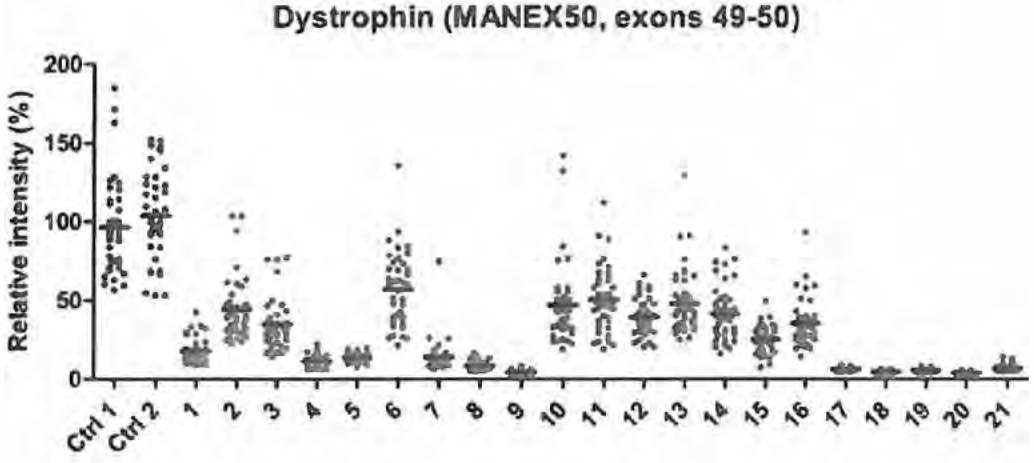
Thanks
Ash

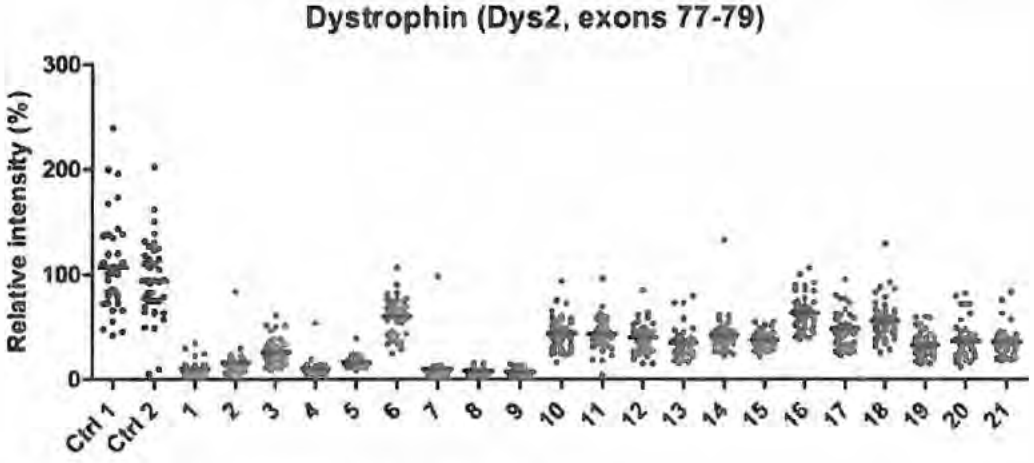
Sent from my BlackBerry 10 smartphone.

From: Farkas, Ronald <Ronald.Farkas@fda.hhs.gov>
Sent: Thursday, May 5, 2016 12:58 PM
To: Bastings, Eric; Dunn, Billy; Rao, Ashutosh; Breder, Christopher D; Unger, Ellis; Temple, Robert
Subject: some additional explanation of dystrophin levels in exon 44 patients

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%. The figure below, from the supplemental material for Anthony et al (2014)[23] 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.



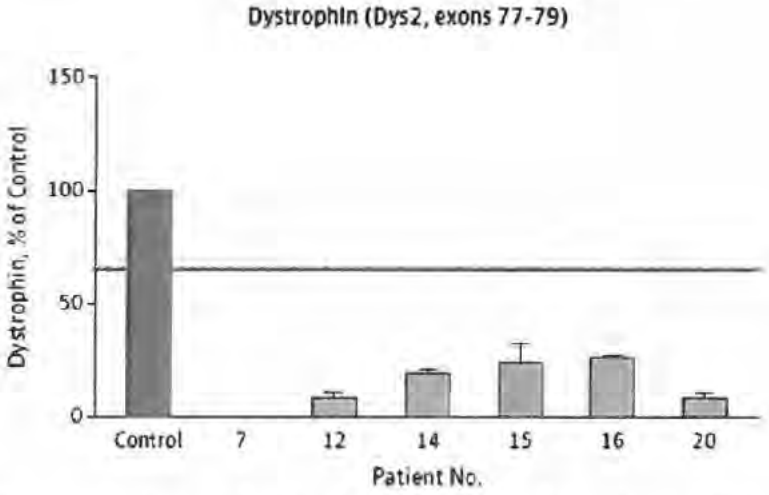




The following are key observations about this data:

- Among both exon 44 and 45 skippable patients, there is a wide range of relative dystrophin intensities. A number of other 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 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, and Patient 5 lost ambulation at age 12.
- Dystrophin levels in patients 3 and 6 (and perhaps patient 2) 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 are available [although not of ideal quality], and 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 2 have immunofluorescence levels similar to the in-frame BMD patients as measured within the same study, it may reasonable to conclude that dystrophin levels by Western blot might also have been, if measured, roughly similar, somewhere between about 10% and 25% of normal. Even considering the potentially large degree of error in such a "cross-method comparison" dystrophin levels were likely substantially higher than 1% of normal in these exon 44 patients with milder clinical course.

From: Behr, Virginia L
To: Woodcock, Janet
Subject: RE: Sarepta
Date: Wednesday, July 06, 2016 11:27:32 AM

Good to know. I'm sure you'll still be invited.

Virginia

From: Woodcock, Janet
Sent: Wednesday, July 06, 2016 11:07 AM
To: Behr, Virginia L
Subject: RE: Sarepta

Sounds like they are going to go with the informal. They will be talking to you. I don't need to attend the meeting with Rob. jw

---

From: Behr, Virginia L
Sent: Wednesday, July 06, 2016 7:14 AM
To: Woodcock, Janet
Subject: RE: Sarepta

Janet,

Acknowledging receipt. I will send you a fuller response by 8:15 this morning, as I understand you have a meeting with key OND leadership on this issue at 9am. I have a call at 9am with Doug T. about another matter, but will make myself available 9:30-10:30am if anyone wants to discuss dispute resolution options (both informal and formal) during your meeting. It may be good for everyone to hear the same information at the same time. I didn't ask to attend your meeting this morning, as I didn't want to interfere with the goals of the meeting. So, feel free to call me at 301 796 3436 during the last hour of your meeting with Ellis, John, etc. I'm teleworking.

Virginia L. Behr
CDER Ombudsman

(301) 796-3436
WO51, Room 6158
http://inside.fda.gov:9003/CDER/OfficeofExecutivePrograms/Ombudsman/default.htm

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From: Woodcock, Janet
Sent: Tuesday, July 05, 2016 8:11 PM
To: Behr, Virginia L
Subject: Sarepta

I ad already notified Rob that OND wanted to talk to him. It is important to get this done

ASAP. Thanks. Jw

From: Choy, Fannie (Yuet)
To: Bastings, Eric; Farkas, Ronald
Subject: RE: Northstar for latest assessment of Study 202
Date: Wednesday, July 06, 2016 7:23:38 AM
Attachments: FW Regulatory Contact re NDA 206488.msg

Hi,

Please see attached email for Week 240 results. Formal submission pending.

Thanks
Fannie

From: Bastings, Eric
Sent: Wednesday, July 06, 2016 1:38 AM
To: Choy, Fannie (Yuet); Farkas, Ronald
Subject: Northstar for latest assessment of Study 202

Did Sarepta submit the latest northstar results for study 202? If not, please request them.
Thanks.

Eric Bastings, MD
Deputy Director
Division of Neurology Products
CDER/OND

From: Califf, Robert
To: Woodcock, Janet
Subject: RE: eteplirsen NDA
Date: Tuesday, July 05, 2016 8:54:23 PM

Janet,

I'm here all week. I have a lunch with John J tomorrow, but I think that should be preserved for broader discussion. Th and Fri are pretty flexible as it now stands.

I want to make sure I have what is written about appeals so that I don't make an inadvertent mistake.

rmc

Robert M Califf MD
Commissioner of Food and Drugs

From: Woodcock, Janet
Sent: Tuesday, July 05, 2016 5:16 PM
To: Califf, Robert
Subject: Fwd: eteplirsen NDA

Would be helpful if you met w them. ASAP. Ellis also thought that the original western blot data was not real so did not expect would be facing this issue. I have been upfront with them all along. Wish someone would show some leadership. Catastrophe-predictions have been made at frequent intervals. Jw.

From: Unger, Ellis <Ellis.Unger@fda.hhs.gov>
Date: July 5, 2016 at 3:36:37 PM EDT
To: Woodcock, Janet <Janet.Woodcock@fda.hhs.gov>
Cc: Jenkins, John K <John.Jenkins@fda.hhs.gov>, Temple, Robert <Robert.Temple@fda.hhs.gov>
Subject: eteplirsen NDA

Janet,

I don't have to tell you how difficult the eteplirsen decision has been for many of us in ODE-I. As you know, we have reached different scientific conclusions on the strength of the data, and in particular, the likelihood that the small increase observed in Becker-type dystrophin is reasonably likely to predict clinical benefit. This decision could be precedent setting with respect to accelerated approval, i.e., where the bar should be set for changes in a pharmacodynamic biomarker that are deemed "reasonably likely to predict clinical benefit." Moreover, to my knowledge, this could be the first time a Center Director has overruled a review team (and an advisory committee) on a question of whether effectiveness has been demonstrated.

I know that Dr. Jenkins has mentioned the possibility of involving Dr. Califf in the eteplirsen decision on at least one occasion, and I would like to request a formal appeal to the Commissioner on this matter.

I'm aware that the Commissioner's official role is to consider the administrative aspects of review decisions and not the science. But given the potential for setting a precedent here, I think he should be aware of the various points of view and consider the potential ramifications of the matter at hand.

I'm also aware that you advised Sarepta that we would be prepared to grant accelerated approval of their NDA within 4 business days of receiving their new data, but there was a provision in the letter that the increase in dystrophin had to be meaningful, and we do not have agreement on this point. Thus, it is my hope that a Commissioner Briefing can be held before an action is taken.

I have discussed the above with Dr. Jenkins, and he supports this course of action.

I propose that we reserve a few minutes at the briefing tomorrow to discuss this matter.

Thank you for your consideration,

Ellis

From: Unger, Ellis
To: Woodcock, Janet
Cc: Jenkins, John K
Subject: RE: eteplirsen NDA
Date: Tuesday, July 05, 2016 7:01:30 PM

Thanks, Janet. We've contacted Virginia Behr to help start the process. Ellis

From: Woodcock, Janet
Sent: Tuesday, July 05, 2016 4:11 PM
To: Unger, Ellis
Subject: RE: eteplirsen NDA

I'm happy to have you brief Rob on this, if he is available. Janet W

From: Unger, Ellis
Sent: Tuesday, July 05, 2016 3:37 PM
To: Woodcock, Janet
Cc: Jenkins, John K; Temple, Robert
Subject: eteplirsen NDA

Janet,

I don't have to tell you how difficult the eteplirsen decision has been for many of us in ODE-I. As you know, we have reached different scientific conclusions on the strength of the data, and in particular, the likelihood that the small increase observed in Becker-type dystrophin is reasonably likely to predict clinical benefit. This decision could be precedent setting with respect to accelerated approval, i.e., where the bar should be set for changes in a pharmacodynamic biomarker that are deemed "reasonably likely to predict clinical benefit." Moreover, to my knowledge, this could be the first time a Center Director has overruled a review team (and an advisory committee) on a question of whether effectiveness has been demonstrated.

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I'm aware that the Commissioner's official role is to consider the administrative aspects of review decisions and not the science. But given the potential for setting a precedent here, I think he should be aware of the various points of view and consider the potential ramifications of the matter at hand.

I'm also aware that you advised Sarepta that we would be prepared to grant accelerated approval of their NDA within 4 business days of receiving their new data, but there was a provision in the letter that the increase in dystrophin had to be meaningful, and we do not have agreement on this point. Thus, it is my hope that a Commissioner Briefing can be held before an action is taken.

I have discussed the above with Dr. Jenkins, and he supports this course of action.

I propose that we reserve a few minutes at the briefing tomorrow to discuss this matter.

Thank you for your consideration,

Ellis

From: Jenkins, John K
To: Woodcock, Janet
Cc: Jenkins, John K
Subject: FW: Sarepta NDA 206488 Inspection Summary
Date: Sunday, July 03, 2016 3:22:51 PM

Janet

Here is a table of the dystrophin data that Ellis made as part of his review. I asked Sharnell to schedule a meeting with you on Wednesday morning to discuss two main issues with the team:

1. Whether the pharmacodynamic data showing an increase in dystrophin in some boys meets the evidentiary standards for "reasonably likely to predict clinical benefit" for AA.

2. The design of PMRs.

I hope to receive the DNP/ODE1 draft reviews including the new data today or early tomorrow. Based on a phone meeting I had with the team on Friday I expect both with recommend CR. I'd like to review their rationale and then hear your perspective. Would you be free for a call tomorrow afternoon or evening?

On the PMR front, a placebo controlled trial in another exon for confirmation is not going to be feasible given the ethics of IV placebo administration to children that may involve some form of central line. So, we need to discuss the dose ranging options for the ongoing, or a new trial, with eteplirsen, and interpretable options for the other exons. A dose ranging trial that "won" would clearly be interpretable, but as you know, a "failure" on clinical endpoints may not be completely interpretable. We need to reach agreement on what we would ask Sarepta to commit to and I asked the team to hold off on further communications with Sarepta on this issue until we meet on Wednesday.

Let me know a good time for us to discuss these issues tomorrow. I can be available pretty much anytime, but prefer afternoon or evening since I'd like to read the DNP/ODE1 reviews before I speak to you.

John

From: Rao, Ashutosh
To: Woodcock, Janet; Moscicki, Richard
Cc: Kozlowski, Steven; Rosenberg, Amy
Subject: Sarepta inspection - draft memo
Date: Wednesday, June 29, 2016 5:34:06 PM
Attachments: NDA 206488 Inspection Summary_v3.docx

Dear Janet and Rich,

I have attached a draft memo based on the inspection of Sarepta's laboratory site visit for your review and feedback. I welcome any suggested edits or comments. Please let me know if there are any questions.

A more comprehensive EIR (inspection report) with OSIS and ORA portions will take a bit longer to put together but I can share with you any of the exhibits (raw data files, notebooks, screenshots, photographs) now for additional clarification if needed. OSIS covered the blinding and shipping. ORA is putting everything together as per their requirements.

Thanks
Ash

IND/BLA/NDA:	IND 077429/ NDA 206488
TO:	Billy Dunn, M.D. (Director, Division of Neurology Products/ODE1)
FROM:	Ashutosh Rao, Ph.D. (Laboratory Chief, DBRR III/OBP/OPQ/CDER)
THROUGH:	Amy Rosenberg, M.D. (Director, DBRR III/OBP/OPQ/CDER) Steven Kozlowski, M.D. (Director, OBP/OPQ/CDER)
SUBJECT:	Review of dystrophin bioassays observed during inspection and related study report SR-CR-16-003
SPONSOR:	Sarepta Therapeutics
PRODUCT:	Eteplirsen (EXONDYS 51) is a phosphorodiamidate morpholino oligomer designed to bind to exon 51 of the human dystrophin pre-mRNA and intended to cause skipping of exon 51 to generate an internally truncated dystrophin protein. It is supplied as a 2 mL vial containing 100 mg (50 mg/mL) and single use 10 mL vial containing 500 mg (50 mg/mL) preservative –free solution.
INDICATION:	For the treatment of Duchenne muscular dystrophy (DMD) in patients with a confirmed gene mutation amenable to exon 51 skipping.
ROUTE OF ADMIN.	Intravenous (IV) infusion
CLINICAL DIVISION:	Division of Neurology Products (ODE1/OND/CDER)

Executive Summary:

The conduct of the western blotting procedure for the biopsy samples from study 4658-301 appeared to be within the scope of the sponsor's predetermined standard operating protocol SR-CR-16-003. The inspection confirmed technical compliance with the methodology, verified sample blinding throughout the procedure, confirmed that the procurement and analysis of raw data with passing acceptance criteria was used for % dystrophin calculations and successfully verified the same data in the study report '4658-301 Week 48 Interim Analysis' submitted to the agency.

The Sponsor could improve upon the robustness of the detection portion of the method by adopting automated and digitized detection systems and reference standards with lower variability in the future.

Background:

A limited, high-priority PDUFA inspection of a Sponsor's Laboratory Testing Site at Corvallis, OR, was conducted between June 20-24, 2016, upon request from the Division of Neurology Products, and per FACTS assignment # 11648400. The inspection assignment requested observation of the laboratory's conduct of a western blotting analytical procedure, real time confirmation of the integrity of the associated data generated from the procedure, as well as an assessment of the firm's adherence to their predefined protocols and blinding procedures. This inspection and the laboratory's performance of the western blotting procedures are associated with the Sponsor's study protocol 4658-301 (PROMOVI) titled, "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". The study is being conducted under IND # 077429, in support of Sarepta Therapeutics, Inc.'s New Drug Application (NDA) # 206488. The inspection was conducted by myself and Young Moon Choi, Ph.D. (Lead Pharmacologist,

OSIS), and Mark Babbit (Investigator, ORA). This summary provided in this memo and requested by the Division of Neurology Products specifically addresses the dystrophin analytical aspects observed on site by me and a review of the report submitted by the Sponsor on 6/27/2016 based on the data obtained during inspection. The inspection did not include an assessment of Good Laboratory Practices or current Good Manufacturing Practices. Please refer to the Establishment Inspection Report (EIR) under the FEI number 3009712573 for a full description of the inspectional items.

For this purposes of this memo, the term 'observation' refers to the observed activities related to the bioassay method and not an objectionable compliance action. No objectionable FDA Form 483 observations were issued to the Sponsor. The first part of this memo summarizes the observations made during inspections regarding the control samples, western blotting procedure, dystrophin quantitation, and data analysis. The second part of the memo describes concurrence with the data set provided in study report SR-CR-16-003. It does not address the clinical efficacy or review the clinical interpretation of the % dystrophin values reported.

1. Summary of inspectional findings at Sarepta's Corvallis, OR, Laboratory Testing Site that conducted an interim dystrophin analyses of biopsy samples from study 4658-301 (PROMOVI) by western blot:

The finalized western blotting protocol SR-CR-16-003 and its appendices were used as a reference during the observation of the analytical procedure with samples from study 4658-301.

The control samples: The normal control NC-5 was originally designated as C14-23 and obtained from (b) (4) (b) (4) tissue bank (b) (4) NC-5 was obtained from the biceps of a 14 year old male at (b) (4) and as per the specimen report provided by the Sponsor, which noted that this subject had no pathological diagnosis. The sample NC-5 (b) (4) was used with the week 180 samples from study 202. (b) (4) (b) (4) (b) (4) The untreated DMD controls were obtained from the PROMOVI study. Six untreated DMD samples were tested and the three with the lowest % dystrophin values were used as a pooled sample of the Negative Control. They were not from the week 48 but from the patients randomized to the week 24, 72, or 96 groups.

A copy of the Biopsy Specimen Collection and Examination Form was reviewed for each of the normal, DMD, and study 301 biopsy samples. The pathological examination was performed by (b) (4). It was noted that all samples were considered acceptable based on physical examination, measurement, absence of evidence of crushing by forceps, absence of freezing artifacts, fibrotic and/or adipose tissue content using H&E stained sections, and fiber orientation. No samples appear to be rejected based on the quality assessment in their tissue allocation SOP. A copy of the exon mutations and patient ages of each of the blinded samples was provided by the Sponsor, without reference to the sample identification.

Sample designation and western blot procedure: Each pair of blinded, individual patient samples were randomized and randomly labeled as either 'Ford' or 'Chevy' (b) (4). The samples were shipped from (b) (4) and stored at -20°C at Corvallis, OR. (b) (4)

(b) (4)

Quantitation of images and data analyses: Each of the films was analyzed for dystrophin band density with ImageQuant (version 8.1) software (b) (4). A PowerPoint presentation with each of the steps involved and as observed on June 21-24 was provided by the Sponsor.

(b) (4)

The Microsoft Excel table print-outs provided by the Sponsor showed the interim analysis with raw numbers of % dystrophin, the R-square value, whether the R-square value was a pass or fail

(b) (4)

At the end of the inspection, two CD-ROMs were provided by Mr. Voss with all raw and analyzed data files. The inspection was closed with a scientific discussion with (b) (4) (b) (4) John Voss, M.S., and (b) (4) about (1) need for improvement of the current western blotting with a more robust detection and quantitation method that allows consistent quantitation at low levels of dystrophin, (2) the need for more robust assays, such as quantitative mass spectrometry, with greater precision and (3) the need for a more reliable reference standard, such as recombinant protein or cell line-based extracts, with lower inherent variability to allow precise quantitation of relative % normal dystrophin. The Sponsor acknowledged the feedback and stated that they are in the process of further developing their protein analyses methods and will be submitting a proposal for using a skeletal muscle myoblast cell line-based reference standard in the near future.

Reviewer's comments: *The western blotting procedure for the biopsy samples appeared to have been conducted within the scope of the sponsor's predetermined standard operating procedures. I and the other FDA inspectors followed the western blotting procedure from the removal of samples from the freezer to the densitometric quantitation and did not observe any inappropriate manipulation. At no point did we have reason to believe that the sample blinding was compromised. The technicians were observed to be diligent and competent in the performance of the bioassay. The Sponsor could improve upon the consistency of the detection portion of the method and was advised to consider other more robust detection systems and reference standards in the future. Each of the additional analyses conducted in our presence, such as the overlaid chromatogram traces, appeared to be obtained with a sound scientific justification of its usefulness to clarify the relative dystrophin levels between the samples as observed with the protocol.*

2. Review of dystrophin bioassay information from study 4658-301 in Sarepta's NDA amendment 42 and study report SR-CR-16-003 submitted on 6/27/2016:

Based on a review of the study report SR-CR-16-003, I was able to match each of the data points that passed acceptance criteria and used for their data table on page 17 (**Appendix 5**). Powerpoint slides were provided to the Division of Neurology Products (Dr. Ron Farkas) on 6/28/2016 showing a line-by-line comparison of each of the data points with QC-checked summary tables we were provided during the on-site inspection.

The following data points from the failed gels didn't match the summary data table I had from the inspection but did match the original worksheet from the technicians. Neither of these data points was used in the analyses by the sponsor because these are from failed gels so they should not impact any of the mean values.

1. Patient ID 301-07, Gel 13, we were given 0 and 0 as the numbers for lane 7 and 8. The sponsor has reported 0.04 and 0.22. The original data worksheet confirms 0.04 and 0.22. This gel failed its R-square acceptance criteria so this data point is not included in the sponsor's analysis.

2. Patient ID 301-12, Gel 24, we were given 0.02 as the value for Lane 7. The sponsor has reported 0.01. The original data worksheet confirms 0.01. This gel failed its R-square acceptance criteria so this data point is not included in the sponsor's analysis.

Reviewer's comments: *The raw % dystrophin data that passed predefined acceptance criteria and submitted by the Sponsor was in agreement with the raw data obtained on site at the Corvallis, OR, testing laboratory. The two exceptions noted above for the data that failed quality control assessments were in agreement with the original data worksheets and not used for calculation of the % dystrophin values and hence should not impact the overall findings.*

The Division of Neurology Products (ODE1/OND) will be conducting a review of the clinical efficacy and interpretation of the clinical implications of the % dystrophin findings.

Appendix 1

Western blot analysis schedule and sample loading sequence of the gels (provided by Sarepta)

WESTERN BLOT ANALYSIS SCHEDULE

DAY 1-2: JUNE 20 & 21 2016

Gel #	Box	Lane									
		1	2	3	4	5	6	7	8	9	10
1	1a	HMW	4%	2%	1%	0.5%	0.25%	Ford-22559 (1.5X)	Chevy-22559 (1.5X)	Neg Ctrl	HMW
2	1b	HMW	4%	2%	1%	0.5%	0.25%	Ford-22559 (1.5X)	Chevy-22559 (1.5X)	Neg Ctrl	HMW
3	2a	HMW	4%	2%	1%	0.5%	0.25%	Ford-27336 (2X)	Chevy-27336 (2X)	Neg Ctrl	HMW
4	2b	HMW	4%	2%	1%	0.5%	0.25%	Ford-27336 (2X)	Chevy-27336 (2X)	Neg Ctrl	HMW
5	3a	HMW	4%	2%	1%	0.5%	0.25%	Ford-24422 (1X)	Chevy-24422 (2X)	Neg Ctrl	HMW
6	3b	HMW	4%	2%	1%	0.5%	0.25%	Ford-24422 (1X)	Chevy-24422 (2X)	Neg Ctrl	HMW
7	4a	HMW	4%	2%	1%	0.5%	0.25%	Ford-27138 (1X)	Chevy-27138 (1X)	Neg Ctrl	HMW
8	4b	HMW	4%	2%	1%	0.5%	0.25%	Ford-27138 (1X)	Chevy-27138 (1X)	Neg Ctrl	HMW
9	5a	HMW	4%	2%	1%	0.5%	0.25%	Ford-28500 (2.5X)	Chevy-28500 (1X)	Neg Ctrl	HMW
10	5b	HMW	4%	2%	1%	0.5%	0.25%	Ford-28500 (2.5X)	Chevy-28500 (1X)	Neg Ctrl	HMW
11	6a	HMW	4%	2%	1%	0.5%	0.25%	Ford-24986 (1X)	Chevy-24986 (2X)	Neg Ctrl	HMW
12	6b	HMW	4%	2%	1%	0.5%	0.25%	Ford-24986 (1X)	Chevy-24986 (2X)	Neg Ctrl	HMW

DAY 3-4: JUNE 22 & 23 2016

Gel #	Box	Lane									
		1	2	3	4	5	6	7	8	9	10
13	1a	HMW	4%	2%	1%	0.5%	0.25%	Ford-20841 (1X)	Chevy-20841 (2X)	Neg Ctrl	HMW
14	1b	HMW	4%	2%	1%	0.5%	0.25%	Ford-20841 (1X)	Chevy-20841 (2X)	Neg Ctrl	HMW
15	2a	HMW	4%	2%	1%	0.5%	0.25%	Ford-22355 (1.5X)	Chevy-22355 (1.5X)	Neg Ctrl	HMW
16	2b	HMW	4%	2%	1%	0.5%	0.25%	Ford-22355 (1.5X)	Chevy-22355 (1.5X)	Neg Ctrl	HMW
17	3a	HMW	4%	2%	1%	0.5%	0.25%	Ford-28907 (2X)	Chevy-28907 (1X)	Neg Ctrl	HMW
18	3b	HMW	4%	2%	1%	0.5%	0.25%	Ford-28907 (2X)	Chevy-28907 (1X)	Neg Ctrl	HMW
19	4a	HMW	4%	2%	1%	0.5%	0.25%	Ford-29648 (2X)	Chevy-29648 (2X)	Neg Ctrl	HMW
20	4b	HMW	4%	2%	1%	0.5%	0.25%	Ford-29648 (2X)	Chevy-29648 (2X)	Neg Ctrl	HMW
21	5a	HMW	4%	2%	1%	0.5%	0.25%	Ford-29727 (1X)	Chevy-29727 (1X)	Neg Ctrl	HMW
22	5b	HMW	4%	2%	1%	0.5%	0.25%	Ford-29727 (1X)	Chevy-29727 (1X)	Neg Ctrl	HMW
23	6a	HMW	4%	2%	1%	0.5%	0.25%	Ford-29751 (1X)	Chevy-29751 (1X)	Neg Ctrl	HMW
24	6b	HMW	4%	2%	1%	0.5%	0.25%	Ford-29751 (1X)	Chevy-29751 (1X)	Neg Ctrl	HMW

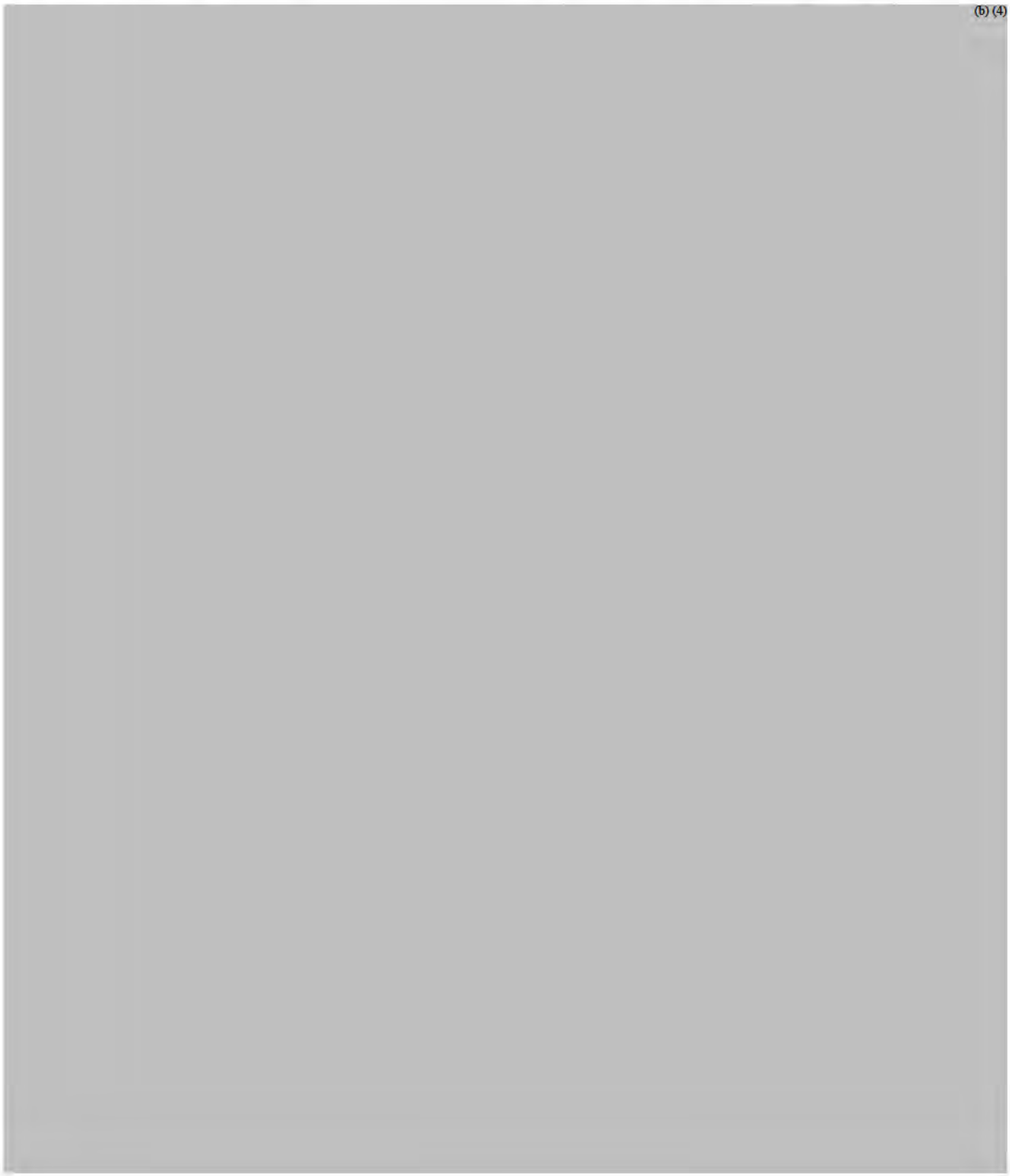
WESTERN BLOT ANALYSIS SCHEDULE

DAY 3-4: JUNE 22 & 23 2016

Gel #	Box	Lane									
		1	2	3	4	5	6	7	8	9	10
25	7a	HMW	4%	2%	1%	0.5%	0.25%	Ford-25715 (1X)	Chevy-25715 (2X)	Neg Ctrl	HMW
26	7b	HMW	4%	2%	1%	0.5%	0.25%	Ford-25715 (1X)	Chevy-25715 (2X)	Neg Ctrl	HMW

Appendix 2

Raw dystrophin antibody-probed membranes for each of the western blot samples (Images provided by Sarepta)



Appendix 3

Three examples of chromatographic traces of the dystrophin quantitation from Lanes 7 and 8 using ImageQuant software (provided by Sarepta)

Image Filename: SR-CR-16-003_Gel#5_DYS1_30min.tif

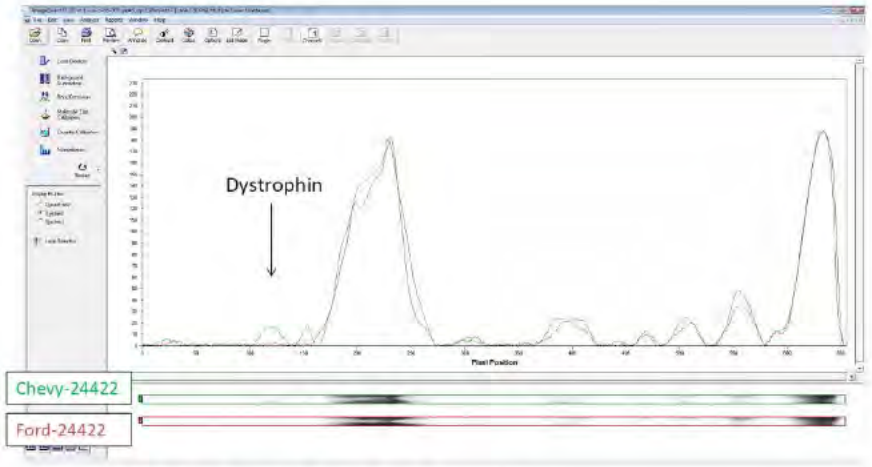


Image Filename: SR-CR-16-003_Gel#9_DYS1_30min.tif

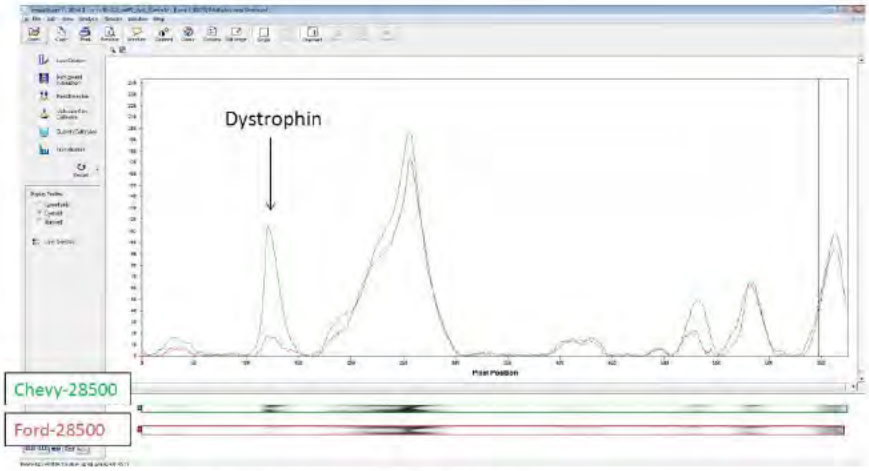
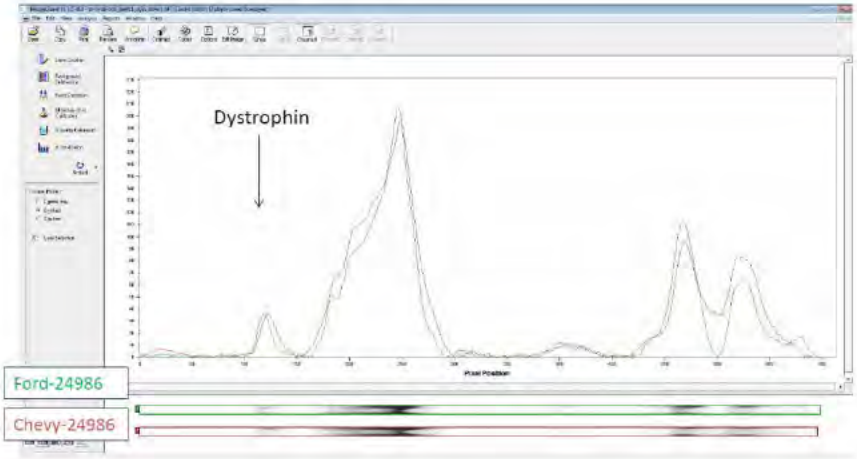


Image Filename: SR-CR-16-003_Gel#11_DYS1_30min.tif



Appendix 4

Summary raw data tables showing all individual data points and whether they passed or failed acceptance criteria from the 15, 20, or 30 minute film exposures (Three tables below provided by Sarepta)

SR-CR-16-003: DYS1 - 15 minute exposure							
Gel	Box	% Dystrophin (Lane 7)	% Dystrophin (Lane 8)	R2 Value	R2 ≥ 0.90	0.25%NC (Neg CT)	Neg CT <0.25%
13	1a	0.00	0.00	0.38	Fail	67389 (47602)	Pass
14	1b	0.17	0.42	0.97	Pass	64418 (58077)	Pass
15	2a	0.08	0.08	0.95	Pass	37476 (58536)	Fail
16	2b	0.14	0.05	0.83	Fail	21696 (20615)	Pass
17	3a	1.17	0.14	0.98	Pass	22073 (35280)	Fail
18	3b	1.57	0.24	0.98	Pass	49106 (37627)	Pass
19	4a	0.11	0.12	0.93	Pass	40030 (8873)	Pass
20	4b	0.05	0.11	0.98	Pass	35884 (39241)	Fail
21	5a	0.31	0.01	0.98	Pass	110706 (48990)	Pass
22	5b	0.63	0.08	0.93	Pass	93278 (52055)	Pass
23	6a	0.09	0.02	0.91	Pass	77556 (51352)	Pass
24	6b	0.02	0.00	0.78	Fail	108111 (64389)	Pass
25	8a	0.34	0.34	0.96	Pass	38943 (83782)	Fail
26	8b	0.18	0.21	0.97	Pass	20460 (19812)	Pass

SR-CR-16-003: DYS1 - 20 minute exposure							
Gel	Box	% Dystrophin (Lane 7)	% Dystrophin (Lane 8)	R2 Value	R2 ≥ 0.90	0.25%NC (Neg CT)	Neg CT <0.25%
1	1a	0.14	0.27	0.99	Pass	14425 (8649)	Pass
2	1b	0.07	0.21	0.96	Pass	39798 (15235)	Pass
3	2a	0.36	0.35	0.99	Pass	26926 (14147)	Pass
4	2b	0.10	0.12	0.90	Pass	47296 (73361)	Fail
5	3a	0.13	0.50	0.98	Pass	22397 (31305)	Fail
6	3b	0.09	0.22	1.00	Pass	64945 (29089)	Pass
7	4a	0.04	0.08	0.95	Pass	44795 (16235)	Pass
8	4b	0.04	0.13	0.89	Fail	60880 (35534)	Pass
9	5a	0.10	1.08	0.80	Fail	86479 (40898)	Pass
10	5b	0.07	0.74	1.00	Pass	33696 (13462)	Pass
11	6a	0.30	0.37	0.97	Pass	31429 (30177)	Pass
12	6b	0.15	0.18	0.94	Pass	23477 (32971)	Fail
13	1a	0.01	0.01	0.11	Fail	87486 (71878)	Pass
14	1b	0.02	0.23	0.96	Pass	114817 (88315)	Pass
15	2a	0.04	0.05	0.90	Pass	82129 (91951)	Pass
16	2b	0.34	0.06	0.72	Fail	41439 (28302)	Pass
17	3a	0.91	0.08	0.99	Pass	78991 (47683)	Pass
18	3b	2.01	0.47	0.79	Fail	72064 (110397)	Fail
19	4a	0.01	0.22	0.91	Pass	78641 (33905)	Pass
20	4b	0.01	0.03	0.93	Pass	79184 (88118)	Fail
21	5a	0.09	0.00	0.75	Fail	187873 (38528)	Pass
22	5b	0.54	0.02	0.94	Pass	127964 (74633)	Pass
23	6a	0.00	0.00	0.80	Fail	131413 (58017)	Pass
24	6b	0.00	0.00	0.10	Fail	133242 (88876)	Pass
25	8a	0.20	0.32	0.65	Fail	33377 (88614)	Fail
26	8b	0.08	0.08	0.91	Pass	39914 (30132)	Pass

SR-CR-16-003: DYS1 - 30 minute exposure							
Gel	Box	% Dystrophin (Lane 7)	% Dystrophin (Lane 8)	R2 Value	R2 ≥ 0.90	0.25%NC (Neg CT)	Neg CT <0.25%
1	1a	0.15	0.22	0.98	Pass	28142 (11562)	Pass
2	1b	0.11	0.29	0.99	Pass	43028 (10859)	Pass
3	2a	0.49	0.50	0.96	Pass	25657 (32471)	Fail
4	2b	0.12	0.26	0.92	Pass	49843 (76849)	Fail
5	3a	0.06	0.50	0.99	Pass	25008 (24738)	Pass
6	3b	0.06	0.24	0.99	Pass	64307 (34135)	Pass
7	4a	0.04	0.10	0.96	Pass	41141 (20779)	Pass
8	4b	0.06	0.19	0.83	Fail	51902 (41555)	Pass
9	5a	0.10	0.92	0.87	Fail	97732 (52789)	Pass
10	5b	0.17	1.02	0.98	Pass	60795 (11082)	Pass
11	6a	0.42	0.48	0.96	Pass	32341 (37122)	Fail
12	6b	0.29	0.46	0.96	Pass	29677 (34028)	Fail
13	1a	0.21	0.33	0.74	Fail	57768 (65008)	Fail
14	1b	0.04	0.77	0.70	Fail	102231 (81450)	Pass
15	2a	0.03	0.03	0.91	Pass	76752 (110138)	Fail
16	2b	0.34	0.04	0.71	Fail	46083 (40425)	Pass
17	3a	1.11	0.08	0.96	Pass	60216 (61057)	Fail
18	3b	3.91	0.48	0.99	Pass	81999 (89679)	Fail
19	4a	0.09	0.15	0.93	Pass	58708 (28376)	Pass
20	4b	0.00	0.05	0.95	Pass	66033 (80195)	Fail
21	5a	0.11	0.00	0.28	Fail	175676 (80890)	Pass
22	5b	0.49	0.03	0.92	Pass	110870 (50983)	Pass
23	6a	0.02	0.00	0.86	Fail	95415 (92390)	Pass
24	6b	0.00	0.00	0.19	Fail	127200 (95678)	Pass
25	8a	0.10	0.11	0.28	Fail	48731 (108832)	Fail
26	8b	0.04	0.08	0.90	Pass	42916 (29626)	Pass

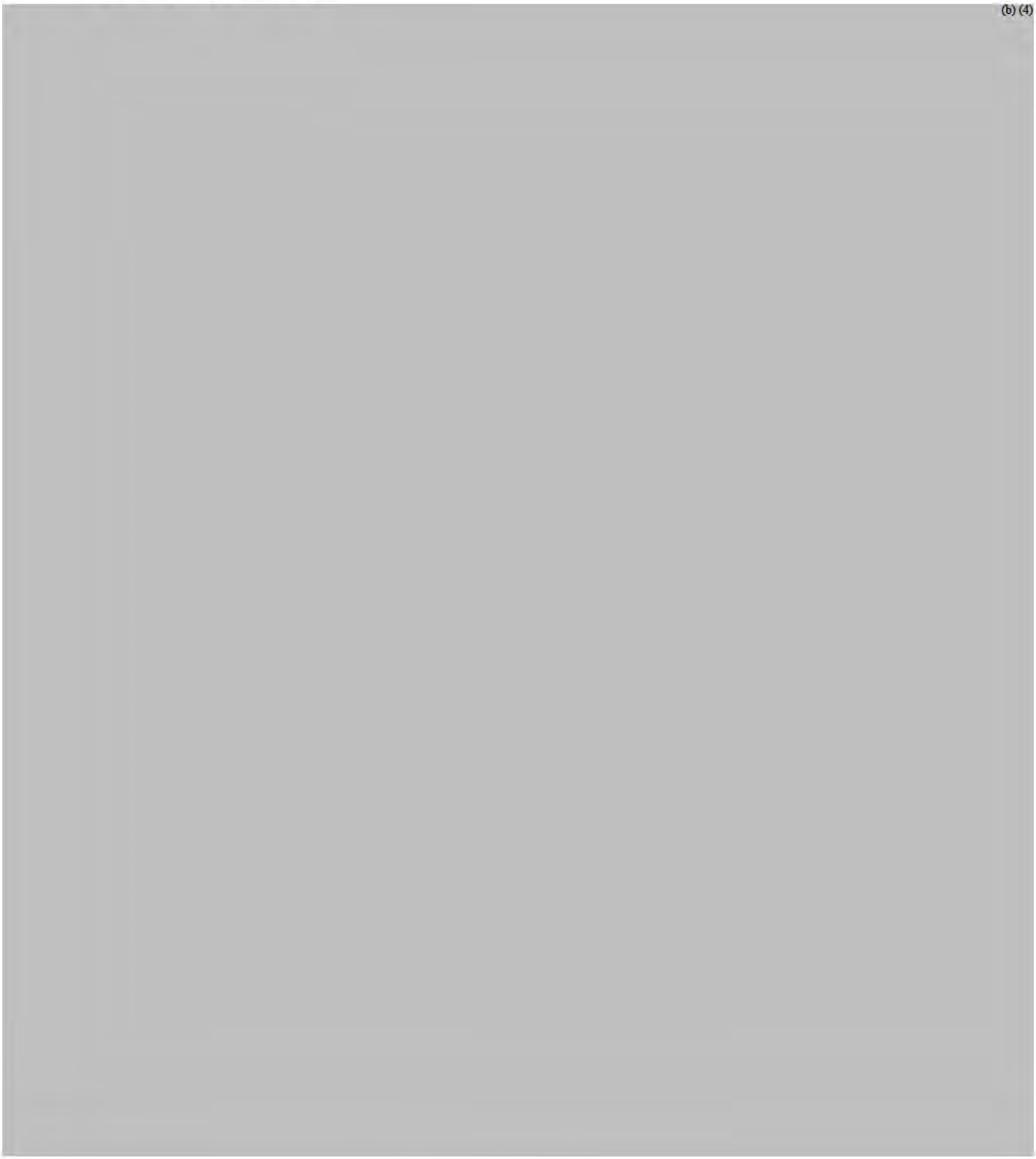
Appendix 5

Data listing from report 4658-301-SR-CR-16 of Dystrophin Western blot results with all raw values (provided by Sarepta)

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

June 27, 2016

(b) (4)



From: Jenkins, John K
To: Woodcock, Janet
Subject: RE: Sarepta
Date: Tuesday, June 28, 2016 6:58:37 PM

Janet

I would like to talk to you tonight or early tomorrow about the path forward on this application. I just finished a long telecon with the ODE1/DNP review team and we need some guidance/input from you on some issues.

I am free for a call until about 10 pm tonight (still recovering from 14 hour time change yesterday, so hopefully I can stay awake that long) or any time after 7 am tomorrow. My home number is (b) (6)

John

From: Woodcock, Janet
Sent: Tuesday, June 28, 2016 8:55 AM
To: Jenkins, John K
Subject: Sarepta

I spoke to Ash and looked at what he sent and some summary data the firm sent me. They said they were submitting the data to the division yesterday but maybe they waited till today

The drug is clearly inducing production of dystrophin in about half of patients at low levels. Ash told me-he was at the award ceremony-that you could even distinguish different deleted exons by the length of the resultant protein (the samples were run in duplicate).

The achieved levels were low but this data can be combined with the four year results

I will be out third and fri but completely reachable. Jw

From: Ligon, Sharnell (CDER)
To: Woodcock, Janet
Subject: Fw: Request for Tcon
Date: Sunday, June 26, 2016 10:22:23 AM

Hi Dr. Woodcock,

Not sure if you saw this, but Shamim is requesting to speak with you today.

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

From: Shamim Ruff <SRuff@Sarepta.com>
Sent: Saturday, June 25, 2016 1:59 PM
To: Woodcock, Janet
Cc: Ligon, Sharnell (CDER)
Subject: Request for Tcon

Dear Dr Woodcock,

We have just received the unblinded data for the PROMOVI baseline and Week 48 Western blot assays from the CRO. We are currently working to prepare a high level summary to send to you tomorrow and would like to follow up with a telephone call to walk you through the results. Is there any possibility to speak to you tomorrow late afternoon/early evening? If that is not possible, we would request to "meet" with you, Dr Moscicki and Dr Rao on Monday. The only time we can't make on Monday is 9-10am as we have a Shareholders meeting.

I apologize for contacting you on a Saturday but Dr Kaye and I believe it is very important that we brief you as soon as possible.

Best regards,
Shamim

Shamim Ruff
SVP Regulatory Affairs and Quality
p 617-274-4009 c (b) (6)
e sruff@sarepta.com

SAREPTA

215 First Street, Cambridge, MA 02142

From: Rao, Ashutosh
To: Woodcock, Janet
Cc: Moscicki, Richard
Subject: RE: URGENT: Follow Up From Telecon This Morning
Date: Friday, June 03, 2016 1:35:14 PM

Dear Janet,

Thanks for your email. Some follow-up thoughts -

1. We should check with the clinical division to make sure they agree with the protocol and suggested deviations. For instance, they say that they will need to unblind the samples and adapt their previous protocol for this testing. The changes in protocol should not undermine the other endpoints in the clinical study for its primary/secondary endpoints, otherwise we may be risking the whole study and its value.
2. If the clinical division is in agreement with the modified approach and risk, I believe it would be best to onsite for a targeted visit between June 20-24 because, according to the flow chart they provided, this is when they will be running the western blots and starting to prepare data tables. I don't think we need to be there for the receipt of consumables, equipment, and other logistical steps but we could have an ORA person there if desired. My task during the visit would be to (1) observe the western blotting procedure for the clinical samples from their ongoing study, (2) confirm compliance with the their pre-established SOP, (3) confirm the documentation to support blinding/unblinding steps (I will need help from OSIS for this), and (4) observe them enter raw data into a format that could be verified when submitted as a formal submission to the Agency.
3. For the inspection, we should have an OSIS (formerly OSI) person go with me because the visit should be appropriately documented and reported. One OSI inspector could go to the Iowa site and one would go with me. Sean Kassim is the OD for OSIS.
4. If this gets a final 'go ahead', I will need help from your office or OSI with expedited travel arrangements and would prefer that this visit is not advertised outside FDA to avoid any unwanted attention during or after the visit. Once completed, the visit and inspection report will become part of the official record.

I have discussed this with Steve and Amy and they are okay with this strategy.

Best,
Ash

From: Woodcock, Janet
Sent: Friday, June 03, 2016 8:46 AM
To: Rao, Ashutosh
Subject: Fwd: URGENT: Follow Up From Telecon This Morning
Importance: High

FYI. I will ask them to send the protocol jw.

From: Shamim Ruff <SRuff@Sarepta.com>
Date: June 2, 2016 at 6:17:35 PM EDT
To: Moscicki, Richard <Richard.Moscicki@fda.hhs.gov>, Woodcock, Janet <Janet.Woodcock@fda.hhs.gov>
Cc: Ed Kaye <EKaye@Sarepta.com>
Subject: URGENT: Follow Up From Telecon This Morning
Importance: High

Dear Dr. Woodcock and Dr Moscicki

Thank you for the discussion this morning. Based on our conversation, if we reduce the dystrophin procedures down to the bare essentials, we could perform the analyses by the end of June; this is assuming the process goes perfectly the first time, without any delays or repeats. We have discussed with our team FDA's request to expedite the dystrophin analysis. In order to meet this request, we need FDA to agree to the following conditions:

1. **We must start the process by June 6, 2016.** There is no room for flexibility with this date due to our dire financial constraints as a result of the ongoing delays.
2. **Dr. Rao will be an observer/advisor throughout the whole process** (3-4 weeks during June in Iowa and Oregon) since he is the only FDA representative with the requisite knowledge, expertise and familiarity with the eteplirsen dystrophin analyses/protocol.
3. Dystrophin assays will be conducted using an adapted, pre-defined protocol based on the Week 180 methodology, e.g. no blinding.
4. A different "normal" control to Week 180 will be used due to lack of availability of previous/Week 180 normal control tissue.
5. Non-GLP facility - the assays will be performed at the Sarepta Corvallis site in Oregon by trained Sarepta personnel. As discussed, our Corvallis site is in the process of being closed down so will not be in an ideal state although the lab is still functional.

Deliverables:

- Success is defined as demonstration of an increase in dystrophin using Western Blot assay.

- FDA will confirm – by June 3, **in writing,** that **Accelerated Approval will be granted by the end of June when an increase in dystrophin is demonstrated based on the assumptions above.**

- Labeling discussions and post-marketing commitments to be conducted concurrently and completed by the end of June or sooner. Any delay, for any reason, past June will significantly impact our ability to continue the ongoing eteplirsen studies (202, 203, 204, PROMOVI).

Regards,

Shamim

From: Shamim Ruff <SRuff@Sarepta.com>
Date: June 1, 2016 at 9:08:37 PM PDT
To: "janet.woodcock@fda.hhs.gov" <janet.woodcock@fda.hhs.gov>, "Rich Moscicki (richard.moscicki@fda.hhs.gov)" <richard.moscicki@fda.hhs.gov>
Cc: Ed Kaye <EKaye@Sarepta.com>
Subject: FW: Request Below From DNP - URGENT Tcon Request

Dear Dr Woodcock and Dr Moscicki

Dr Kaye and I would like to request an urgent telephone call with you both to discuss the Division's request for additional dystrophin data.

Please see below a request for additional dystrophin data from the ongoing PROMOVI study. We want to emphasize that we cannot meet their request in a timely manner. Please note that even if a protocol amendment is not required, it would take us several months to analyze the PROMOVI samples.

Regards,
Shamim

Shamim Ruff
SVP Regulatory Affairs and Quality
p 617-274-4009 c (b) (6)
e sruff@sarepta.com

<image001.jpg>
215 First Street, Cambridge, MA 02142

From: Choy, Fannie (Yuet) [mailto:Fannie.Choy@fda.hhs.gov]
Sent: Wednesday, June 01, 2016 10:54 AM
To: Shamim Ruff <SRuff@Sarepta.com>
Cc: Matthew Rael <MRael@Sarepta.com>; Choy, Fannie (Yuet) <Fannie.Choy@fda.hhs.gov>
Subject: FDA Information: re: NDA 206488 / eteplirsen
Importance: High

Dear Shamim:

We refer to NDA 206488 for eteplirsen submitted on June 26, 2015.

As you know, we were unable to complete our review by the PDUFA date of May 26, but we are committed to completing our review process in a timely manner. A critical component of our ongoing review is whether there is substantial evidence that eteplirsen increases the production of dystrophin, as such a finding could potentially support an accelerated approval. As you know, the dystrophin biomarker data from Study 201/202 include only two pre/post biopsy samples from boys originally randomized to Study 201, and these samples, and all but one of the samples from external control boys, were obtained from a different muscle group. On May 5, you responded to our request for information about completed biopsies from the ongoing Promovi trial. You reported that baseline biopsies have been obtained from 62 boys in the eteplirsen-treated arm and that 10 boys each have undergone a biopsy following 24 and 48 weeks of eteplirsen treatment. Analysis of the data for immunohistochemistry and Western blotting from these additional biopsies would substantially enhance our assessment of whether eteplirsen treatment leads to dystrophin production. You suggested that a protocol amendment would require 3-6 months, because of the time needed to amend the protocol, distribute it to the sites, and gain IRB approvals. We are eager to work with you to explore ways we can collaborate to expedite the timeline for making these data available for review and will do all we can to assist you in this effort. We would like to schedule a teleconference with you in the next day or two to explore the most efficient options to obtain these analyses.

Please confirm receipt of email.

Regards,

Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products
ODE I/OND/CDER
Food and Drug Administration
10903 New Hampshire Avenue, WO22 Rm. 4215
Silver Spring, MD 20993-0002
301-796-2899 phone
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From: Moscicki, Richard
To: Woodcock, Janet
Subject: FW: URGENT: Follow Up From Telecon This Morning
Date: Friday, June 03, 2016 10:31:33 AM
Attachments: PROMOVI Dystrophin Assay June 2016 SR.pptx
Importance: High

I assume you saw this, Ed Kaye called me as well to propose this idea. How did the review division respond? I wonder if Ash can do this? How can I help, shall I speak with Ash? Rich.

From: Shamim Ruff [mailto:SRuff@Sarepta.com]
Sent: Thursday, June 02, 2016 6:17 PM
To: Woodcock, Janet; Moscicki, Richard
Cc: Ed Kaye
Subject: URGENT: Follow Up From Telecon This Morning
Importance: High

Dear Dr. Woodcock and Dr Moscicki

Thank you for the discussion this morning. Based on our conversation, if we reduce the dystrophin procedures down to the bare essentials, we could perform the analyses by the end of June; this is assuming the process goes perfectly the first time, without any delays or repeats. We have discussed with our team FDA's request to expedite the dystrophin analysis. In order to meet this request, we need FDA to agree to the following conditions:

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Shamim

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Shamim

Shamim Ruff
SVP Regulatory Affairs and Quality
p 617-274-4009 c (b) (6)
e sruff@sarepta.com

<image001.jpg>
215 First Street, Cambridge, MA 02142

From: Choy, Fannie (Yuet) [mailto:Fannie.Choy@fda.hhs.gov]
Sent: Wednesday, June 01, 2016 10:54 AM
To: Shamim Ruff <SRuff@Sarepta.com>
Cc: Matthew Rael <MRael@Sarepta.com>; Choy, Fannie (Yuet) <Fannie.Choy@fda.hhs.gov>
Subject: FDA Information: re: NDA 206488 / eteplirsen
Importance: High

Dear Shamim:

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Please confirm receipt of email.

Regards,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products
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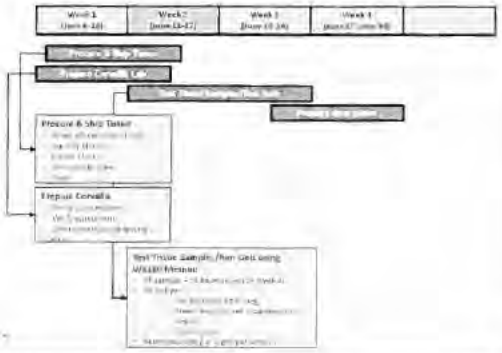
SAREPTA

PROMOVI DYSTROPHIN ASSAY TIMELINE

02June2016

WORKFLOW AND TIMELINE OVERVIEW



ASSUMPTIONS

- **We must start the process by June 6, 2016.** There is no room for flexibility with this date.
- **Dr. Rao will be an observer/advisor <u>throughout the whole process</u>** (3-4 weeks during June in Iowa and Oregon) since he is the only FDA representative with the requisite knowledge, expertise and familiarity with the eteplirsen dystrophin analyses/protocol.
- Dystrophin assays will be conducted using an adapted, pre-defined protocol based on the Week 180 methodology, e.g. no blinding.
- A different "normal" control to Week 180 will be used due to lack of availability of previous/Week 180 normal control tissue.
- Non-GLP facility - the assays will be performed at the Sarepta Corvallis site in Oregon by trained Sarepta personnel. As discussed, our Corvallis site is in the process of being closed down so will not be in an ideal state although the lab is still functional.

DELIVERABLES FOR ACCELERATED APPROVAL

- Success is defined as demonstration of an increase in dystrophin, using Western Blot assay.
- FDA will confirm – by June 3, **<u>in writing</u>**, that Accelerated Approval will be granted by the end of June when an increase in dystrophin is demonstrated.
- Labeling discussions and post-marketing commitments to be conducted concurrently and completed by the end of June or sooner.

From:	Rao, Ashutosh
To:	Woodcock, Janet; Unger, Ellis; Dunn, Billy
Cc:	Choy, Fannie (Yuet); Kozlowski, Steven; Rosenberg, Amy
Subject:	RE: Sarepta
Date:	Thursday, June 02, 2016 4:54:53 PM

Dear Janet,

Please see below for a few strategies that Sarepta could try for testing their ongoing study samples. The strategy with the best likelihood of delivering quality results in a short time may be to push Sarepta to use the labs at Nationwide Children's and get the testing done expeditiously if this is what the review team needs for recommending accelerated approval. I have proposed a few other options, each with its pros/cons. It isn't possible to predict who can do it within the next 2 months or so without discussing with them. As you know, even the best of CLIA labs can mess up samples or take a long time to process them, neither of which would be acceptable with these precious samples.

Happy to discuss further or help in any way we can.

Thanks
Ash

Strategy #1. Sarepta could send their technicians to NCH (Columbus, OH) to run the western blots. OBP can provide oversight using the inspectional pathway in collaboration with ORA, OSI, and OND.

Pros: Both Sarepta and NCH should be very motivated to move this drug development program forward so we could make a strong recommendation for them to make lab time available to do this. The labs at NCH are well-equipped to hit the ground running with established and validated protocols.
Cons: The lab's PI would need to agree to making the lab available. If Jerry Mendell/Zarif Sahenk are unable or unwilling to accommodate, another PI at NCH that has published on dystrophin is Kevin Flanigan. Dr. Flanigan was a also a site co-investigator for Sarepta during study 201/202.

#2. Sarepta sends blinded samples to a different academic lab that is familiar with DMD and dystrophin protein analyses. Some possible names are listed below.

Eric Hoffman (Children's National Medical Center, Washington DC)
Kristy Brown and Yetrib Hathout (Children's National Medical Center, Washington DC) – work closely with Hoffman
K Nagaraju (Children's National Medical Center, Washington DC) - works with murine dystrophin models
Rabi Tawill (University of Rochester)
Jeff Chamberlain (University of Washington School of Medicine) (serves on Sarepta's advisory board)

Pros: These labs are familiar with dystrophin, its caveats and western blotting. They should be set up for such studies. They could do it faster as an informal "collaboration" rather than a CDA, CRADA, or contract that might otherwise be required with for-profit entities.
Cons: They may need an IRB approval before running the samples.

#3. Sarepta (b) (4) **. If not, they could use a different CTL such as Bioreliance, PPD, Covance. They could also approach Biomarin/GSK/Prosensa.**

Pros: These CROs may have experience with the Western blot method. Flagship is specifically familiar with dystrophin, DMD, and related caveats. Biomarin/GSK/Prosensa have validated western blot protocols for measuring dystrophin.
Cons: Other than Flagship, the CTLs may not be familiar with dystrophin and each of these entities will need time to negotiate/amend a contract/CRADA/CDA with sarepta

#4. A CLIA -certified NIH lab could help analyze the samples. Below is a list of either senior leadership points-of-contact or directors of a few known CLIA labs at NIH (there may be others).

Stephen Katz (Director, NIAMS, and Chair of the inter-agency muscular dystrophy coordinating committee) and Avi Nath (Clinical Director, NINDS) – senior-level points of contact for labs at NIAMS or NINDS that can test for dystrophin. Carsten Bonnemann and Ken Fishbeck are two possible labs at NINDS. Francis Collins's office might also be a good resource for providing options. Glen Nuckolls at NINDS could also suggest other names, he is no longer lab-based but runs the extramural grant program related to DMD.
Sergio Rosenzweig, NIH NIAD – a CLIA certified lab at NIH that can potentially do Western blots although they don't do it routinely for such large proteins. This recommendation was made by Daniela Verthelyi in OBP.
Harvey Klein, Laboratory Director, NIH/Clinical Center – a CLIA certified lab at NIH that is certified for general immunology, serology, and hematology testing. Would need verification if they can do Western blots.
Michael Baseler, Director, SAIC Frederick (NCI) a CLIA lab that does a range of clinical testing. Western blot isn't listed as service but 'functional assays' are listed. -
https://ncifrederick.cancer.gov/programs/science/csp/default.asp
Carl Oberholtzer, Chief, Laboratory of Pathology, NCI – a CLIA lab that does perform neuropathology assessments; would need verification if they can do Western blots
https://home.ccr.cancer.gov/LOP/Clinical/lpstaff.asp

Pros: The labs are CLIA certified so there is some assurance of quality work
Cons: They may not be familiar with dystrophin and they may not be willing to rush the samples because they also serve the NIH clinical center and other ongoing study protocols.

#5. Sarepta sends blinded protein lysates (not tissue) to an FDA lab for analyses.

Pros: The testing would be done by biochemists knowledgeable on assay validation requirements, protein analytics, and dystrophin. Our labs are familiar with 'regulatory research' projects and the study would be carried out in-house and in consultation with OND.
Cons: We would need to either order specific reagents (SOP-specific antibodies, buffers, positive/intermediate/negative controls) or obtain the entire set of required reagents from Sarepta. Our lab ordering process currently takes 2-3 weeks to deliver items. An informal method transfer exercise would need to take place where we discuss the SOP with the original lab and do a test run with healthy and patient samples. I am not sure Sarepta/we could publish the findings or use it in any fashion other than as a 'confirmatory assessment for FDA review purposes only' without a more formal agreement in place, this could get messy from an intellectual property/legal standpoint. An IRB approval would be needed to receive and analyze the samples.

From: Woodcock, Janet
Sent: Thursday, June 02, 2016 10:44 AM
To: Unger, Ellis; Dunn, Billy; Rao, Ashutosh
Cc: Choy, Fannie (Yuet)
Subject: Sarepta

I spoke to the firm. They would take a long time with the tech transfer (they have a CRO lined up) to do the analysis of 20 WB- 10 baseline and 10 48 week. However, I proposed: what if they gave "us" the samples and we had someone run them. They are considering that. Ash, this would mean a lab with some experience would be needed, and would need to drop everything in the next week or so.

They don't have enough normal muscle control left for all the runs. Another normal sample would need to be validated against the current standard and they have enough for that. That would also need to be done by "us".

The samples are all stored by University of Iowa, who may be able to do the blinding before sending.

I propose that the Division hold off talking to them until they determine if they are willing to do this.

I guess I would be ok if NIH did this (if they were willing) under our supervision.

The firm should get back to us "soon"... jw

From: Matthew Rael
To: Choy, Fannie (Yuet)
Cc: Shamim Ruff
Subject: RE: FDA Information: re: NDA 206488 / eteplirsen
Date: Wednesday, June 01, 2016 11:20:43 AM

Hi Fannie,

I acknowledge receipt.

We'll get back to you as soon as possible.

Regards,

Matt

Matthew Rael, MS
Senior Manager, Regulatory Affairs
p 617.274.4029 c (b) (6) f 617.812.0509
e mrael@sarepta.com

SAREPTA THERAPEUTICS

215 First Street, Cambridge, MA 02142 USA

From: Choy, Fannie (Yuet) [mailto:Fannie.Choy@fda.hhs.gov]
Sent: Wednesday, June 01, 2016 10:54 AM
To: Shamim Ruff <SRuff@Sarepta.com>
Cc: Matthew Rael <MRael@Sarepta.com>; Choy, Fannie (Yuet) <Fannie.Choy@fda.hhs.gov>
Subject: FDA Information: re: NDA 206488 / eteplirsen
Importance: High

Dear Shamim:

We refer to NDA 206488 for eteplirsen submitted on June 26, 2015.

As you know, we were unable to complete our review by the PDUFA date of May 26, but we are committed to completing our review process in a timely manner. A critical component of our ongoing review is whether there is substantial evidence that eteplirsen increases the production of dystrophin, as such a finding could potentially support an accelerated approval. As you know, the dystrophin biomarker data from Study 201/202 include only two pre/post biopsy samples from boys originally randomized to Study 201, and these samples, and all but one of the samples from external

control boys, were obtained from a different muscle group. On May 5, you responded to our request for information about completed biopsies from the ongoing Promovi trial. You reported that baseline biopsies have been obtained from 62 boys in the eteplirsen-treated arm and that 10 boys each have undergone a biopsy following 24 and 48 weeks of eteplirsen treatment. Analysis of the data for immunohistochemistry and Western blotting from these additional biopsies would substantially enhance our assessment of whether eteplirsen treatment leads to dystrophin production. You suggested that a protocol amendment would require 3-6 months, because of the time needed to amend the protocol, distribute it to the sites, and gain IRB approvals. We are eager to work with you to explore ways we can collaborate to expedite the timeline for making these data available for review and will do all we can to assist you in this effort. We would like to schedule a teleconference with you in the next day or two to explore the most efficient options to obtain these analyses.

Please confirm receipt of email.

Regards,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products
ODE I/OND/CDER
Food and Drug Administration
10903 New Hampshire Avenue, WO22 Rm. 4215
Silver Spring, MD 20993-0002
301-796-2899 phone
fannie.choy@fda.hhs.gov

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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.

/s/

YUET L CHOY
06/03/2016
At the request of Dr. Billy Dunn, DNP Director

From: Unger, Ellis
To: Woodcock, Janet
Cc: Jenkins, John K
Subject: RE: Message to Sarepta: re: additional dystrophin data
Date: Wednesday, June 01, 2016 10:25:24 AM

Will do.

Also – I have a question for you. In your memo you discuss the possibility that exon 52 carries prognostic implications, and you say that subject 6 was the only patient in study 201 who was noted to be unable to rise without external support at baseline. I wasn't aware of that, and wondered where you found that information.

Ellis

From: Woodcock, Janet
Sent: Wednesday, June 01, 2016 9:01 AM
To: Unger, Ellis
Subject: RE: Message to Sarepta: re: additional dystrophin data

Yes, well maybe not do the immunofluourescence since that is more complicated, in my opinion. Not sure what it adds. I think it is best to talk to them as well as the written communications. jw

---

From: Unger, Ellis
Sent: Wednesday, June 01, 2016 8:03 AM
To: Woodcock, Janet
Subject: RE: Message to Sarepta: re: additional dystrophin data

Janet,

We'll remove the sentence "[redacted (b) (5)] "

With respect to your suggestion that they might be able to run the samples from Week 48 more expeditiously, I would caution that running a set of samples from a particular time point, separate from the others, would likely lead us to the same type of conundrum we're in now, with uncontrolled data.

So I suggest we talk with them, and get a sense of the amount of time they think they'd need to get the analyses run. If you are comfortable with that time frame, then we'll work out an analytical plan.

Ellis

From: Woodcock, Janet
Sent: Wednesday, June 01, 2016 6:51 AM
To: Unger, Ellis

Subject: Re: Message to Sarepta: re: additional dystrophin data

Ellis I can't go along with the sentence about the agency considers it critical...before" because I don't agree w that

Also the most key might just be the WB on the 10 boys at 48 weeks. Maybe that could be faster. Also suggest you call them after sending this. Jw.

From: Unger, Ellis <Ellis.Unger@fda.hhs.gov>
Date: May 31, 2016 at 7:53:29 PM EDT
To: Woodcock, Janet <Janet.Woodcock@fda.hhs.gov>
Cc: Bastings, Eric <Eric.Bastings@fda.hhs.gov>, Temple, Robert <Robert.Temple@fda.hhs.gov>, Dunn, Billy <Billy.Dunn@fda.hhs.gov>, Jenkins, John K <John.Jenkins@fda.hhs.gov>
Subject: FW: Message to Sarepta: re: additional dystrophin data

Hi Janet,

Below is our proposed message for Sarepta. Please let us know if you have comments or edits.

Thanks in advance,

Ellis

- - - -

As you know, we were unable to complete our review by the PDUFA date of May 26, but we are committed to completing our review process in a timely manner. A critical component of our ongoing review is whether there is substantial evidence that eteplirsen increases the production of dystrophin, as such a finding could potentially support an accelerated approval. As you know, the dystrophin biomarker data from Study 201/202 include only two pre/post biopsy samples from boys originally randomized to Study 201, and these samples, and all but one of the samples from external control boys, were obtained from a different muscle group. On May 5, you responded to our request for information about completed biopsies from the ongoing Promovi trial. You reported that baseline biopsies have been obtained from 62 boys in the eteplirsen-treated arm and that 10 boys each have undergone a biopsy following 24 and 48 weeks of eteplirsen treatment. Analysis of the data for immunohistochemistry and Western blotting from these additional biopsies would substantially enhance our assessment of whether eteplirsen treatment leads to dystrophin production. You suggested that a protocol amendment would require 3-6 months, because of the time needed to amend the protocol, distribute it to the sites, and gain IRB approvals. We are eager to work with you to explore ways we can collaborate to expedite the timeline for making these

data available for review and will do all we can to assist you in this effort. [redacted] (b) (5) [redacted] We would like to schedule a teleconference with you in the next day or two to explore the most efficient options to obtain these analyses.

From: Unger, Ellis
To: Woodcock, Janet
Cc: Jenkins, John K; Temple, Robert; Dunn, Billy; Bastings, Eric
Subject: FW: Message to Sarepta: re: additional dystrophin data
Date: Tuesday, May 31, 2016 7:53:30 PM

Hi Janet,

Below is our proposed message for Sarepta. Please let us know if you have comments or edits.

Thanks in advance,

Ellis

- - - -

As you know, we were unable to complete our review by the PDUFA date of May 26, but we are committed to completing our review process in a timely manner. A critical component of our ongoing review is whether there is substantial evidence that eteplirsen increases the production of dystrophin, as such a finding could potentially support an accelerated approval. As you know, the dystrophin biomarker data from Study 201/202 include only two pre/post biopsy samples from boys originally randomized to Study 201, and these samples, and all but one of the samples from external control boys, were obtained from a different muscle group. On May 5, you responded to our request for information about completed biopsies from the ongoing Promovi trial. You reported that baseline biopsies have been obtained from 62 boys in the eteplirsen-treated arm and that 10 boys each have undergone a biopsy following 24 and 48 weeks of eteplirsen treatment. Analysis of the data for immunohistochemistry and Western blotting from these additional biopsies would substantially enhance our assessment of whether eteplirsen treatment leads to dystrophin production. You suggested that a protocol amendment would require 3-6 months, because of the time needed to amend the protocol, distribute it to the sites, and gain IRB approvals. We are eager to work with you to explore ways we can collaborate to expedite the timeline for making these data available for review and will do all we can to assist you in this effort. (b) (4) [redacted] We would like to schedule a teleconference with you in the next day or two to explore the most efficient options to obtain these analyses.

From: Throckmorton, Douglas C
To: Woodcock, Janet
Cc: Moscicki, Richard
Subject: Sarepta dct.docx
Date: Tuesday, May 24, 2016 11:30:37 PM
Attachments: Sarepta dct.docx

Here are some suggestions. Thanks, Doug

Center Director Decisional Memo

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(b) (5)

[redacted]

5 pages of draft language have been withheld as b(5) immediately following this page

From: Temple, Robert
To: Woodcock, Janet
Cc: Temple, Robert; Blount, Aprile
Subject: Sarepta
Date: Monday, May 23, 2016 11:22:10 AM
Attachments: Message to Sarepta_EU RF_RT.DOC

JW,

This was meant to go Friday, but it did not. It was to explain why I thought it was critical to get the new dystrophin data, which could be done very rapidly. I'm very sorry it did not get out and you probably know all this and have considered these matters. I expect it will be part of any discussions this week, but I did want to state my reasons. As your note said, things can be waived but you still need to conclude that dystrophin levels are increased.

Attached is the communication to Sarepta proposed by the Division, ODE 1, and OND. It is clearly more insistent than what you suggested in your E-mail of May 8. There are 2 main reasons for this. We believe, based on the reviews by Ellis, Ash, and others, that:

1. The existing biopsy samples can be tested by Western blot and immunohistochemistry in a short time (a few weeks to a month) and we would plan to work very closely with them to accomplish this. The contention that 3-6 months are needed for IRB clearance (with more time afterward to do the samples) is not credible.
2. Good evidence of increased dystrophin is critical to the possible accelerated approval. There can be judgment as to the "reasonably likely to predict outcome" part of AA but the evidence for the surrogate should be strong and there are, as the reviews show, substantial doubts about whether the existing data show this. The new data should leave no uncertainty at all, either way. I should note that, without saying what the final reviews will say, that there is considerable support for the pathway you proposed at the AC meeting (AA based on dystrophin with various sources of support for reasonable likelihood) even if the dystrophin levels are not very high. The proposed message to Sarepta more or less says that and it should be quite encouraging, if they are convinced that the levels are indeed increased.

Message to Sarepta:

(b) (5)

(b) (5)

4 pages of draft language have been withheld as b(5) immediately following this page

From: Jenkins, John K
To: Woodcock, Janet
Cc: Jenkins, John K
Subject: RE: Action
Date: Monday, May 23, 2016 8:55:13 AM

FYI, the division will not have preliminary labeling to send today, so we will notify about the missed user fee date today and let them know the preliminary labeling is coming soon (probably Wednesday). Since we don't know the nature of the action you propose, the preliminary labeling comments will not include clinical issues like indication, D&A, clinical studies, etc. We cannot draft those until we understand your thinking better.

John

From: Woodcock, Janet
Sent: Monday, May 23, 2016 8:31 AM
To: Jenkins, John K
Subject: RE: Action

The wording is fine. I expect they will call me, I will tell them to keep responding to FDA requests.
jw

From: Jenkins, John K
Sent: Monday, May 23, 2016 8:28 AM
To: Woodcock, Janet
Subject: FW: Action

This is what I just sent.

From: Jenkins, John K
Sent: Monday, May 23, 2016 8:24 AM
To: Woodcock, Janet
Cc: Jenkins, John K
Subject: RE: Action

As for the communication with the sponsor, please let me know your comments on the draft text below. I developed this without input from the division/ODE, but time is short, so we need to get a message to them today or tomorrow.

(b) (5)

[redacted]

I tried to walk a fine line between providing information while not telegraphing what the action on the application might be. Sending even preliminary labeling comments before the review team has

a chance to read your review and meet with you to discuss your conclusions makes me uncomfortable since it may be interpreted by the review that a final decision has been made prior to completing the Equal Voice process. Communicating on labeling at this point may also be interpreted by the sponsor and investors as a signal of pending approval.

Please let me know how you would like to edit the proposed statement.

John

From: Woodcock, Janet
Sent: Monday, May 23, 2016 7:55 AM
To: Jenkins, John K
Subject: RE: Action

Sure. I will send a draft review around in the next few days. We can then schedule a meeting. I think the team should start the label negotiations with the firm. I do not think that doing more analyses of biopsies in a hurry would be a good idea, although I understand the rationale behind it. There have been enough controversies over the the data as it is. We need to have a rock solid agreement on what will be done. I heard some people mention independent substantiation of the assays, for example. That is not currently planned for, and would have to be put into a plan before the assays were done. jw

---

From: Jenkins, John K
Sent: Monday, May 23, 2016 7:51 AM
To: Unger, Ellis; Woodcock, Janet
Cc: Temple, Robert; Jenkins, John K
Subject: RE: Action

Janet

Given the unusual nature of the eteplirsen situation, I think it would be a good idea for you to meet with the review team to discuss your approach/conclusions related to this application. Such a meeting is specified in the CDER MaPP on Equal Voice. I think this would be best held after the team has a chance to read your draft review.

We will need to communicate to Sarepta that we will not meet the PDUFA goal date. I think Bob communicated the team's proposed text last week that would have included asking that they provide the biopsy data from the ongoing PROMOVI trial before FDA made its decision. I assume that message was not communicated. How do you wish us to communicate the delay to them now?

John

From: Unger, Ellis
Sent: Sunday, May 22, 2016 2:00 PM
To: Woodcock, Janet
Cc: Jenkins, John K; Temple, Robert
Subject: Re: Action

Janet,

I'm in St. Louis now. I'll be driving all day tomorrow (from St. Louis to DC) and expect to get home Monday evening. I can file my memo on Tuesday. Could we talk on Tuesday instead of Monday?

Ellis

Sent from my BlackBerry.

From: Woodcock, Janet
Sent: Sunday, May 22, 2016 10:13 AM
To: Unger, Ellis
Cc: Jenkins, John K
Subject: Action

I have finished my review of all the Sarepta data and am unchanged in my opinion. Ellis I note your memo is on draft. Do you know when you will finish? I would like to talk to you, tomorrow am if possible.

If you are concerned about precedent here, I would point out 314.126 which authorizes the Center Director to waive certain elements going into an assessment of adequate and well controlled, although the trials must still be controlled

I expect to finalize my decisional memo in the next few days. Jw

From: Jenkins, John K
To: Woodcock, Janet
Subject: FW: Action
Date: Monday, May 23, 2016 8:28:06 AM

This is what I just sent.

From: Jenkins, John K
Sent: Monday, May 23, 2016 8:24 AM
To: Woodcock, Janet
Cc: Jenkins, John K
Subject: RE: Action

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(b) (5)

[redacted]

I tried to walk a fine line between providing information while not telegraphing what the action on the application might be. Sending even preliminary labeling comments before the review team has a chance to read your review and meet with you to discuss your conclusions makes me uncomfortable since it may be interpreted by the review that a final decision has been made prior to completing the Equal Voice process. Communicating on labeling at this point may also be interpreted by the sponsor and investors as a signal of pending approval.

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From: Jenkins, John K
Sent: Monday, May 23, 2016 7:51 AM
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Cc: Temple, Robert; Jenkins, John K
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Ellis

Sent from my BlackBerry.

From: Woodcock, Janet
Sent: Sunday, May 22, 2016 10:13 AM
To: Unger, Ellis
Cc: Jenkins, John K
Subject: Action

I have finished my review of all the Sarepta data and am unchanged in my opinion. Ellis I note your memo is on draft. Do you know when you will finish? I would like to talk to you, tomorrow am if possible.

If you are concerned about precedent here, I would point out 314.126 which authorizes the Center Director to waive certain elements going into an assessment of adequate and well controlled, although the trials must still be controlled

I expect to finalize my decisional memo in the next few days. Jw

From: Jenkins, John K
To: Unger, Ellis; Woodcock, Janet
Cc: Temple, Robert; Jenkins, John K
Subject: RE: Action
Date: Monday, May 23, 2016 7:50:40 AM

Janet

Given the unusual nature of the eteplirsen situation, I think it would be a good idea for you to meet with the review team to discuss your approach/conclusions related to this application. Such a meeting is specified in the CDER MaPP on Equal Voice. I think this would be best held after the team has a chance to read your draft review.

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I expect to finalize my decisional memo in the next few days. Jw

From: Unger, Ellis
To: Woodcock, Janet
Subject: Re: Action
Date: Sunday, May 22, 2016 2:30:32 PM

Thanks. In fact, I didn't know you didn't want me to tell the team, and I told them an hour ago.

Their response is that they think they can get the label in shape quickly. I presume this will be an AA, right?

Ellis

Sent from my BlackBerry.

From: Woodcock, Janet
Sent: Sunday, May 22, 2016 1:13 PM
To: Unger, Ellis
Subject: Re: Action

Of course. I want to move on this and talk about how to relay the decision to the team. Safe travels. Thanks. Jw

From: Unger, Ellis <Ellis.Unger@fda.hhs.gov>
Date: May 22, 2016 at 1:59:58 PM EDT
To: Woodcock, Janet <Janet.Woodcock@fda.hhs.gov>
Cc: Temple, Robert <Robert.Temple@fda.hhs.gov>, Jenkins, John K <John.Jenkins@fda.hhs.gov>
Subject: Re: Action

Janet,

I'm in St. Louis now. I'll be driving all day tomorrow (from St. Louis to DC) and expect to get home Monday evening. I can file my memo on Tuesday. Could we talk on Tuesday instead of Monday?

Ellis

Sent from my BlackBerry.

From: Woodcock, Janet
Sent: Sunday, May 22, 2016 10:13 AM
To: Unger, Ellis
Cc: Jenkins, John K

Subject: Action

I have finished my review of all the Sarepta data and am unchanged in my opinion. Ellis I note your memo is on draft. Do you know when you will finish? I would like to talk to you, tomorrow am if possible.

If you are concerned about precedent here, I would point out 314.126 which authorizes the Center Director to waive certain elements going into an assessment of adequate and well controlled, although the trials must still be controlled

I expect to finalize my decisional memo in the next few days. Jw

From: Guidos, Robert
To: Woodcock, Janet
Subject: IMPORTANT
Date: Wednesday, May 18, 2016 1:34:24 PM
Attachments: NCHR letter to Commissioner re FDA approval standards for rare diseases.pdf
Importance: High

Janet, see below and attached.
Bob

From: Sher, Rachel
Sent: Wednesday, May 18, 2016 1:26 PM
To: Guidos, Robert
Subject: FW: Concerns about upcoming FDA decision
Importance: High

Can you route this to whoever should get it in CDER?

From: Diana Zuckerman [mailto:dz@center4research.org]
Sent: Thursday, May 12, 2016 6:13 PM
To: FDA Commissioner
Cc: Sher, Rachel
Subject: Concerns about upcoming FDA decision
Importance: High

Dear Commissioner Califf,

Since you suggested I contact you by email, I am taking this opportunity to do so. I don't plan on making it a habit to send you letters regarding specific medical products, but this one FDA decision will send a very clear message about whether FDA is making decisions based on science or not, as well as whether FDA respects the analyses of its scientists and Advisory Committees. The letter is below and attached. I bolded some points to make it easier to skim.

Thank you for considering our views on this important precedent.

Sincerely,
Diana Zuckerman, Ph.D.
President
National Center for Health Research
Cancer Prevention and Treatment Fund
1001 Connecticut Ave, NW, Ste. 1100
Washington, DC 20036
(202) 223-4000
www.center4research.org
www.stopcancerfund.org

May 12, 2016

Robert M. Califf, M.D.

Commissioner
Food and Drug Administration
10903 New Hampshire Ave
Silver Spring, MD 20993-0002

Dear Commissioner Califf,

I am writing to express our concerns about the April 25th meeting of the FDA's Peripheral and Central Nervous System Drugs Advisory Committee to discuss the approval of Sarepta's eteplirsen for the treatment of Duchenne muscular dystrophy.

One of our Center's Senior Fellows, Dr. Laura Gottschalk, spoke during the public comment period in support of the FDA scientists' views regarding the poor quality of the eteplirsen study design and data. She was the only one of 52 public speakers to do so. She pointed out that with only 12 patients, inadequate control groups, and variations in natural disease progression in historical controls as well as the patients in the study, approval of eteplirsen would only be appropriate if there is a very clear benefit to its use. Unfortunately, Sarepta failed to prove that eteplirsen meets a scientific standard of effectiveness. **FDA approval of eteplirsen on such insufficient data would set a dangerously low bar for approval of drugs in the future.**

We have great empathy for the parents of children with Duchenne Muscular Dystrophy who participated in the Sarepta trial, one of whom we know and respect. We also know that **Sarepta has misled the parents about their data, misrepresented FDA's previous warnings about the inadequacy of the Sarepta trial, and threatened parents that if eteplirsen isn't approved soon, the children will no longer have any access to the drug.** As a result of these misstatements and threats, the patient advocates at the meeting were extremely well-organized and determined.

We understand that Dr. Janet Woodcock wanted to assure patients that she understood their point of view, but we were appalled when she implied that the Advisory Committee should ignore the inadequacy of the science and approve the drug through the accelerated approval pathway despite no scientific evidence that it works. **We have never seen any CDER or CDRH Director so inappropriately try to influence an FDA Advisory Committee at the more than 200 FDA meetings that I and my staff have attended or analyzed.** Despite the hostile and inappropriate behavior of the audience (as described below), Dr. Woodcock repeatedly expressed her sympathy with the audience and reinforced their behavior by making herself available for photographs with the advocates (see Facebook for one example: https://www.facebook.com/terri.wesleyellsworth/posts/10248379642200051).

We are especially concerned that **Sarepta justified their lack of a randomized control group based on their "ethical concerns" about depriving patients of an effective treatment, given that there was no clear evidence that eteplirsen is effective.** The company used that justification to explain why they disregarded FDA's repeated warnings that their sample size was too small and that the lack of an adequately sized control group would make approval unlikely. If the FDA were to approve eteplirsen despite the Advisory Committee's recommendation to reject it, it would send a **clear message to companies that they can ignore FDA's repeated warnings about the need for an adequate control group based on questionable claims that a control group is unethical.**

Since the FDA's goal is to approve a new drug if it is effective, and require more research if the results are ambiguous, **we strongly urge the FDA to defer a decision until it carefully reviews the existing data on the effectiveness of eteplirsen in the approximately 21-48 new patients who have been taking the drug for almost a year.** At the FDA's urging, the company began enrolling more than 100 patients in a larger confirmatory trial, PROMOVI (unfortunately, also without an appropriate control group). It should be unacceptable to the FDA that Sarepta did not discuss their preliminary efficacy results from the PROMOVI trial, even though they provided safety data for those patients during the meeting that was not included in the materials provided to the Advisory Committee. Based on the slides that Sarepta presented, they should have had data on 21-48 new patients who had been taking eteplirsen for approximately 11 months at the time of the FDA meeting, and presumably even more patients who have taken the drug for at least 6 months. Rather than basing a decision on the efficacy data for only 12 patients, FDA should insist on reviewing efficacy and safety data on any PROMOVI patients who have been enrolled in the study for at least 6 months.

A related concern is that the April 25th Advisory Committee **meeting created a hostile environment for scientists who questioned whether eteplirsen was proven effective.** During the FDA presentation, the **audience repeatedly and loudly scoffed or laughed at the clinical team leader of the Division of Neurology Products** as he presented his data. The **audience booed the Deputy Director of the Division of Neurology Products** during his concluding remarks. In contrast, the speaker representing the Jett Foundation received a standing ovation after her comments on behalf of Sarepta. During the open public hearing, there was raucous applause after each of the 51 speakers in favor of eteplirsen.

By law, FDA Advisory Committee meetings should be a place for Committee members to openly discuss the drug or device under consideration. Although stakeholders may have differing goals and perspectives, the meeting as a whole should be an unbiased forum to discuss scientific and clinical data, providing the FDA and the public with the **unbiased opinions of Committee members**. Our two staff members, Dr. Laura Gottschalk and Mr. Paul Brown, have each attended many FDA Advisory Committee meetings, and usually if audience members respond positively or negatively to presentations, they are sternly warned that they will be removed if they do not obey the rules, including no applause or booing. In contrast, at the Sarepta meeting, the moderator made a few weak attempts to ask the audience to hold their applause until all the speakers had presented, but to no avail.

This out-of-control atmosphere had a clear impact on Advisory Committee members who had concerns about the drug. They quickly realized that any tough questions would be met with hostility, whereas positive comments about eteplirsen would be applauded. There is a natural urge to please a crowd that is cheering and booing in response to what is being said. Under the circumstances, it is nothing short of miraculous that most Advisory Committee members voted against approval. Even so, there were three abstentions. Based on our experience evaluating more than 200 FDA Advisory Committee meetings, **three abstentions is extremely rare** and suggests a reluctance to displease the audience.

Due to the frightening atmosphere, our staff decided to leave immediately after our public comment. We were particularly worried because the only guards at the meeting were protecting the panel members and FDA staff, not anyone in the audience. There were no metal detectors or other safeguards at the hotel, as there are for meetings held at FDA. As we

left the room after our statement, people from the audience **swore loudly at our staff** and "gave us the finger." One of the men in the audience followed us out of the building while swearing at us, and **threatened to punch Mr. Brown in the face**.

We have been told by panel members, that after the meeting was over, some (if not all) of the **panel members who had voted against approval were escorted out by an armed guard**, in response to their request.

We support FDA's efforts to include patients' perspectives at public meetings, but this **should never be at the expense of the scientific integrity of the process or the safety of panel members**. And, in our experience, the FDA has been much more accommodating to patients who support approval of a product (such as eteplirsen and flibanserin, for example) than for those who have been harmed by one (Essure and breast implants, for example).

By law, the FDA vets its Advisory Committee members to minimize conflicts of interest that could possibly bias their views. However, if the FDA wants honest, unbiased feedback from all panel members, then they need to ensure that the meeting encourages that, rather than discourages it. We strongly urge you to consider the precedent that the FDA will set if the agency approves eteplirsen based on an open label trial of only 12 patients with such ambiguous results that were reviewed at a public meeting that demeaned the agency and its scientists.

Sincerely,

Diana Zuckerman, PhD
President

Scientific Dispute Regarding Approval of Sarepta Therapeutics' Eteplirsen – Commissioner's Decision

(b) (5)

11 pages of draft language have been withheld as b(5) immediately following this page

Scientific Dispute Regarding Approval of Sarepta Therapeutics' Eteplirsen – Commissioner's Decision

(b) (5)

10 pages of draft language have been withheld as b(5) immediately following this page

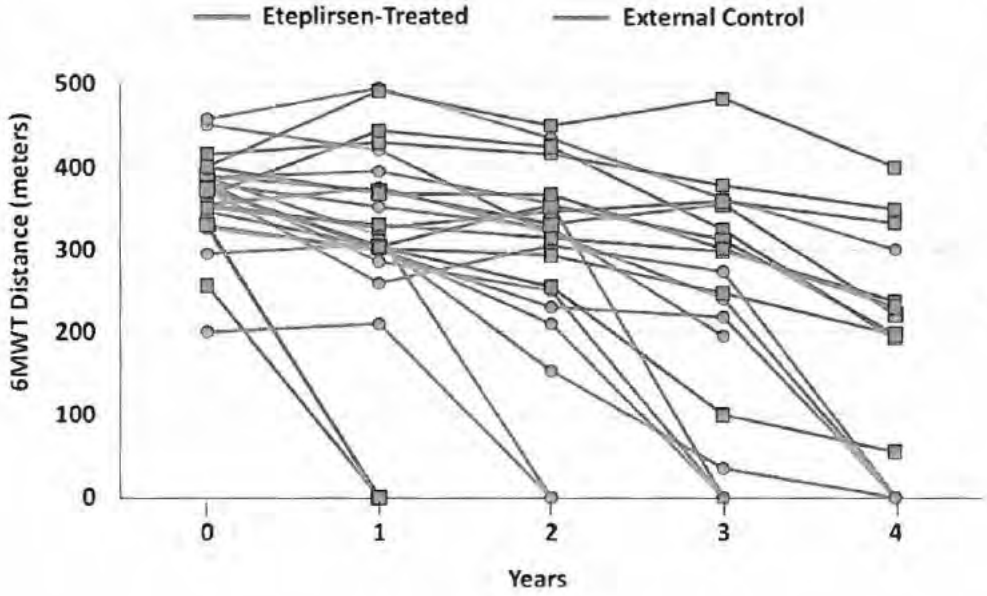
Scientific Dispute Regarding Approval of Sarepta Therapeutics' Eteplirsen – Commissioner's Decision

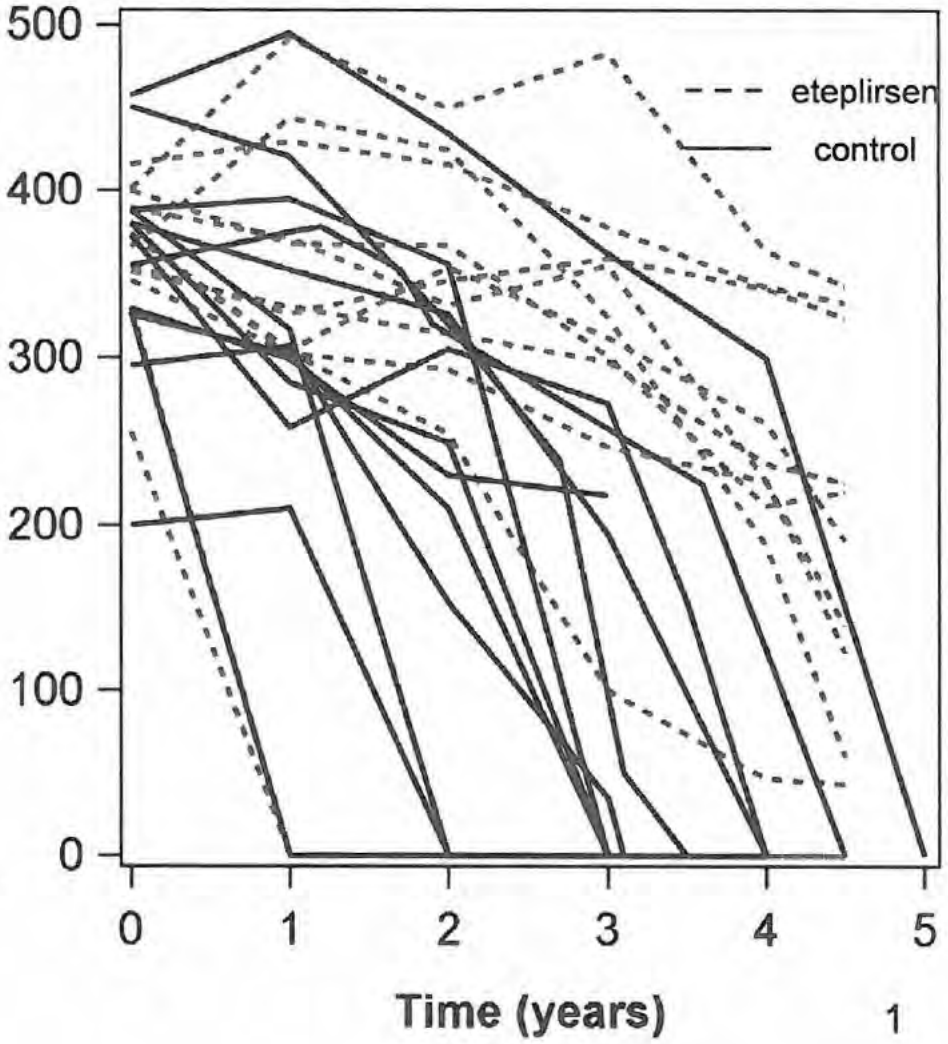
(b) (5)

10 pages of draft language have been withheld as b(5) immediately following this page

Data affected by truncation and misalignment

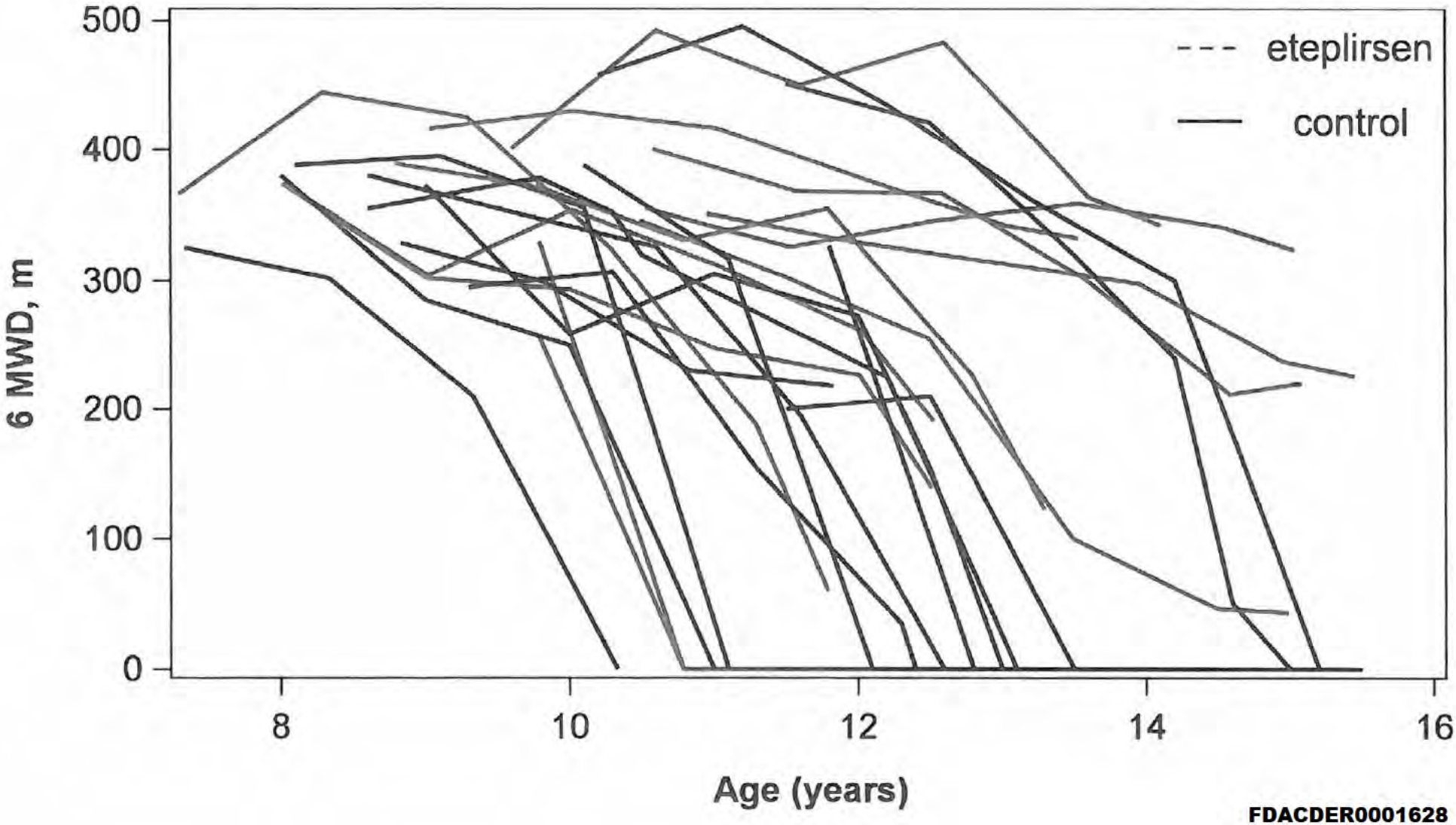
Year 4 Individual 6MWT, Eteplirsen-Treated vs. External Control (Exon 51)



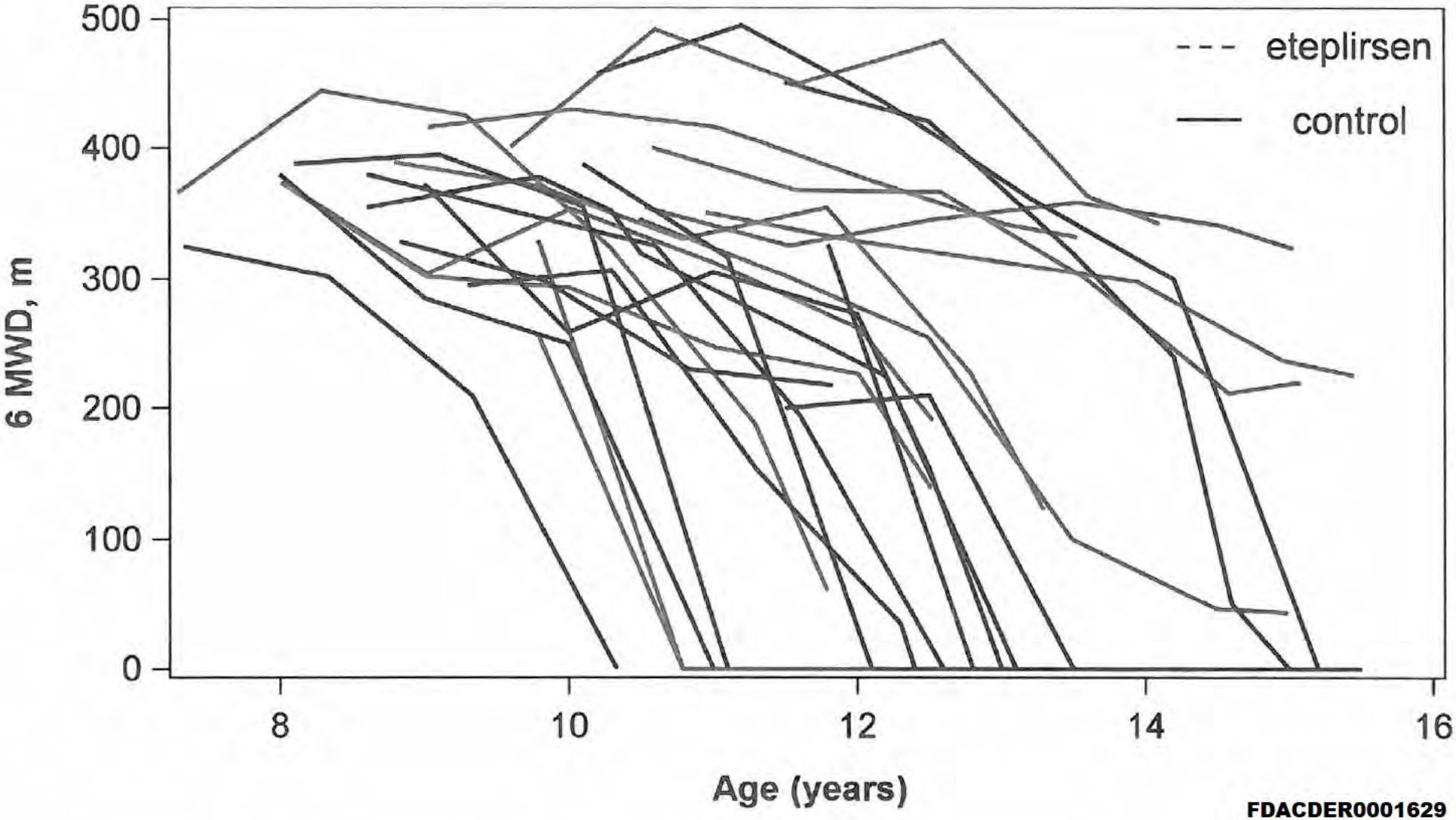


1

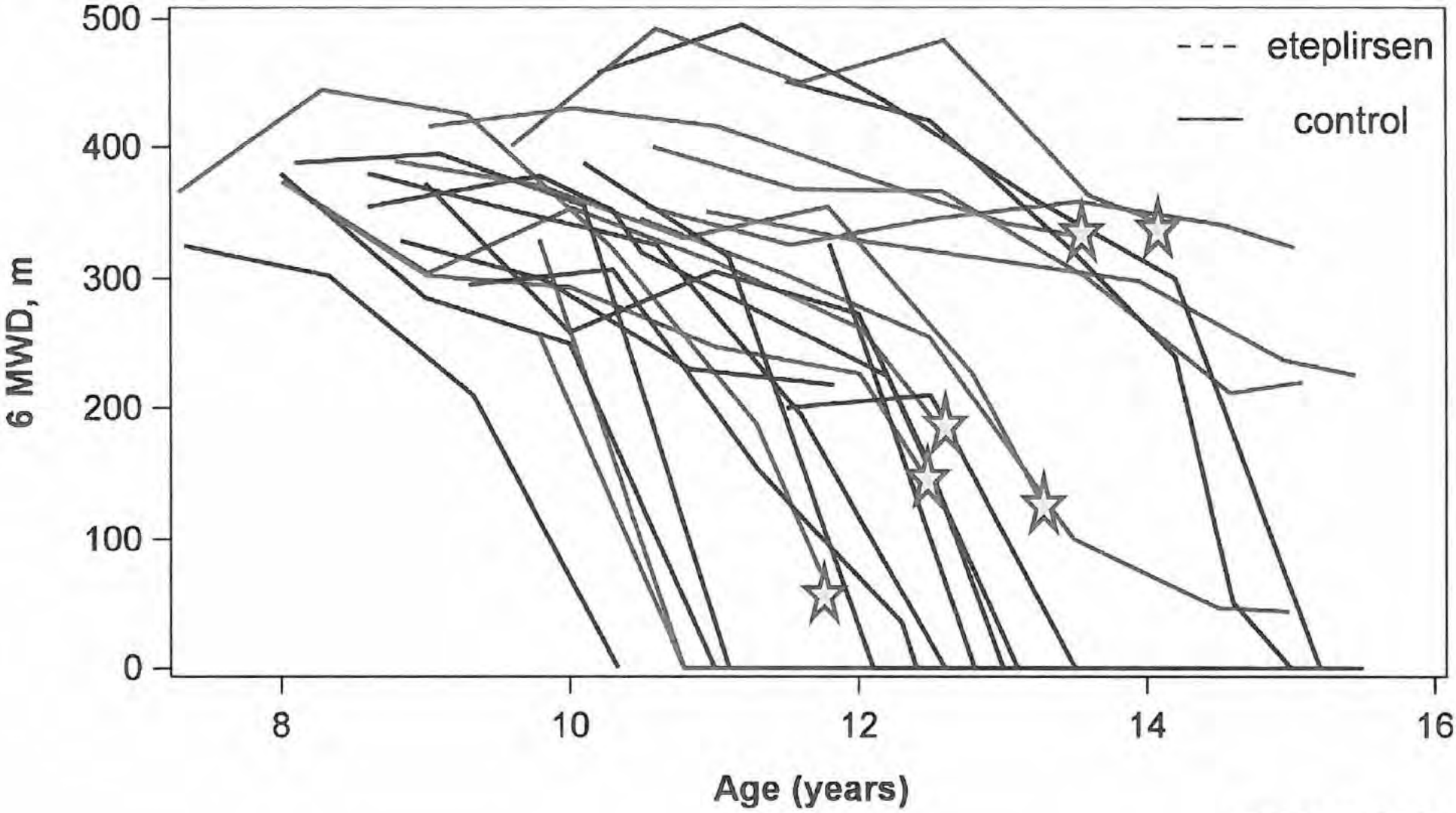
Eteplirsen patients and controls not matched more than grossly by age



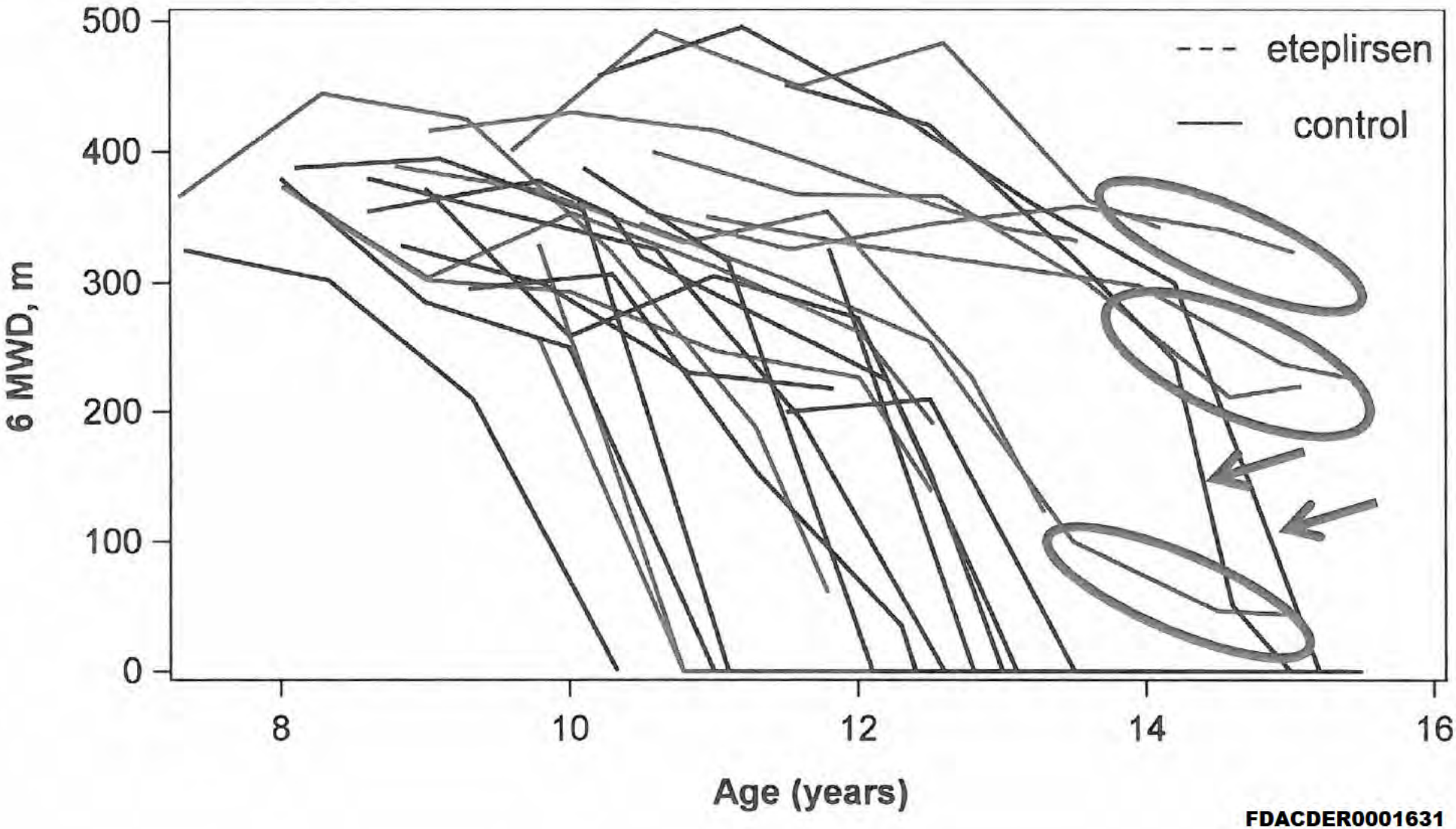
Viewed against age, the importance of data truncation becomes clearer



Eteplirsen patients indicated by a star decline in 6MWT closely overlapping controls, but truncated



Some eteplirsen patients have less steep decline in final year measured (circles) vs controls (arrows)



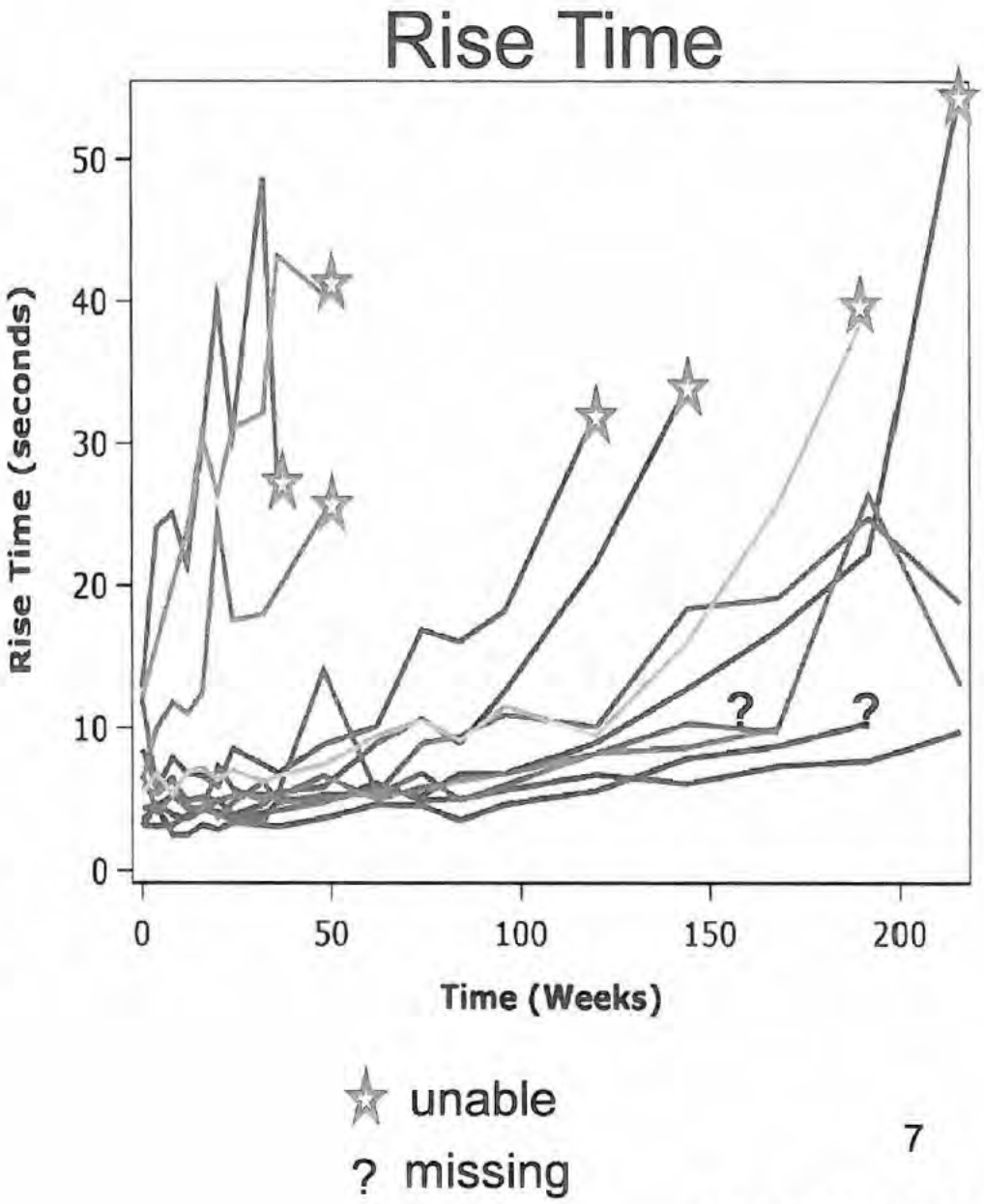
Critical to understand that ambulation not a "hard" (black and white) endpoint

Depends on conscious care decisions

- "Transition to a wheelchair usually is a gradual process" *
- "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"°

*www.mda.org/disease/duchenne-muscular-dystrophy/signs-and-symptoms
°http://www.parentprojectmd.org/site/PageServer?pagename=Care_stage_nonambulatory

- Majority of eteplirsen patients unable, or nearly unable to rise (and several now with 6MWT of less than 100 m)
- <u>patients outside of efficacy studies may have chosen wheelchair use</u>



Findings on another endpoint, the North Star Ambulatory Assessment (NSAA), further suggest that underlying disease progression was similar in all eteplirsen-treated patients and historical patients

North Star Ambulatory Assessment (NSAA)

- Because of the problems with use of 6MWT in historically controlled trials described earlier, FDA advised the applicant to present data from a measure that might more directly represent muscle strength

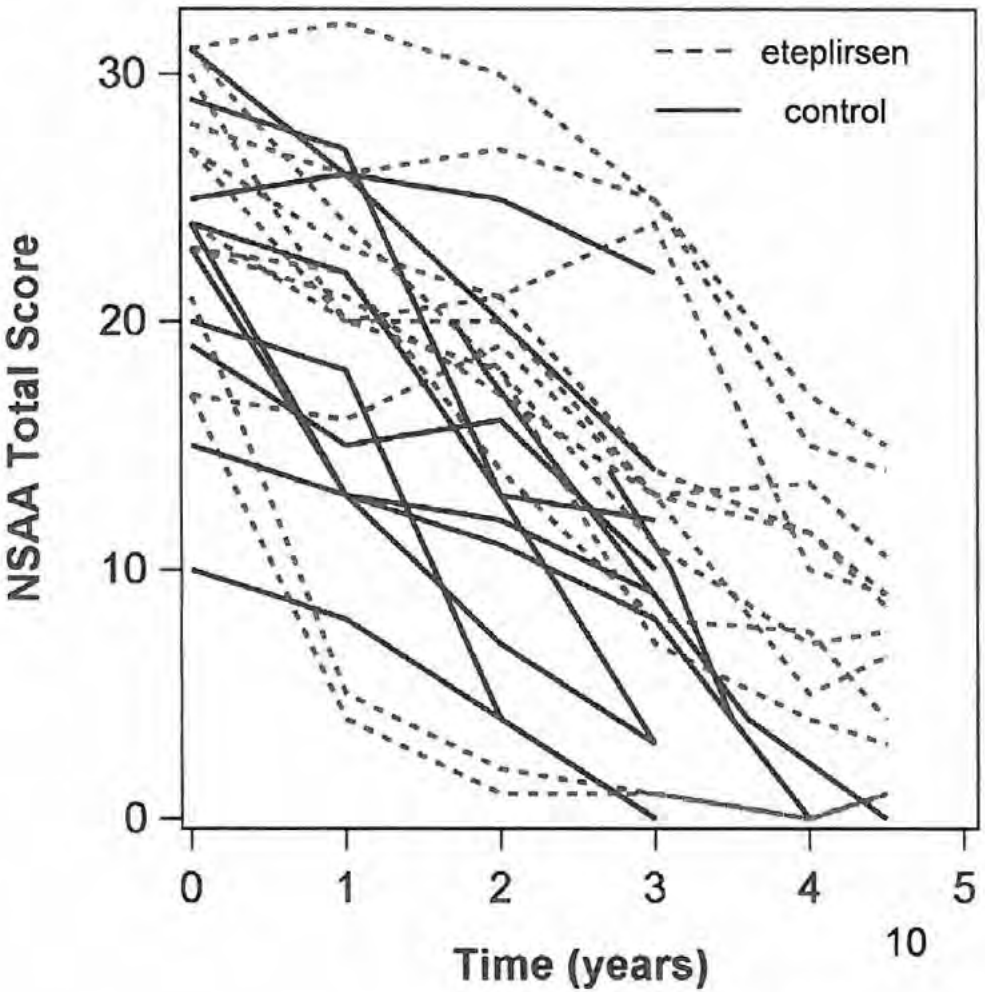
- NSAA a measure of underlying ambulatory **abilities**, such as 'rise from floor' that support specific **activities** such as walking

- Similar to 6MWT, score still depends on supportive care and effort, and is partially based on subjective assessment of evaluator and therefore susceptible expectation bias in open-label studies

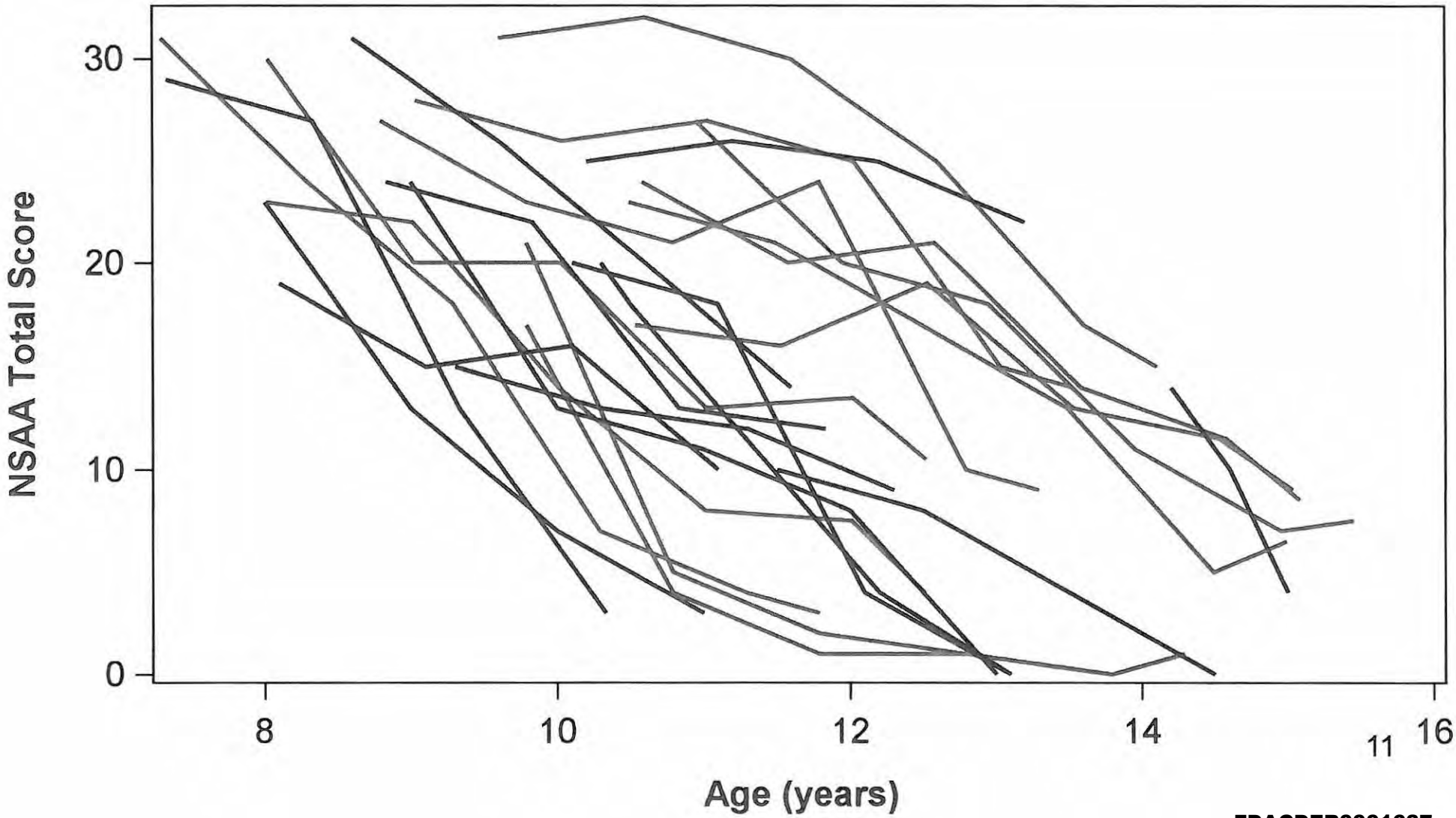
NSAA
Eteplirsen vs. Applicant's Historical Controls

- Notable that the course of all eteplirsen patients was roughly similar to controls for NSAA

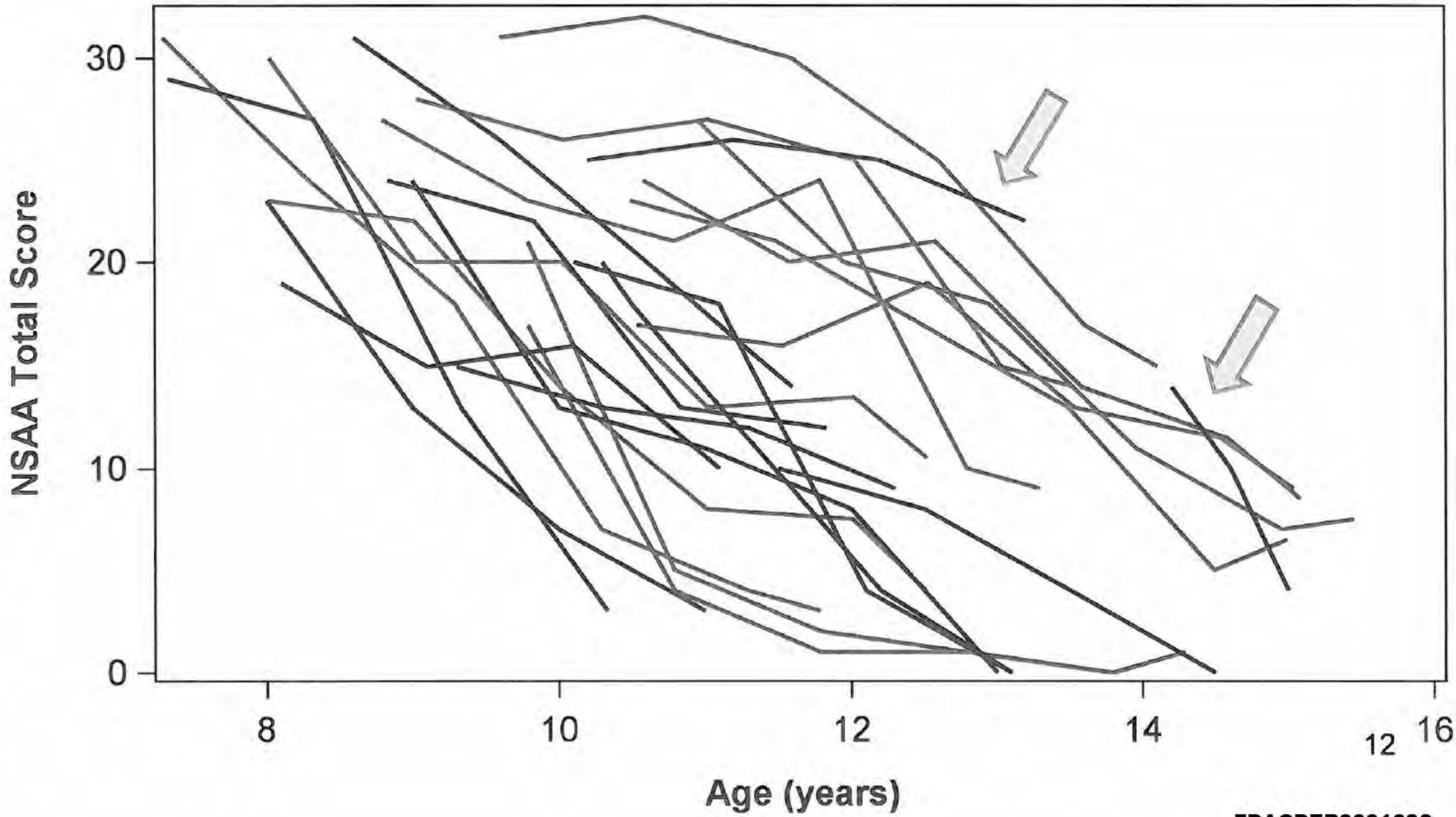
- All patients, treated and controls, decline steeply



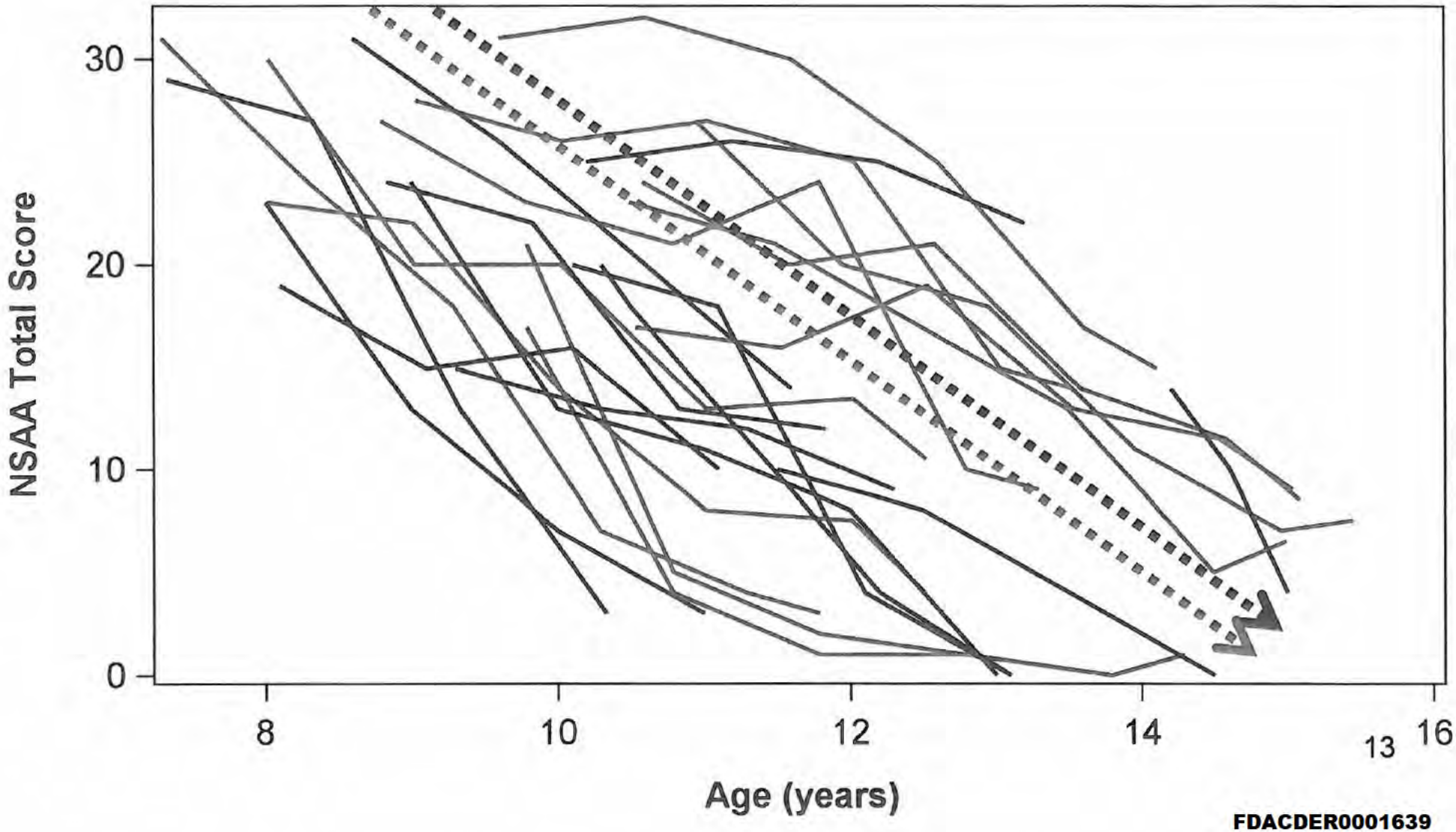
Viewed against age, lines "shift" relative to viewing by years of observation



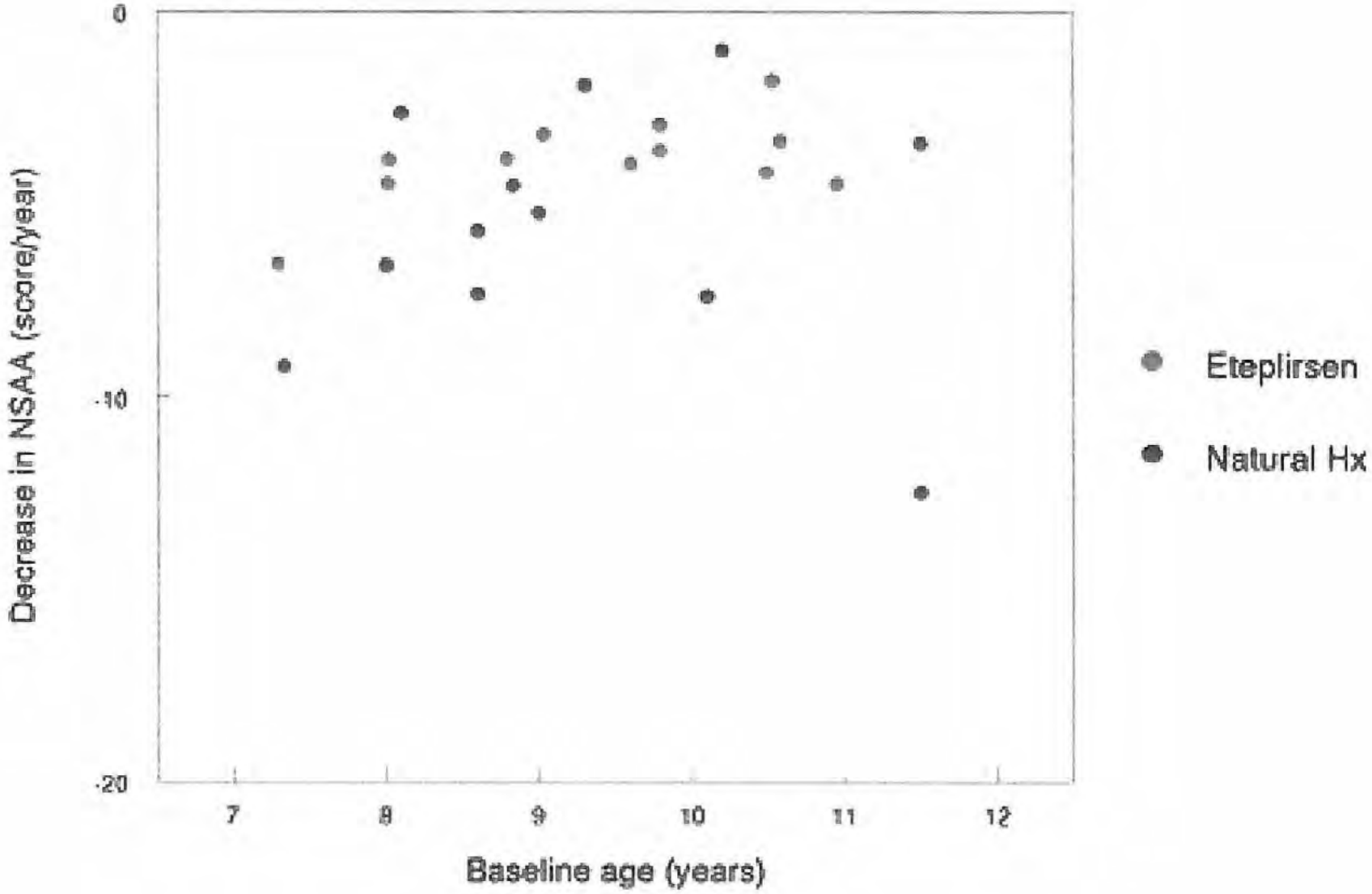
Viewed against age, eteplirsen patients bounded by controls (arrows) (more red lines right from lack of matched controls)



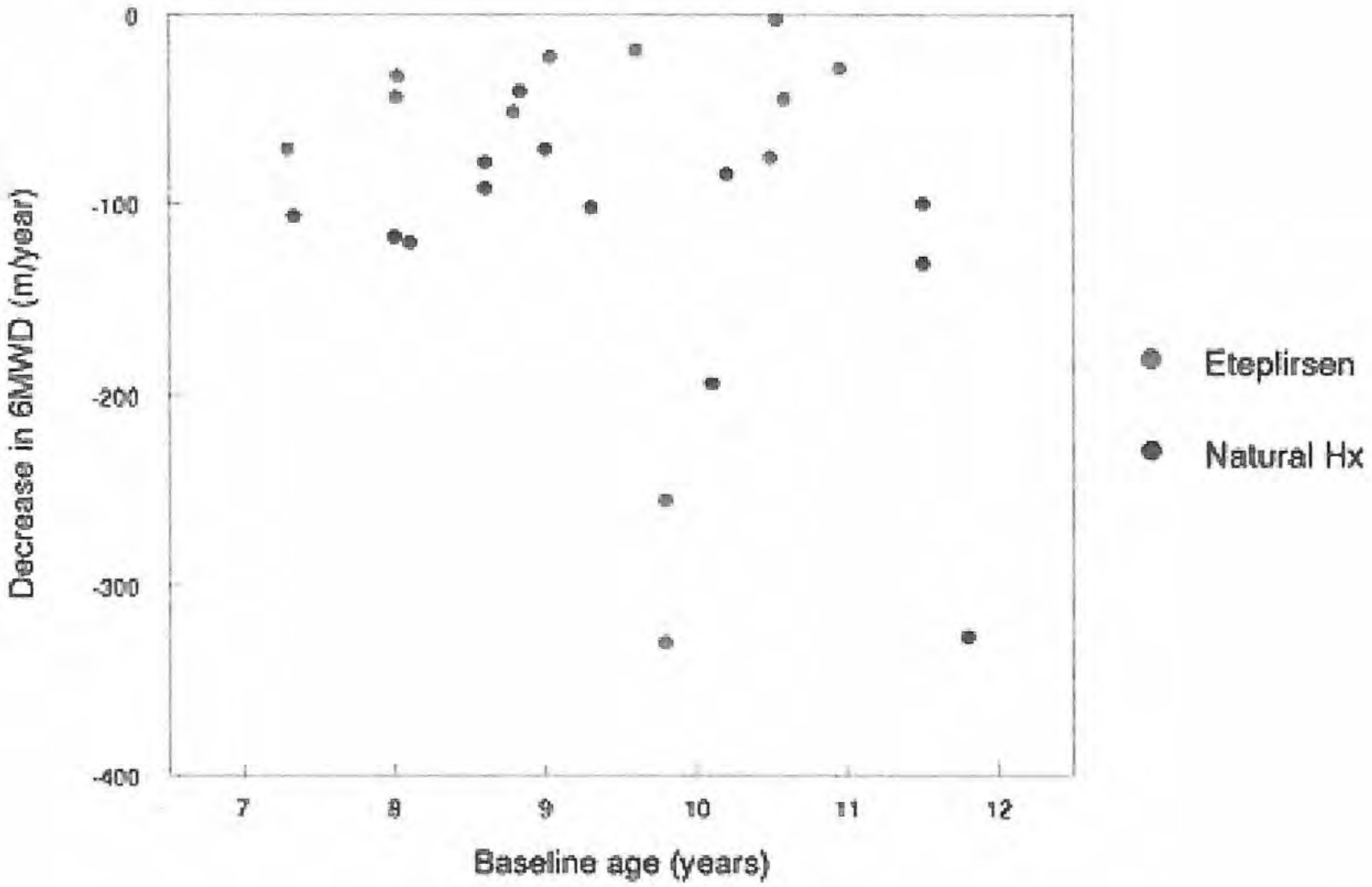
Similar change of NSAA shown for all patients - declining from "upper left" to "lower right" (arrows for illustration only)



Slope analysis, NSAA



Slope analysis, 6MWT



From:	Choy, Fannie (Yuet)
To:	Woodcock, Janet
Cc:	Jenkins, John K; Unger, Ellis; Temple, Robert; Dunn, Billy; Bastings, Eric; Choy, Fannie (Yuet)
Subject:	Eteplirsen Package (NDA 206488)
Date:	Tuesday, May 17, 2016 7:49:50 PM
Attachments:	Stat_206488 Eteplirsen Stats review_final.pdf OCP_Eteplirsen_OCP_Review.pdf Farkas CDTL review eteplirsen file.pdf 20160507b Eteplirsen N206488S000 Clinical Review.pdf Eteplirsen Draft Office Director Memo_5-17-16.pdf NDA 206488 (eteplirsen) Division Director memo.pdf

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Dr. Woodcock:

Attached please find the reviews and memos for NDA 206488 / eteplirsen for your review.

Office Director

Memo Director

Memo CDTL Memo

Clinical Review

Biostat Review OCP Review

Thank you,

Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products

CLINICAL PHARMACOLOGY REVIEW

NDA Number:	206488
Applicant Name:	Sarepta Therapeutics, Inc.
Submission Dates:	08/20/2015, 11/02/2015
Brand Name:	EXONDYS 51
Generic Name	Eteplirsen
Dosage Form:	Aqueous solution for intravenous infusion
Dosage Strengths:	Single use 2 mL vials containing 100 mg (50 mg/mL) of eteplirsen and single use 10 mL vials containing 500 mg (50 mg/mL) of eteplirsen
Proposed Indication:	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
OCP Division (s):	Division of Pharmacometrics, Division of Clinical Pharmacology 1, Genomics and Targeted Therapy
Primary Reviewers:	Atul Bhattaram, Ta-Chen Wu, Bart Rogers
Team Leaders:	Kevin Krudys, Angela Men, Christian Grimstein

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY

Eteplirsen is an exon skipping phosphorodiamidate morpholino oligomer (PMO) which is expected to restore the mRNA reading frame to induce dystrophin protein production and is proposed to be indicated 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 proposed treatment regimen is 30 milligrams per kilogram of body weight (30 mg/kg) once weekly as an intravenous infusion over 35 to 60 minutes.

The findings from the Office of Clinical Pharmacology are as follows:

- A relationship between eteplirsen dose (30, 50 mg/kg/wk) and changes in 6 minute walk distance (6MWD) cannot be characterized based on the clinical study (Study 201/202) in 12 patients.
- Comparison of changes in 6MWD and NSAA total score in eteplirsen treated patients with historical controls from Italian DMD Registry and Leuven Neuromuscular Reference Center [NMRC, Belgium] did not provide clear evidence of efficacy. The analyses included 3 year follow up data submitted on 5/20/2015, 4th year update from eteplirsen treated patients submitted on 12/14/2015, updates on historical controls submitted on 12/17/2015, 01/08/2016 and correction in 6MWD from a patient in historical controls submitted on 04/01/2016. Issues with DMD historical controls from Italy and Belgium are well documented in the review by Dr. Ronald Farkas (Clinical Team Leader, Division of Neurology Products (DNP), CDER, FDA).
- 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 t1/2 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.
- When found to be safe and effective, eteplirsen should be indicated for all mutations amenable to exon-51 skipping.

1.1 Recommendations

The Office of Clinical Pharmacology (OCP) has reviewed the submission (NDA 206488) and recommends that robust evidence on the effectiveness of eteplirsen needs to be generated by the sponsor prior to approval. The recommendations from OCP are discussed below:

- The sponsor should conduct a double-blind, placebo-controlled study in patients with mutations that are amenable to exon-51 skipping and who are likely to be ambulant for 1 year. The ability of such a trial to provide substantial evidence of effectiveness is based on the (A) reported 6MWD changes in 10 out of 12 patients (non-ITT) who remained ambulatory at 48 weeks in Study 201/202 and (B) testimonies about beneficial effects by patients and caregivers at PCNS (Peripheral and Central Nervous System) advisory committee meeting held on April 25th, 2016. The sponsor should demonstrate evidence of effectiveness using appropriate clinical endpoints that are

based on baseline upper or lower body strength in patients whose age is between 4 and 12 years.

- 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. An example of an alternative regimen would be eteplirsen administered twice weekly for 6 months followed by once weekly for 6 months. A more frequent dosing regimen could help to increase the production of dystrophin. The immunogenicity of eteplisen can be further assessed in future clinical trial(s) as well.

1.2 Summary of Important Clinical Pharmacology Findings

Effectiveness of Eteplirsen in DMD Patients (Age 7-12 years)

The efficacy and safety of eteplirsen was evaluated in 1 clinical study (Study 201/202) that enrolled 12 patients. Briefly, in the clinical study (Study 201):

2 patients received placebo for 24 weeks followed by eteplirsen 30 mg/kg for 4 weeks
2 patients received placebo for 24 weeks followed by eteplirsen 50 mg/kg for 4 weeks
4 patients received eteplirsen 30 mg/kg for 28 weeks
4 patients received eteplirsen 50 mg/kg for 28 weeks

The primary efficacy endpoint in Study 201 was the change from baseline in the percentage of dystrophin-positive fibers as measured in muscle biopsy tissue using immunohistochemistry (IHC) at Week 12 for the 50 mg/kg/wk eteplirsen and matching placebo groups and at Week 24 for the 30 mg/kg/wk eteplirsen and matching placebo groups. The sponsor also collected data on clinical endpoints such as 6 minute walk distance (6MWD), rise time and NorthStar Ambulatory Assessment total score (NSAA). No significant differences in 6MWD were detected between the eteplirsen and placebo groups at the end of 24 weeks (Refer to the review by Dr. Xiang Ling, Division of Biometrics 1, Office of Biostatistics, CDER, FDA).

Figure 1 (Left graph) shows 6MWD by individual patient and treatment group up to 48 weeks in Study 201/202. Figure 1 (Right graph) shows the mean change in 6MWD by treatment group up to 48 weeks. Two boys (twins) randomized to the 30 mg/kg group lost ambulation within 24 weeks. Sponsor attributes the early loss of ambulation to low 6MWD at baseline.

Figure 2 (Left graph) shows the mean change in 6MWD in 8 patients (ITT population) who received eteplirsen at baseline (early start) and 4 patients who received eteplirsen after 24 weeks (delayed start). Figure 2 (Right graph) shows the mean change in 6MWD in 10 ambulatory patients at 48 weeks (non-ITT population) who received eteplirsen at baseline (early start) and those who received eteplirsen after 24 weeks (delayed start). Sponsor attributes the apparent stabilization of 6MWD in the delayed start group at 36 weeks to dystrophin production time.

The recommended dose of eteplirsen, 30 mg/kg administered by weekly IV infusion, was chosen based on results from combined Studies 201/202 which showed no apparent difference in biological and clinical efficacy compared to 50 mg/kg as assessed by the percentage of dystrophin-positive fibers and clinical outcome measures including the 6MWD.

For greater details on dystrophin production, please refer to the review by Dr. Ashutosh Rao (Acting Branch Chief, Office of Biotechnology Products, CDER), Dr. Christopher Breder (Medical Officer, Division of Neurology Products, CDER) and Dr. Ronald Farkas (Team Leader, Division of Neurology Products, CDER). Several issues relating to amount of dystrophin formed, controls to compare pre- and post-baseline dystrophin

levels, correlations between dystrophin and clinical endpoints are discussed in their reviews.

Figure 1. (Left) 6MWD vs Time by Treatment Group and Individual Patient (Right) Mean Change in 6MWD vs Time by Treatment Group. Patients Were Switched From Placebo to Eteplirsen at 24 Weeks (Shown in Reference Line at 24 Weeks)

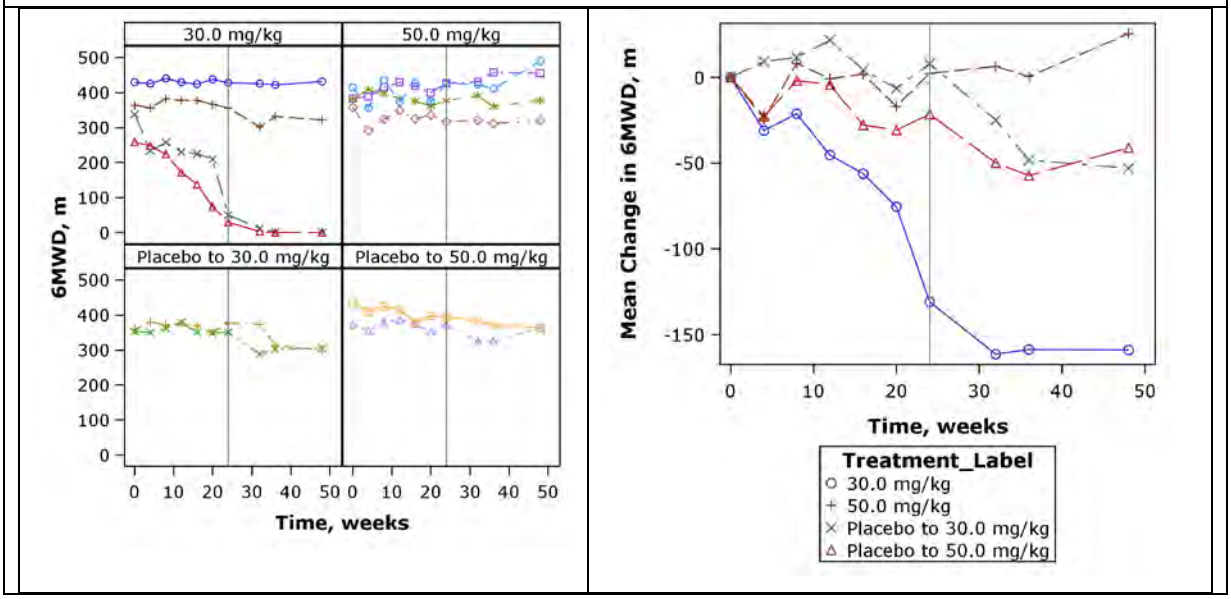
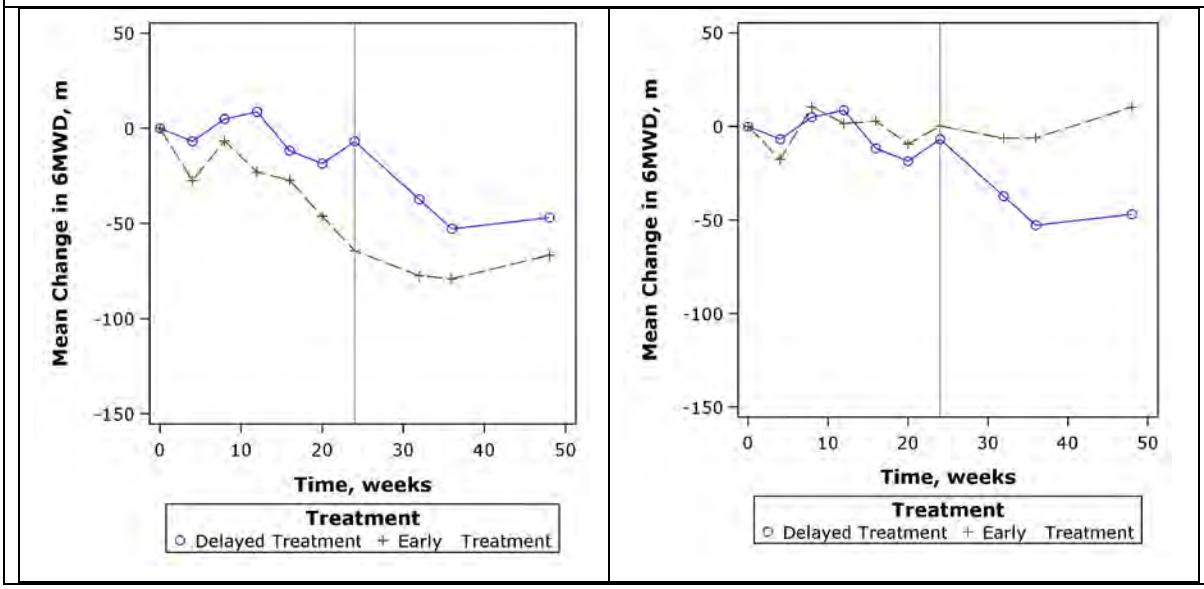


Figure 2. (Left) Mean Change in 6MWD vs Time by Early Treatment and Delayed Treatment Group in ITT population. (N=12) (Right) Mean Change in 6MWD vs Time by Early Treatment and Delayed Treatment Group in non-ITT population (N=10).



Patients receiving eteplirsen or placebo during the first 24 weeks continued to receive eteplirsen for the next 4 years in an open-label setting (Study 202). At the end of 4 years (~216 weeks), changes in 6MWD and NSAA total scores between eteplirsen-treated patients (Study 201/202) and historical controls were compared by the sponsor. The following filters were applied to allow for the identification of a matched patient historical control cohort:

1. Corticosteroid use at Baseline

2. Sufficient longitudinal data for 6MWT available (defined as including a baseline value and at least one valid post-baseline value)

3. Age ≥7

4. Genotype amenable to any exon skipping therapy

5. Genotype amenable to exon 51 skipping therapy

For information on statistical analysis of the comparisons, refer to the review by Dr. Xiang Ling, Division of Biometrics 1, Office of Biostatistics, CDER, FDA. The reviewer (Dr Atul Bhattaram, Division of Pharmacometrics, Office of Clinical Pharmacology, CDER, FDA) conducted additional exploratory analysis to address issues raised by the clinical team (Division of Neurology Products (DNP), CDER, FDA). Specifically, the clinical team was interested in exploring changes in 6MWD, NSAA total score and other endpoints like rise time with age in eteplirsen treated patients and historical controls. Also, the clinical team was interested in comparing changes in 6MWD from eteplirsen treated patients with patients who received placebo in other controlled clinical trials. The findings from these analyses are discussed below:

Figure 3 shows the individual and mean level changes in 6MWD and NSAA total scores with time in eteplirsen-treated patients and patients in natural history studies. Figure 3 would suggest that 10 out of 12 patients treated with eteplirsen have not lost ambulation compared to 12 out of 13 historical controls. Further exploration of the data was conducted using age of the patient instead of time since enrollment in the study. This decision was taken because time since enrollment in the study does not account for baseline age of the patient.

Figure 4 shows the individual level changes in 6MWD and NSAA total scores with age in eteplirsen treated patients and patients in natural history studies. Figure 5 shows the individual level changes in rise time and 10 meter run/walk time with age in eteplirsen treated patients and patients in natural history studies. It should be noted that NSAA total scores, rise time and 10 meter run/walk time are not available from all patients in natural history studies. The review team concluded that, in general, changes in the clinical scores in eteplirsen treated patients are in the range of changes in historical control, while recognizing changes in clinical scores in some individual eteplirsen treated patients around 14-15 years appear different from natural history controls.

The review team also identified several issues with historical controls (refer to review by Dr Ronald Farkas, DNP, CDER, FDA). These issues were discussed at PCNS AC meeting on April 25th, 2016.

Figure 3. (**Top, Left**) Individual Patient Level Changes in 6MWD With Time in Eteplirsen Treated Patients (—) and Historical Controls (—). (**Top, Right**) 6MWD (Mean±SD) Changes With Time in Eteplirsen Treated Patients and Historical Controls. (**Bottom, Left**) Individual Patient Level Changes in Total NSAA score With Time in Eteplirsen Treated Patients and Historical Controls. (**Top, Right**) Total NSAA score (Mean±SD) Changes With Time in Eteplirsen Treated Patients and Historical Controls.

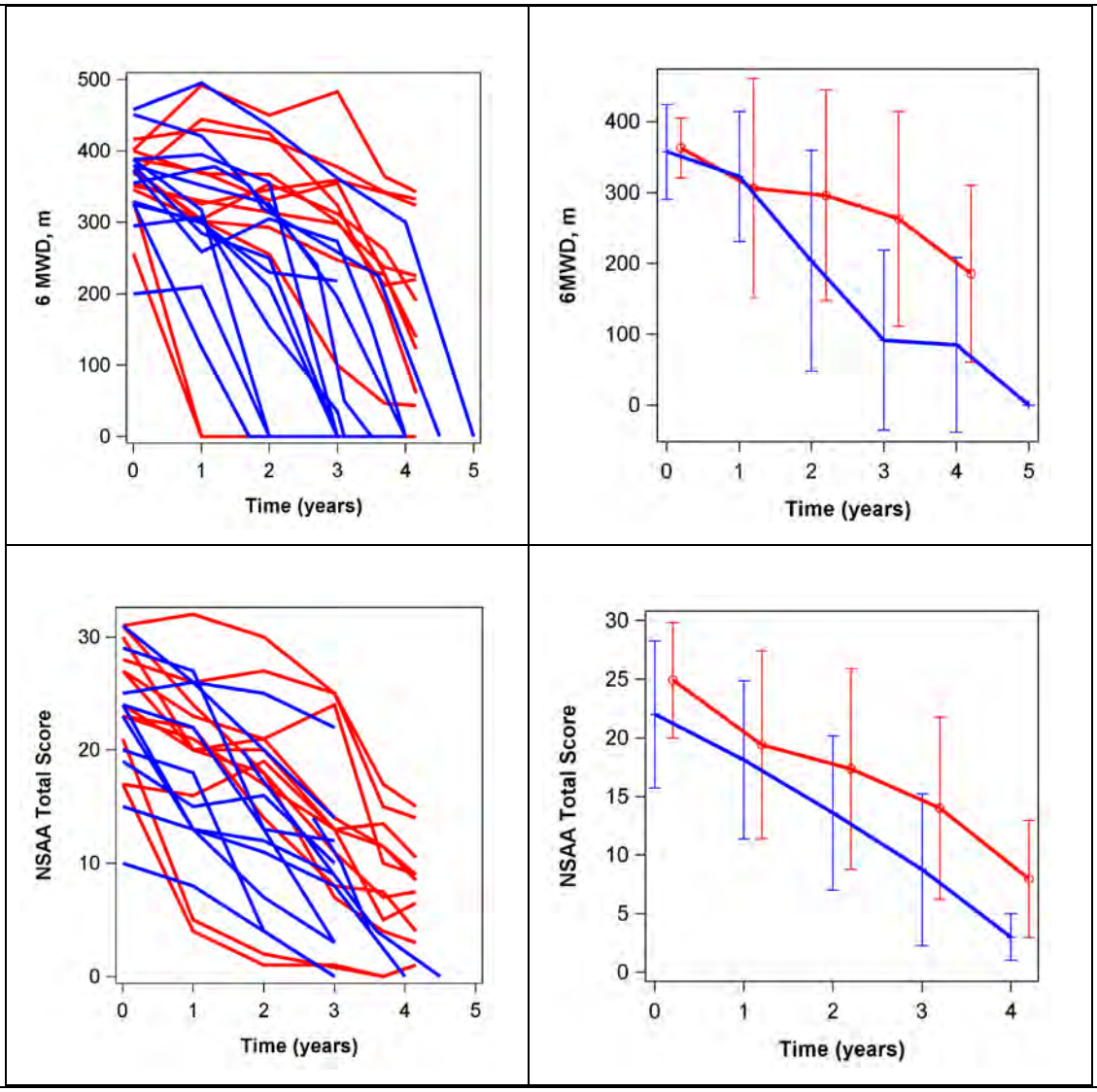
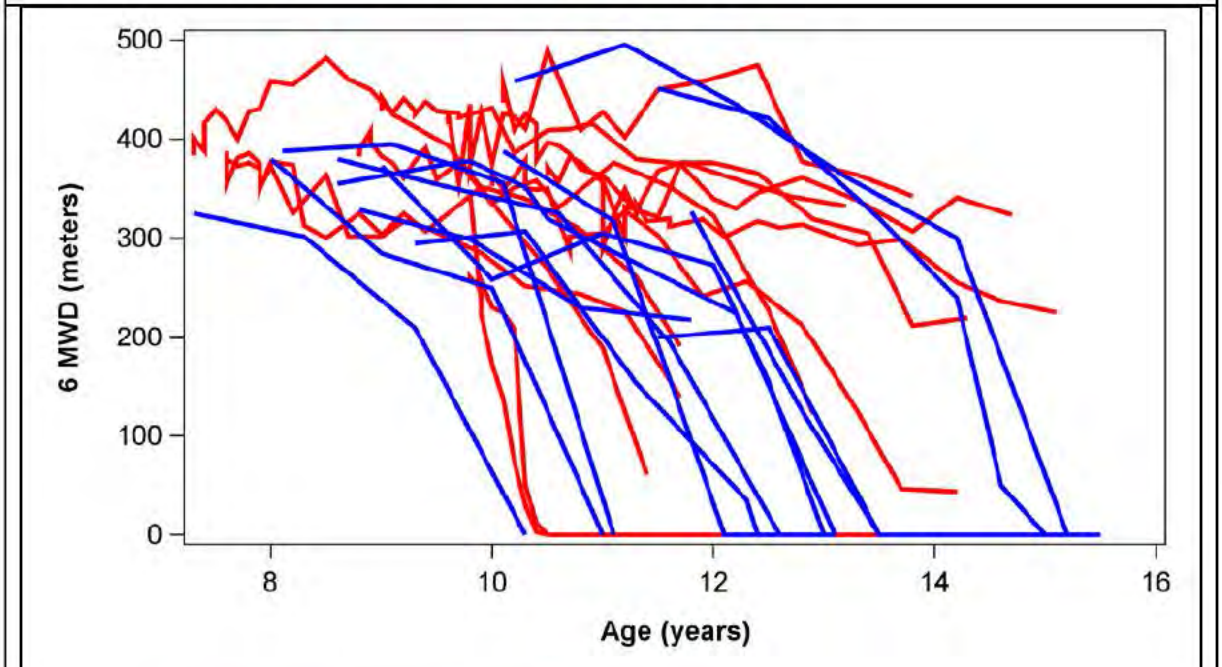


Figure 4. (Top) Changes in 6MWD in 12 Eteplirsen Treated Patients (—) and 13 Historical Controls (—) With Age (Bottom) Changes in NSAA Total Score in 12 Eteplirsen Treated Patients and 12 Historical Controls With Age.



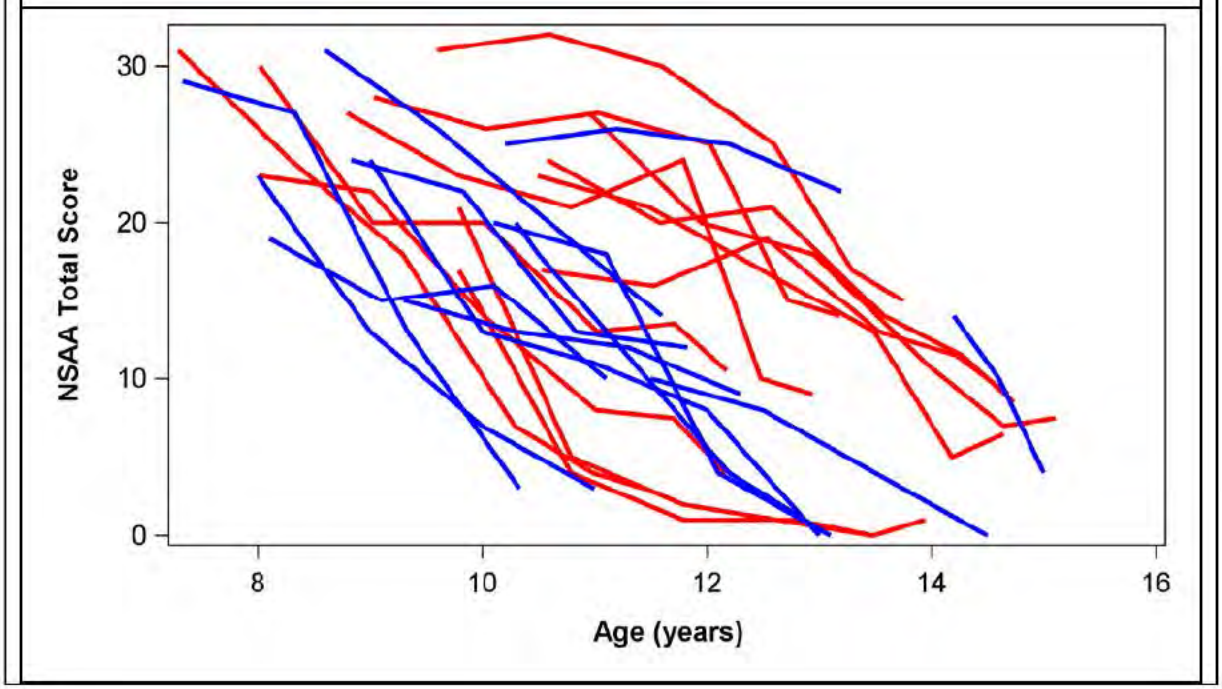
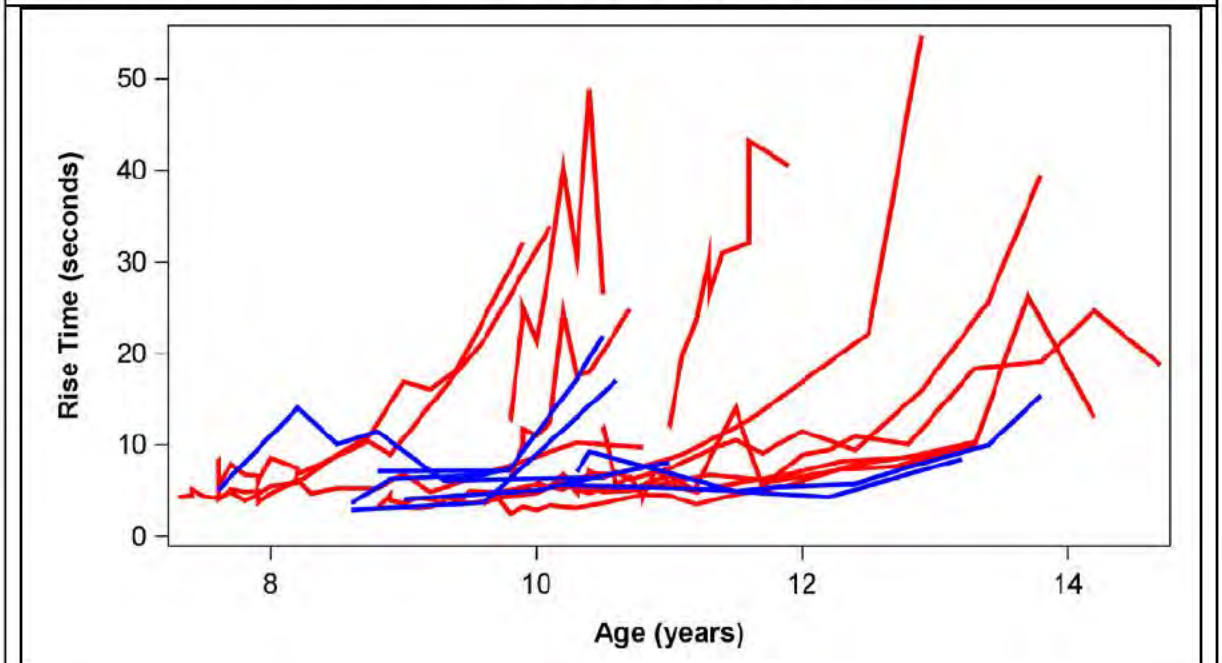
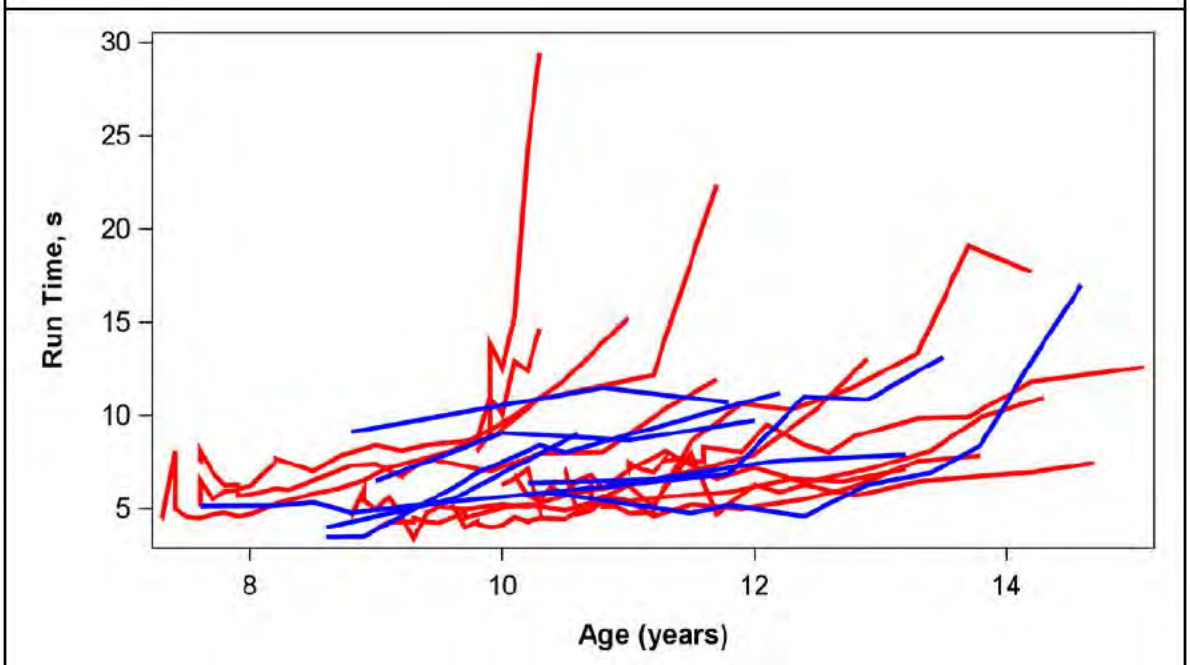


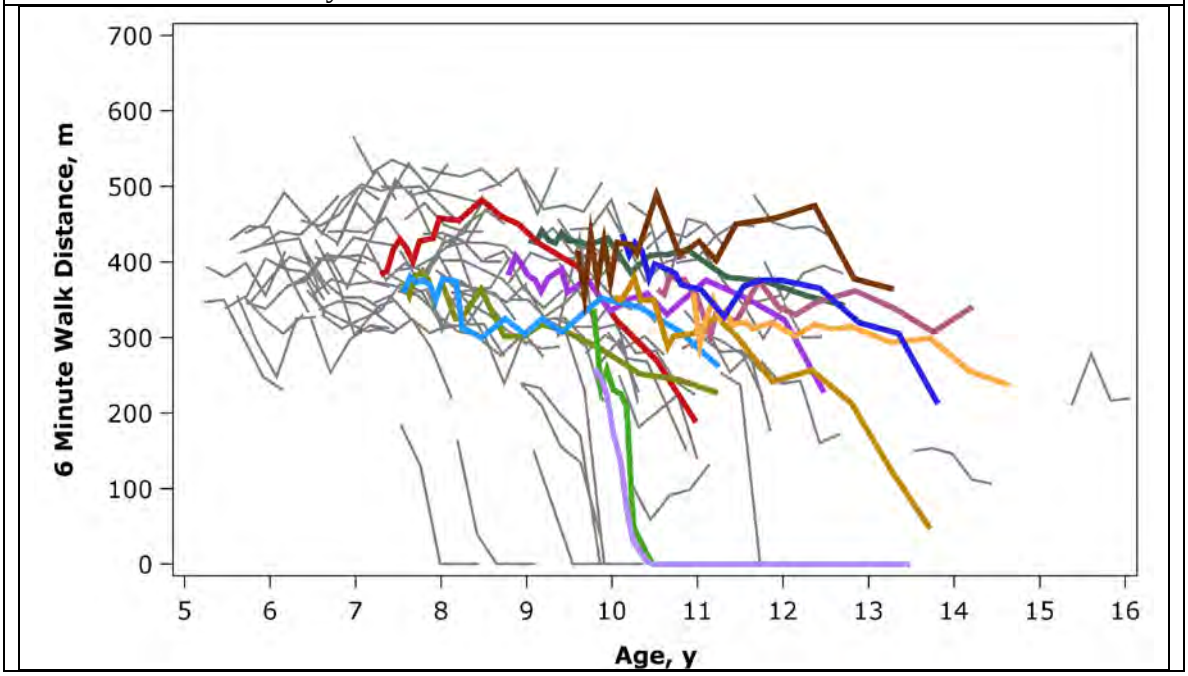
Figure 5. (Top) Changes in Rise Time in 12 Eteplirsen Treated Patients (—) and 8 Historical Controls (—) With Age (Bottom) Changes in 10 meter Run Time in 12 Eteplirsen Treated Patients and 7 Historical Controls With Age.





Due to issues with the historical controls (refer to review by Dr. Ronald Farkas, Clinical Team Leader, DNP, CDER, FDA), the review team compared 6MWD changes in eteplirsen-treated patients with patients receiving placebo in well-controlled clinical trials conducted by another sponsor. The findings are shown in Figure 6. The data suggests that the 6MWD changes in eteplirsen-treated patients are within natural course of the disease. However, it should be noted that there are few patients in the range of 13-16 years for comparison purposes. For discussion on how these findings influence decision on overall evidence of effectiveness, refer to the review by Dr. Ronald Farkas , DNP, CDER, FDA.

Figure 6. Changes in 6MWD With Age. Data From 12 Eteplirsen-Treated Patients are Shown in Colored Lines. Data From Patients in Placebo Group From Other Controlled Trials are Shown in Grey Lines



Pharmacokinetics:

- Approximate dose-proportionality and linearity in PK properties were observed following multiple-doses (0.5~20 mg/kg/wk) in Phase 1 studies and multiple-doses (30 and 50 mg/kg/wk) in efficacy trials. There was insignificant drug accumulation following weekly dosing across this dose range of 0.5~50 mg/kg.
- Following single or multiple IV infusion, the peak plasma concentrations (Cmax) of eteplirsen occurred near the end of infusion and plasma concentration-time profiles of eteplirsen were generally similar and showed multi-phasic decline, whereas the majority of drug elimination occurred within 24 hours.
- Plasma protein binding of eteplirsen in humans is relatively low, ranging 6.1~16.5% and is independent of concentration studied.
- Distribution or cellular uptake of eteplirsen into peripheral tissues is supported by the volume of distribution (Vd) values obtained following single or multiple doses (e.g., approximately 19 L/31.5kg after 30 mg/kg/week doses in Study 201).
- Eteplirsen was found to be metabolically stable in vitro with no evidence of metabolism or metabolite.
- 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, total clearance of eteplirsen of 339 and 319 mL/hr/kg, and renal clearance of 221 and 234 mL/hr/kg, respectively. Elimination t1/2 was 3.3~3.5 and 3.2~3.8 hours on average for 30 and 50 mg/kg, respectively. To note, elimination t1/2 ranged 1.6~3.6 hours across the lower 0.5~20 mg/kg/wk dose range.
- The inter-subject variability of eteplirsen is considered to be moderate, generally in the range of 20~55% for exposure measures (Cmax and AUCs) as well as other key PK parameters.

Intrinsic factors:

Mutations Amenable to Exon 51 Skipping:
The sponsor has studied six different DMD mutations amenable to exon-51 skipping therapy. Additional DMD mutations (e.g. 19-50, 52-63) are known to exist, however they are ultra-rare (1-2 subjects in database) in nature. While there may be some differences in functionality of the exon-51 skipped transcripts; restoring the reading frame to produce dystrophin even if it may be different between DMD mutations is warranted.

Extrinsic factors:

Drug-Drug Interaction (DDI)
In vitro studies:
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:

- Eteplirsen had insignificant inhibitory effects for CYP2B6, CYP2C8, CYP2D6, CYP3A4/5, CYP1A2, CYP2C9, or CYP2C19 in human liver microsomes. There was no metabolism-dependent inhibition observed with any of the CYPs tested.
- Eteplirsen at the concentration range studied did not show significant enzyme inducing capability for CYP1A2, CYP2B6, and CYP3A4 in human hepatocytes.
- Eteplirsen is not a substrate and/or an inhibitor of major human drug transporters OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, and BSEP in transfected CHO cells, Caco-2 monolayers, or inside-out human membranes.

Safety Findings

Please refer to the review by Dr. Christopher Breder (Medical Officer, Division of Neurology Products, CDER)

2 QUESTION BASED REVIEW

2.1 General Attributes of the Drug

2.1.1 What are the proposed mechanism of action and therapeutic indications?

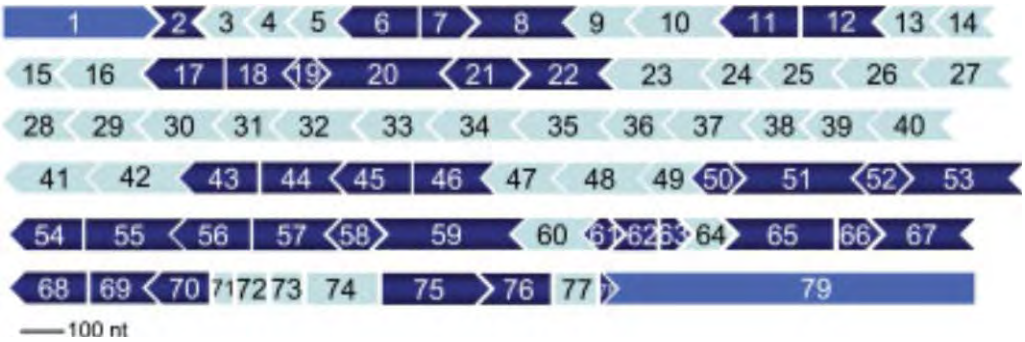
Eteplirsen is an exon skipping phosphorodiamidate morpholino oligomer (PMO) which is intended to restore the mRNA reading frame to induce a truncated dystrophin protein production. The proposed indication is for the treatment of Duchenne Muscular Dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping.

2.1.2 Are the studied populations in the sponsor's clinical trials representative of the to-be labeled population?

No. The sponsor has studied six different DMD mutations amenable to exon-51 skipping therapy. Eteplirsen is to be indicated for all mutations amenable to skipping exon 51.

Additional DMD mutations (e.g. 19-50, 52-63) are known to exist, however they are ultra-rare (1-2 subjects in database) in nature. A search of the Leiden DMD database (www.dmd.nl) using the known exon splicing (Figure 7)**,** identified subjects composing of ten additional DMD mutations (i.e., 3-50, 13-50, 17-50, 19-50, 29-50, 38-50, 40-50, 43-50, 52-58, 52-63) that may be amenable to exon-51 skipping based on the mechanism of action of eteplirsen. Amenable mutations are those in which skipping of exon-51 would, in theory, restore the reading frame. For instance, in Figure 1, a subject with a deletion of exons 44-50 would not be amenable to exon-51 skipping as exons 43 and 52 cannot be spliced together, whereas, a deletion of exons 43-50 can be successfully spliced by exon-51 skipping.

Figure 7. Depiction of the 79 Exons of the Dystrophin Gene and Splicing



Source: PMID 19156838

Note: In-frame exons are in light blue, out-of-frame in dark blue. Deletions are considered in-frame when the exons flanking the deletion "fit."

2.1.3 Should eteplirsen be indicated for patients amenable to exon-51 skipping who were not studied in the clinical development program?

Yes. Despite not all DMD mutations amenable to exon-51 skipping being represented in the clinical development program, if eteplirsen is ultimately found to be safe and effective to warrant approval, then eteplirsen should be indicated for all exon-51 amenable mutations.

2.2 General Clinical Pharmacology

2.2.1 Are the active moieties in plasma appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes. Eteplirsen is the active moiety in plasma and in urine and are measured using validated HPLC methods.

2.2.2 Immunogenicity

The Sponsor reported no detected biologically meaningful effects of eteplirsen on the immune system in nonclinical studies. In Study AVI-4658-28, patients had undetectable levels of anti-dystrophin antibody following treatment of eteplirsen. Most of the patients in the 10.0 and 20.0 mg/kg dose groups showed decreases in CD3, CD4 and CD8 counts, which is consistent the nonclinical findings. Because of the very limited number of subjects in various studies, any pertinent effect on the PK could not be meaningfully assessed.

2.2.3 What are the PK characteristics of the drug?

2.2.3.1 What are the single and multiple dose PK parameters?

The PK characteristics of eteplirsen following 60-minute IV infusion in male patients with DMD (5-15 years of age) were evaluated in the multiple-dose, dose-rangingStudy AVI-4658-28 (Weeks 1, 6, 12), pivotal Studies AVI-4658-201 (Weeks 1, 12, 24/25), and long-term extension Studies AVI-4658-202 (Week 8 (cumulative Week 36) and Week 124 (cumulative Week 152)). Plasma samples for eteplirsen were collected up to 24 hours post-end of infusion. Urine samples for PK characterization were also collected for 24 hours in these studies.

As illustrated in Figure 8, 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. Plasma PK parameters for eteplirsen are summarized in Table 1, Table 2, Table 3, whereas representative urinary PK parameters are summarized in Table 4.

Figure 8. Plasma concentration-time profiles (mean (SD) of eteplirsen from the end of the 60-minute infusion for each dose cohort averaged across in Study AVI-4658-28 (Weeks 1, 6, and 12) and in Study AVI-4658-201 (Week 12).

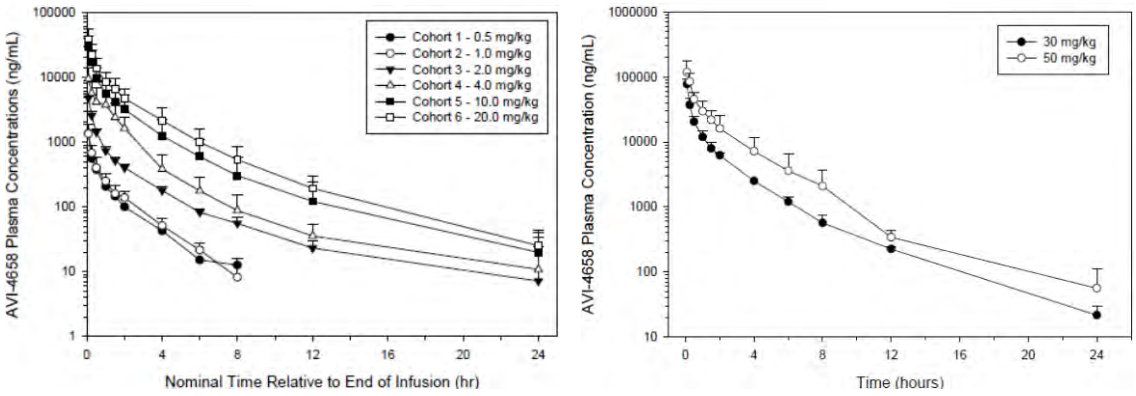


Table 1. Eteplirsen plasma PK parameters across 0.5~20 mg/kg dose range at Weeks 1, 6, and 12 (Study AVI-4658-28)

	eteplirsen dose (mg/kg)					
Parameter Statistic	0.5 (N=4)	1.0 (N=2)	2.0 (N=2)	4.0 (N=3)	10 (N=4)	20 (N=4)
T_{max} (hr)						
n	2	5	6	8	12	12
Mean (SD)	1.09 (NA)	1.16 (0.060)	1.12 (0.100)	1.22 (0.310)	1.07 (0.060)	1.10 (0.040)
C_{max} (ng/mL)						
n	2	5	6	8	12	12
Mean (SD)	1360 (NA)	1340 (549)	4820 (804)	9500 (4290)	29400 (14300)	39000 (16900)
AUC_{0-24} (hr*ng/mL)						
n	2	5	6	8	12	12
Mean (SD)	1570 (NA)	1730 (541)	6200 (586)	15400 (8310)	39700 (20900)	57100 (24400)
$AUC_{0-\infty}$ (hr*ng/mL)						
n	2	5	6	6	12	12
Mean (SD)	1570 (NA)	1740 (537)	6220 (555)	18600 (6920)	39800 (21000)	57300 (24500)
CL_{tot} (mL/hr/kg)						
n	2	5	6	6	12	12
Mean (SD)	320 (NA)	615 (173)	324 (29.3)	233 (61.6)	317 (144)	404 (148)
V_{ss} (mL/kg)						
n	2	5	6	6	12	12
Mean (SD)	482 (NA)	981 (305)	703 (285)	450 (136)	556 (212)	862 (308)
$t_{1/2}$ (hr)						
n	2	2	6	6	12	12
Mean (SD)	1.62 (NA)	1.62 (0.317)	2.58 (0.304)	2.11 (0.531)	3.27 (1.25)	3.60 (0.376)
MRT_{∞} (hr)						
n	2	5	6	6	12	12
Mean (SD)	1.50 (NA)	1.58 (0.116)	2.16 (0.853)	1.92 (0.347)	1.85 (0.327)	2.15 (0.223)

* N represents sum of the number of subject datasets evaluable across the 3 study weeks and does not represent distinct individuals.

Table 2. Eteplirsen plasma PK parameters at Week 12 (Study AVI-4658-201)

Treatment Group	Statistic	T_{max} hr	C_{max} ng/mL	AUC_{0-24} hr*ng/mL	$AUC_{0-\infty}$ hr*ng/mL	CL_{PL} mL/hr/kg	V_{ss} mL/kg	$t_{½}$ hr
Eteplirsen 30 mg/kg	N	4	4	4	4	4	4	4
	Mean	1.08	77,200	91,040	91,170	339	601	3.30
	SD	0.01	15,568	16,713	16,755	75.8	157	0.341
	CV%	1.26	20.2	18.4	18.4	22.3	26.1	10.3
Eteplirsen 50 mg/kg	N	4	4	4	4	4	4	4
	Mean	1.14	124,600	180,825	181,162	319	638	3.17
	SD	0.08	54,898	87,698	88,040	125	224	0.249
	CV%	6.58	44.1	48.5	48.6	39.1	35.1	7.85

Table 3. Eteplirsen plasma PK parameters at Week 152 (Study AVI-4658-202)

Treatment Group	Statistic	T_{max} hr	C_{max} ng/mL	AUC_{0-24} hr*ng/mL	$AUC_{0-\infty}$ hr*ng/mL	CL_{PL} mL/hr/kg	V_{ss} mL/kg	$t_{1/2}$ hr
Eteplirsen 30 mg/kg	n	6	6	6	6	6	6	6
	Mean	1.12	85,067	127,457	127,810	243.9	526.2	3.543
	SD	0.08	15,913	25,798	25,906	54.9	91.5	0.643
	CV%	7.31	18.71	20.24	20.27	22.51	17.39	18.15
Eteplirsen 50 mg/kg	n	6	6	6	6	6	6	6
	Mean	1.11	125,750	192,618	193,181	322.1	690.0	3.775
	SD	0.06	64,610	106,879	107,442	150.0	339.7	0.628
	CV%	5.84	51.38	55.49	55.62	46.58	49.24	16.64

Table 4. Eteplirsen urinary PK parameters at Week 12 (Study 201)

Treatment Group	Subject	Body Weight (kg)[a]	Urine Volume (mL)	Urine Concentration (µg/mL)	Amount Excreted (mg)	Percent of Dose Excreted	CL_R (mL/hr/kg)	CL_{PL} (mL/hr/kg)	Percent CL_R/CL_{PL}
Eteplirsen	n	4	4	4	3[b]	3[b]	3[b]	4	3[b]
30 mg/kg	Mean	37.6	1,746	602	701	64.1	221	339	64.2
	SD	9.0	569	299	329	13.8	53.1	75.8	13.8
	CV%	23.9	32.6	49.7	46.9	21.5	24.0	22.3	21.5
Eteplirsen	n	4	4	4	4	4	4	4	4
50 mg/kg	Mean	30.8	786	1,378	1,026	69.4	234	319	69.5
	SD	8.0	301	378	270	24.7	154	125	24.7
	CV%	26.0	38.3	27.4	26.3	35.6	65.7	39.1	35.5

a. Body weight at Week 11 was used to determine infusion amount for Week 12 dose
b. Excluding an outlier at the 30 mg/kg dose level

Detailed information and discussion are available in the following Sections.

2.2.3.2 How does the PK of the drug and its major metabolites in healthy adults compare to that in patients?

The parent drug eteplirsen is the only known active moiety and was measured across the studies. Pharmacokinetics were characterized in pediatric patients with DMD only, not in healthy subjects.

2.2.3.3 What are the characteristics of drug absorption, distribution, metabolism and elimination?

Absorption:

The bioavailability is assumed 100% because of the proposed route of drug administration (i.e., IV infusion). Following single or multiple IV infusions, the peak plasma concentrations (Cmax) of eteplirsen occurred near the end of infusion. i.e., approximately 1.07~0.22 hours over 0.5-20 mg/kg/wk doses and 1.08~1.14 hours at 30 and 50 mg/kg/wk doses studied. Approximate dose-proportionality and linearity in PK properties were observed following multiple-doses (0.5~20 mg/kg/wk) in Phase 1 studies and multiple-doses (30 and 50 mg/kg/wk) in efficacy trials.

Distribution:
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 [see Section 2.4.2.1]. 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.

Metabolism:
Eteplirsen was found to be metabolic stable in human liver microsomes (Study 4658 PKD 002) with no evidence of metabolism or metabolite.

Elimination:
In Study AVI-4658-28, PK urine samples were collected up to 24 hours at Weeks 1, 6, and 12 to assess the renal route of elimination. The mean percent of dose excreted unchanged in the urine ranged from 32.1% to 63.7% across 0.5~20 mg/kg/week dose range, with higher 63.7% and 60.3% excreted for the 10 and 20 mg/kg doses,

respectively. Renal clearance ranged 116~229 mL/hr/kg (or 62.6~119.4 mL/min), with higher 198 and 229 mL/hr/kg for the 10 and 20 mg/kg dose, respectively. Elimination t1/2 ranged 1.6~3.6 hours across 0.5~20 mg/kg/wk dose range.

In Study AVI-4658-201, the 24-hour urine sampling following 12 weeks of dosing resulted in 64.1% and 69.4% of mean percent of dose excreted unchanged in the urine for 30 and 50 mg/kg dose, respectively. The total clearance of eteplirsen was 339 and 319 mL/hr/kg following 12 weeks doses of 30 and 50 mg/kg, respectively. The renal clearance of eteplirsen was 221 and 234 mL/hr/kg following 12 weeks of 30 and 50 mg/kg, respectively. Renal clearance of eteplirsen accounted for 64.1% or approximately two-thirds of total systemic clearance. Elimination $t_{1/2}$ was 3.3 and 3.2 hours on average for 30 and 50 mg/kg, respectively. To note, similar systemic and urinary PK parameters were obtained for the extension study (Study AVI-4658-202), with elimination $t_{1/2}$ being 3.5 and 3.8 hours on average for 30 and 50 mg/kg, respectively.

2.2.3.4 Based on PK parameters, what is the degree of linearity in the dose-concentration relationship?

Eteplirsen dose proportionality was evaluated in a multiple dose study (Study AVI-4658-202) with mean Cmax, AUC0-24, and AUC0-∞ across a weekly dose range of 0.5~20 mg/kg. These parameters increased in a slightly less than proportional manner, with exponents of the power curve being 0.80, 0.82, and 0.78 for Cmax, AUC0-24, and AUC0-∞, respectively (<1.0 for dose-proportionality).

In Study AVI-4658-201, between the 30 and 50 mg/kg/wk dose levels (1.67-fold), Cmax increased approximately dose-proportionally (~1.61-fold), whereas AUCs increased more than dose-proportionally (~1.99-fold). In Study AVI-4658-202 with same doses, Cmax increased approximately 1.48-fold, whereas AUC0-∞ increased approximately 1.51-fold.

2.2.3.5 How do the PK parameters change with time following chronic dosing?

The key plasma and urinary PK parameters remained similar following weekly dosing across a dose range of 0.5 to 20 mg/kg for 12 weeks and for 30 and 50 mg/kg through the long-term extension (cumulative week 152) in clinical trials. Furthermore, there was no or minimum observed drug accumulation (ratio of 1.02 and 1.25 for the 30 and 50 mg/kg dose levels, respectively) following weekly dosing across the dose range studied, which is anticipated in view of the short t1/2 and the weekly dosing interval.

2.2.3.6 What is the inter- and intra-subject variability of PK parameters in volunteers and patients?

The inter-subject variability of eteplirsen is considered to be moderate. The mean inter-subject variability for exposure measures (Cmax and AUCs) as well as other key PK parameters (such as CL and Vd) were generally in the range of 20~55%. Of note, there was limitation in numbers of subjects for certain dose cohorts or study.

2.3 Intrinsic Factors

2.3.1 What intrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

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.

2.3.2 Based upon what is known about E-R relationships and their variability, what dosage regimen adjustments are recommended for each group?

2.3.2.1 Elderly

Duchenne muscular dystrophy is a rare degenerative neuromuscular disorder with a worldwide incidence of approximately 1 in 3500 neonatal boys irrespective of geographical region, race, or population density. Patients typically develop a waddling gait as toddlers, have trouble walking by the age of 8, become wheelchair dependent by 10~14 years of age, and die of respiratory or cardiac failure in their 20s ~30s. Therefore, no study has been conducted in the elderly subjects to examine the age effect on PK or clinical consequence.

2.3.2.2 Pediatric Patients

The pharmacokinetic characterization and clinical studies of eteplirsen were conducted in male pediatric DMD patients only.

2.3.2.3 Race

Studies were conducted mostly in Caucasians (for example, all were Caucasians except one each in Study AVI-4658-28 and Study AVI-4658-201/202 were Asian). Potential impact of ethnicity is not known.

2.3.2.4 Renal Impairment

The effect of renal impairment was not assessed.

2.3.2.5 Hepatic Impairment

The effect of hepatic impairment was not assessed.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The in vitro investigation on major CYP isozymes and transporters did not reveal the need for additional in vivo study in humans.

2.4.2 What are the drug-drug interactions?

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

No. Based on results of in vitro investigation on microsomal metabolism, plasma protein binding, inhibition or induction of major CYP isozymes, or major drug transporters, eteplirsen is expected to have a low potential for adverse drug-drug interactions in humans, as summarized below.

Metabolism by CYP: Study 4658 PKD 002 investigated the extent of metabolism and the metabolic profile of ^{14}C-eteplirsen using human hepatic microsomes. ^{14}C-Eteplirsen was found to be metabolically stable under the conditions tested. Similar results were found in the animal species.

Protein-binding:
Study 4658 PKD 001 investigated the extent of binding of ^{14}C-eteplirsen to human plasma proteins in vitro. Overall, protein binding of ^{14}C-eteplirsen to human plasma was low, ranging 6.1~16.5%, and was not shown to be concentration-dependent across range of 8~800 µg/mL of eteplirsen concentration. Clinically significant drug-interaction via protein displacement is unlikely.

Inhibition potential:
Study 4658 PKD 004 investigated the direct (reversible) and metabolism-dependent inhibitory potential of eteplirsen on activities of CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) in pooled human hepatic microsomes, using recommended probe substrates for the CYPs. Results showed that eteplirsen at the concentrations up to 6.66 mg/mL had little or no evidence of direct inhibition for CYP2B6, CYP2C8, CYP2D6, or CYP3A4/5, whereas eteplirsen exhibited inhibitory potential of CYP1A2, CYP2C9, and CYP2C19 at high concentrations of 6.52, 2.75, and 1.16 mg/mL, respectively. There was no metabolism-dependent inhibition observed with any of the CYPs tested.

The Cmax of eteplirsen observed following 30 mg/kg/wk dosing in pivotal clinical study (Study 201) was approximately 77200 ng/mL (or 0.0772 mg/mL). Significant risk of adverse drug-drug interaction via CYP1A2, CYP2C9 and CYP2C19 inhibition is likely to be low following 30 mg/kg/wk doses, based on the following considering: (1) the highest eteplirsen concentration employed in the in vitro study for examining the direct inhibition is 6.62 mg/mL which is approximately 86-fold of observed Cmax of the target dose, (2) the IC50 values for CYP1A2, CYP2C9 and CYP2C19 are approximately 85.7, 35.6 and 15-fold, respectively, of observed Cmax, (3) 0.0533 and 0.160 mg/mL eteplirsen concentrations, which encompasses the observed Cmax, had minimum or no effects on enzymes, (4) in comparison, positive control inhibitors of these enzymes nearly or completely depleted the enzyme activities.

Induction potential:
The induction potential of human CYP isoenzymes (CYP1A2, CYP2B6, and CYP3A4) by eteplirsen was investigated in cryopreserved human hepatocyte suspensions (Study

4658 PKD 003). Assessment of CYP enzyme induction was performed by measuring (1) mRNA levels (gene expression) using real time polymerase chain reaction (RT-PCR) and (2) activities of CYP1A2 (phenacetin O-deethylase), CYP2B6 (bupropion hydroxylase), and CYP3A4/5 (testosterone 6β-hydroxylase), compared to the positive controls. Eteplirsen at the concentration range (0.00146~6.66 mg/mL) did not show significant enzyme inducing capability for these three CYPs, although slight induction or dose-response was observed for mRNA gene expression or enzyme activity. Considering the co-induction of CYP3A and CYP2C isozymes via activation of the Pregnane X receptor (PXR), result of insignificant CYP3A induction would be applicable to CYP2C isozymes.

2.4.2.2 Is the drug an inhibitor and/or an inducer of transport activities of major human transporters?

In vitro assessments (Study 4658 PKD 003) were conducted to determine if ^{14}C-eteplirsen is a substrate and/or an inhibitor of major human drug transporters OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, and BSEP in transfected CHO cells, Caco-2 monolayers, or inside-out human membranes. Results of uptake of ^{14}C-AVI-4658 showed that ^{14}C-AVI-4658 is not a substrate or potent inhibitor of the uptake transporters or efflux transporters tested in the study over the concentration range tested. Among all uptake transporters tested, AVI-4658 at 80 and 800 µg/mL showed weak inhibition of OCT1 and OATP1B1 only.

2.4.2.3 Does the label specify co-administration of another drug?

Yes. Patients will receive concomitant corticosteroid therapy (deflazacort, prednisone, prednisolone).

2.4.2.4 What other co-medications are likely to be administered to the target population?

In addition to corticosteroids, beta blockers, ACE inhibitors, and medications to manage pain and other co-morbidities are likely co-medications for DMD patients.

2.4.2.5 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

No study for in vivo drug-drug interaction has been conducted, as findings of in vitro investigation did not suggest a need for further in vivo study.

2.4.2.6 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions?

Not known

2.4.2.7 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

There appears to be no unresolved questions that are likely to pose significant adverse clinical consequences based on the in-vitro investigation and the available information for the likely co-medications.

2.1 General Biopharmaceutics

2.1.1 What is the relative bioavailability of the proposed to-be-marketed formulation to the immediate release formulation?

Not applicable

2.2 Analytical Section

2.2.1 What bioanalytical methods are used to assess plasma eteplirsen concentrations?

Validated anion exchange high performance liquid chromatography (HPLC) (b) (4) (b) (4) was used to quantify eteplirsen in human plasma and urine samples. The ranges of the assay are 10-1000 ng/mL in plasma and 10-1000 ng/mL or 25 -2500 µg/mL in urine samples. An analog internal standard (IS), (b) (4), was included in the assays. Summaries of the performance characteristics and validation attributes for plasma and urine eteplirsen are provided in the Table 5, Table 6, Table 7 and Table 8 below.

Table 5: Validated (b) (4) Assay Specifications for Eteplirsen in Human Plasma (Studies 4658-28, 4658-us-201, and 4658-us-202/Week 8)

Matrix	Human plasma	
Sample volume	100 µL	
Sample preparation	Protein precipitation	
Instrumental analysis	Anion exchange HPLC with fluorescence detection	
Regression, weighting	Linear, 1/x	
Standard curve (range)	10 - 1000 ng/mL	
Quality control eteplirsen concentrations	10.0 ng/mL (QC-LLOQ), 30 ng/mL (QC-Low), 150 ng/mL (QC-Mid), 750 ng/mL (QC-High), and 100000 ng/mL (DiQC)	
Accuracy and precision	Accuracy	Precision
Intra-batch (N=6)	-6.0 ~ 13.3%	2.6 ~ 8.5%
Inter-batch (N=18)	-4.7 ~ 7.0%	3.8 ~ 9.2%
Dilution linearity	10000 ng/mL (dilution factor = 100)	
Short-term stability	26 hours (room temperature)	
Freeze/thaw stability	4 Cycles	

Reinjection peproducibility	3 days (room temperature)
Processed sample stability	3 days (room temperature)
Short-term stability (stock solution)	70 hours (room temperature) and 51 days (2-8°C)
Long-term stability	27 days (-80°C) and 192 days (-20°C)

Table 6: Validated [redacted] (b) (4) Assay Specifications for Eteplirsen in Human Plasma (Low Range and High Range) for Study 4658-us-202/Week 124 (Cumulative Week 152)

	Low Range		High Range	
Matrix	Human plasma		Human plasma	
Sample volume	100 µL		100 µL	
Sample preparation	Protein precipitation		Protein precipitation	
Instrumental analysis	Anion exchange HPLC with fluorescence detection		Anion exchange HPLC with fluorescence detection	
Regression, weighting	Linear, 1/x		Linear, 1/x	
Standard curve (range)	10 - 1000 ng/mL		1 - 200 µg/mL	
Quality control eteplirsen concentrations	10.0 ng/mL (QC-LLOQ), 30 ng/mL (QC-Low), 150 ng/mL (QC-Mid), 750 ng/mL (QC-High)		3 µg/mL (QC-Low), 100 µg/mL (QC-Mid), 160 µg/mL (QC-High)	
Accuracy and precision	Accuracy	Precision	Accuracy	Precision
Intra-batch (N=6)	-4.7 ~ 7.3%	1.0 ~ 9.5%	-10.2 ~ 3.1%	0.5 ~ 5.2%
Inter-batch (N=18)	0.6 ~ 4.0%	2.9 ~ 7.9%	-6.2 ~ 1.9%	1.7 ~ 606%
Dilution linearity	50000 ng/mL (dilution factor = 100)		500 µg/mL (dilution factor = 10)	
Short-term stability	26 hours (room temperature)		26 hours (room temperature)	
Freeze/thaw stability	4 Cycles		4 Cycles	
Reinjection peproducibility	3 days (room temperature)		113 hours (room temperature)	
Processed sample stability	3 days (room temperature)		74 days (room temperature)	
Short-term stability (stock and working solution)	2 days (room temperature) and 124 days (2-8°C)		6 hours (room temperature) and 37 hours (2-8°C)	
Long-term stability	155 days (-80°C) and 192 days (-20°C)		168 days (-80°C and -20°C)	

Table 7: Validated [redacted] (b) (4) Assay Specifications for Eteplirsen in Human Urine (Study 4658-28)

Matrix	Human urine
Sample volume	100 µL
Sample preparation	Protein precipitation

Instrumental analysis	Anion exchange HPLC with fluorescence detection	
Regression, weighting	Linear, 1/x	
Standard curve (range)	10 - 1000 ng/mL	
Quality control eteplirsen concentrations	10.0 ng/mL (QC-LLOQ), 30 ng/mL (QC-Low), 150 ng/mL (QC-Mid), 750 ng/mL (QC-High)	
Accuracy and precision	Accuracy	Precision
Intra-batch (N=6)	-10.0 ~ 14.3	3.0 ~ 14.3
Inter-batch (N=18)	-5.6 ~ 9.3	5.5 ~ 12.1
Dilution linearity	5000 ng/mL (dilution factor = 20)	
Short-term stability	28 hours (room temperature)	
Freeze/thaw stability	7 Cycles	
Reinjection peproducibility	9 days (room temperature)	
Processed sample stability	4 days (room temperature)	
Short-term stability (stock solution)	70 hours (room temperature) and 51 days (2-8°C)	
Long-term stability	248 days (-80°C) and 52 days (-20°C)	

Table 8: Validated (b) (4) Assay Specifications for Eteplirsen in Human Urine (Study 4658-201)

Matrix	Human plasma	
Sample volume	100 µL	
Sample preparation	Protein precipitation	
Instrumental analysis	Anion exchange HPLC with UV detection	
Regression, weighting	Linear, 1/x	
Standard curve (range)	25 - 2500 µg/mL	
Quality control eteplirsen concentrations	25 µg/mL (QC-Low), 75 µg/mL (QC-Mid), 2000 µg/mL (QC-High)	
Accuracy and precision	Accuracy	Precision
Intra-batch (N=6)	-13.0 ~ -3.2	0.7 ~ 5.8
Inter-batch (N=18)	-10.4 ~ -5.9	3.4 ~ 4.6
Dilution linearity	4000 µg/mL (dilution factor = 50)	
Short-term stability	24 hours (room temperature)	
Freeze/thaw stability	6 Cycles	
Reinjection peproducibility	4 days (room temperature)	
Processed sample stability	5 days (room temperature)	
Short-term stability (stock and working solution)	24 hours (room temperature) and 51 days (2-8°C)	

Long-term stability and storage temperature (untreated)	195 days (-80°C and -20°C)
Long-term stability and storage temperature (treated with 0.3% acetic acid)	174 days (-80°C and -20°C)

3 DETAILED LABELING RECOMMENDATIONS

The Office of Clinical Pharmacology (OCP/DPM, DCP-1, GTTG) has reviewed the package insert labeling for NDA 206031 and finds it acceptable pending the following revisions shown below.

(b) (4)

8 USE IN SPECIFIC POPULATIONS

8.6 Patients with Renal or Hepatic Impairment (b) (4)

EXONDYS 51 has not been studied in patients with renal or hepatic impairment (b) (4)

(b) (4)

12 CLINICAL PHARMACOLOGY

12.3 Pharmacokinetics

(b) (4)

(b) (4) Following single or multiple IV infusion of EXONDYS 51 in male pediatric DMD patients, plasma concentration-time profiles of eteplirsen were generally similar and showed multi-phasic decline, whereas the majority of drug elimination occurred within 24 hours. Approximate dose-proportionality and linearity in PK properties were observed following multiple-doses (0.5 to 20 mg/kg/wk) in Phase 1 studies and multiple-doses (30 and 50 mg/kg/wk) in efficacy trials. There was insignificant drug accumulation following weekly dosing across this dose range of 0.5 to 50 mg/kg. The inter-subject variability of eteplirsen is considered to be moderate, generally in the range of 20 to 55% for exposure measures (Cmax and AUCs) as well as other key PK parameters. (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4) Following single or multiple IV infusion of EXONDYS 51, the peak plasma concentrations (Cmax) of eteplirsen occurred at near the end of infusion (i.e., 1.1 to 1.2 hours across a dose range of 0.5 to 50 mg/kg/wk doses studied).

Distribution

In vitro investigation suggested that plasma protein binding of eteplirsen in human is relatively low, ranging 6.1 to 16.5% and is concentration-independent under the study condition.

The mean apparent volume of distribution (Vss) of eteplirsen was approximately (b) (4) mL/kg following weekly IV infusion of EXONDYS 51 (b) (4) 30 mg/kg (b) (4)

(b) (4)

~~**Elimination**~~

~~The total clearance (CL_{PL}) of eteplirsen was 339 mL/hr/kg following 12 weeks of therapy with 30 mg/kg~~ (b) (4)

(b) (4)

Metabolism

(b) (4)

(b) (4)

(b) (4) Eteplirsen did not appear to be metabolized by hepatic microsomes of any species tested, including humans. (b) (4)

(b) (4)

Excretion

The total clearance of eteplirsen was approximately 339 mL/hr/kg following 12 weeks of therapy with 30 mg/kg. Renal clearance of eteplirsen accounts for approximately two-thirds of the administered dose within 24 hours of IV administration. Elimination half-life ($t_{1/2}$) of eteplirsen was 3 to 4 hours.

Specific (b) (4) Populations

Age:

The pharmacokinetics of eteplirsen has been evaluated in male pediatric DMD patients. There is no experience with the use of EXONDYS 51 in patients 65 years of age or older.

Gender:

Gender effect is not known since all the subjects in studies are male patients.

Race:

Potential impact of race is not known since nearly all the patients in studies are Caucasians.

Renal or Hepatic Impairment:

EXONDYS 51 has not been studied in patients with renal or hepatic impairment.

Drug Interactions

In vitro data showed that eteplirsen did not significantly inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5). Eteplirsen did not induce CYP2B6 and CYP3A4, and induction of CYP1A2 was substantially less than the prototypical inducer, omeprazole. Eteplirsen was not a substrate nor did it have any major inhibitory potential for any of the key human transporters tested (OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2 and BSEP).

Based on *in vitro* data on plasma protein binding, CYP or drug transporter interactions, and microsomal metabolism study results, eteplirsen is expected to have a low potential for drug-drug interactions in humans.

4. APPENDICES

4.1 Individual Study Reviews

Study Report #	**Study AVI-4658-28**
Title	Dose-Ranging Study of AVI-4658 to Induce Dystrophin Expression in Selected Duchenne Muscular Dystrophy (DMD) Patients
Investigator/ Center	Professor F. Muntoni, at the University College London (UCL) Institute of Child Health, and Great Ormond Street Children's Hospital (GOSH), London, U.K. (Site 01) Professor K. Bushby, at the International Centre for Life (ICFL), Royal Victoria Infirmary (RVI), Newcastle Upon Tyne, U.K (Site 02)
Study Dates	January 07, 2009 – June 08, 2010
Objectives	• To assess the safety of escalating doses of eteplirsen when administered by 12 weekly doses in boys with DMD • To evaluate the PK of eteplirsen in DMD patients • To evaluate the efficacy of eteplirsen over 12 weeks of dosing
Formulation	• Eteplirsen (in phosphate buffered solution) of 100 mg/mL was supplied in single-use vials. • Eteplirsen was diluted up to 50 mL with normal saline solution into a syringe and administered IV over a 60-min period. • Lot numbers: 44GD-DE01 and 60GD-DE01
Patient Population	• A minimum of 18 and a maximum of 24 patients were planned. • Males between the ages of 5~15 years; 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; had a muscle biopsy analysis showing <5% revertant fibers present • Permitted concomitant medications (including oral steroids such as prednisolone, prednisone, and deflazacort) should be kept dosing constant before and during the study. • A total of 19 patients were enrolled and treated across the 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).
Study Design	• A Phase 1b, open-label, multiple-dose, dose-ranging study to assess the safety, tolerability, PK, and exploratory efficacy of eteplirsen in the treatment of boys with confirmed genotypic DMD who were amenable to treatment with exon 51 phosphorodiamidate morpholino oligomer (PMO) • Eligible patients were sequentially allocated to 1 of 6 dose cohorts (N= 2~4 per cohort) to receive eteplirsen IV infusion for 12 weeks, with weekly doses ranging from 0.5 to 20.0 mg/kg. • Dose escalation proceeded after review of safety result. <u>Screening</u> (12 wk):

	• Within 12 weeks pre-treatment: medical history, genetic analysis (if not already available), a skin biopsy for subsequent in vitro dystrophin assessment, muscle biopsy (unless a sample was available from within the previous 24 months), psychological assessment, physical examination (PE), vital signs, safety laboratory tests (hematology, clinical chemistry, urinalysis, coagulation, and lymphocyte and anti-dystrophin antibody analysis), electrocardiogram (ECG), echocardiography (ECHO), and pulmonary function tests (PFTs). • Evaluation of muscle function, including daily movement (by StepWatch Activity Monitor [SAM]), quantitative muscle testing [QMT], North Star Ambulatory Assessment [NSAA], and the 6-minute walk test [6MWT]), was conducted within 1 week of the start of treatment. <u>Treatment</u> (12 wk: selected to ensure sufficient time for production of *de novo* dystrophin): • 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. <u>Follow-up</u> (14 wk): • 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). Efficacy, safety and PK assessments were performed as scheduled.
Exploratory Efficacy	• Primary dystrophin expression analysis: the percentage of dystrophin-positive fibers as measured in the muscle biopsy tissue using immunohistochemistry (IHC) at Week 14 compared to Baseline • Dystrophin intensity (as assessed by IHC) at Week 14 compared to Baseline • Dystrophin protein level (as assessed by Western blot) at Week 14 compared to Baseline • Number and proportion of patients achieving a ≥10% level of internally shortened dystrophin production (measured as a percentage of dystrophin-positive fibers) at Week 14 compared to Baseline • Exon skipping (as assessed by reverse transcription polymerase chain reaction [RT-PCR]) at Week 14 compared to Baseline • 6MWT, QMT, NSAA, SAM, and dystrophin detection
Safety	AEs, vital signs, heart rate (HR) and oxygen saturation (SaO2), safety

<table>
<tr><td>Assessments</td><td>laboratory tests (hematology and coagulation, clinical chemistry, urinalysis, and anti-dystrophin antibodies), immune cell infiltration (presence of CD3, CD4, and CD8 cells in biopsied muscle), PFTs, ECGs, and ECHO (EF and/or fractional shortening [FS]) by comparison to baseline status for each patient, and tolerability</td></tr>
<tr><td>PK Assessments</td><td>• PK blood/plasma samples: pre-dose and at 5, 15, 30, 60, and 90 minutes; and 2, 4, 6, 8, 12, and 24 hours post-dose at Weeks 1, 6, and 12.
• PK urine samples: pre-dose, 0-4 hours post dose, 4-8 hours post dose, 8-12 hours post dose, and 12-24 hours post-dose, at Weeks 1, 6, and 12.
• PK parameters: Cmax, Tmax, AUC0-14, AUC0-last, AUC0-∞, AUC%Extrap, t½, MRT∞, CLtot, Vss, and CLrenal</td></tr>
<tr><td>Statistical Analysis</td><td>• All patients who provided at least 1 PK sample were included for PK evaluation. The reportable PK population included those patients with at least Cmax, Tmax, and AUC0-24 computed from 1 or more of the 3 sampling days (1st, 6th, 12th dose [Weeks 1, 6, and 12]).
• Mean plasma concentrations for plots and summarization were computed based on nominal elapsed sampling times measured from the end of infusion.
• Individual patient and mean plots were prepared with both a linear and a logarithmic y-axis.
• PK parameters were calculated (with WinNonlin Professional v.5.2.1) using a standard non-compartmental analysis method.
• Dose proportionality for Cmax, AUC0-24, and AUC0-∞ was assessed by plotting and fitting with a linear regression curve and a power curve. The power curve assesses the relationship between exposure and dose in the form PK = Dosea or ln(PK) = a x ln(Dose), where PK is Cmax, AUC0-24, or AUC0-∞. The exponent “a” is the proportionality constant and when close to 1.0 indicates dose-proportionality. If less than or greater than 1.0, exposure increases in a lesser or greater manner with dose increment, respectively.</td></tr>
<tr><td>Bioanalytical Methods</td><td>Quantitation of eteplirsen (AVI-4658):
• Anion exchange high performance liquid chromatography with fluorescence detection (b) (4) (b) (4) an analog internal standard (IS), (b) (4) was included.
Table. Assay performance for AVI-4658
<table>
<tr><th>Analyte</th><th></th><th>AVI-4658 (plasma)</th><th>AVI-4658 (urine)</th></tr>
<tr><td>Method:</td><td></td><td>HPLC-FL</td><td>HPLC-UV</td></tr>
<tr><td>Standard Curve:</td><td>Range:</td><td>10-1000 ng/mL</td><td>10-1000 ng/mL</td></tr>
<tr><td></td><td>Precision:</td><td>3.3-5.9%</td><td>3.8-8.7%</td></tr>
</table></td></tr>
</table>

<table>
<tr><td></td><td>
<table>
<tr><td></td><td>Accuracy:</td><td>96.0-109%</td><td>96.2-102%</td></tr>
<tr><td>LLOQ:</td><td></td><td>10 ng/mL</td><td>10 ng/mL</td></tr>
<tr><td>ULOQ:</td><td></td><td>1000 ng/mL</td><td>1000 ng/mL</td></tr>
<tr><td>LQC:</td><td></td><td>30 ng/mL</td><td>30 ng/mL</td></tr>
<tr><td></td><td>Precision:</td><td>5%</td><td>8.9%</td></tr>
<tr><td></td><td>Accuracy:</td><td>99.3%</td><td>110.7%</td></tr>
<tr><td>MQC:</td><td></td><td>150 ng/mL</td><td>150 ng/mL</td></tr>
<tr><td></td><td>Precision:</td><td>6.7%</td><td>8.1%</td></tr>
<tr><td></td><td>Accuracy:</td><td>96.7%</td><td>95.3%</td></tr>
<tr><td>HQC:</td><td></td><td>750 ng/mL</td><td>750 ng/mL</td></tr>
<tr><td></td><td>Precision:</td><td>7.3%</td><td>4.7%</td></tr>
<tr><td></td><td>Accuracy:</td><td>101.1%</td><td>103.2%</td></tr>
</table>
Note: These assays are considered validated and acceptable.
</td></tr>
<tr><td>Population/ Demographics</td><td>

15 patients (out of 19 enrolled) received all 12 infusions.
3 patients experienced venous access difficulties and received only 10 or 11 of the planned doses; while a 4th patient in the 4.0 mg/kg/wk group discontinued treatment after 7 doses due to an AE of asymptomatic deterioration in pre-existing cardiomyopathy (assessed as possibly related to study drug).
Pre- and post-treatment biopsies were available for all patients, except 2 of the 4 patients who received <12 doses did not have a post-treatment muscle biopsy;
The mean age, weight, and height for the 19 patients (1 Asian and 18 White) was 8.7 years (6-13 years), 34.5 kg (21.2-62 kg), and 124.5 cm (107.5-143.5 cm), respectively. Age at the time of DMD diagnosis ranged 1-6 years.

</td></tr>
<tr><td>PK Results</td><td>
Note: Only PK results are presented in this individual study review.

Plasma concentration-time profiles of eteplirsen (AVI-4658) showed multi-phasic decline as shown in Figure 1 below. Plasma concentrations of AVI-4658 fell below detection limit by 12 h for lowest 2 doses. Plasma and urine PK results are summarized in Table 1 and Table 2, respectively.

Figure 1. Mean (SD) plasma concentration-time profiles of AVI-4658 averaged across Weeks 1, 6, and 12 following IV infusion of 6 dose cohorts
</td></tr>
</table>

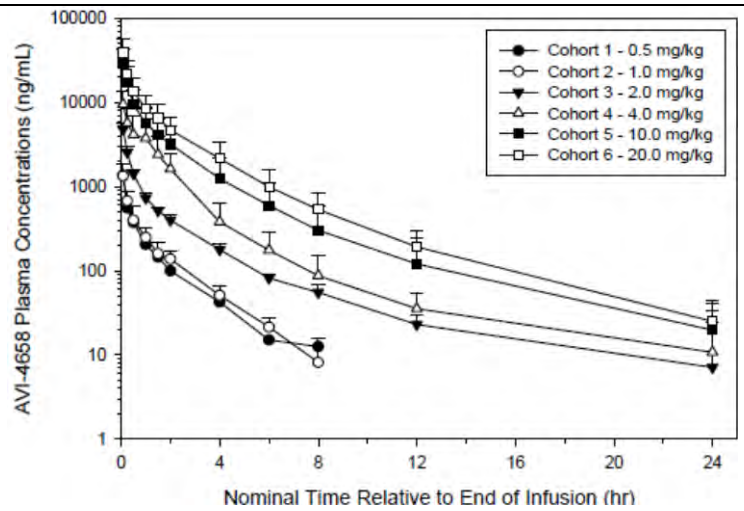


Table 1. Mean PK parameters of AVI-4658 averaged across Weeks 1, 6, and 12

	eteplirsen dose (mg/kg/wk)					
Parameter Statistic	**0.5 (N=4)**	**1.0 (N=2)**	**2.0 (N=2)**	**4.0 (N=3)**	**10 (N=4)**	**20 (N=4)**
T_{max} (hr)						
n	2	5	6	8	12	12
Mean (SD)	1.09 (NA)	1.16 (0.060)	1.12 (0.100)	1.22 (0.310)	1.07 (0.060)	1.10 (0.040)
C_{max} (ng/mL)						
n	2	5	6	8	12	12
Mean (SD)	1360 (NA)	1340 (549)	4820 (804)	9500 (4290)	29400 (14300)	39000 (16900)
AUC_{0-24} (hr*ng/mL)						
n	2	5	6	8	12	12
Mean (SD)	1570 (NA)	1730 (541)	6200 (586)	15400 (8310)	39700 (20900)	57100 (24400)
$AUC_{0-\infty}$ (hr*ng/mL)						
n	2	5	6	6	12	12
Mean (SD)	1570 (NA)	1740 (537)	6220 (555)	18600 (6920)	39800 (21000)	57300 (24500)
CL_{tot} (mL/hr/kg)						
n	2	5	6	6	12	12
Mean (SD)	320 (NA)	615 (173)	324 (29.3)	233 (61.6)	317 (144)	404 (148)
V_{ss} (mL/kg)						
n	2	5	6	6	12	12
Mean (SD)	482 (NA)	981 (305)	703 (285)	450 (136)	556 (212)	862 (308)
$T_{1/2}$ (hr)						
n	2	2	6	6	12	12
Mean (SD)	1.62 (NA)	1.62 (0.317)	2.58 (0.304)	2.11 (0.531)	3.27 (1.25)	3.60 (0.376)
MRT_{∞} (hr)						
n	2	5	6	6	12	12
Mean (SD)	1.50 (NA)	1.58 (0.116)	2.16 (0.853)	1.92 (0.347)	1.85 (0.327)	2.15 (0.223)

Dose-proportionality:
The mean Cmax, AUC0-24 h and AUC0-∞ increased slightly with doses, with exponents of the power curve were 0.7994, 0.8187 and 0.7838, respectively, as illustrated in the Figure 2 below.

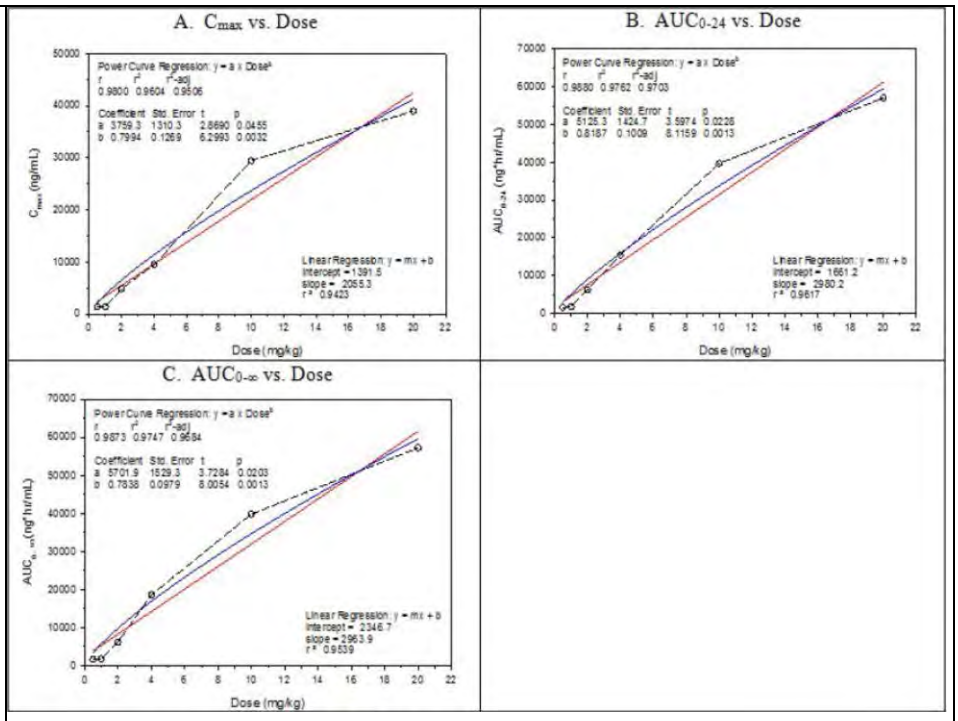


Table 2. Mean AVI-4658 renal clearance parameters across Weeks 1, 6, 12

Cohort/ Dose (mg/kg)		Amt Excreted 0-24 hr (mg)	% of Dose Recovered in Urine	CL_r (mL/hr/kg)	CL_{tot} (mL/hr/kg)	CL_r/CL_{tot} (%)
1/0.5	N	2	2	2	2	2
	Mean	5.85	36.5	116	320	36.5
	SD	.	.	.	.	.
2/1	N	5	5	5	5	5
	Mean	7.61	32.1	184	615	32.3
	SD	4.91	17.7	79.4	173	17.7
3/2	N	6	6	6	6	6
	Mean	39.6	46.2	148	324	46.2
	SD	17.2	15.8	45.3	29.3	15.5
4/4	N	7	7	7	6	6
	Mean	66.0	39.9	122	233	36.8
	SD	43.0	19.2	111	61.6	18.9
5/10	N	12	12	12	12	12
	Mean	220	63.7	198	317	63.8
	SD	119	22.1	97.3	144	22.2
6/20	N	12	12	12	12	12
	Mean	409	60.3	229	404	60.5
	SD	189	14.0	57.7	148	14.0

The urinary recovery as well as contributions from CL_R generally increased with dose increases, noticeably at the higher 10 and 20 mg/kg/wk doses. The remaining fraction may be attributed to non-renal elimination process or potential tissue retainment.

Safety	• No discontinuations or withdrawals from the study due to AEs. • Once weekly IV infusions of eteplirsen, at doses of 0.5 to 20.0 mg/kg/wk for 12 weeks appeared safe and well tolerated in this small sample of boys with DMD. • The most frequently reported AEs related to study treatment

	included headache and tachycardia in 37% and 16% of patients, respectively, with mild to moderate in intensity. • SAEs were reported in 2 patients (11%) during the follow-up period; however, neither was assessed as treatment-related.
Conclusion	*Pharmacokinetics:* • Concentration-time profiles were similar for Weeks 1, 6 and 12 with no accumulation observed between study weeks. This observation is anticipated considering the rapid decline in plasma concentrations over time and the short elimination t1/2 (1.62 - 3.60 h). • Eteplirsen is also characterized by rapid CL_{tot} (233 to 615 mL/hr/kg) and large Vss (450 to 981 mL/kg) across 0.5-20 mg/kg/wk doses. Both CL_{tot} and Vss are similar across Weeks 1, 6, and 12, with 1 mg/kg/wk dose having the highest values. • Plasma exposure increased in slightly less than proportional manner with dose for Cmax, AUC0-24, and AUC0-∞. The lightly less-than dose-proportional increases observed for Cmax, AUC0-24 h and AUC0-∞ are thought not to have significant clinical consequence since these study doses are (much) lower than the proposed or targeted 30 mg/kg/wk dose • Renal clearance of unchanged eteplirsen accounted for 32.1% and 46.2% of total clearance (an increasing trend) at lower doses between 0.5 and 4.0 mg/kg/wk. At the 2 highest 10.0 and 20.0 mg/kg/wk doses, renal clearance accounted for 63.8% and 60.5% of total clearance, respectively, similar to those obtained for the 30 and 50 mg/kg/wk doses studied in the Phase 2 clinical trials. • Renal clearance ranged from 116 to 229 mL/hr/kg (or 62.6 mL/min to 119.4 mL/min) across dose levels, similar to GFR in healthy boys between 5 to 15 years of age.

Study Report #	**Study 4658-us-201**
Title	A Randomized, Double-Blind, Placebo-Controlled, Multiple Dose Efficacy, Safety, Tolerability, and Pharmacokinetics Study of AVI-4658 (Eteplirsen), a Phosphorodiamidate Morpholino Oligomer, Administered Over 28 Weeks in the Treatment of Ambulant Subjects with Duchenne Muscular Dystrophy
Investigator/ Center	Jerry R. Mendell MD; Nationwide Children's Hospital, Columbus, Ohio, USA
Study Dates	July 18, 2011 - February 29, 2012
Objectives	To assess the efficacy, safety, tolerability, and pharmacokinetics (PK) of eteplirsen (AVI-4658) at 50 and 30 mg/kg/week(wk) doses in patients diagnosed with Duchenne muscular dystrophy (DMD).
Formulation	• Eteplirsen (in phosphate buffered solution) of 100 mg/mL was supplied in single-use vials.

<table>
<tr><td></td><td>

Eteplirsen was diluted up to 50 mL with normal saline solution into a syringe and administered IV over a 60-min period.
Lot numbers: 60GD-DE01 and 68GD-DE01

</td></tr>
<tr><td>Patient Population</td><td>

A total of 12 patients were planned and enrolled.
Males between the ages of 7-13 years, inclusive; 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]; be receiving stable dose of treatment with oral corticosteroids for at least 24 weeks before study entry; have intact right and left biceps muscles or an alternative upper arm muscle group; achieve an average distance within 200 m and 400 m ±10% (i.e. within 180 m and 440 m) while walking independently over 6 min.

</td></tr>
<tr><td>Study Design</td><td>

A Phase 2, randomized, single-center, double-blind, placebo-controlled, multiple-dose study to assess the efficacy, safety, tolerability, and PK of once-weekly IV infusions of eteplirsen in patients with genotypically confirmed DMD with an appropriate genetic lesion.
Eligible patients were randomized to receive 50 or 30 mg/kg/wk eteplirsen or placebo, blinded.
After 24 weeks, patients in placebo group were further randomized to Groups 3a and 3b for 4 weeks so the 4 treatment groups are shown below:

<table>
<tr><th>Group</th><th>Treatment/Dose of Eteplirsen</th><th>N</th></tr>
<tr><td>1</td><td>50 mg/kg/wk eteplirsen for 28 weeks</td><td>4</td></tr>
<tr><td>2</td><td>30 mg/kg/wk eteplirsen for 28 weeks</td><td>4</td></tr>
<tr><td>3a</td><td>Placebo for 24 weeks followed by 50 mg/kg/wk eteplirsen for 4 weeks</td><td>2</td></tr>
<tr><td>3b</td><td>Placebo for 24 weeks followed by 30 mg/kg/wk eteplirsen for 4 weeks</td><td>2</td></tr>
</table>

Efficacy, safety and PK assessments were described below.

</td></tr>
<tr><td>Efficacy</td><td>
Primary efficacy endpoint: the change from baseline in the percentage of dystrophin-positive fibers as measured in muscle biopsy tissue using immunohistochemistry (IHC) at Week 12 for the 50 mg/kg/wk eteplirsen and matching placebo groups (Groups 1 and 3a) and at Week 24 for the 30 mg/kg/wk eteplirsen and matching placebo groups (Groups 2 and 3b).

Additional biopsy-related endpoints included change from baseline to Week 12 for Groups 1 and 3a and to Week 24 for Groups 2 and 3b in:

Dystrophin intensity levels as measured by IHC
Total dystrophin protein levels as measured by Western blot analysis
Exon skipping as measured by reverse transcription polymerase chain reaction (RT-PCR)

</td></tr>
</table>

	• CD3, CD4, and CD8 lymphocyte counts as measured by IHC Functional efficacy endpoints included change from baseline to week 24 in the 6-Minute Walk Test (6MWT) • Timed 4-Step Test • Maximum Voluntary Isometric Contraction Test (MVICT) • North Star Ambulatory Assessment (NSAA) total score, and NSAA components including the Timed 10-Meter Run and rise time • 9-Hole Peg Test • Pulmonary Function Testing (PFT) including forced vital capacity (FVC), percent predicted FVC (%FVC), forced expiratory volume in 1 second (FEV1), percent predicted FEV1 (%FEV1), FEV1/FVC ratio; maximal inspiratory pressure (MIP), and maximal expiratory pressure (MEP) Change from baseline to week 24 on the Pediatric Quality of Life Inventory (PedsQL) was an additional endpoint.
Safety Assessments	Frequency and severity of AEs, SAEs, discontinuations due to AEs, safety laboratory tests including hematology, coagulation, and serum chemistry assays (including serum cystatin C) and urinalysis (including urinary cystatin C and KIM-1), immune response to dystrophin by enzyme-linked immunosorbent spot assay (ELISPOT), vital signs, physical examinations, 12-lead ECGs, and ECHO
PK Assessments and Analysis	• PK blood/plasma samples: pre-dose and at 5, 15, 30, 60, and 90 min; and 2, 4, 6, 8, 12, and 24 h post-dose at Weeks 12. • Single PK blood samples: at 5-min post-end of infusion on Weeks 1, 12, 24, and 25. • PK urine samples: pre-dose, and up to 24 h from end of infusion. • PK parameters: Tmax, Cmax, Cmax,ss, Ctrough, Cavg,ss, Vdss, t½, AUC0-24, AUC0-last, AUC0-∞, %AUC∞,ex, CL_{PL}, MRT, Ae, CL_R, and %Extended. • PK parameters for eteplirsen were calculated (with WinNonlin Professional v.5.2) using non-compartmental analysis and were presented with summary statistics. Actual sampling times were used in all final PK analyses. Per protocol times were used to calculate mean plasma concentrations for graphical displays. Individual patient and mean plots were prepared with both a linear and a logarithmic y-axis.
Bioanalytical Methods	Quantitation of eteplirsen (AVI-4658): • Plasma samples: anion exchange high performance liquid chromatography with fluorescence (b) (4) (b) (4); range of the assay: 10-1000 ng/mL • Urine samples: anion exchange high performance liquid chromatography with UV (b) (4); range of the assay: 25 -2500 µg/mL

<table>
<tr><td></td><td>
• These assays are considered validated and acceptable.
Table. Assay performance for AVI-4658
<table>
<tr><th>Analyte</th><th></th><th>AVI-4658 (plasma)</th><th>AVI-4658 (urine)</th></tr>
<tr><td>Method:</td><td></td><td>HPLC-FL</td><td>HPLC-UV</td></tr>
<tr><td>Standard Curve:</td><td>Range:</td><td>10-1000 ng/mL</td><td>25-2500 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>6.4-9.8%</td><td>*</td></tr>
<tr><td></td><td>Accuracy:</td><td>97.0-107%</td><td>96.6-102.4%</td></tr>
<tr><td>LLOQ:</td><td></td><td>10 ng/mL</td><td>25 µg/mL</td></tr>
<tr><td>ULOQ:</td><td></td><td>1000 ng/mL</td><td>2500 µg/mL</td></tr>
<tr><td>LQC:</td><td></td><td>30 ng/mL</td><td>75 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>31.6%ǂ</td><td>*</td></tr>
<tr><td></td><td>Accuracy:</td><td>101%</td><td>102%</td></tr>
<tr><td>MQC:</td><td></td><td>150 ng/mL</td><td>1000 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>18.2%</td><td></td></tr>
<tr><td></td><td>Accuracy:</td><td>108%</td><td>103%</td></tr>
<tr><td>HQC:</td><td></td><td>750 ng/mL</td><td>2000 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>4.9%</td><td>*</td></tr>
<tr><td></td><td>Accuracy:</td><td>96.5%</td><td>103%</td></tr>
</table>
ǂ Precision >25%, outside of acceptable range
* Not reported
</td></tr>
<tr><td>Population/ Demographics</td><td>
• All 12 patients received all scheduled treatments and completed the study. Data were available from all subjects for PK analysis.
• The mean age, weight, height and BMI for the patients (1 Asian and 11 White) was 8.8 years (7-10 years), 31.5 kg (22.1-39.8 kg), and 123.7 cm (116-138 cm), and 20.4 kg/m^2 (16.4-25.6 kg/m^2), respectively.
• Exclusion for PK analysis: (outliers)
  • Subject 1002 treated at 30 mg/kg active, the 12 h concentration
  • Subject 1003 treated at 50 mg/kg active, the 8 h concentration
</td></tr>
<tr><td>PK Results</td><td>
Note: Only PK results are presented in this individual study review.

Plasma profiles of eteplirsen (AVI-4658) showed multi-phasic decline (possibly bi- or tri-phasic) as shown in Figure 1 below. Plasma and urine PK results are summarized in Tables 1 and 2.

Figure 1. Mean (SD) plasma concentration-time profiles of AVI-4658 at Week 12
</td></tr>
</table>

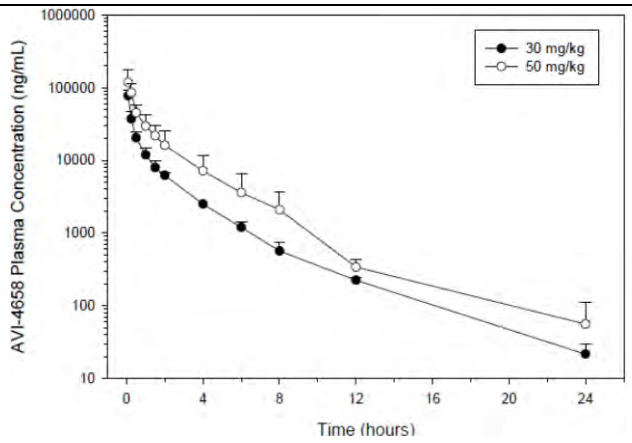


Table 1. Summary of plasma PK for AVI-4658 in ng/mL at Visit 13 (Week 12)

Treatment Group	Subject	T_{max}	C_{max}	$AUC_{0\text{-last}}$	$AUC_{0\text{-}\infty}$	$AUC_{0\text{-}24}$	AUC% ext	CL_{PL}	V_{ss}	Half-Life ($t_{1/2}$)	R^2 adjusted	MRT_{∞}
		hr	ng/mL	hr*ng/mL	hr*ng/mL	hr*ng/mL	%	mL/hr/kg	mL/kg	hr		hr
AVI-4658 30mg/kg	1002	1.10	54,400	66,193	66,253	66,177	0.091	453	836	3.03	0.9878	1.85
	1006	1.08	87,800	102,101	102,278	102,061	0.173	293	519	3.79	0.9813	1.77
	1009	1.08	80,100	99,113	99,215	99,089	0.102	302	535	3.26	0.9812	1.77
	1010	1.07	86,500	96,851	96,934	96,835	0.086	309	516	3.11	0.9953	1.67
	N	4	4	4	4	4	4	4	4	4	4	4
	Mean	1.08	77,200	91,065	91,170	91,040	0.113	339	601	3.30	0.9864	1.76
	SD	0.01	15,568	16720	16,755	16,713	0.041	75.8	157	0.341	0.0067	0.074
	CV%	1.26	20.2	18.4	18.4	18.4	35.9	22.3	26.1	10.3	0.68	4.18
AVI-4658 50mg/kg	1003	1.13	70,400	107,414	107,531	107,385	0.108	465	911	3.13	0.9652	1.96
	1004	1.25	101,000	144,441	144,613	144,401	0.118	346	716	3.24	0.9912	2.07
	1012	1.08	199,000	307,758	308,453	307,582	0.226	162	401	3.44	0.9760	2.48
	1015	1.10	128,000	163,962	164,051	163,933	0.054	305	523	2.85	0.9943	1.71
	N	4	4	4	4	4	4	4	4	4	4	4
	Mean	1.14	124,600	180,894	181,162	180,825	0.127	319	638	3.17	0.9817	2.06
	SD	0.08	54,898	87,767	88,040	87,698	0.072	125	224	0.249	0.0136	0.318
	CV%	6.58	44.1	48.5	48.6	48.5	56.7	39.1	35.1	7.85	1.38	15.5

- Five-minute concentrations across Weeks 1~25 averaged 84600 ±19783 ng/mL for the 30 mg/kg dose (CV% ranging 18.4-20.2% for Cmax and AUCs) and 132386 ± 49357 ng/mL for 50 mg/kg dose (CV% ranging 44.1-48.6% for Cmax and AUCs). The reported concentration ratio (50 vs. 30 mg/kg) was 1.56, a similar proportion to doses.
- The reported average of concentration ratios of eleplirsen across Weeks were 1.024 and 1.254 for the 30 and 50 mg/kg dose levels, respectively, suggesting a minimum accumulation after weekly dosing.
- Between two doses (1.67-fold), Cmax increased approximately dose-proportionally (~1.61-fold), whereas AUCs increased more than dose-proportionally (~1.99-fold).

Table 2. Summary of urinary secretion and renal clearance for AVI-4658 at Visit 13 (Week 12)

	<table><tr><th>Treatment Group</th><th>Subject</th><th>Body Wt (kg) *</th><th>Urine Volume (mL)</th><th>Urine Concentration (µg/mL)</th><th>Amount Excreted (mg)</th><th>Percent of Dose Excreted</th><th>CL_R (mL/hr/kg)</th><th>CL_{PL} (mL/hr/kg)</th></tr><tr><td>AVI-4658 30 mg/kg</td><td>1002</td><td>25.5</td><td>1,587</td><td>278</td><td>441</td><td>57.7</td><td>261</td><td>453</td></tr><tr><td></td><td>1006</td><td>36.1</td><td>1,220</td><td>485</td><td>592</td><td>54.6</td><td>161</td><td>293</td></tr><tr><td></td><td>1009</td><td>44.7</td><td>1,620</td><td>661</td><td>1,071</td><td>79.9</td><td>242</td><td>302</td></tr><tr><td></td><td>1010</td><td>44.2</td><td>2,555</td><td>985</td><td>2,517</td><td>190</td><td>588</td><td>309</td></tr><tr><td></td><td>N</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr><tr><td></td><td>Mean</td><td>37.6</td><td>1,746</td><td>602</td><td>1,155</td><td>95.5</td><td>313</td><td>339</td></tr><tr><td></td><td>SD</td><td>9.0</td><td>569</td><td>299</td><td>947</td><td>63.9</td><td>189</td><td>75.8</td></tr><tr><td></td><td>CV%</td><td>23.9</td><td>32.6</td><td>49.7</td><td>81.9</td><td>66.9</td><td>60.2</td><td>22.3</td></tr><tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>AVI-4658 50 mg/kg</td><td>1003</td><td>24.5</td><td>600</td><td>1,920</td><td>1,152</td><td>94.0</td><td>438</td><td>465</td></tr><tr><td></td><td>1004</td><td>29.3</td><td>460</td><td>1,350</td><td>621</td><td>42.4</td><td>147</td><td>346</td></tr><tr><td></td><td>1012</td><td>42.5</td><td>1,024</td><td>1,140</td><td>1,167</td><td>54.9</td><td>89.3</td><td>162</td></tr><tr><td></td><td>1015</td><td>27.0</td><td>1,058</td><td>1,100</td><td>1,164</td><td>86.2</td><td>263</td><td>305</td></tr><tr><td></td><td>N</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr><tr><td></td><td>Mean</td><td>30.8</td><td>786</td><td>1,378</td><td>1,026</td><td>69.4</td><td>234</td><td>319</td></tr><tr><td></td><td>SD</td><td>8.0</td><td>301</td><td>378</td><td>270</td><td>24.7</td><td>154</td><td>125</td></tr><tr><td></td><td>CV%</td><td>26.0</td><td>38.3</td><td>27.4</td><td>26.3</td><td>35.6</td><td>65.7</td><td>39.1</td></tr></table> Note: • Subject #1010 had unexplainable high amount excreted (data in bold) and was considered an outlier in analysis. • After excluding Subject 1010 as outlier: %Ae = 64.1%, CL_R = 221 mL/h/kg, % of renal excretion accounted for 64.1% of total clearance.
Conclusion	*Pharmacokinetics:* • The PK profiles and the PK parameters of 30 and 50 mg/kg/wk eteplirsen observed in this study are similar to that previously reported for the lower doses in DMD patients as observed in study AVI-4658-28. • The similar plasma concentrations at 5 minutes post-end of infusion across weeks suggest that there is minimum accumulation at either dose level after once-weekly dosing. • Between the 30 and 50 mg/kg/wk dose levels, Cmax increased approximately proportional with dose, whereas AUC increased greater than dose-proportional. The greater than dose-proportional increase in exposure at higher dose might be attributed to the saturable process for the non-renal elimination. • Renal clearance of eteplirsen accounted for 64.1% or approximately two-thirds of total systemic clearance.

Study Report #	**Study 4658-us-202**
Title	Open-Label, Multiple-Dose, Efficacy, Safety, and Tolerability Study of Eteplirsen in Patients with Duchenne Muscular Dystrophy who Participated in Study 4658-us-201
Investigator/ Center	Jerry R. Mendell MD; Nationwide Children's Hospital, Columbus, Ohio, USA
Study Dates	February 29, 2012 - November 04, 2014
Objectives	• To assess the ongoing efficacy, safety, and tolerability of an additional 212 weeks of treatment of eteplirsen (AVI-4658) in

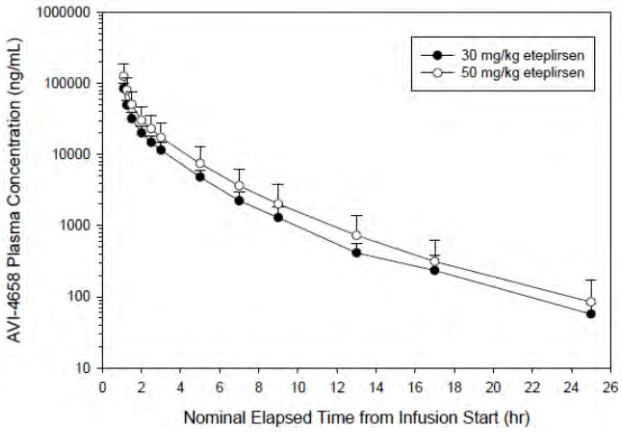
<table>
<tr><td></td><td>DMD patients who have successfully completed the 28-week study (4658-us-201).
• To explore the link between biomarkers for DMD at the clinical status</td></tr>
<tr><td>Formulation</td><td>• Eteplirsen (in phosphate buffered solution) of 100 mg/mL was supplied in single-use vials.
• Eteplirsen was diluted with normal saline solution into a syringe and administered IV over a 60-min period.</td></tr>
<tr><td>Patient Population</td><td>• A total of 12 DMD patients who completed Study 4658-us-201. [Refer to individual study review for Study 4658-us-201 for more details]</td></tr>
<tr><td>Study Design</td><td>• Eligible patients from Study 4658-us-201 continued to receive once-weekly 50 or 30 mg/kg/wk eteplirsen for an additional 212 weeks.
• Treatment Groups 1, 2, 3a and 3b remained the same:
<table>
<tr><th>Group</th><th>Treatment / Dose in Study 4658-us-201</th><th>Dose of Eteplirsen For Study 4658-us-202</th></tr>
<tr><td>1</td><td>50 mg/kg eteplirsen for 28 weeks</td><td>50 mg/kg eteplirsen</td></tr>
<tr><td>2</td><td>30 mg/kg eteplirsen for 28 weeks</td><td>30 mg/kg eteplirsen</td></tr>
<tr><td>3a</td><td>Placebo for 24 weeks followed by 50 mg/kg eteplirsen for 4 weeks</td><td>50 mg/kg eteplirsen</td></tr>
<tr><td>3b</td><td>Placebo for 24 weeks followed by 30 mg/kg eteplirsen for 4 weeks</td><td>30 mg/kg eteplirsen</td></tr>
</table>
• Efficacy, safety, PK, and biomarker assessments were performed at scheduled visits.
• All subjects underwent muscle biopsies for analysis of exon skipping, dystrophin expression, and inflammatory markers at Week 20; biopsies were performed within 24 to 96 hours following the completion of the eteplirsen infusion.</td></tr>
<tr><td>Efficacy</td><td>Primary biological efficacy endpoint: the change from Baseline at Week 48 (cumulative Study 4658- us-201 + 4658-us-202) in the percent of dystrophin positive fibers (type = anti-dystrophin antibody MANDYS106) in muscle biopsy tissue as measured by immunohistochemistry (IHC).

Primary functional efficacy endpoint: the change from Baseline on the 6-Minute Walk Test (6MWT).

Additional exploratory and supportive efficacy endpoints evaluated changes from Baseline in:
• Dystrophin intensity per fiber (per IHC) and total dystrophin protein (as determined by Western blot)
• CD3, CD4, and CD8 lymphocyte count in muscle biopsy tissue
• Exon skipping in muscle biopsy tissue as assessed by reverse transcriptase polymerase chain reaction (RT-PCR)
• Pulmonary function test results including forced vital capacity</td></tr>
</table>

<table>
<tr><td></td><td>(FVC), percent predicted FVC, forced expiratory volume in 1 second (FEV1), percent predicted FEV1, FEV1/FVC ratio, maximum inspiratory pressure (MIP), percent predicted MIP, maximum expiratory pressure (MEP) and percent predicted MEP
• Timed 4-Step Test
• North Star Ambulatory Assessment (NSAA)
• Maximum voluntary isometric contraction test (MVICT)
• 9-Hole Peg Test</td></tr>
<tr><td>Safety Assessments</td><td>Frequency and severity of AEs, SAEs, discontinuations due to AEs, safety laboratory tests including hematology, coagulation, and serum chemistry assays (including serum cystatin C) and urinalysis (including urinary cystatin C and KIM-1), immune response to dystrophin by enzyme-linked immunosorbent spot assay (ELISPOT), vital signs, physical examinations, 12-lead ECGs, and ECHO</td></tr>
<tr><td>PK Assessments and Analysis</td><td>• PK blood/plasma samples: pre-dose and at 5, 15, 30, 60, and 90 min; and 2, 4, 6, 8, 12, and 24 h post-dose at Week 124 (or Weeks 152 from the start of Study 4658-us-201).
• PK urine samples were not collected.
• PK parameters: Tmax, Cmax, Vdss, t½, AUC0-24, AUC0-last (Week 152 only), AUC0-∞, %AUC∞,ex, CL_{PL} (Weeks 36 and 152), MRT, and %Extended. [Refer to individual study review for Study 4658-us-201 for detail statistical analysis]</td></tr>
<tr><td>Bioanalytical Methods</td><td>Quantitation of eteplirsen (AVI-4658):
• Plasma samples: anion exchange high performance liquid chromatography with fluorescence (b) (4) (b) (4)

Table. Assay performance for AVI-4658
<table>
<tr><th>Analyte</th><th></th><th>AVI-4658 (plasma)</th><th>AVI-4658 (plasma)</th></tr>
<tr><td>Method:</td><td></td><td>HPLC-FL</td><td>HPLC-FL</td></tr>
<tr><td>Standard Curve:</td><td>Range:</td><td>10.0-1000 ng/mL</td><td>1.00-200 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>*</td><td>0.7-8.1%</td></tr>
<tr><td></td><td>Accuracy:</td><td>95-98.5%</td><td>97.5-101.1%</td></tr>
<tr><td>LLOQ:</td><td></td><td>10.0 ng/mL</td><td>1.00 µg/mL</td></tr>
<tr><td>ULOQ:</td><td></td><td>1000 ng/mL</td><td>200 µg/mL</td></tr>
<tr><td>LQC:</td><td></td><td>30 ng/mL</td><td>3 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>*</td><td>4.6%</td></tr>
<tr><td></td><td>Accuracy:</td><td>115.7%</td><td>102%</td></tr>
<tr><td>MQC:</td><td></td><td>500 ng/mL</td><td>100 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>*</td><td>0.8%</td></tr>
<tr><td></td><td>Accuracy:</td><td>99%</td><td>101%</td></tr>
<tr><td>HQC:</td><td></td><td>750 ng/mL</td><td>160 µg/mL</td></tr>
<tr><td></td><td>Precision:</td><td>*</td><td>0.6%</td></tr>
<tr><td></td><td>Accuracy:</td><td>99.6%</td><td>125%</td></tr>
</table>
* Precision not reported</td></tr>
<tr><td>Population/</td><td>• All 12 patients received all scheduled treatments and completed the</td></tr>
</table>

Demographics	study (N=6 each received 30 mg/kg and 50 mg/kg). Data were available from all subjects for PK analysis. [Refer to individual study review for Study 4658-us-201 for detail on demographic characteristics]
PK Results	Note: Only PK results are presented in this individual study review. Plasma profiles of eteplirsen (AVI-4658) and plasma PK results were shown in Figure 1 and Table 1, respectively. Figure 1. Mean (SD) plasma concentration-time profiles of AVI-4658 at Week 124  Table 1. Summary of plasma PK for AVI-4658 in ng/mL at Week 124

Treatment Group	Subject	T_{max}	C_{max}	$AUC_{0\text{-}last}$	$AUC_{0\text{-}\infty}$	$AUC_{0\text{-}24}$	AUC% ext	CL_{PL}	V_{ss}	Half-Life ($t_{1/2}$)	R^2 adjusted	MRT_{∞}
		hr	ng/mL	hr*ng/mL	hr*ng/mL	hr*ng/mL	%	mL/hr/kg	mL/kg	hr	-	hr
30 mg/kg eteplirsen	S01002	1.08	68,900	92,146	92,253	92,123	0.116	325.2	627.5	3.469	0.9961	1.930
	S01006	1.28	89,300	127,562	127,929	127,482	0.287	234.5	489.4	4.214	0.9994	2.087
	S01007	1.08	63,300	100,183	100,432	100,139	0.247	298.7	645.8	4.245	0.9967	2.162
	S01008	1.08	100,000	145,883	146,120	145,817	0.162	205.3	415.8	2.536	0.9252	2.025
	S01009	1.08	102,000	152,489	152,904	152,400	0.271	196.2	466.5	3.576	0.9770	2.378
	S01010	1.08	86,900	146,869	147,221	146,785	0.239	203.8	512.3	3.219	0.9735	2.514
	N	6	6	6	6	6	6	6	6	6	6	6
	Mean	1.12	85,067	127,522	127,810	127,457	0.220	243.9	526.2	3.543	0.9780	2.183
	SD	0.08	15,913	25,821	25,906	25,798	0.067	54.9	91.5	0.643	0.0281	0.222
	CV%	7.31	18.71	20.25	20.27	20.24	30.43	22.51	17.39	18.15	2.87	10.17
	Median	1.08	88,100	136,723	137,024	136,649	0.243	219.9	500.9	3.523	0.9866	2.125
50 mg/kg eteplirsen	S01003	1.03	92,700	147,894	148,089	147,846	0.132	337.6	746.5	2.832	0.9731	2.211
	S01004	1.08	165,000	219,796	220,280	219,713	0.220	227.0	445.4	4.040	0.9922	1.962
	S01005	1.15	148,000	199,269	199,487	199,215	0.109	250.6	460.1	3.335	0.9990	1.836
	S01012	1.08	223,000	386,723	388,212	386,445	0.383	128.8	345.4	4.031	0.9964	2.682
	S01013	1.08	76,800	107,010	107,263	106,968	0.236	466.1	910.2	4.644	0.9516	1.953
	S01015	1.22	49,000	95,563	95,753	95,519	0.198	522.2	1232.1	3.770	0.9849	2.360
	N	6	6	6	6	6	6	6	6	6	6	6
	Mean	1.11	125,750	192,709	193,181	192,618	0.213	322.1	690.0	3.775	0.9829	2.167
	SD	0.06	64,610	106,966	107,442	106,879	0.097	150.0	339.7	0.628	0.0179	0.317
	CV%	5.84	51.38	55.51	55.62	55.49	45.66	46.58	49.24	16.64	1.83	14.61
	Median	1.08	120,350	173,582	173,788	173,531	0.209	294.1	603.3	3.900	0.9885	2.087

- The key PK parameters and 5-min concentrations between two doses showed similar results to those in Study 4658-us-201.
- Between two doses (1.67-fold), Cmax increased approximately 1.48-fold, whereas AUC0-∞ increased approximately 1.51-fold.

Conclusion	*Pharmacokinetics:* • Similar concentration-time profiles with rapid decline in concentrations over 24 h and short t1/2 (3-4 h), with minimum accumulation following weekly 1-h IV infusion, were observed. • The key PK parameters and exposure results (Cmax and AUCs)

	between two doses showed similar results to those in Study 4658-us-201. • Both Cmax and AUC0-∞ were reported to increase in a slightly less than dose proportional manner between the 30 and 50 mg/kg doses.

Study Report #	**4658 PKD 001**
Title	In Vitro Plasma Protein Binding of ^{14}C-AVI-4658 in Mouse, Rat, Monkey, Human
Objectives	To determine, in vitro; the extent of binding of ^{14}C-AVI-4658 to plasma proteins of mouse, rat, monkey, and human.
Study Design	Note: This review focuses on part using human biomaterial only. The extent of radiolabeled ^{14}C-AVI-4658 (or ^{14}C- Eteplirsen) binding to human plasma proteins (from pooled plasma of healthy males) was assessed (b) (4). Various concentrations of ^{14}C-AVI-4658 (8, 24, 80, 240, and 800 µg/mL) was added to plasma samples and fortified plasma samples were loaded (b) (4) (b) (4) and then centrifuged (b) (4) (b) (4) Summary of the experimental design is provided in the following table:

Matrix	Experiment	Concentration (µg/mL)
	Equilibrium Dialysis	
Plasma (human)	Time-to-Equilibrium	800 (2, 4, 8, 16, and 24 hours)
DPBS	Time-to-Equilibrium	800 (1, 4, 8, and 24 hours)
	(b) (4)	
Plasma (human)	Preliminary (Nonspecific)	80 and 800
DPBS	Preliminary (Nonspecific)	80 and 800
Plasma (all species)	Concentration Dependence,	8, 24, 80, 240, 800

Results of percentages of unbound and bound ^{14}C-AVI-4658 at various drug concentrations in human plasma (concentration dependence (b) (4)) are presented in the Table below.

	^{14}C-AVI-4658	Percentage of Radioactivity						
		Unbound			Bound		Recovered	
Species	(µg/mL)	Replicate	Mean	SD[a]	Replicate	Mean	Mean	SI
Human	8	86.3	84.4	1.65	13.7	15.6	91.4	1.71
		83.2			16.8			
		83.7			16.3			
	24	83.3	83.5	0.626	16.7	16.5	87.1	3.47
		83.1			16.9			
		84.3			15.7			
	80	91.9	93.9	1.91	8.1	6.1	94.4	2.80
		94.0			6.0			
		95.7			4.3			
	240	85.0	85.9	1.34	15.0	14.1	96.1	3.87
		87.4			12.6			
		85.3			14.7			
	800	86.2	85.9	1.49	13.8	14.1	97.6	1.18
		84.3			15.7			
		87.2			12.8			

Conclusion	Overall, ^{14}C-AVI-4658 had low protein binding in human plasma, ranging 6.1 to 16.5%, with recovery ≥87.1%. In addition, the protein

	binding in human plasma was not concentration-depending across range of 8~800 µg/mL of drug concentration. Similar low protein binding was also observed in animal species.
Comment	The drug concentration range chosen did not adequately cover the observed plasma concentrations in patients at 39 and 50 mg/kg/wk doses, noticeably drug concentrations <8 µg/mL.

Study Report #	**4658 PKD 002**
Title	Metabolism of 14C-AVI-4658 in Mouse, Rat, Monkey, and Human Hepatic Microsomes
Objectives	To determine the extent of metabolism and metabolic profile of ^{14}C-AVI-4658 in vitro using mouse, rat, monkey, and human hepatic microsomes
Study Design	Note: This review focuses on part using human biomaterial only. Pooled hepatic microsomes from human subjects were obtained, with protein concentrations were 20 mg/mL for each microsomal preparation. ^{14}C-AVI-4658 (8 and 80 µg/mL) was then incubated with mouse, rat, monkey, and human hepatic microsomes (1 mg/mL) in the presence of nicotinamine adenine dinucleotide phosphate (NADPH, 1 mM) at 37°C for 0, 30, 45, 60, and 120 min, all in triplicate. Metabolic controls were conducted by incubating ^{14}C-AVI-4658 of the same concentrations in microsomes in the absence of NADPH at 0 and 120 min to determine the stability of the test article. The radiochromatograms of ^{14}C-AVI-4658 incubation samples were generated by high performance liquid chromatography (HPLC) with radiometric detection.
Results	In the presence of NADPH (1 mM), no changes in radiopurity were observed for all the incubation samples and different incubation times, as shown in the Table below. The results indicate that ^{14}C-AVI-4658 was stable in mouse, rat, monkey, and human hepatic microsomes under the study conditions. Table. Metabolism of ^{14}C-AVI-4658 (8 and 80 µM) incubated with human hepatic microsomes in the absence and presence of NADPH:

	<table><tr><th rowspan="3">Incubation Time (Minutes)</th><th colspan="6">Percentage of Radioactivity</th></tr><tr><th colspan="3">8 µM ^{14}C-AVI-4658</th><th colspan="3">80 µM ^{14}C-AVI-4658</th></tr><tr><th>Replicate</th><th>Mean</th><th>SD</th><th>Replicate</th><th>Mean</th><th>SD</th></tr><tr><td>No NADPH</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>0</td><td>98.8</td><td>98.7</td><td>0.197</td><td>98.6</td><td>98.7</td><td>0.0964</td></tr><tr><td></td><td>98.8</td><td></td><td></td><td>98.7</td><td></td><td></td></tr><tr><td></td><td>98.5</td><td></td><td></td><td>98.8</td><td></td><td></td></tr><tr><td>120</td><td>99.0</td><td>99.1</td><td>0.272</td><td>98.7</td><td>98.5</td><td>0.207</td></tr><tr><td></td><td>99.4</td><td></td><td></td><td>98.3</td><td></td><td></td></tr><tr><td></td><td>98.8</td><td></td><td></td><td>98.6</td><td></td><td></td></tr><tr><td>NADPH</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>0</td><td>99.0</td><td>98.3</td><td>0.991</td><td>97.6</td><td>97.8</td><td>1.00</td></tr><tr><td></td><td>98.7</td><td></td><td></td><td>98.8</td><td></td><td></td></tr><tr><td></td><td>97.1[a]</td><td></td><td></td><td>96.8[b]</td><td></td><td></td></tr><tr><td>30</td><td>91.4</td><td>95.8</td><td>3.93</td><td>98.5</td><td>98.3</td><td>0.482</td></tr><tr><td></td><td>99.0</td><td></td><td></td><td>97.8</td><td></td><td></td></tr><tr><td></td><td>96.9[c]</td><td></td><td></td><td>98.6</td><td></td><td></td></tr><tr><td>45</td><td>98.6</td><td>98.4</td><td>0.244</td><td>98.5</td><td>98.5</td><td>0.640</td></tr><tr><td></td><td>98.5</td><td></td><td></td><td>97.8</td><td></td><td></td></tr><tr><td></td><td>98.1</td><td></td><td></td><td>99.1</td><td></td><td></td></tr><tr><td>60</td><td>98.5</td><td>98.7</td><td>0.196</td><td>98.8</td><td>98.5</td><td>0.261</td></tr><tr><td></td><td>98.9</td><td></td><td></td><td>98.4</td><td></td><td></td></tr><tr><td></td><td>98.7</td><td></td><td></td><td>98.4</td><td></td><td></td></tr><tr><td>120</td><td>98.3</td><td>98.4</td><td>0.135</td><td>98.2</td><td>98.3</td><td>0.163</td></tr><tr><td></td><td>98.6</td><td></td><td></td><td>98.5</td><td></td><td></td></tr><tr><td></td><td>98.4</td><td></td><td></td><td>98.2</td><td></td><td></td></tr></table>
Conclusion	No evidence for the metabolism of ^{14}C-AVI-4658 was observed in human hepatic microsomes under the study conditions. Similar results were found for the animal species.

Study Report #	**4658 PKD 003**
Title	Evaluation of Cytochrome P450 Induction Following Exposure of Primary Cultures of Human Hepatocytes to Eteplirsen
Objectives	To measure the extent of induction of specific human CYP1A2, CYP2B6, and CYP3A4 following exposure of cryopreserved human hepatocytes to eteplirsen
Study Design	Assessment of CYP enzyme induction was performed by measuring (1) mRNA levels (gene expression) using real time polymerase chain reaction (RT-PCR) as the primary endpoint and (2) activities of CYP1A2 (phenacetin O-deethylase), CYP2B6 (bupropion hydroxylase), and CYP3A4/5 (testosterone 6β-hydroxylase) as the secondary endpoint. Cytotoxicity and stability were also assessed to show no evidence of cytotoxicity of eteplirsen after 72 h or loss of eteplirsen from the hepatocyte incubations by metabolism or degradation over 24 h in the presence of hepatocytes. No evidence for non-specific binding of eteplirsen to the hepatocyte incubation vessels was also first established. <u>Final eteplirsen concentrations</u>: • 0.00145905, 0.1038375, 0.20022, 0.399375, 0.79875, 1.5975, 3.35475, and 6.65625 mg/mL for assessing cytotoxicity, stability, and non-specific binding experiments, and gene expression • 0.00145905, 0.79875, and 6.65625 mg/mL for assessing enzyme activity

Prototypical inducers and non-inducer dosing solutions:

CYP Enzyme Induced	Prototypical Inducer / Non-Inducer	Vehicle for Preparation of Dosing Solution	Concentration (μM)
CYP1A2	Omeprazole	1% ACN in sHMM	50
CYP2B6	Phenobarbital	1% ACN in sHMM	1000
CYP3A4	Rifampicin	1% ACN in sHMM	20
Non-Inducer	Flumazenil	1% ACN in sHMM	20

- Dosing was repeated every 24 h so that the hepatocytes were exposed for a total of 72 h.

Incubation with probe CYP substrates:
1 h incubation with probe substrates shown in the following Table:

CYP Isoenzyme	Substrate	Concentration (μM)
CYP1A2	Phenacetin	100
CYP2B6	Bupropion	500
CYP3A4/5	Testosterone	250

Gene expression: Relative concentrations of mRNA) for CYP1A2, CYP2B6, and CYP3A4 were determined by Real-Time (RT)-PCR, using TaqMan®:

CYP Activities: Quantified by the production of isoenzyme specific metabolites

CYP Isoenzyme	Metabolite ID	Enzyme
CYP1A2	Acetaminophen	Phenacetin *O*-deethylase
CYP2B6	Hydroxybupropion	Bupropion hydroxylase
CYP3A4/5	6β-Hydroxytestosterone	Testosterone 6β-hydroxylase

Results

Effect of Eteplirsen on CYP1A2:

CYP1A2 mRNA:

- The fold induction following exposure to omeprazole ranged 46.5-90.5.
- The fold induction following exposure to flumazenil (non-inducer) ranged 2.41-6.11.
- The fold induction following exposure to eteplirsen ranged 0.675-6.39.
- Only Donor 2 showed dose-response.

CYP1A2 Activity:

- The fold induction following exposure to omeprazole ranged 21.7-57.6.
- The fold induction following exposure to flumazenil (non-inducer) ranged 0.891-1.55.
- The fold induction following exposure to eteplirsen ranged 0.927-7.51, suggesting an induction.
- There was evidence of dose-response for all 3 donors.
- Percent of positive control ranged -0.140-21.2% from 3 donors over the drug concentration range tested.

	Effect of Eteplirsen on CYP2B6: CYP2B6 mRNA: • The fold induction following exposure to phenobarbital ranged 9.02-14.8. • The fold induction following exposure to flumazenil (non-inducer) ranged 0.298-6.18. • The fold induction following exposure to eteplirsen ranged 0.675-6.39. • Only Donor 3 showed dose-response. CYP2B6 Activity: • The fold induction following exposure to phenobarbital ranged 10.3-22.2. • The fold induction following exposure to flumazenil (non-inducer) ranged 1.07-1.32. • The fold induction following exposure to eteplirsen ranged 0.972-1.97. • There was evidence of dose-response for all 3 donors. • Percent of positive control ranged -0.276-13.3% from 3 donors over the drug concentration range tested. **Effect of Eteplirsen on CYP3A4/5:** CYP3A4/5 mRNA: • The fold induction following exposure to rifampicin ranged 13.0-346. • The fold induction following exposure to flumazenil (non-inducer) ranged 0.960-2.04. • The fold induction following exposure to eteplirsen ranged 0.332-12.3. • Only Donor 2 showed dose-response. CYP3A4/5 Activity: • The fold induction following exposure to rifampicin ranged 19.9-25.0. • The fold induction following exposure to flumazenil (non-inducer) ranged 1.07-1.32. • The fold induction following exposure to eteplirsen ranged 0.822-1.46. • There was evidence of dose-response for all 3 donors. • Percent of positive control ranged -0.910-2.71% from 3 donors over the drug concentration range tested.
Conclusion	• Eteplirsen in the in vitro study using human hepatocytes did not show significant enzyme inducing capability for CYP1A2, CYP2B6 and CYP3A4/5, although slight induction or dose-response was observed for mRNA gene expression or enzyme activity. • The levels of induction in the presence of eteplirsen at the concentration range (0.00145905~6.65625 mg/mL) were low,

	compared to the positive control, as shown in the Results section above.
Comments	• Considering that these test concentrations are much higher than the clinically observed plasma concentration, except for the 0.00145905 mg/mL, we agree that results from this study did not suggest induction of all three CYP isozymes by eteplirsen in vivo. • Considering the co-induction of CYP3A and CYP2C isozymes via activation of the Pregnane X receptor (PXR), result of insignificant CYP3A induction would eliminate the need for the in vitro or in vivo induction studies for CYP2C isozyme.

Study Report #	**4658 PKD 004**
Title	Inhibitory Potential of Eteplirsen Towards Human Hepatic Microsomal Cytochrome P450 Isoenzymes
Objectives	To characterize the direct (reversible) and metabolism-dependent inhibitory potential of eteplirsen on activities of CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5) in human hepatic microsomes
Study Design	Test system: • Pooled human hepatic microsomes from 50 individuals (25 males and 25 females) were obtained from vendor, which have been characterized and validated for total protein and selected enzyme/CYP activities. • Details of the incubation conditions and probe substrates for each assay are presented in the following table. All sample and control incubations were performed in triplicate.

Activity Assay (Cytochrome P450)	Substrate (µM)	Protein (mg/mL)	Time (Minutes)	Analyte	Positive Control (µM)
Phenacetin *O*-deethylase (CYP1A2)	30	0.1	15	Acetaminophen	Fluvoxamine (1)
Bupropion hydroxylase (CYP2B6)	65	0.1	15	Hydroxybupropion	ThioTEPA (100)
Amodiaquine *N*-deethylase (CYP2C8)	1.0	0.025	10	Desethylamodiaquine	Montelukast (0.1)
Diclofenac 4′-hydroxylase (CYP2C9)	3.5	0.025	10	4′-Hydroxydiclofenac	Sulfaphenazole (3)
S-mephenytoin 4′-hydroxylase (CYP2C19)	25	0.1	15	4′-Hydroxymephenytoin	Nootkatone (30)
Bufuralol 1′-hydroxylase (CYP2D6)	11	0.1	15	1′-Hydroxybufuralol	Quinidine (0.3)
Testosterone 6β-hydroxylase (CYP3A4/5)	45	0.25	5	6β-Hydroxytestosterone	Ketoconazole (0.2)
Midazolam 1′-hydroxylase (CYP3A4/5)	2.0	0.1	5	1′-Hydroxymidazolam	Ketoconazole (0.2)

Direct (reversible) inhibition:

- Assays of CYP-selective enzyme activities were performed in the absence and presence of 0.001491, 0.005325, 0.015975, 0.05325, 0.15975, 0.639, 2.13, and 6.65625 mg/mL final assay concentrations of eteplirsen.

	• Single probe substrate concentration for each CYP was used. • Concentration of eteplirsen that inhibits 50% of the activity (IC50) of each specific isoenzyme of CYP was calculated when >50% inhibition was observed (Part I). • For CYP isoenzymes (i.e., CYP1A2, CYP2C9 and CYP2C19) in which >50% inhibition was observed, the inhibition constant (Ki) and the type of inhibition were further assessed with eteplirsen concentrations (0, 1.065, 2.13, 4.26, 8.52, and 10.65 mg/mL final assay concentrations). <u>Metabolism-dependent inhibition</u>: • The same CYP-selective activities were tested after pre-incubation of 0.639, 2.13, and 6.65625 mg/mL eteplirsen in the presence and absence of NADPH and 10-fold concentrated suspension of pooled human hepatic microsomes for 30 min [the final eteplirsen concentrations after dilution were 0.0639, 0.213, and 0.665625 mg/mL, respectively].
Results	<u>Direct (reversible) inhibition</u>: • Inhibition <50% was observed for CYP2B6, CYP2C8, CYP2D6, CYP3A4/5, or CYP3A4/5 at eteplirsen concentrations up to 6.65625 mg/mL => little or no evidence of direct inhibition. • Inhibition >50% was observed for CYP1A2 at eteplirsen concentrations at 6.65625 mg/mL (49.6% activity remaining), for CYP2C9 at 6.65625 mg/mL (22.2% activity remaining), and for CYP2C19 at the highest 2.13 (30.3% activity remaining) and 6.65625 mg/mL (11.4% activity remaining) => some evidence of direct inhibition, more noticeably for CYP2C9 and CYP2C19 at these high eteplirsen concentrations. • Summary of inhibition on human hepatic CYP isoenzymes by eteplirsen is presented in the following table:

	Direct Inhibition			
CYP Isoenzyme	Conclusion	IC_{50} (mg/mL)	K_i (mg/mL)	Type of Inhibition
CYP1A2	Yes	6.52	7.58 ± 0.63	Competitive[a]
CYP2B6	No	NA	NA	NA
CYP2C8	No	NA	NA	NA
CYP2C9	Yes	2.75	0.676 ± 0.048	Competitive
CYP2C19	Yes	1.16	0.553 ± 0.029	Competitive
CYP2D6	No	NA	NA	NA
CYP3A4/5[b]	No	NA	NA	NA
CYP3A4/5[c]	No	NA	NA	NA

[a] The applicant stated that the Ki value may be slightly inaccurate because of the highest 10.65 mg/mL eteplirsen concentration employed. Also, mixed or noncompetitive inhibition cannot be ruled out for CYP1A2.
[b] Midazolam 1′-hydroxylase.
[c] Testosterone 6β-hydroxylase

<u>Metabolism-dependent inhibition</u>:

	• The CYP enzyme activity, as reflected by % activity remaining, remained similar, suggesting no metabolism-dependent inhibition by eteplirsen was observed in these experiments.
Conclusion	• Eteplirsen in the in vitro study using human hepatocytes showed little or no evidence of direct inhibition was observed for CYP2B6, CYP2C8, CYP2D6, CYP3A4/5, or CYP3A4/5 in the presence of eteplirsen under the study conditions. • In vitro results showed some evidence of direct or competitive inhibition for CYP1A2, CYP2C9 and CYP2C19 at the highest eteplirsen concentrations used in the study. • No metabolism-dependent inhibition by eteplirsen was observed
Comments	• The Cmax of eteplirsen observed following 30 mg/kg/wk dosing in pivotal clinical study (Study 201) was approximately 77200 ng/mL (= 0.0772 mg/mL). Significant drug interaction potential via CYP1A2, CYP2C9 and CYP2C19 inhibition is likely to be low following 30 mg/kg/wk doses, based on the following considering: (1) the highest eteplirsen concentration employed in the in vitro study for examining the direct inhibition is 6.62 mg/mL which is approximately 86-fold of observed Cmax of the target dose, (2) the IC50 values for CYP1A2, CYP2C9 and CYP2C19 are approximately 85.7, 35.6 and 15-fold, respectively, of observed Cmax, (3) 0.05325 and 0.15975 mg/mL eteplirsen concentrations, which encompasses the observed Cmax, had minimum or no effects on enzymes, (4) in comparison, positive control inhibitors of these enzymes nearly or completely depleted the enzyme activities. • In the clinical trials, the higher 50 mg/kg/wk doses, which resulted in higher eteplirsen plasma concentrations, have been studied in DMD patients and may provide additional safety assurance or exposure coverage concerning any potential drug-drug interactions.

Study Report #	**4658 PKD 005**
Title	Evaluation of AVI-4658 as a Substrate and Inhibitor of a Panel of Human Drug Transporters
Objectives	To determine if AVI-4658 (eteplirsen) is a substrate and/or an inhibitor of OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, and BSEP
Study Design	Test system: • Chinese hamster ovary (CHO) cells, stably transfected individually with vector pCMV6, OAT1-pCMV6, OAT3-pCMV6, OCT1-pCMV6, OCT2-pCMV6, OATP1B1-pCMV6, and OATP1B3-pCMV6 were used for uptake transporter assays. • Caco-2 monolayers (grown on (b) (4) membrane inserts, with an initial density of 4 x 10 cells/cm) were used for P-gp and BCRP assays. The apparent permeability of 14C-

mannitol (a paracellular marker) and 14C-caffeine (a transcellular marker), and the efflux ratios of ^{3}H-digoxin (a known P-gp substrate) and ^{3}H-estrone-3-sulfate (a known BCRP substrate) were determined for the suitability of the monolayers.

- Inside-out human BSEP membranes prepared from insect cells (Sf9), as well as MRP2 membranes were also obtained. MK-571 (100 μM) was used as a selective inhibitor of MRP2.

Assessment of AVI-4658 as a substrate or an inhibitor of uptake transporters:

- Uptake of ^{14}C-AVI-4658 (8 and 80 μg/mL) by each uptake transporter and the vector control was conducted for 5, 15, and 30 minutes, in the absence and presence or a known inhibitor for each uptake transporter.
- Uptake of the known substrate by each transporter was conducted in the presence and absence of a known inhibitor and AVI-4658 (80 and 800 μg/mL) according to the uptake incubation procedure.
- Uptake transporter substrates and inhibitors are summarized in the following table.

Transporter	Known Substrate (μM)	Known Inhibitor (μM)
OAT1	^{14}C-para-Aminohippurate (1)	Probenecid (200)
OAT3	^{3}H-Estrone-3-sulfate (1)	Probenecid (200)
OCT1	^{14}C-Tetraethylammonium (1)	Quinidine (256)
OCT2	^{14}C-Tetraethylammonium (1)	Quinidine (256)
OATP1B1	^{3}H-Estradiol-17β-D-glucuronide (0.5)	Cyclosporine A (10)
OATP1B3	^{3}H-Cholecystokinin octapeptide (1)	Cyclosporine A (10)

Assessment of AVI-4658 as a substrate or an inhibitor of P-gp and BCRP:

- The apparent permeability in both the apical-to-basolateral (A to B) direction and basolateral-to-apical (B to A) direction was determined.
- The apparent permeability of ^{14}C-AVI-4658 (8 and 80 μg/mL) was determined in the presence of vehicle and known inhibitor according to the efflux incubation procedure for 1, 2, 3, and 4 h. The apparent permeability of each known substrate was assessed in the presence of vehicle and each known inhibitor as controls. [zosuquidar (2 μM, a selective inhibitor of P-gp, but not BCRP); Ko143 (1 μM, a selective inhibitor of BCRP, but not P-gp)]
- For each transporter, the apparent permeability of known substrate was determined in the presence of vehicle, known inhibitor, and AVI-4658 (80 and 800 μg/mL) for 1 h according to the efflux incubation procedure.
- Efflux transporter substrates and inhibitors are shown in the following table:

	<table><tr><th>Transporter</th><th>Known Substrate (μM)</th><th>Known Inhibitor (μM)</th></tr><tr><td>P-gp</td><td>^{3}H-Digoxin (1)</td><td>Zosuquidar (2) Ko143[a] (1)</td></tr><tr><td>BCRP</td><td>^{3}H-Estrone-3-sulfate (0.1)</td><td>Ko143 (1) Zosuquidar[a] (2)</td></tr></table> Assessment of AVI-4658 as a substrate or an inhibitor of MRP2: • MRP2 ATPase activity was determined in the presence of vehicle, probe substrate probenecid (1000 μM), and AVI-4658 (80 and 800 μg/mL). Control incubations were conducted in the absence and presence of DPBS. • Uptake of 3H- leukotriene C4 (LTC4, 0.1 μM) by MRP2 was determined in the presence of vehicle and AVI-4658 (80 and 800 μg/mL). MK-571 (100 μM) was used as a positive inhibitor. Control incubations were conducted in the absence and presence of DPBS. Assessment of AVI-4658 as a substrate or an inhibitor of BSEP: • Uptake of ^{14}C-AVI-4658 (8 and 80 μg/mL) into BSEP membranes was conducted according to the BSEP incubation procedure. Uptake of 3H- taurocholate (TCA, 1 μM) was performed as a positive control in the absence and presence of DPBS. • Uptake of 3H-TCA (1 μM) was conducted in the presence of AVI-4658 (80 and • 800 μg/mL), vehicle, and known inhibitor (bosentan, 200 μM) in the absence and presence of DPBS according to the BSEP incubation procedure.
Results	AVI-4658 as a substrate or an inhibitor of uptake transporters: • Results of uptake of 14C-AVI-4658 showed that that ^{14}C-AVI-4658 was not a substrate of the uptake transporters tested in the study. • The fold uptake over the vector control was ≤2.07 the highest for OAT1 (at 8 μg/mL at 30 min) and <2 for other uptake transporters. However, uptake of ^{14}C-AVI-4658 by OAT1 (with fold uptake over vector control ≥2) was not observed under different assay conditions (different incubation times and/or different final ^{14}C-AVI-4658 concentration). • The presence of selective inhibitors did not significantly change the fold uptake. AVI-4658 as an inhibitor of uptake transporters: • Among all uptake transporters tested, AVI-4658 showed weak inhibition of OCT1 and OATP1B1 only. • The remaining OCT1 activities were 74.3 and 86.1% of the solvent control in the presence of AVI-4658 at 80 and 800 μg/mL, respectively. • The remaining OATP1B1 activities were 82.2 and 67.8 of the solvent control, in the presence of AVI-4658 at 80 and 800 μg/mL,

	respectively. <u>AVI-4658 as a substrate an inhibitor of P-gp or BCRP</u>: • The apparent permeability and efflux ratio of ^{14}C-AVI-4658 were ≤0.0987 x 10^{-6} cm/s (low apparent permeability) and ≤1.43, respectively. These parameters were not markedly changed in the presence of selective inhibitor of either P-gp or BCRP. <u>AVI-4658 as an inhibitor of P-gp or BCRP</u>: • AVI-4658 (80 and 800 μg/mL) did not inhibition the transport of 3H-digoxin mediated by P-gp or 3H-estrone-3-sulfate mediated by BCRP. <u>Assessment of AVI-4658 as a substrate or an inhibitor of MRP2</u>: • AVI-4658 at 80 and 800 μg/mL showed weak stimulation of MRP2 ATPase activity. The signal-to-noise ratios were 1.93 and 1.41 (both <2), respectively, which is not qualified to be a substrate of MRP2. • AVI-4658 (80 and 800 μg/mL) did not inhibit the MRP2 uptake of 3H-leukotriene C4. The signal-to-noise ratio was 18.3 (vs. 1.93 by MK-571) and the remaining activity was ≥99.8%. <u>Assessment of AVI-4658 as a substrate or an inhibitor of BSEP</u>: • AVI-4658 (8 and 80 μg/mL) was not actively taken up by BSEP membranes with signal-to-noise ratios ≤1.17 (vs. 14.4 by 3H-TCA). • AVI-4658 did not inhibit BSEP with the remaining activity ≥105%.
Conclusion	• The results indicate that AVI-4658 was not a substrate of OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, or BSEP over the concentration range investigated. • AVI-4658 showed weak inhibition of OCT1 and OATP1B1, but not of OAT1, OAT3, OCT2, OATP1B3, P-gp, BCRP, MRP2, or BSEP over the concentration range investigated. The weak inhibition of OCT1 and OATP1B1 is not likely to warrant an in vivo study or result in clinically significant drug-drug interaction.

4.2 Pharmacometrics Review

Background

The pharmacometrics review focused on exploring changes in various clinical endpoints such as 6 minute walk distance (6MWD), total NSAA scores, rise time, 10 meter run/walk time in eteplirsen treated patients with age. Graphical comparisons were made to historical natural history data. Interpretation of clinical and natural history data requires an interdisciplinary approach, so many of the analyses in this review were conducted in response to specific questions raised from reviewers in the Division of Neurology Products (DNP). The team was also interested in comparing 6MWD data from eteplirsen treated patients and patients administered placebo in controlled clinical trials. The analyses included 3 year follow up data submitted on 5/20/2015, 4th year update from eteplirsen treated patients submitted on 12/14/2015, updates on historical controls submitted on 12/17/2015, 01/08/2016 and correction in 6MWD from a patient in historical controls submitted on 04/01/2016.

The analysis results are discussed in Section 1.2 of this review.

Data

Historical Controls From Italy and Belgium

Patient-level data were provided by Professor Eugenio Mercuri, MD, PhD (Catholic University, Rome, Italy) on behalf of the Italian DMD Registry database, and from Professor Nathalie Goemans, MD (University Hospitals, Leuven, Belgium). The Italian DMD Registry includes patient data from 11 neuromuscular care centers in Italy (b) (4) (b) (4). The patients in Dr. Goemans' registry attended the Leuven Neuromuscular Reference Center (NMRC) for clinical care and management.

The following filters were applied to allow for the identification of appropriately matched patient historical control cohort:

1. Corticosteroid use at Baseline
2. Sufficient longitudinal data for 6MWT available (defined as including a Baseline value and at least one valid post-baseline value)
3. Age ≥7
4. Genotype amenable to any exon skipping therapy
5. Genotype amenable to exon 51 skipping therapy

The Italian DMD cohort contained the 6MWT results at Baseline (Month 0) and at Months 12, 24, and 36, with age and steroid use entered for each visit and with genotype

information for 97 patients. Of these patients, 87 valid cases were identified based on applying filters 1 and 2; 62 when filters 1, 2, and 3 were applied; 34 when filters 1, 2, 3, and 4 are applied; and 10 when all filters are applied.

The NMRC dataset contained 6MWT results at various time points, the patient's age and steroid use at the same time points, and genotype information for 89 patients. However, discrete visit designations (ie, Baseline, Month 12, etc) were not identified in the dataset. In order to maximize the utilization of these data, time points with non-zero meters on the 6MWT assessment for patients who were ≥ 7 years of age and on a steroid, were designated as the Baseline visit. If no 6MWT assessment was available for the time points that would correspond to time elapsed of 12, 24, or 36 months (ie, discrete Month), the assessment within 3.5 months of the corresponding discrete month was used. If there were two such assessments (ie, within the 3.5 month boundary of the discrete month), the one that was closest to the corresponding month was used. As such, of the 89 patients that were included in the NMRC dataset, only 29 had data based on applying filters 1 and 2 or applying filters 1, 2 and 3; 16 patients had data when filters 1, 2, 3 and 4 were applied, and 3 patients had data when all filters were applied.

Controls from Clinical Trials

A total of 92 DMD patients with mutations amenable to exon 51 skipping, age greater than 5 years and taking stable dose of steroids at baseline were administered placebo for 48 weeks in controlled clinical trials. The 6MWD data from these patients were discussed at a previous PCNS AC meeting held on November 24th, 2015.

Software

All analyses were done using SAS® (Version 9.3) and R Studio (Version 0.97.551)

Listing of Analyses Codes and Output Files

File Name	Description	Location in \\cdsnas\pharmacometrics\
Sarepta_PooledData.sas	SAS code for comparing eteplirsen patients with historical controls	

4.3 Genomics and Targeted Therapy Review

OFFICE OF CLINICAL PHARMACOLOGY
GENOMICS and TARGETED THERAPY GROUP REVIEW

NDA/BLA Number	206488
Submission Date	06/26/2015
Applicant Name	Sarepta Therapeutics
Generic Name	Eteplirsen
Proposed Indication	Treatment of Duchenne Muscular Dystrophy
Primary Reviewer	Hobart Rogers Pharm.D, Ph.D.
Secondary Reviewer	Christian Grimstein Ph.D.

EXECUTIVE SUMMARY

Eteplirsen is a synthetic antisense oligonucleotide (AON) that is targeted against exon 51 of the dystrophin gene. Eteplirsen is being developed for the treatment of Duchenne Muscular Dystrophy (DMD) in individuals who possess deletion mutations amenable to the skipping of exon-51 to restore the reading frame and produce an internally-deleted dystrophin protein. Individuals with these internally-deleted dystrophins have on average a much milder form of disease known as Becker Muscular Dystrophy (BMD). The sponsor is seeking an approval of eteplirsen for all mutations amenable to skipping exon-51 in the *DMD* gene, however not all mutations were studied in the clinical trials.

The purpose of this review is to evaluate whether eteplirsen should be approved for all mutations amenable to exon-51 skipping by eteplirsen. The review concluded that given the lack of available subjects for study, coupled with inherent heterogeneity in disease, along with the unknowns regarding the functionality of the internally-deleted dystrophin; determining efficacy in patients with ultra-rare DMD mutations amenable to exon-51 skipping is difficult. Furthermore, there are no reasons to believe that the safety of eteplirsen is in any way different in these ultra-rare populations of patients. Hence, it is reasonable to conclude that the restoration of the reading frame by eteplirsen should be beneficial for all DMD mutations amenable to exon-51 skipping. The findings of this review indicate that eteplirsen, if found to be safe and effective in the studied population, should be indicated for all mutations amenable to exon-51 skipping.

1 Background

Duchenne Muscular Dystrophy (DMD) is characterized by an absence of the protein dystrophin. Dystrophin is a rod shaped cytoplasmic protein that connects the cytoskeleton of a muscle fiber the surrounding extracellular matrix through the cell membrane. Functionally, dystrophin acts to stabilize the sarcolemma membrane against

the stress imposed by muscle contraction. The lack of dystrophin in DMD results in a severe disease observed in the first years of life with patients typically losing ambulation around the age of 12 years and the need for mechanical ventilation around 18 years of age. Another related genetic disease is Becker Muscular Dystrophy (BMD), where an internally-deleted dystrophin is produced. BMD results in a much milder phenotype with many patients remaining ambulant throughout life or even asymptomatic.

The stark contrast between DMD and BMD phenotype is the presence of dystrophin. In DMD the reading-frame of the mRNA is disrupted and little to no dystrophin is produced, whereas in BMD, the reading frame is intact and an internally-deleted, but somewhat functional dystrophin protein is produced.

The gene for dystrophin is one of the largest in the human genome consisting of 79 exons. DMD is an X-linked disorder; mutations occur in about 1 in every 3500 male births. There are a large variety of mutations, with one out of three mutations occurring *de novo*. Over 4500 pathogenic mutations are known to cause DMD. Large deletions are present in about 60% of patients, large duplications in about 10% and point mutations (confined mostly to coding exons) in about 30% of patients (PMID: 219693337). Of the deletion mutations, approximately 66% of patients carry a deletion of one or more exons, of which 70% cluster between exon 45 and 55 (PMID: 19156838).

Eteplirsen is a synthetic chemically modified phosphorodiamidate morpholino oligomer (PMO) RNA antisense oligonucleotide composed of 30 nucleotides in a sequence specific for exon 51 of the dystrophin pre-mRNA. Eteplirsen binds to exon 51 of the dystrophin pre-mRNA causing exon skipping during processing and restoring the reading frame to produce a truncated internally-deleted dystrophin. In theory, this exon 51 skipping would restore the reading frame of the mRNA to allow an internally-deleted dystrophin protein to be expressed. The resultant protein, while not complete, is expected to convert DMD patients to the less severe BMD phenotype.

2 Submission Contents Related to Genomics

The sponsor submitted the following labeling language for eteplirsen:

Indications and Usage:
Eteplirsen is an exon skipping oligonucleotide inducer of dystrophin synthesis indicated for the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing.

The sponsor's submitted data included the underlying DMD mutation for all patients. The sponsor's to-be labeled population compared to the studied population will be the focus of this review. The sponsor's proposed labeling states that the drug will be indicated for subjects with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing.

Of the DMD mutations amenable to treatment with eteplirsen, the sponsor has studied six (45-50, 47-50, 48-50, 49-50, 50, and 52) different DMD deletion mutations in their clinical program (Table 1).

Table 1. DMD Mutations Present in Sponsor's Safety Population

		Eteplirsen							
	Placebo (N=4)	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=40)	50 mg/kg IV (N=6)	All IV (N=65)	All Eteplirsen (N=72)
Mutation [d]									
45-50	0	2 (28.6%)	3 (27.3%)[d]	0	3 (75.0%) [d]	14 (35.0%)	1 (16.7%)	21 (32.3%)	23 (31.9%)
47-50	0	0	0	1 (25.0%) [d]	0	0	0	1 (1.5%)	1 (1.4%)
48-50	0	2 (28.6%)	4 (36.4%)[d]	1 (25.0%) [d]	0	7 (17.5%)	0	12 (18.5%)	14 (19.4%)
49-50	3 (75.0%)	1 (14.3%)	3 (27.3%)[d]	2 (50.0%) [d]	1 (25.0%) [d]	9 (22.5%)	3 (50.0%)	18 (27.7%)	19 (26.4%)
50	1 (25.0%)	2 (28.6%)	0	0	0	5 (12.5%)	1 (16.7%)	6 (9.2%)	8 (11.1%)
52	0	0	1 (9.0%)[d]	0	0	4 (10.0%)	1 (16.7%)	6 (9.2%)	6 (8.3%)
Unknown	0	0	0	0	0	1 (2.5%)[e]	0	1 (1.5%) [e]	1 (1.4%) [e]

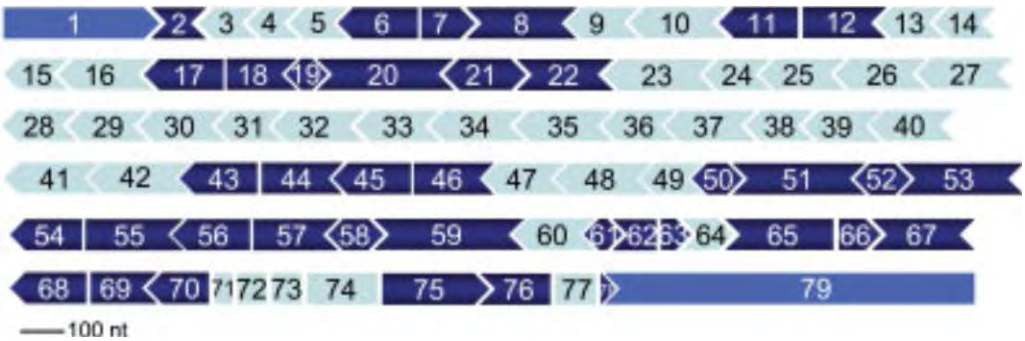
Source: Modified from page 49 Summary of Clinical Safety

3 Key Questions and Summary of Findings

3.1 Are the studied populations in the sponsor's clinical trials representative of the to-be labeled population?

No. The sponsor has studied six different DMD mutations amenable to exon-51 skipping therapy. Eteplirsen is to be indicated for all mutations amenable to skipping exon 51. Additional DMD mutations (e.g. 19-50, 52-63) are known to exist, however they are ultra-rare (1-2 subjects in database) in nature. A search of the Leiden DMD database (www.dmd.nl) using the known exon splicing ***(Figure 1),*** *identified subjects composing of ten additional DMD mutations (i.e., 3-50, 13-50, 17-50, 19-50, 29-50, 38-50, 40-50,43-50, 52-58, 52-63) that may be amenable to exon-51 skipping based on the mechanism of action of eteplirsen. Amenable mutations are those in which skipping of exon-51 would, in theory, restore the reading frame. For instance, in Figure 1, a subject with a deletion of exons 44-50 would not be amenable to exon-51 skipping as exons 43 and 52 cannot be spliced together, whereas, a deletion of exons 43-50 can be successfully spliced by exon-51 skipping.*

Figure 1. Depiction of the 79 Exons of the Dystrophin Gene and Splicing



Source: PMID 19156838
Note: In-frame exons are in light blue, out-of-frame in dark blue. Deletions are considered in-frame when the exons flanking the deletion "fit."

3.2 ***Should Eteplirsen be indicated for patients amenable to exon-51 skipping who were not studied in the clinical development program?***

Yes. Despite not all DMD mutations amenable to exon-51 skipping being represented in the clinical development program, if eteplirsen is ultimately found to be safe and effective to warrant approval, then eteplirsen should be indicated for all exon-51 amenable mutations.

Reviewer comment: In theory, restoring the reading frame by skipping exon-51 may result in a milder form of the disease (i.e. transition from DMD phenotype towards a BMD phenotype); therefore it has the potential to be efficacious for patients with all amenable mutations. However, given the ultra-rare occurrence of some exon-51 amenable mutations (e.g. 43-50 deletions) it is exceedingly difficult to find adequate numbers of patients for clinical studies. Moreover, given the strict inclusion criteria for the eteplirsen clinical trials, these patients may have been ineligible to participate (e.g. non-ambulatory). Furthermore, given the inherent variability in disease, studying these ultra-rare mutation subsets may be challenging for determining efficacy or lack thereof.

Many unknowns remain in how the internally-deleted dystrophin can impact disease, both in quantity and quality. Successful exon-51 skipping in the case of each DMD deletion mutation would create a different internally-deleted dystrophin protein. For some mutations amenable to exon-51 skipping we have BMD subjects with the same internally-deleted "in-frame" mutations to infer some degree of functionality of that protein (PMID: 25633150, 22102647). BMD patients are generally less severe, however there can be a large heterogeneity in disease phenotype (PMIDs: 25633150, 2404853). While in-frame deletions in the proximal regions of the protein (exons 20-40) tend to be milder than those in the distal part (exons 40-55), it is still difficult to predict exactly what the functionality of the skipped dystrophin protein may be (PMIDs: 19156838,16770791,17041910). For example, a case report of a patient missing exons 17-48 only resulted in mild BMD, with the patient being ambulant at 61 years of age (PMID: 2404210). Thus, it is clear that the amount of exons present

isn't directly correlated with functionality. Hence, while we can infer some functionality of an exon-51 skipped product, many unknowns remain on how it can affect clinical phenotype.

Determining efficacy in single patients with a specific exon-51 skipping amenable mutation is difficult for the following reasons: a lack of available subjects for study, coupled with inherent heterogeneity in disease, along with the unknowns regarding the functionality of the internally-deleted dystrophin. Moreover, there are no reasons to believe that the safety of eteplirsen is in any way different in these ultra-rare populations of patients. Thus, 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.

3.3 Is there a difference in the functionality of the exon-skipped truncated dystrophin produced by treatment with eteplirsen?

Potentially. Given the significant intra- and inter-subject variation in disease phenotype, it is likely that large numbers of DMD patients with different mutations would need to be studied in order to determine efficacy. Given the small numbers of subjects in the sponsor's submission with specific DMD deletions, numerical comparisons can only be made for a few of the exon-51 skipping amenable groups.

3.3.1 Sponsor's analysis

The sponsor did not perform any efficacy analyses by DMD mutation type. Given the overall small numbers of subjects enrolled in their clinical program, further subgroup analysis is likely underpowered.

3.3.2 Reviewer's analysis

The sponsor enrolled six different DMD deletion mutations that were amenable to exon-51 skipping in their clinical development program. The goal of eteplirsen treatment is to restore the reading frame and produce a truncated dystrophin protein similar to patients with BMD. In theory, each DMD mutation amenable to exon-51 skip will produce a different internally-deleted dystrophin. It is unlikely that an amenable mutation would not respond to treatment with eteplirsen. Given the heterogeneity in disease phenotype DMD mutations, it is difficult to ascertain whether differences in DMD mutation affected efficacy. While there may be some differences in functionality of the exon-51 skipped transcripts; restoring the reading frame to produce dystrophin even if it may be different between DMD mutations is warranted.

4 Summary and Conclusions

Eteplirsen is being sought for the indication of the treatment of DMD in all mutations amenable to exon-51 skipping. There were six different DMD mutations represented in the sponsor's clinical development program; however one mutation (47-50) only had only one representative subject. Although eteplirsen was not studied in all DMD mutations amenable to exon-51 skipping, it may be reasonable to extrapolate efficacy to ultra-rare populations (i.e., mutations with only one or two known subjects), given the inherent variability in disease, and our understanding of the mechanism of action in restoring the reading frame. Last, there are no reasons to believe that the safety of eteplirsen is in any way different in these ultra-rare populations of patients. Hence, given the challenges of studying these ultra-rare populations of disease, coupled with the lack of any unique safety concerns, it is reasonable to approve eteplirsen for all DMD mutations amenable to exon-51 skipping, if found to be safe and effective in the studied population.

5 Recommendations

It is the finding of this review that eteplirsen, if found to be safe and effective to warrant approval, is likely to benefit all mutations amenable to exon-51 skipping and should be labeled accordingly.

Post-marketing studies

None.

5.1 Labeling recommendations

No additional labeling recommendations.
Eteplirsen is an exon skipping phosphorodiamidate morpholino oligomer (PMO) which restores the mRNA reading frame to induce dystrophin protein production and is indicated 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. This indication is approved on an intermediate endpoint demonstrating delayed disease progression as measured by the 6 minute walk test [see Clinical Studies (14)]. Continued benefit will be evaluated through confirmatory trials. (1)

CLINICAL PHARMACOLOGY FILING FORM

Application Information			
NDA/BLA Number	206488	**SDN**	2
Applicant	Sarepta	**Submission Date**	6/26/2015
Generic Name	Eteplirsen	**Brand Name**	Eteplirsen
Drug Class	Antisense Oligonucleotide		
Indication	Duchenne Muscular Dystrophy		
Dosage Regimen	30 mg/kg infusion over 35 to 60 minutes once weekly		
Dosage Form	Injection		
OCP Division	DCP-1, DPM	**OND Division**	DNP

OCP Review Team	Primary Reviewer(s)	Secondary Reviewer/ Team Leader
Division	Ta-Chen Wu	Angela Men
Pharmacometrics	Venkatesh Atul Bhattaram	Kevin Krudys
Genomics	Hobart Rogers	Christian Grimstein

Review Classification	☐ Standard ☑ Priority ☐ Expedited		
Filing Date	8/4/2015	**74-Day Letter Date**	9/8/2015
Review Due Date	11/27/2015	**PDUFA Goal Date**	2/26/2016

Application Fileability

Is the Clinical Pharmacology section of the application fileable?
☑ Yes
☐ No
If no list reason(s)

Are there any potential review issues/ comments to be forwarded to the Applicant in the 74-day letter?
☐ Yes
☑No
If yes list comment(s)

Is there a need for clinical trial(s) inspection?
☐ Yes
☑ No
If yes explain

Clinical Pharmacology Package

Tabular Listing of All Human Studies	☑ Yes ☐ No	Clinical Pharmacology Summary	☑ Yes ☐ No
Bioanalytical and Analytical Methods	☐ Yes ☑ NA	Labeling	☑ Yes ☐ No

Clinical Pharmacology Studies			
Study Type		**Count**	**Comment(s)**
In Vitro Studies			
☐ Metabolism Characterization		1	Study 4658-PKD_002: Metabolism in human hepatic microsomes
☐ Transporter Characterization		1	Study 4658-PKD_005: AVI-4658 as substrate and inhibitor of human drug transporters
☐ Distribution		1	Study 4658-PKD_001: In vitro plasma protein binding of 14C-AVI-4658
☐ Drug-Drug Interaction		2	Study 4658-PKD_003: Induction potential for CYPs in human hepatocytes Study 4658-PKD_004: Inhibitory potential for CYPs in human liver microsomes
In Vivo Studies			
Biopharmaceutics			
☐ Absolute Bioavailability			
☐ Relative Bioavailability			
☐ Bioequivalence			
☐ Food Effect			
☐ Other (b) (4)		13	2 bioanalytical validation reports (plasma and urine samples) 11 bioanalytical reports (plasma and urine samples; stability)
Human			
Healthy Subjects	☐ Single Dose		
	☐ Multiple Dose		
Patients	☐ Single Dose		
	☐ Multiple Dose		
☐ Mass Balance Study			
☐ Other (e.g. dose proportionality)			
Intrinsic Factors			

☐ Race		
☐ Sex		
☐ Geriatrics		
☐ Pediatrics		
☐ Hepatic Impairment		
☐ Renal Impairment		
☐ Genetics		
Extrinsic Factors		
☐ Effects on Primary Drug		
☐ Effects of Primary Drug		
Pharmacodynamics		
☐ Healthy Subjects		
☑ Patients		
Pharmacokinetics/Pharmacodynamics		
☐ Healthy Subjects		
☑ Patients	7	Study AVI-4658-33: Phase 1/2, Investigator-Sponsored, Proof of Concept Study in Patients with DMD Study AVI-4658-28: Phase 1b, Multiple-Dose Study of Eteplirsen IV in Patients with DMD Study 4658-us-201: Phase 2, double-blind, placebo-controlled, multiple-dose (N=12) Study 4658-us-202: Extension of Study 201 Study 4658-us-301: Pivotal confirmatory Phase 3, Open-label, multi-center vs untreated control group (i.e., DMD patients not amenable to exon 51 skipping) Study 4658-us-204: Open-label, multi-center in advanced stage DMD SR-15-031: Observational; historical control
☐ QT		In vitro and studies in animals
Pharmacometrics		
☐ Population Pharmacokinetics		
☐ Exposure-Efficacy		
☐ Exposure-Safety		
☑ Natural History Analysis	1	Effect of Eteplirsen on 6-Minute Walk Test and North Star Ambulation Assessment Total Score in

		DMD Patients as Compared to Matched Historical Controls		
Total Number of Studies	**In Vitro**	5	**In Vivo**	7+1
Total Number of Studies to be Reviewed				

Criteria for Refusal to File (RTF)		
RTF Parameter	**Assessment**	**Comments**
1. Did the applicant submit bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	☐Yes ☐No ☑N/A	
2. Did the applicant provide metabolism and drug-drug interaction information? (Note: RTF only if there is complete lack of information)	☐Yes ☑No ☐N/A	Only in vitro study information
3. Did the applicant submit pharmacokinetic studies to characterize the drug product, or submit a waiver request?	☐Yes ☑No ☐N/A	
4. Did the applicant submit comparative bioavailability data between proposed drug product and reference product for a 505(b)(2) application?	☐Yes ☐No ☑N/A	
5. Did the applicant submit data to allow the evaluation of the validity of the analytical assay for the moieties of interest?	☑Yes ☐No ☐N/A	
6. Did the applicant submit study reports/rationale to support dose/dosing interval and dose adjustment?	☑Yes ☐No ☐N/A	
7. Does the submission contain PK and PD analysis datasets and PK and PD parameter datasets for each primary study that supports items 1 to 6 above (in .xpt format if data are submitted electronically)?	☑Yes ☐No ☐N/A	
8. Did the applicant submit the module 2 summaries (e.g. summary-clin-pharm, summary-biopharm, pharmkin-written-summary)?	☑Yes ☐No ☐N/A	
9. Is the clinical pharmacology and	☑Yes ☐No	(Navigation within reports

biopharmaceutics section of the submission legible, organized, indexed and paginated in a manner to allow substantive review to begin? If provided as an electronic submission, is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work leading to appropriate sections, reports, and appendices?	☐N/A	is tricky; no hyperlink to reports such as bioanalytical reports or datasets, though they can be located in separate locations)
Complete Application **10.** Did the applicant submit studies including study reports, analysis datasets, source code, input files and key analysis output, or justification for not conducting studies, as agreed to at the pre-NDA or pre-BLA meeting? If the answer is 'No', has the sponsor submitted a justification that was previously agreed to before the NDA submission?	☑Yes ☐No ☐N/A	
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality) Checklist		
Data		
1. Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	☑Yes ☐No ☐N/A	
2. If applicable, are the pharmacogenomic data sets submitted in the appropriate format?	☑Yes ☐No ☐N/A	Mutation information in natural history studies have been submitted.
Studies and Analysis		
3. Is the appropriate pharmacokinetic information submitted?	☑Yes ☐No ☐N/A	(PK dataset for Study 301 needs to be confirmed)
4. Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	☐Yes ☐No ☑N/A	
5. Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	☐Yes ☐No ☑N/A	

6. Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	☐Yes ☐No ☑N/A	
7. Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?	☐Yes ☐No ☑N/A	
General		
8. Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	☑Yes ☐No ☐N/A	
9. Was the translation (of study reports or other study information) from another language needed and provided in this submission?	☐Yes ☑No ☐N/A	

Filing Memo
The sponsor compared effects of eteplirsen on 6MWT and NSAA with historical controls (Natural history). This data will be analyzed by the pharmacometrics reviewer.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

VENKATESH A BHATTARAM
05/06/2016

TA-CHEN WU
05/06/2016

CHRISTIAN GRIMSTEIN on behalf of HOBART ROGERS
05/06/2016

CHRISTIAN GRIMSTEIN
05/06/2016

YUXIN MEN
05/06/2016

KEVIN M KRUDYS
05/06/2016

MEHUL U MEHTA
05/06/2016

Cross-Discipline Team Leader Review

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

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	twenty-fifth year. • 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.	
Current Treatment Options	There are no FDA-approved treatments for DMD. • 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. • 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.	There is high unmet medical need for treatment of DMD to slow functional decline and prolong survival.
Benefit	• 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. • 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. • 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	Substantial evidence of efficacy was not provided for clinical or biomarker (dystrophin) endpoints. 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.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	forms of dystrophinopathy.	
Risk	• The clinical safety database for eteplirsen is small: 114 total patients exposed, with only 36 exposed for ≥24 weeks and 12 exposed for ≥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. • 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.	The safety database was small, but would be sufficient to support approval if there was demonstration of substantial evidence of efficacy.
Risk Management	• Safety risks have not been identified that would require risk management beyond standard pharmacovigilance.	Standard pharmacovigilance is recommneded

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 Reglatory 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)

[redacted]. 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 [redacted] method used during drug product manufacture.
    3. Revalidate the robustness of the in-process [redacted] method in terms of [redacted].
    4. Investigate the consistent bias in the in-process [redacted] results and the release [redacted] results.

    As noted in an addendum to the OPQ review, on 3/10/2016, a potential OAI alert for the DS manufacturing facility, [redacted] was activated as a result of a routine GMP surveillance inspection from [redacted] The inspection resulted in a 14-item FDA-483 and was initially classified OAI by the field investigators. The [redacted] 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 [redacted] 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 (Cmax 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 25th 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[1] 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**.

[1]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 4th 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 4th 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 1/10th 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.[2] 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)

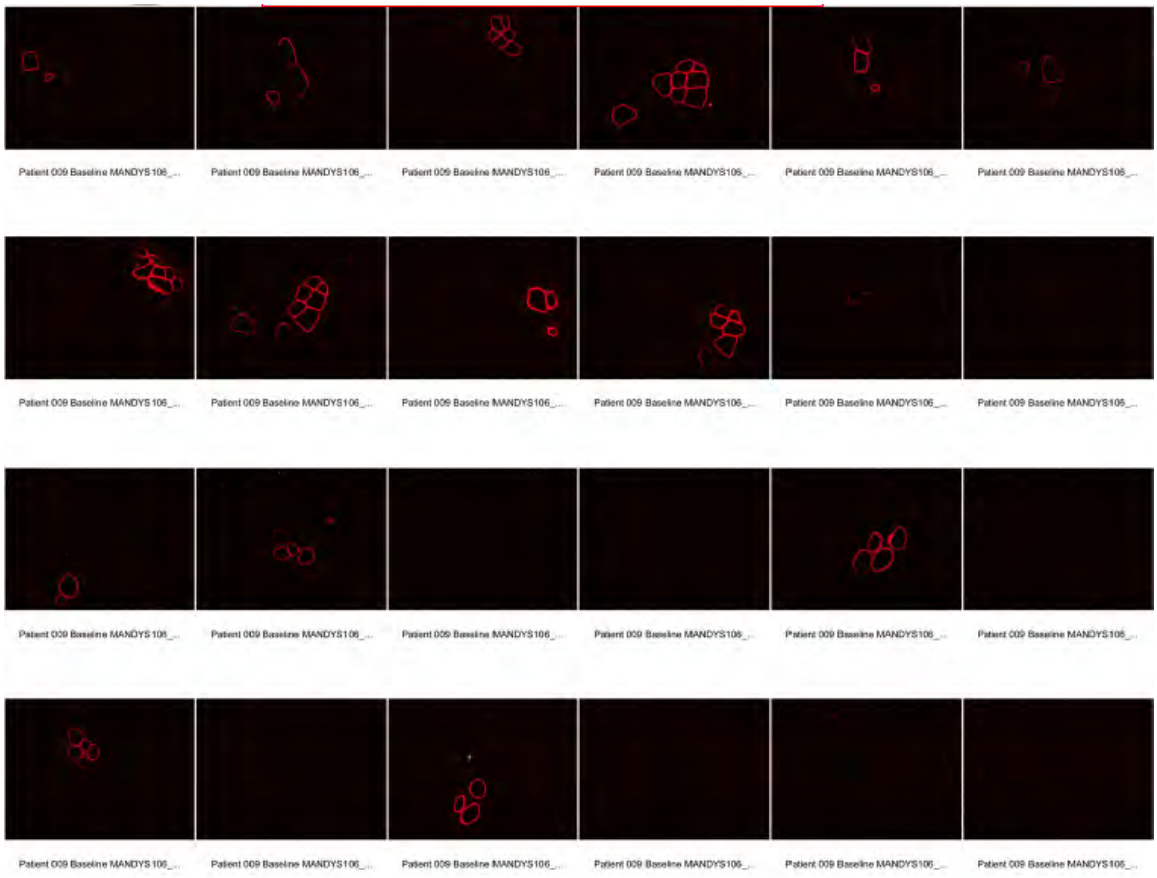
The measurement of total dystrophin immunofluorescence by Bioquant was first carried out on blinded baseline, Week 12, and Week 24 images, captured at 20x magnification. The results showed essentially no change in intensity for any patient. Negative results were obtained both when the study was conducted 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 4th biopsy. Bias in field selection may have resulted in preferential capture of bright fibers that appear similar to revertant fibers.

[2] 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



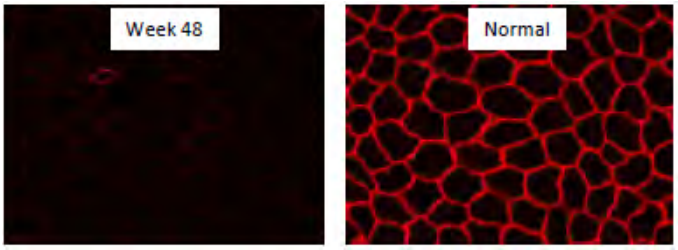
Week 48 samples were processed separately for dystrophin immunofluorescence from earlier samples, and had higher background staining. As a consequence, valid comparison is not possible with earlier time points for percent positive fibers or total immunofluorescence because the higher background staining, 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

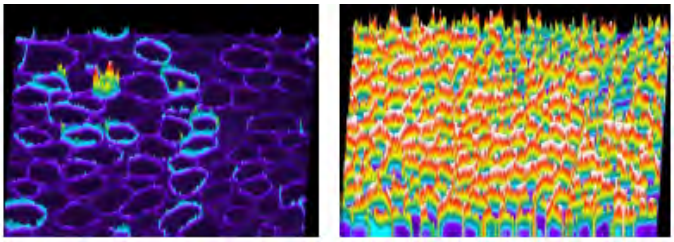
Figure 3. The top two images show the intensity as originally captured, and the bottom two images show the intensity converted to "heatmap" images that represent the observed (unmodified) pixel intensity as color, from low intensity blue to high intensity red and white.

Figure 3: Dystrophin Immunofluorescence vs. Normal Control

Original Image



"Heat map"



It is important to note that the applicant digitally processed[3] 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."*

[3] 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 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 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.4 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,5 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),[6] dystrophin levels averaged about 0.3% of normal, but ranged from undetectable to ≈ 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%.[7] However, such a small sample size may not provide a reliable

[4] Pradhan S (2002) Valley sign in Duchenne muscular dystrophy: importance in patients with inconspicuous calves. Neurol India. 50,184-186.

[5] 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.

[6] FDA Advisory Committee presentation for drisapersen, slide 43.

[7] 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,[8] and may occur in DMD[9] (one study in DMD did not find expansion of revertant fibers with age, but appears to have had low sensitivity for detecting change[10]). 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.[11]*

- **Lack of independent confirmation**: The applicant has not obtained independent confirmation of dystrophin findings.[12]

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

[8] 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.

[9] Fanin et al (1995) Dystrophin-positive fibers in Duchenne dystrophy: origin and correlation to clinical course. Muscle and Nerve. 18:1115-1120.

[10] Arechavala-Gomeza, V et al (2010) Revertant fibres and dystrophin traces in Duchenne muscular dystrophy: implications for clinical trials. Neuromuscul Disord. 20:295-301.

[11] Taylor LE (2012) Quantification of dystrophin immunofluorescence in dystrophinopathy muscle specimens. Neuropathology and Applied Neurobiology. 38:591-601.

[12] 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 4th 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 4th biopsy**: Prior to conduct of biomarker studies on the 4th 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, 4th 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[13] (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 (R2 >0.9) of sensitivity extending as low as 0.25% of normal."*

FDA response*: As quoted above, prior to analysis of the 4th 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.[14] The applicant's discussion of the variability of the Western blot method does not consider these potentially large sources of biological variability.*

[13] 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.

[14] 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% x 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 4th 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[15] 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,[16] including those directly involved in the development of eteplirsen,[17] have stated that levels less than 3% of that of normal

[15] 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"

[16] Flanigan KM (2014) Duchenne and Becker muscular dystrophies. Neurol Clin. 32,l 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." [18]

Dystrophin levels in exon-51 model BMD patients have been observed to be much higher than these estimates, roughly 80% of normal on average.[19] 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,[20] 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%,[21] 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.[22] 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[23]) or on the percentage of fibers staining for dystrophin (staining in nearly 100% of fibers occurs in at least some exon 44 skippable patients[24]). 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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[17] 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.

[18] Wilton SD, Veedu RN, Fletcher S (2015) The emperor's new dystrophin: finding sense in the noise. Trends in Molecular Medicine. 21, 417-426.

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

[20] Flanigan KM (2014) Duchenne and Becker muscular dystrophies. Neurol Clin 32:671-688.

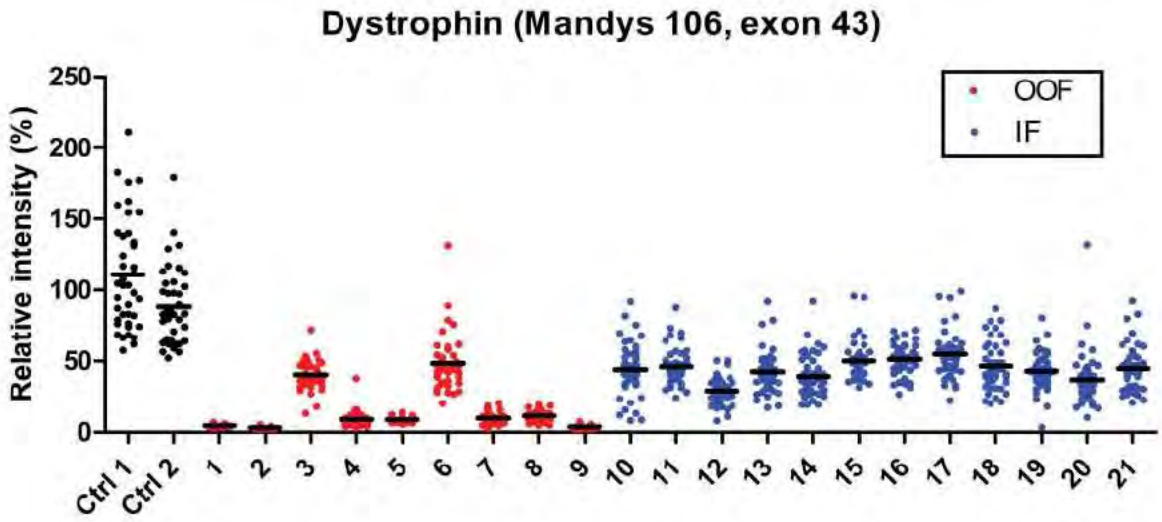
[21] Nicholson, LVB (1993) Functional significance of dystrophin positive fibers in Duchenne muscular dystrophy. Archives of Diseases in Childhood. 68:632-636.

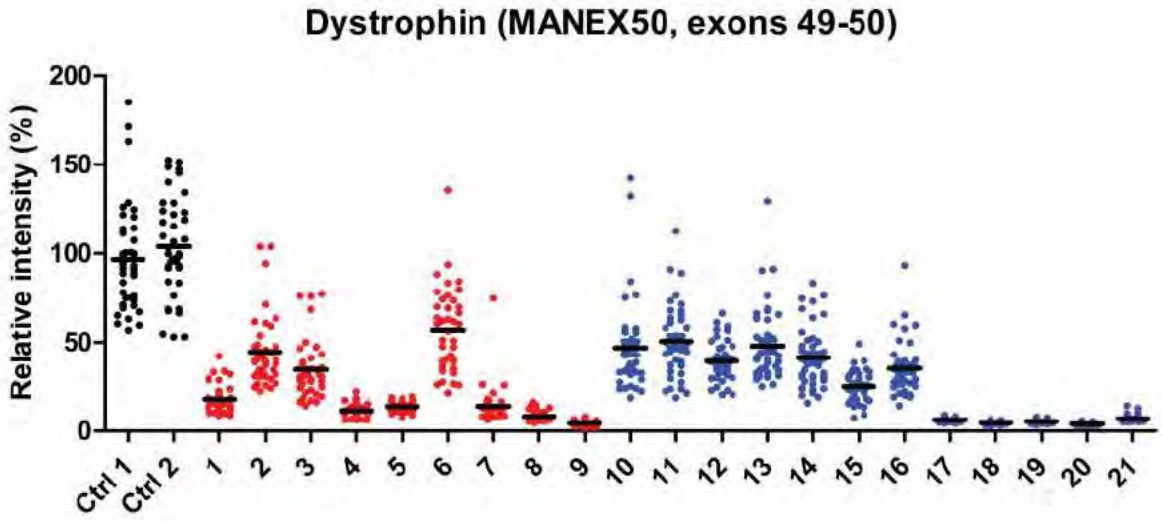
[22] Fanin et al (1995) Dystrophin-positive fibers in Duchenne dystrophy: origin and correlation to clinical course. Muscle and Nerve. 18:1115-1120.

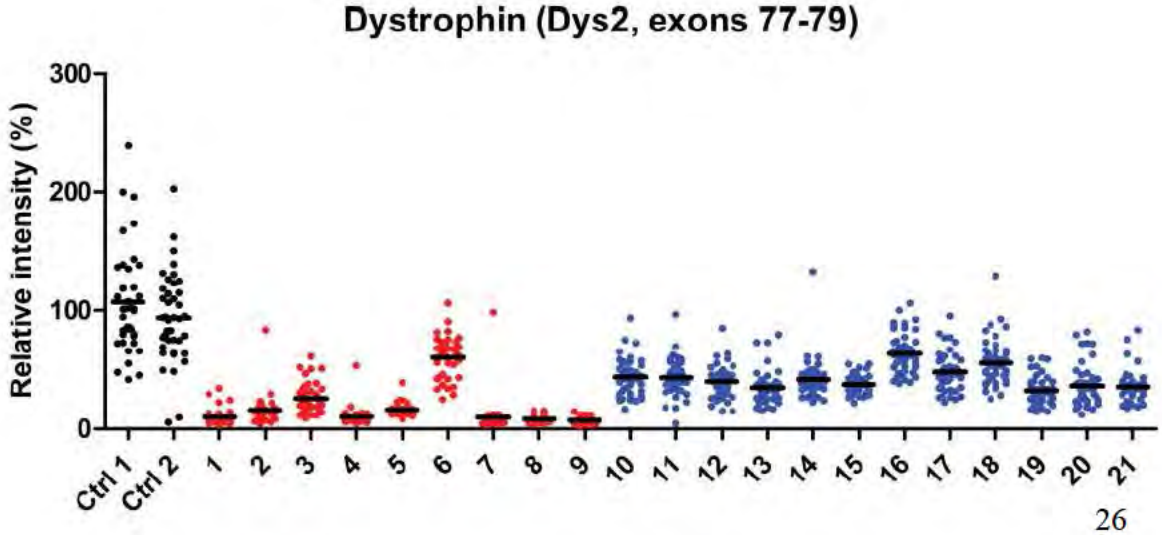
[23] Anthony K, et al (2014) Biochemical characterization of patients with in-frame or out-of-frame DMD deletions pertinent to exon 44 or 45 skipping. JAMA Neurol. 71:32—40.

[24] 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)[23] 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.

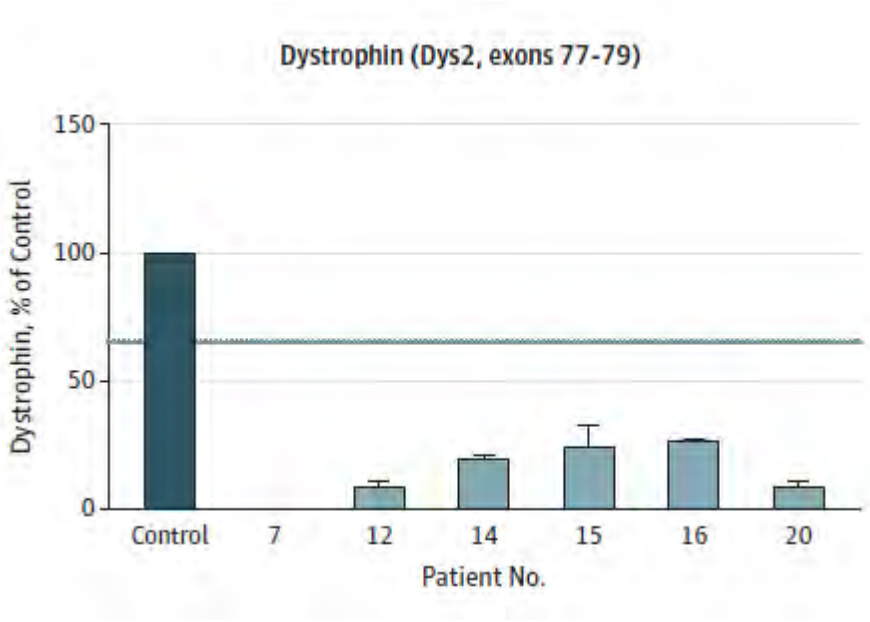






The following are key observations about this data:

- 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 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"[25] for reasons including the pro-inflammatory environment that develops in DMD.[26]

Functionality: The exact dystrophin mutation affects the clinical phenotype in BMD,[27] 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."[28]

Localization: In BMD, dystrophin is typically present in all or most fibers[29,30] 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,[31] 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.[32]

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

[25] Wilton SD, Fletcher S, Flanigan KM(2014) Dystrophin as a therapeutic biomarker: Are we ignoring data from the pase? Neuromuscular Disorder. 24, 463-466.
[26] Rosenberg et al (2015) Immune-mediated pathology in Duchenne muscular dystrophy. Sci Transl Med 7,299rv4.
[27] Nicolas et al (2015) Becker muscular dystrophy severity is linked to the structure of dystrophin. Human Molecular Genetics. 24:1267-1279.
[28] Van den Bergen JC et al (2014) Dystrophin levels and clinical severity in Becker muscular dystrophy patients. Neurol Neurosurg Psychiatry. 85, 747-753.
[29] Arahata et al (1989) Dystrophin diagnosis: comparison of dystrophin abnormalities by immunofluorescence and immunoblot analysis. Proc Natl Acad Sci. 86,7154-7158.
[30] Morandi et al (1995) Dystrophin characterization in BMD patients: correlation of abnormal protein with clinical phenotype. Journal of Neurological Sciences 132,146-155.
[31] Arechavala-Gomeza et al (2010) Revertant fibres and dystrophin traces in Duchenne muscular dystrophy: implications for clinical trials. Neuromuscul Disord. 20,295-301.
[32] 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[33] 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

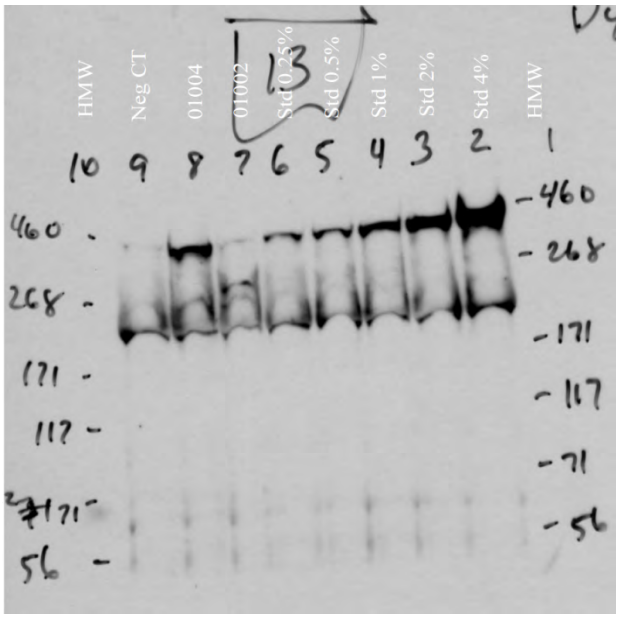
Considerable confusion can be created by the fact that a number of different methods have been used to quantify dystrophin expression, some more quantitative than others, and some producing higher absolute numbers than others. As discussed above, immunofluorescence is mainly informative of dystrophin 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.

[33] 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.

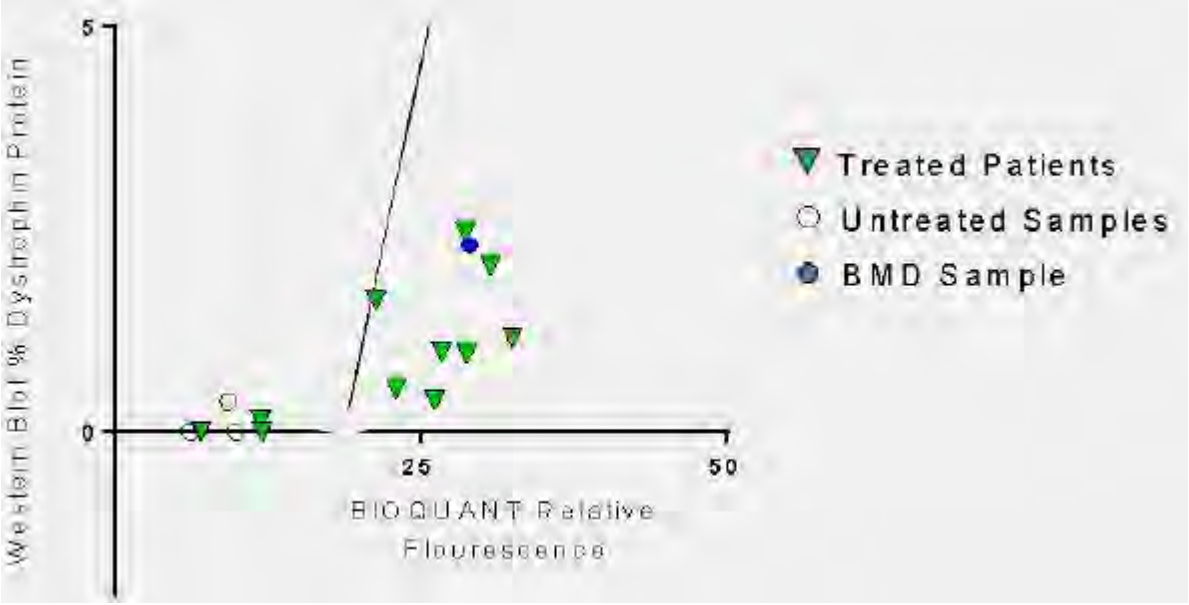
Figure 4: Western blot, 4th Biopsy, Study 202



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,[34] there is justifiable interest in

[34] 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,[35] 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."

[35] 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,[36] 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[37] 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[38] reported that in data collected by the Cooperative International Neuromuscular Research Group (CINRG) about 25% boys[39] similarly treated with deflazacort were ambulatory at age 16 years (N = 80).

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

[37] 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.

[38] 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

[39] 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.[40] Gentle exercise appears to provide additional benefit, including delay of functional deterioration.[41] 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,[42] 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.

[40] 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

[41] 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

[42] 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) [43] "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"[44]
> - "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"[45]

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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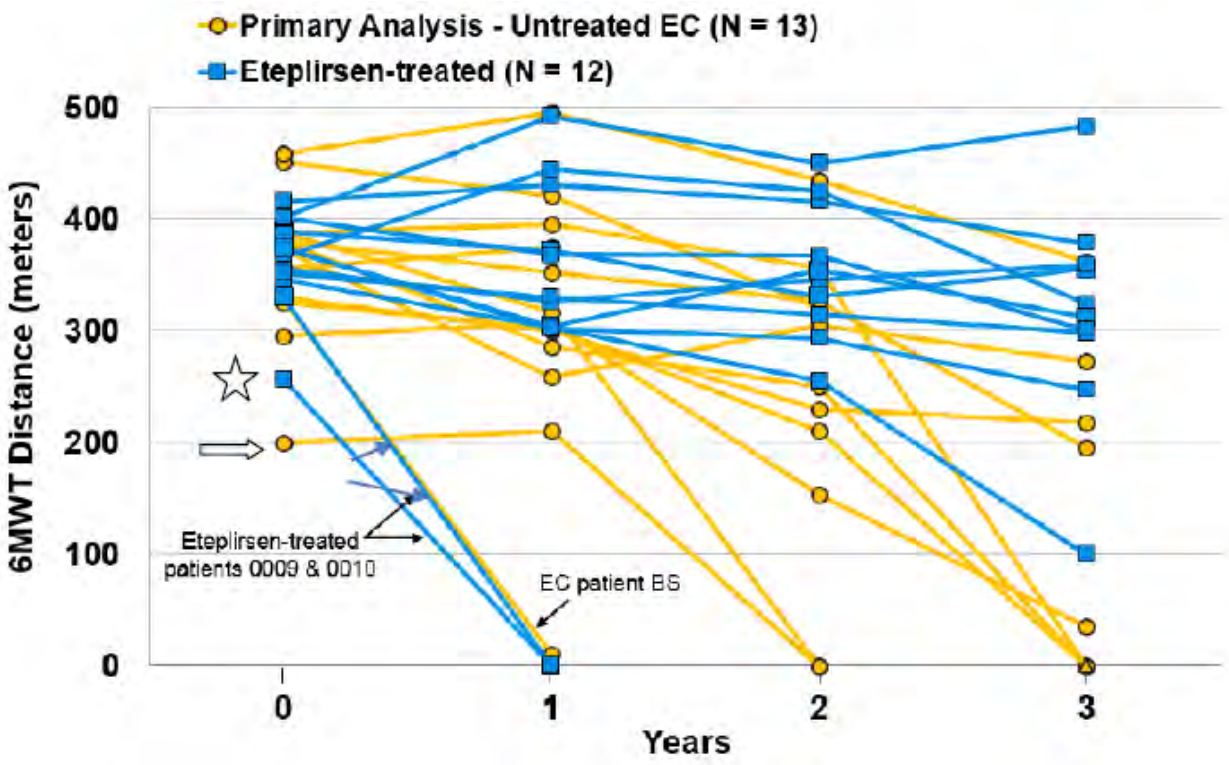
[43] Mendell et al. (2007) Challenges in drug development for muscle disease: a stakeholders meeting. Muscle Nerve 35:8-16.

[44] www.mda.org/disease/duchenne-muscular-dystrophy/signs-and-symptoms. Accessed 9 May 2016.

[45] 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[46] 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.*

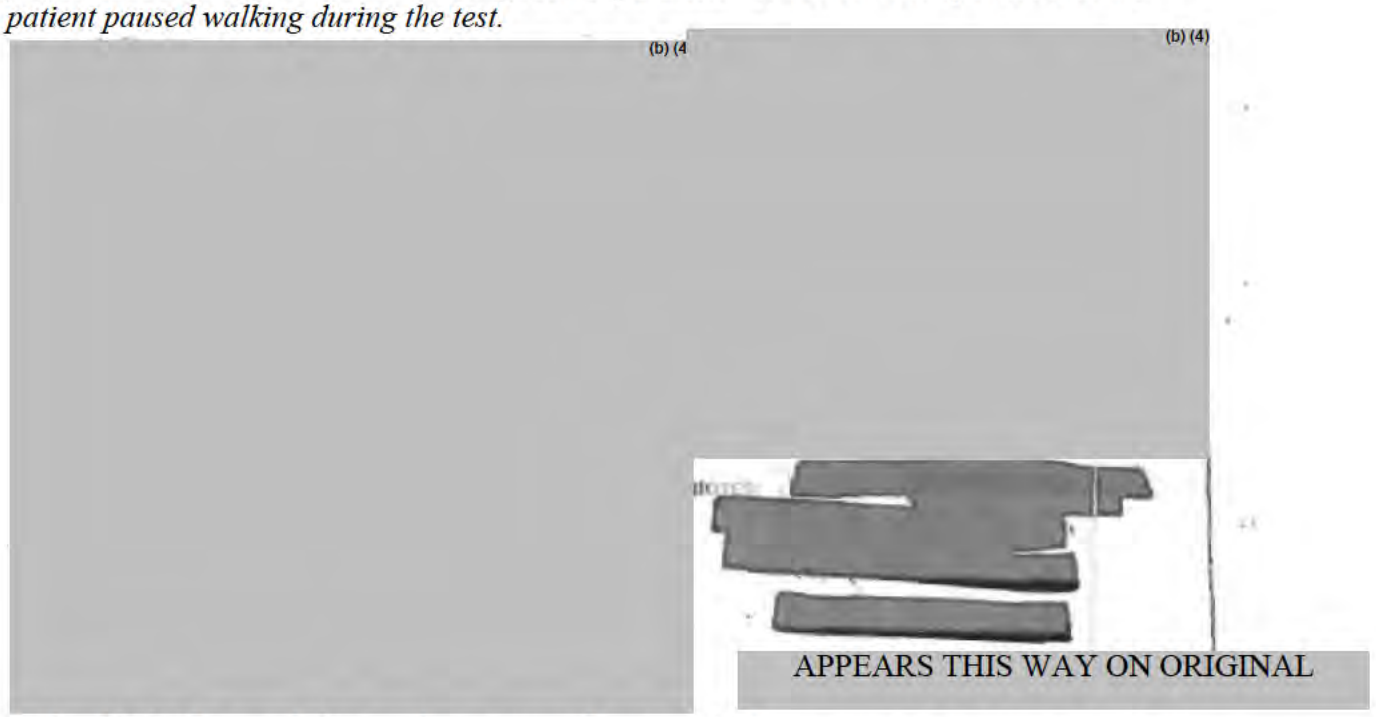
- *At age 10, one exon-51 skippable control patient from Italy was recorded as having a 6MWT of 356 m, and a 10 m walk time of 10 s. One year later, at age 11, 10 m*

[46] 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[47] 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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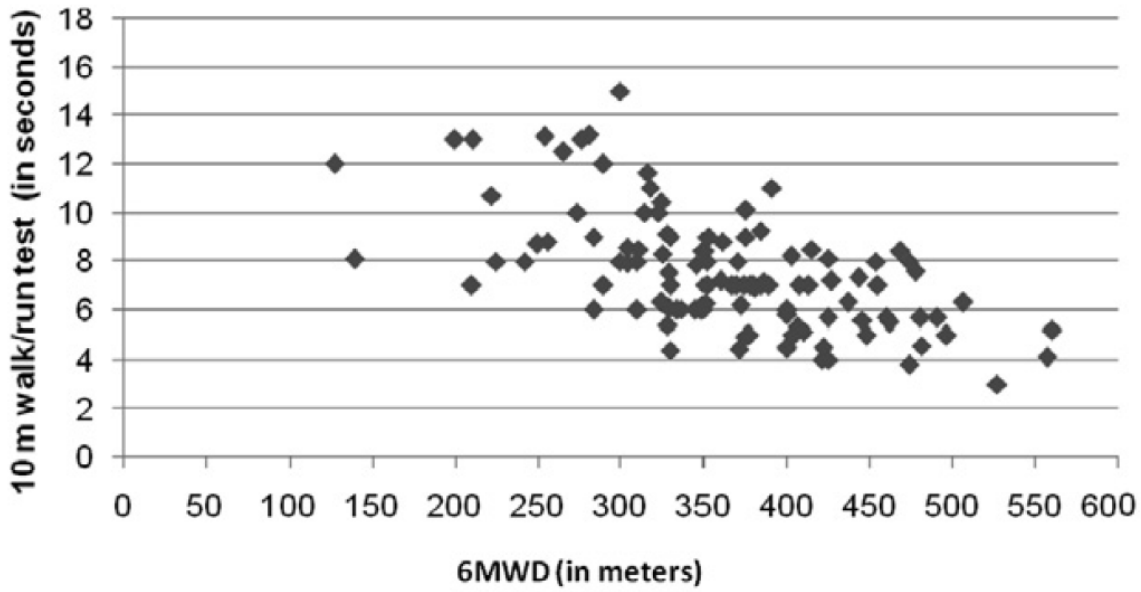
[47] Per applicant addendum for February 23, 2016 AC meeting: "patients (b) (6) were subsequently reported to have loss of ambulation with "0" meters on the 6MWT at ~4.5 years. In addition, external control patient (b) (6) was known to have lost ambulation with a 6MWT of "0" at 4.8 years"

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[48] 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.[49] 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

[48] 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.

[49] 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,[50] 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 predicator of loss of ambulation. At the most recent study visit, 6MWT was less than 250 m for the 7 out 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.

[50] 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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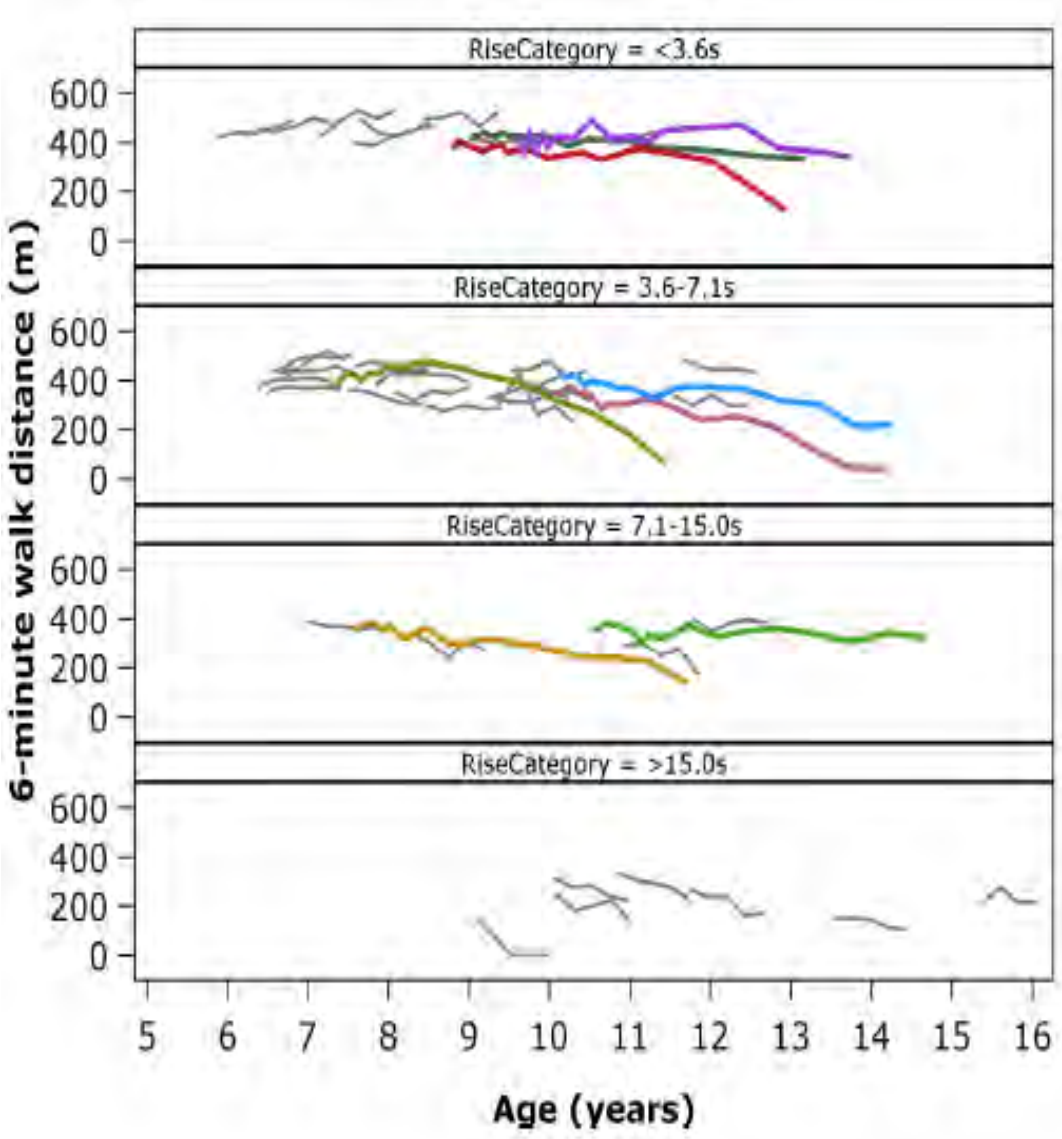


Figure 9: 6MWT, Prednisone-treated patients

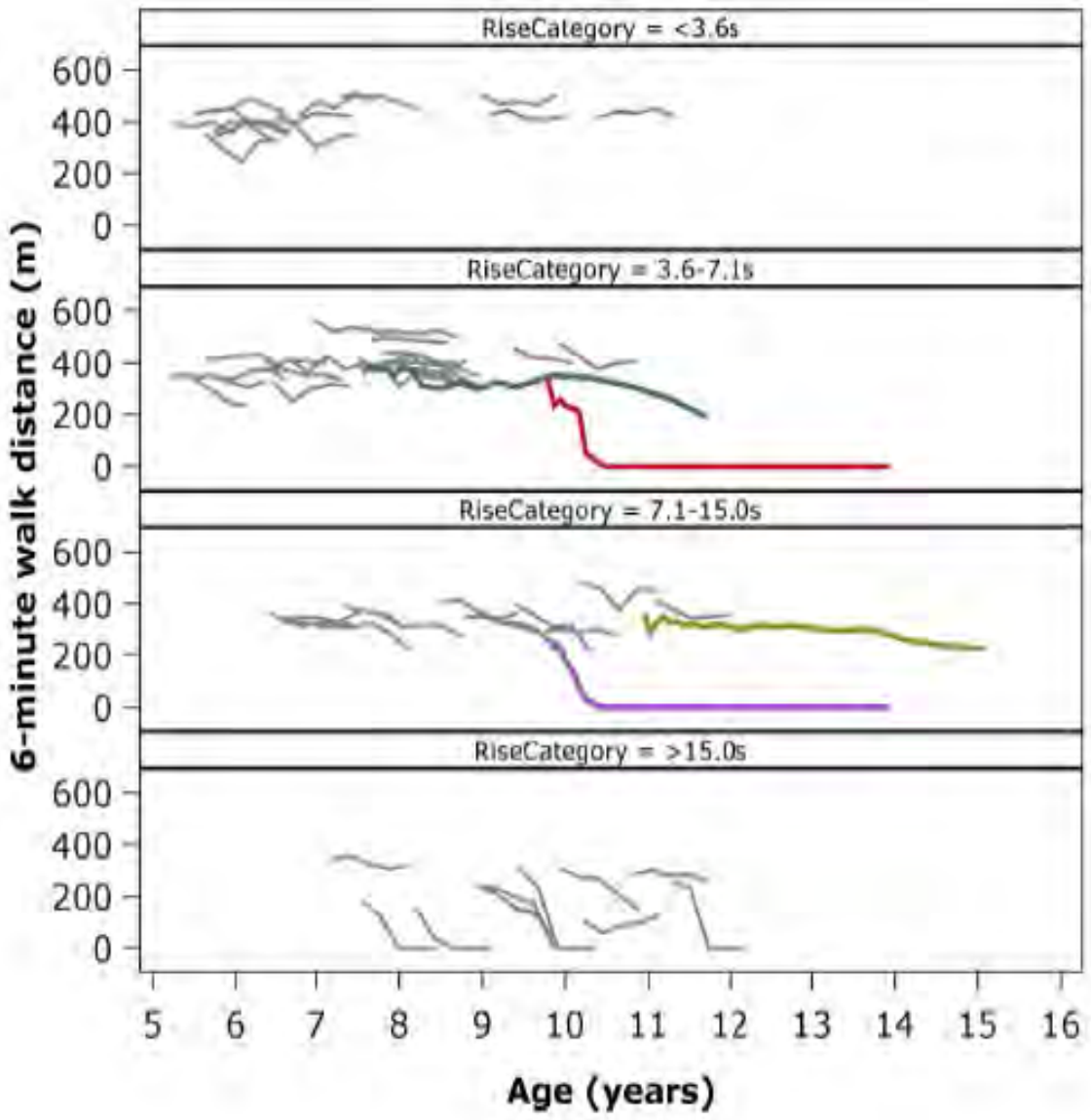
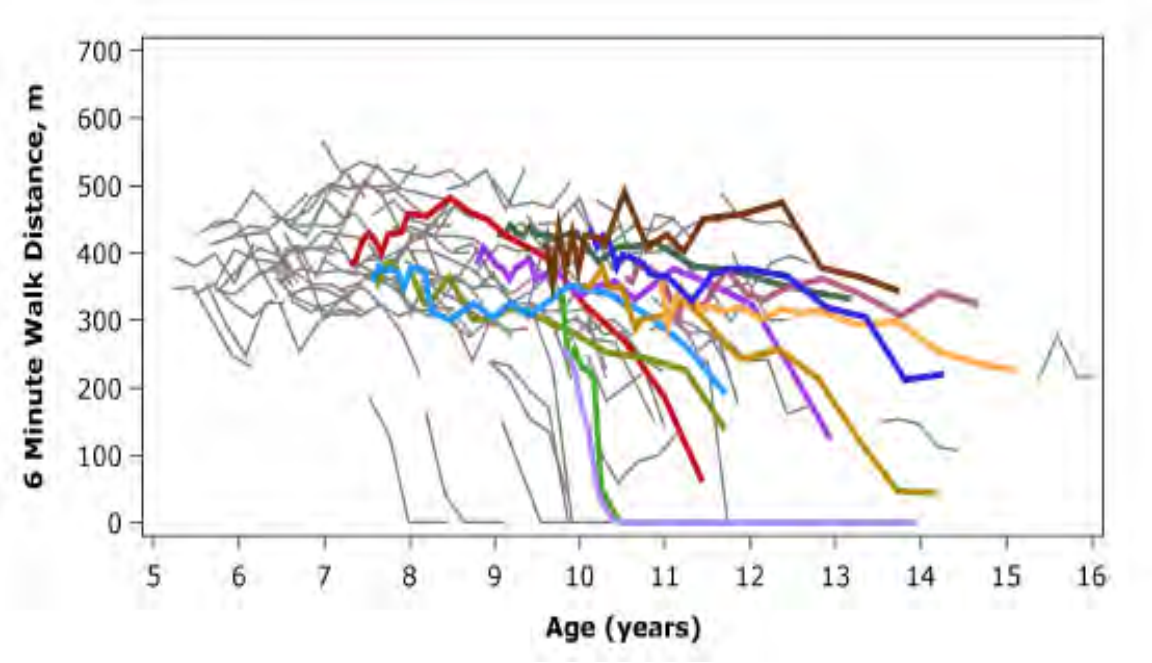


Figure 10: 6MWT, eteplirsen vs controls on placebo, all patients



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., ≥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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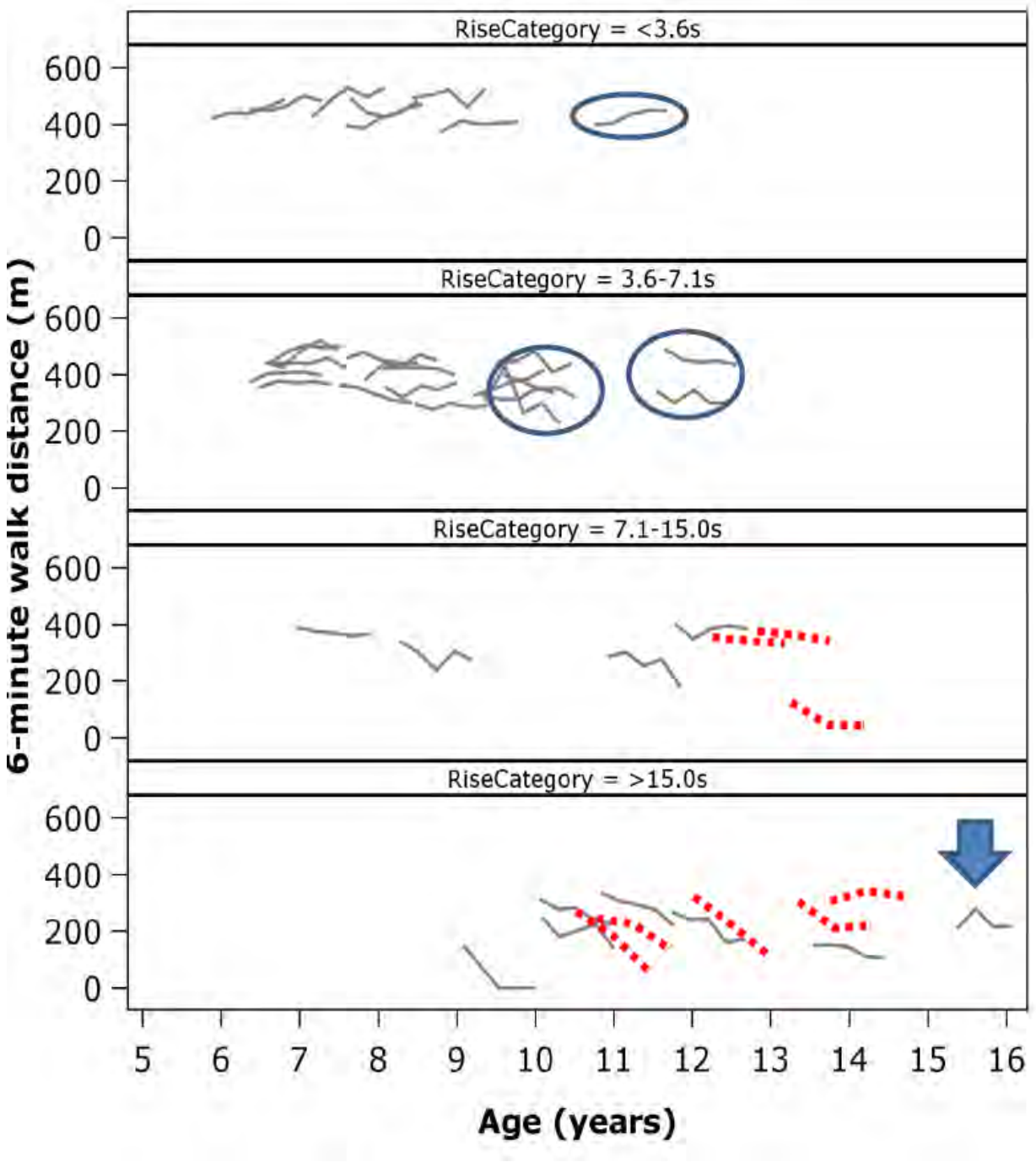


Sarepta: "*Comparison of eteplirsen-treated patients to the appropriately matched external control shows that more than one year is required to observe a divergence in disease progression between the two groups"*

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.

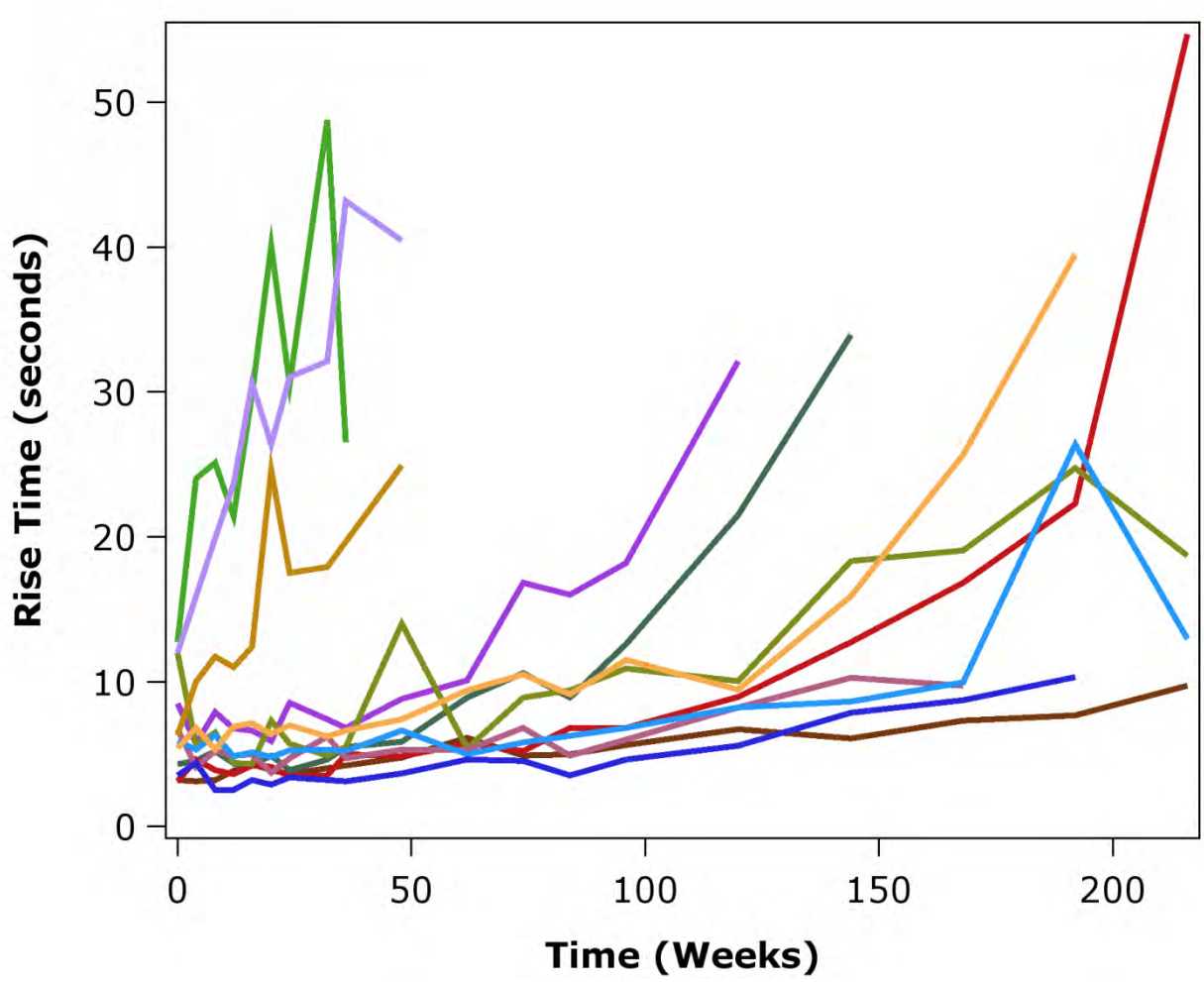
Because evidence that even a few eteplirsen patients might have progressed markedly differently than expected by natural history would be of interest, a few additional observations about these data are important. Assignment of eteplirsen patients to rise-time category is affected by random noise in the 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

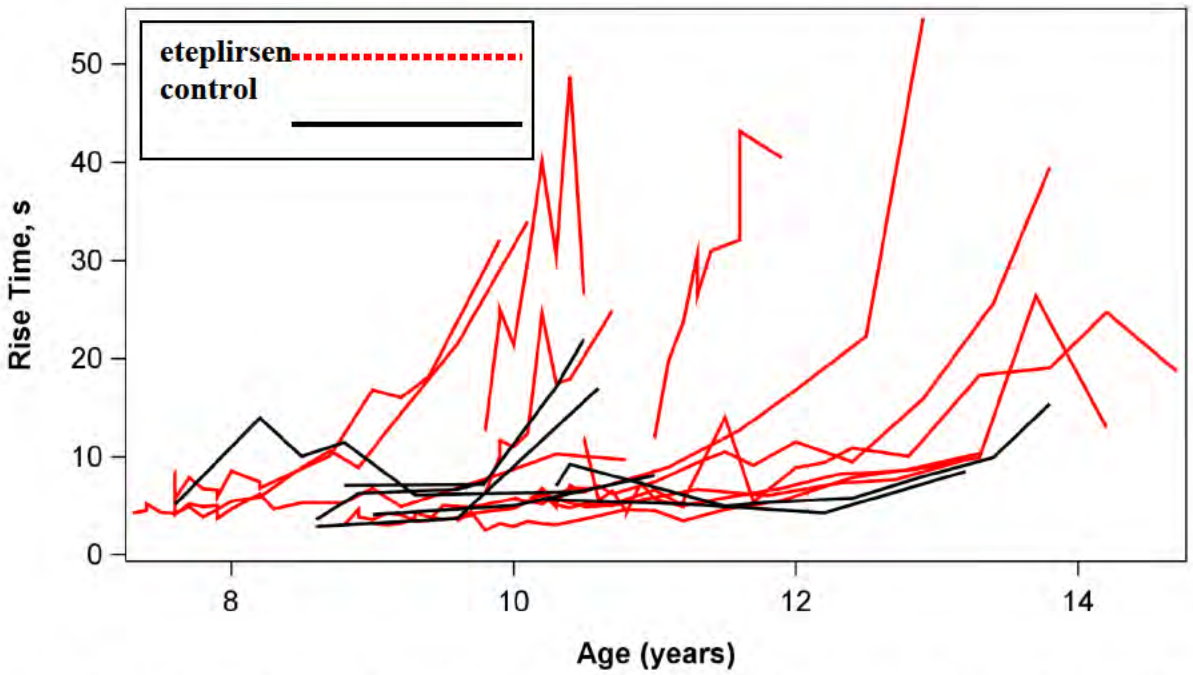
Figure 12 shows rise time from floor for the eteplirsen patients. Three eteplirsen patients lost the ability to rise from the floor in the first year of Study 201. The applicant has, at times, proposed that after an initial time period in which dystrophin levels from eteplirsen accumulated, disease progression 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



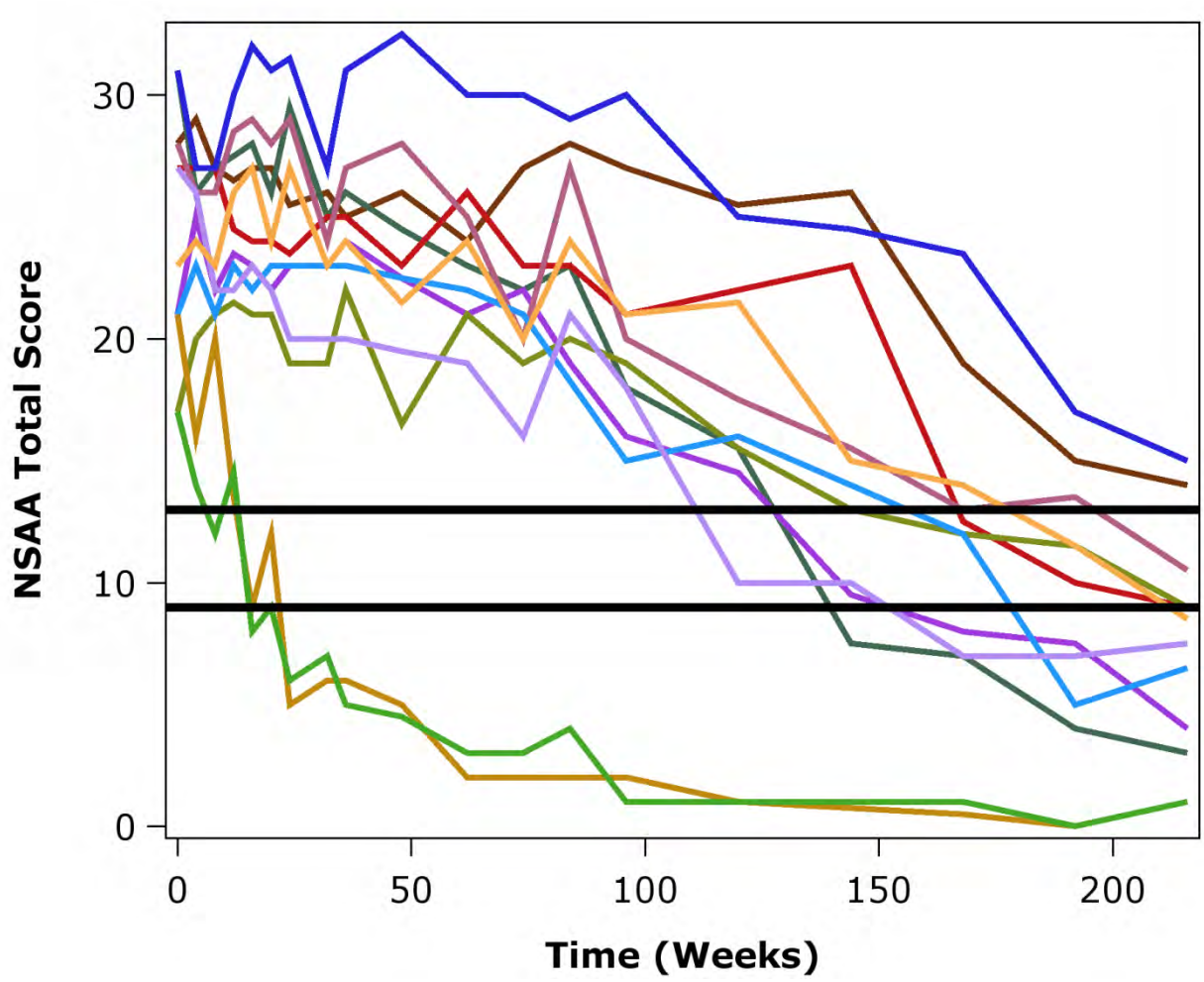
The applicant has emphasized a time-to-loss analysis for rise time but, similar to 6MWT, the recording of when a function is lost is partly subjective, and may be substantially affected by the level of disability at which the examiner concludes that attempting the test of function is no longer warranted. 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.[51] 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

[51] 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 fascioscapulohumoral muscular dystrophy Statland et al.[52] 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."[53] 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.[54] 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*

[52] Statland JM et al (2013) Reevaluating measures of disease progression in facioscapulohumeral muscular dystrophy. Neuromuscul Disord. 23,306-12.
[53] 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.
[54] 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,[55] 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.[56] The CINRG investigators caution that "differences in standards of care and dosing complicated

[55] 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

[56] 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

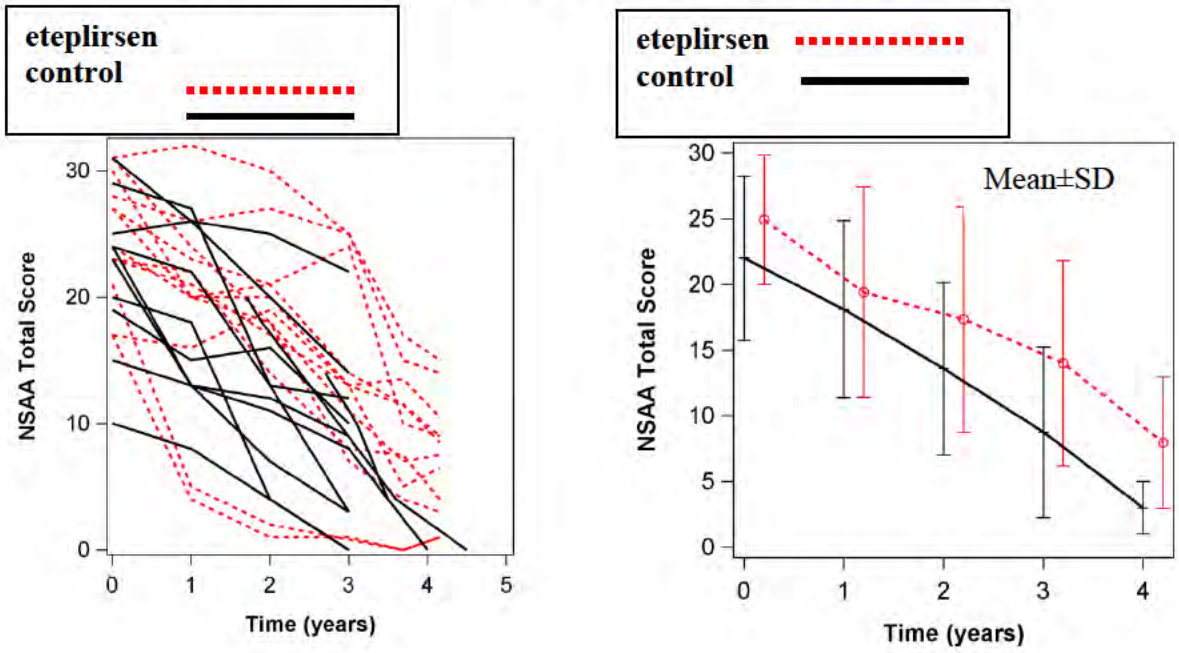
e. NSAA, Eteplirsen vs. Applicant's Controls

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

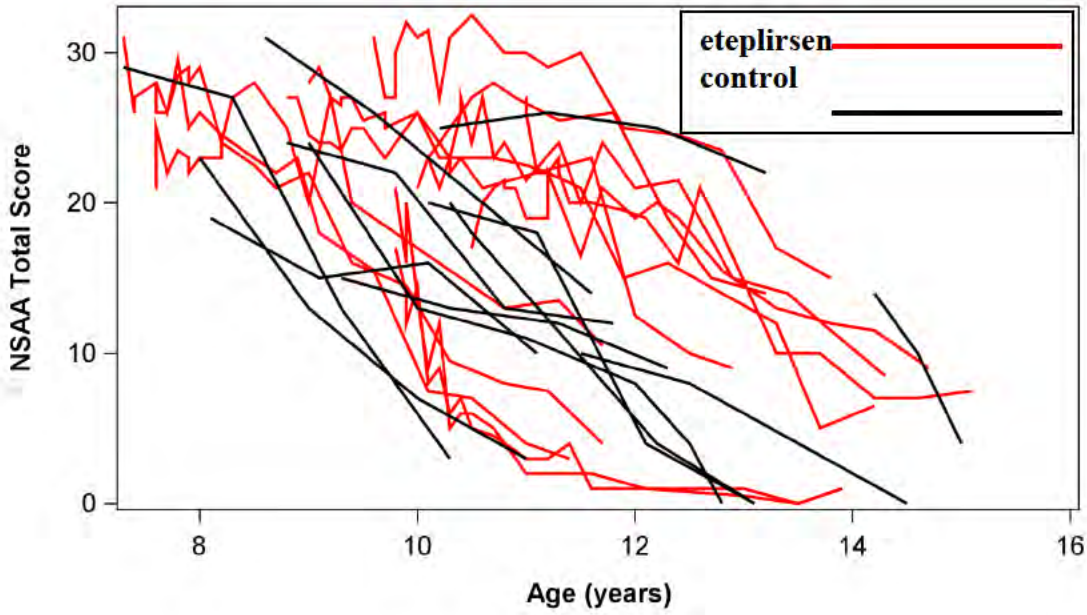


Because muscle function is strongly correlated with age in DMD,

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.*

APPEARS THIS WAY ON ORIGINAL

Figure 16: NSAA by Patient Age

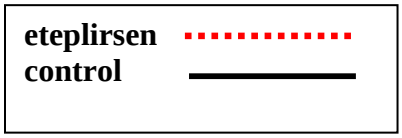


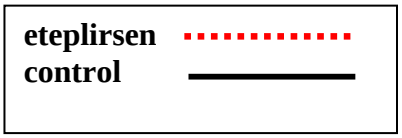
f. 6MWT, Eteplirsen vs. Applicant's Controls

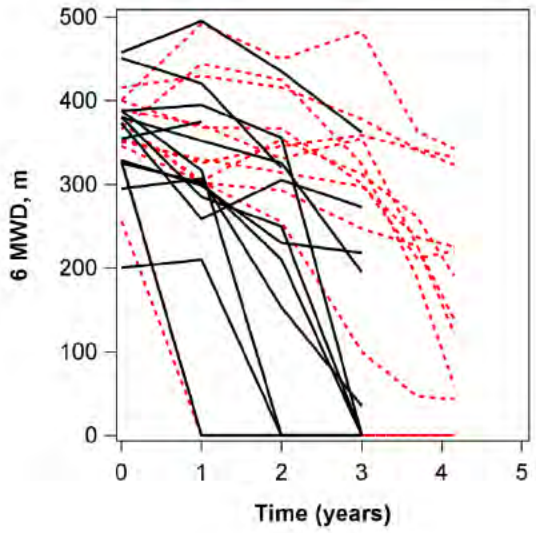
Comparison of eteplirsen patients (red) to the applicant's historical controls (black) is shown for 6-minute walk distance in

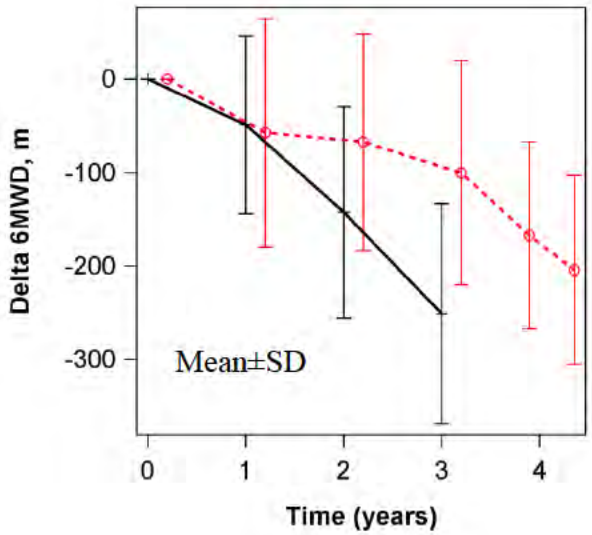
Figure 17, for all patients (left) and mean for each group (right). As discussed above, FDA has long expressed concern to the applicant that the 6MWT is particularly susceptible to bias, and unreliable in Study 202. Importantly, whereas the difference in 6-minute walk distance shown would be of clinical 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



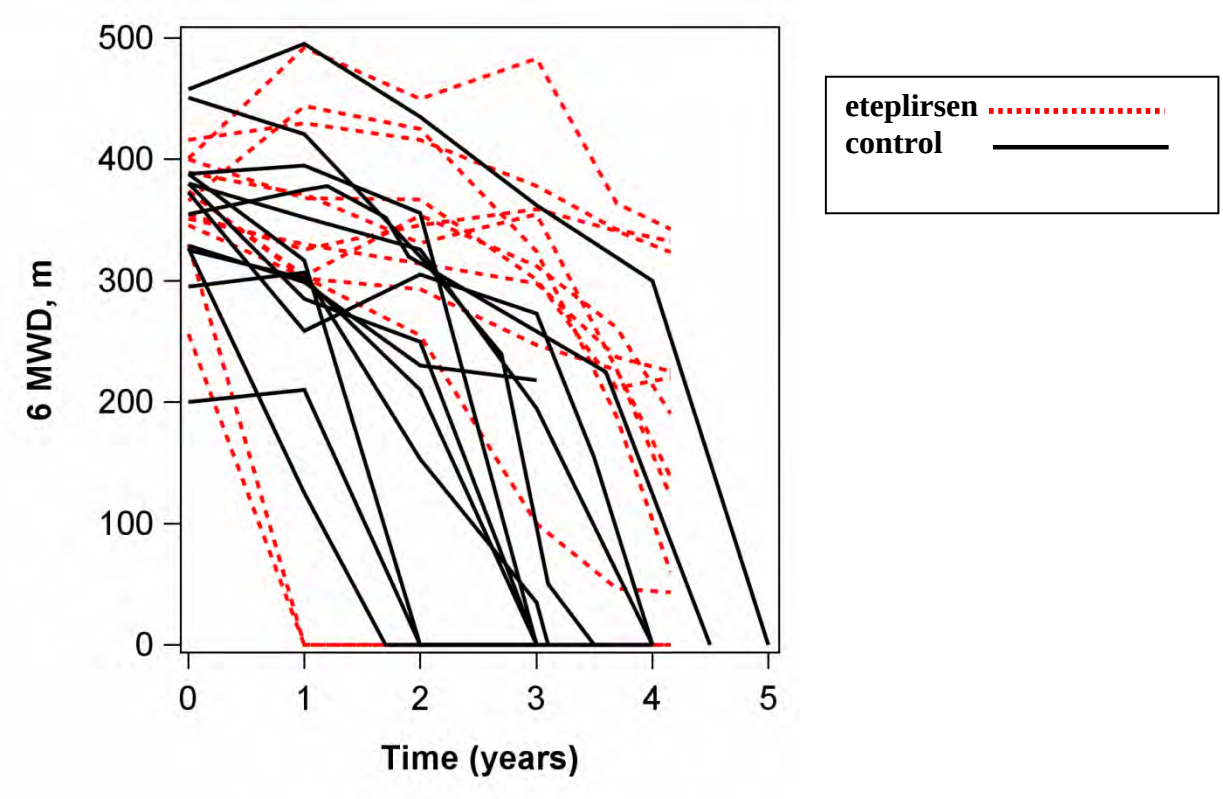



6 MWD, m
500
400
300
200
100
0
0
1
2
3
4
5
Time (years)


Delta 6MWD, m
0
-100
-200
-300
Mean±SD
0
1
2
3
4
Time (years)

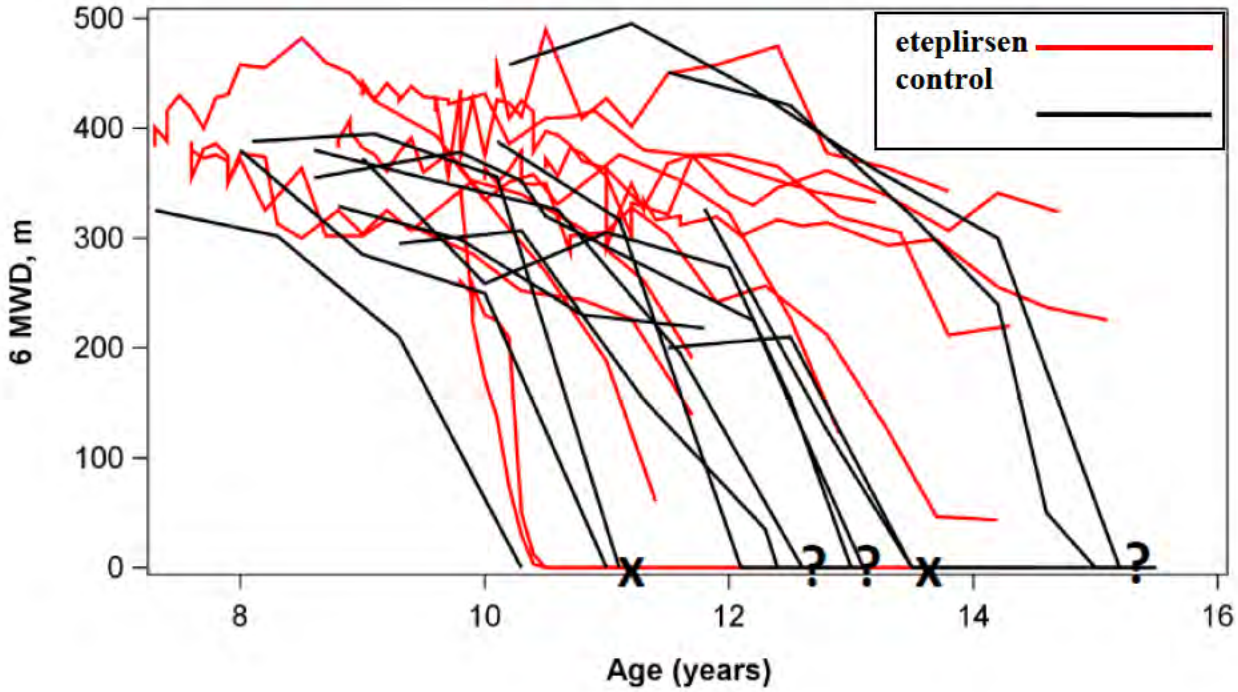
An updated version of 6MWT vs. time on treatment/observation is shown in Figure 18.

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.

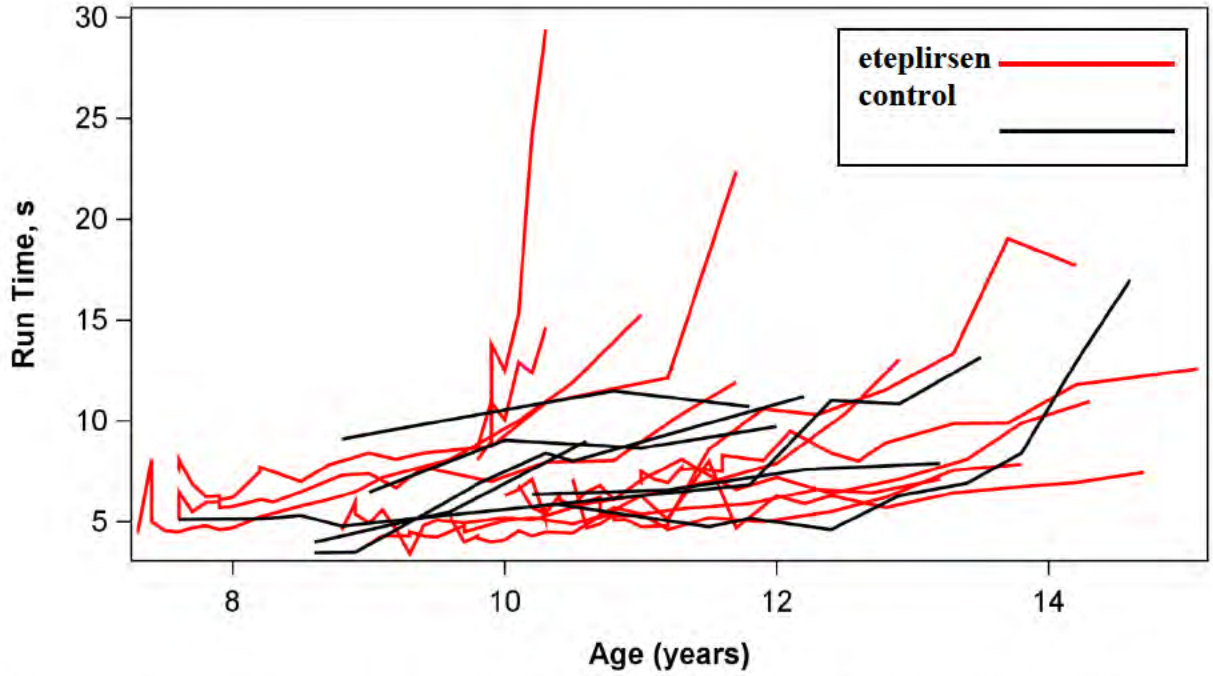
Table 1: Study 201/202: 6MWT Results[1] through Study Week 240

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[2]	~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
[1] Day 1 6MWT results (meters)
[2] 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.*

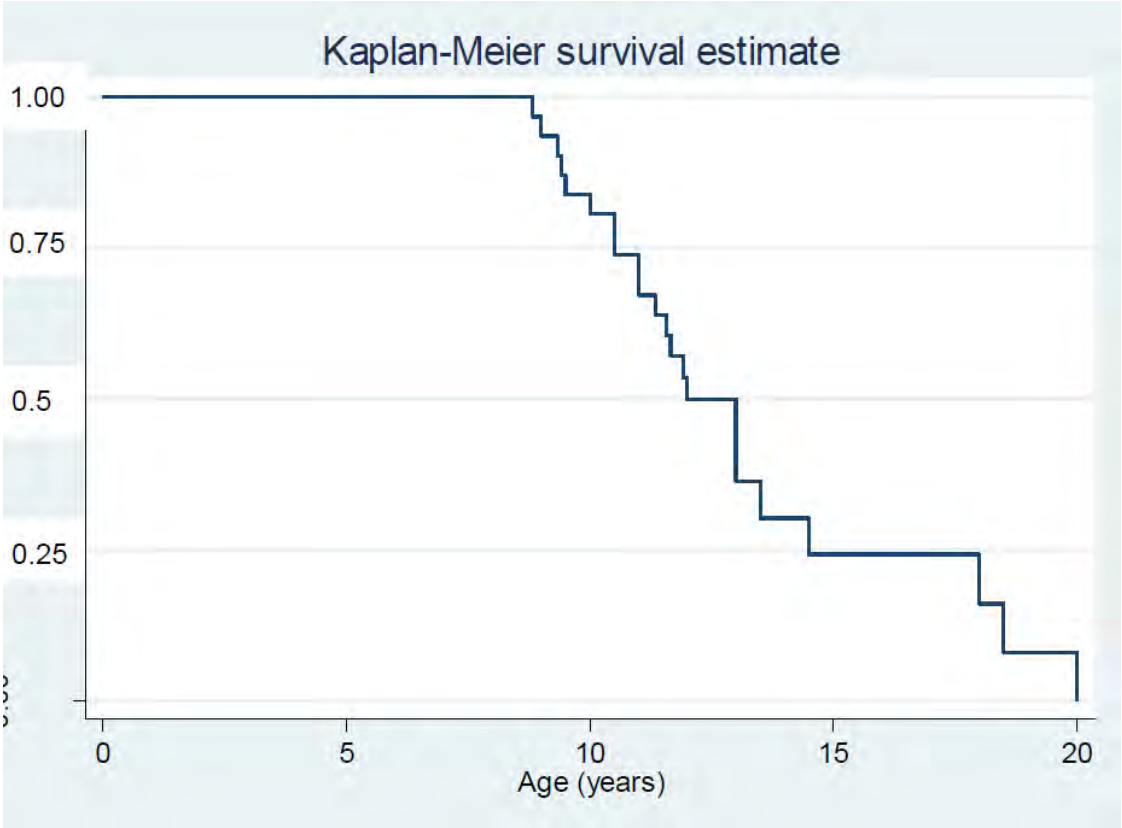
New CINRG Data: Age Range of Ambulation of Exon-51 amenable patients

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

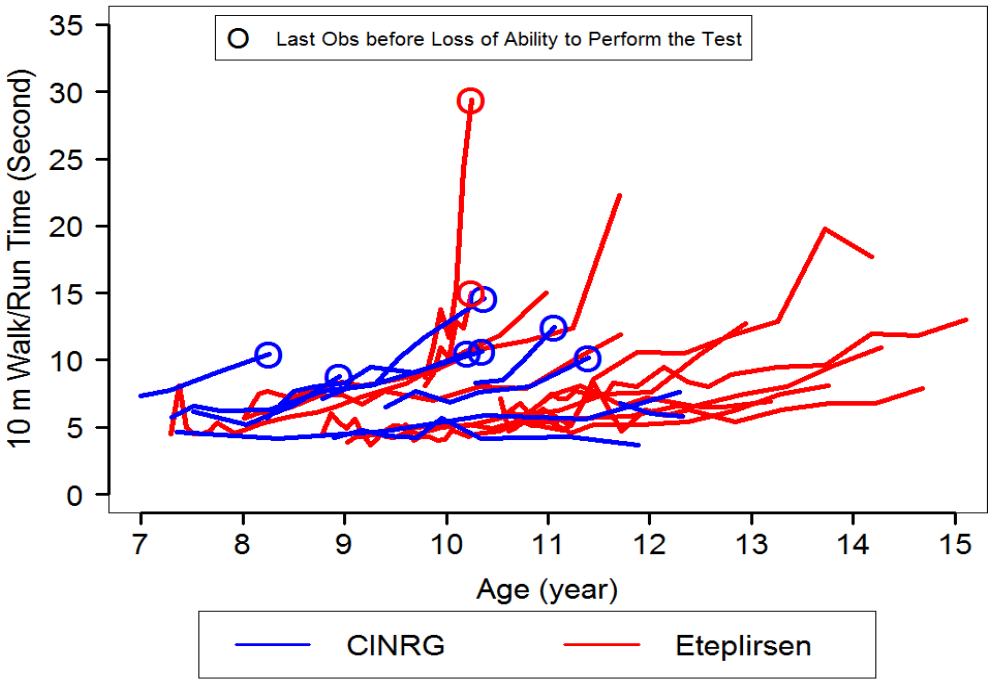
After release of the FDA memo prior to the PCNS AC meeting additional analyses were done on data that had been recently received from CINRG for 10 meter run/walk, rise time, and 4 step climb.

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:

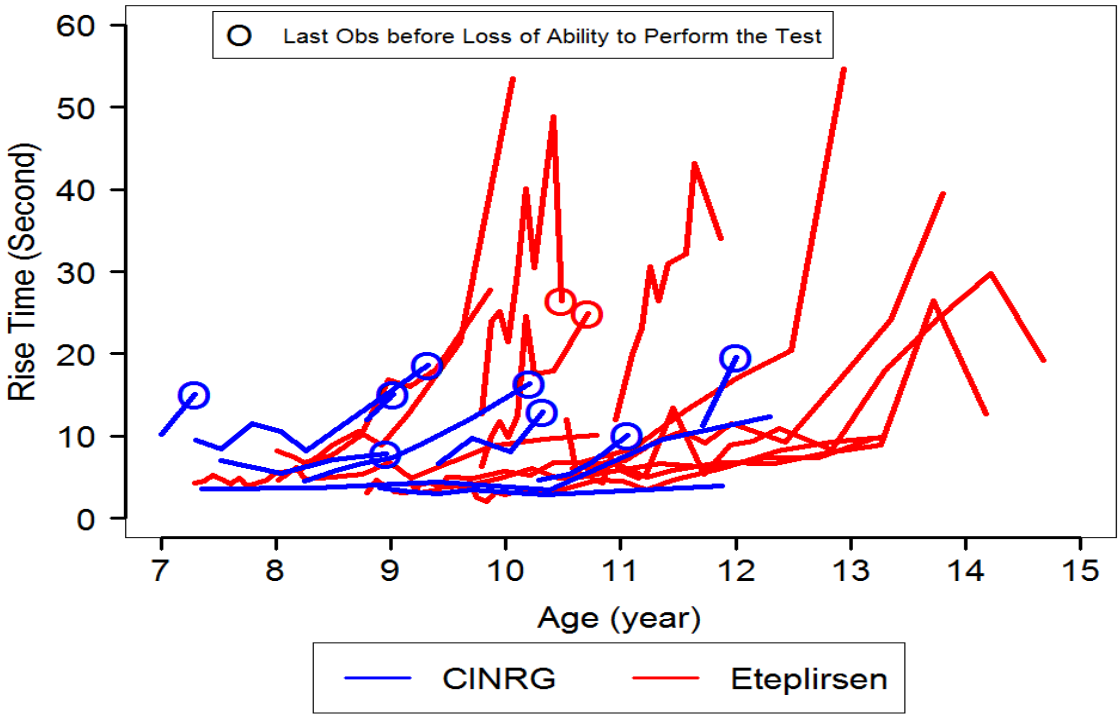
- Exon-51 skippable
- Ambulatory at baseline
- Baseline age 6-12 years
- 10m run/walk time less than 10 seconds

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."

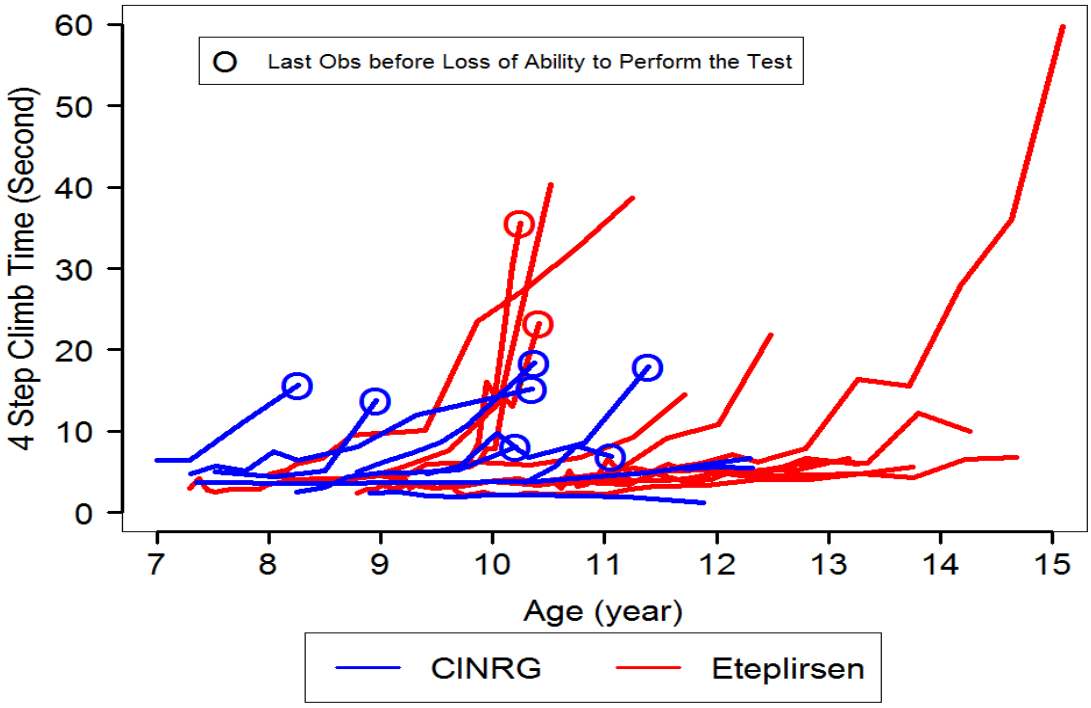
10 Meter Run/Walk



Rise Time



4-Step Climb



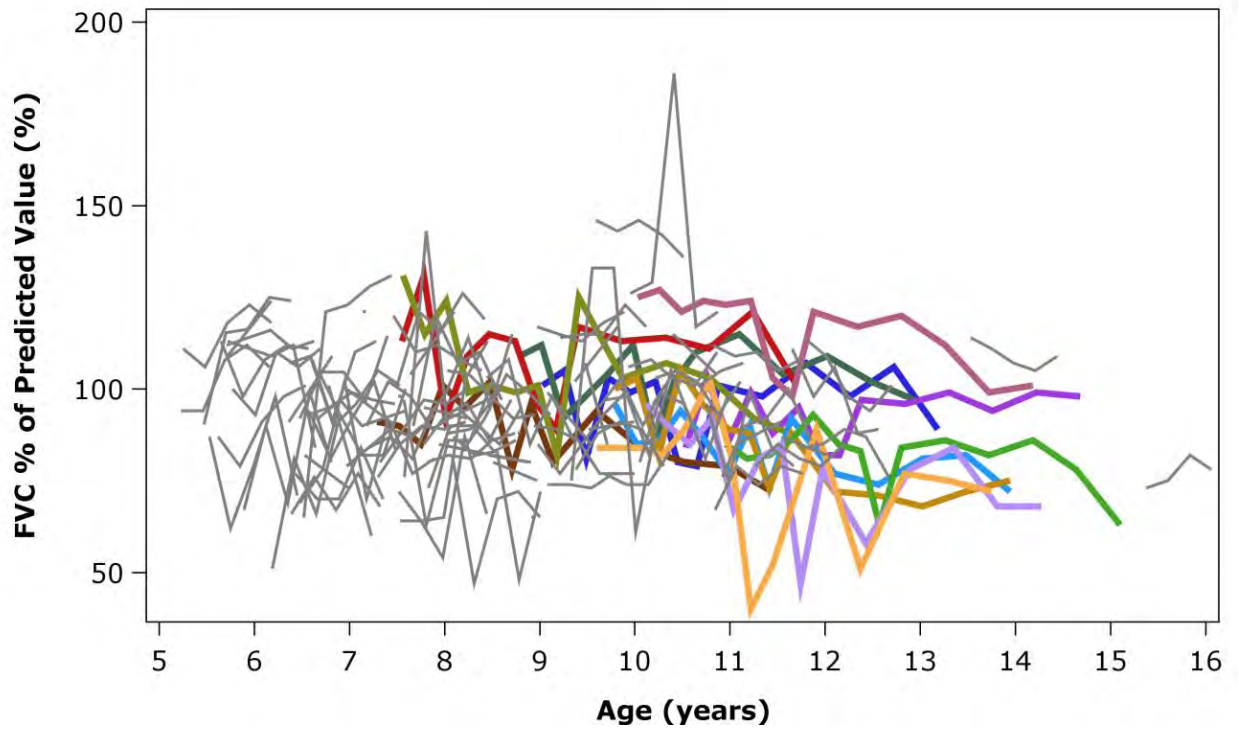
Pulmonary Function

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. [57,58]

> *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.* [59] *The applicant's analyses regarding pulmonary function therefore appear to be confounded and uninterpretable.*

[57] Biggar WD et al (2001) Deflazacort treatment of Duchenne muscular dystrophy. Journal of Pediatrics. 138, 45-50.
[58] Machado DL et al (2012) Lung function monitoring in patients with Duchenne muscular dystrophy on steroid therapy. BMC Research Notes. 5,435
[59] 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[60] 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

[60] 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 ≈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, ≈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 ≥24 weeks and 12 exposed for ≥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%[61,62, 63] 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.[64] 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
  - Eterplirsen 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

[61] McDonald et al. (2002) Fracture prevalence in Duchenne muscular dystrophy. Dev Med Child Neurol. 44:695-8.
[62] Vestergaard et al (2001) Fracture risk in patients with muscular dystrophy and spinal muscular atrophy. J Rehabil Med 33:150-155.
[63] King WM et al (2007) Orthopedic outcomes of long-term daily corticosteroid treatment in Duchenne muscular dystrophy. Neurology. 68:1607-1613.
[64] 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 (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. Bryne'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 (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 (b) (4)
  - Unexpected stability in eteplirsen-treated boys

- John Day, MD, PhD (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.[65]

- 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),[66] in exon-51 skippable patients, dystrophin levels averaged about 0.3% of normal, but ranged from undetectable to ≈ 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

[65] 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.

[66] 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.

<u>Biomarker Evidence</u>

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% ± 0.84% of normal (mean ± 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.*

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 4th 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 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 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.

APPEARS THIS WAY ON ORIGINAL

Dystrophin Analytical Methodology:

FDA Statement	Sarepta Clarification
"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." (FDA BD page 29 of PDF)	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.
"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." (FDA BD page 30 of PDF)	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 ($p<0.001$).
"Random measurement error can be large in comparison to the estimated amount of dystrophin." (FDA BD page 31 of PDF)	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 Statement	Sarepta Clarification
"There is no simple or reliable way to compare estimates of dystrophin amount derived from immunofluorescence with estimates derived from Western blot." (FDA BD page 35 PDF).	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).
"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." (FDA BD page 34 of PDF)	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.

Potential Clinical Impact:

FDA Statement	Sarepta Clarification
"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." (FDA BD page 7 of PDF)	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. • 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). • 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.
"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."	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): Baseline characteristics are not comparable between eteplirsen and the proposed placebo group: • Placebo group included boys <7 years old

FDA Statement	Sarepta Clarification
(FDA BD page 13 of PDF)	• Placebo group included many patients with baseline 6MWT >440 meters which is outside the eteplirsen trial's inclusion criteria Placebo patients were followed for only one year, whereas eteplirsen-treated patients were followed for 3 or more years: • 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. • 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. • 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.
"The robustness of the study result is a concern since a single patient could change the results substantially." (FDA BD page 69 of PDF)	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.
"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." (FDA BD page 47 of PDF)	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 available data • Last Observation Carried Forward imputation (conservative analysis assuming that the 2 control patients did not decline)

Regulatory Feedback:

FDA Statement	Sarepta Clarification
"As the duration of exposure in Study 202 increased, the applicant proposed comparing the clinical course of treated patients to historical controls." (FDA BD page 38 of PDF)	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).

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/s/

RONALD H FARKAS
05/17/2016

CLINICAL REVIEW

Application Type	New Drug Application
Application Number(s)	206488
Priority or Standard	Priority
Submit Date(s)	June 26, 2015
Received Date(s)	June 26, 2015
PDUFA Goal Date	May 26, 2016 (1 extension for Major Amendment)
Division/Office	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)
Reviewer Names	Christopher D. Breder, MD PhD Ashutosh Rao, PhD (Dystrophin bioassays)
Review Completion Date	May 06, 2016
Established Name	Eteplirsen
(Proposed) Trade Name	EXONDYS 51
Applicant	Sarepta Therapeutics Inc.
Formulation(s)	Solution
Dosing Regimen	Intravenous Injection
Proposed Indication(s)	Treatment of Duchenne Muscular Dystrophy
Intended Population(s)	Patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping
Recommendation on Regulatory Action	Complete Response
Recommended Indication(s) (if applicable)	N/A

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1 Executive Summary

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[1] 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.**

[1] 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
Analysis of Condition	• 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.	DMD is a serious and life threatening disease where a therapy with a true and meaningful treatment effect would be beneficial.
Current Treatment Options	• 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.	There is a substantial unmet need for therapies in DMD.
Benefit	• 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: ○ Comparisons to placebo in the 24 week portion of Study 201 / 202 were negative on tests of clinical function (6MWT) and biomarker data. ○ Data from the long-term biomarker data seemed biased in terms of selection of controls. The effect after 36 Weeks of producing < 1% of normal does not seem reasonably likely to me to predict a clinical benefit. ○ 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	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.

	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).	
Risk	• 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.	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.
Risk Management	• If eteplirsen is approved, the following risk management approaches are recommended: o Future clinical trials should be adequately designed to evaluate the safety profile of eteplirsen. o The maximal tolerated dose should be determined and evaluated in a controlled clinical trial. o 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. o The potential for immunogenicity and rhabdomyolysis must be evaluated.	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.

2 Therapeutic Context

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

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 <u>appropriately matched</u> 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±73.9 m/year; McDonald [;Mcdonald *et al.* 2010]: 57±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

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

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 [a] 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 [b] 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	Eteplirsen: 0.09 mg or 0.9 mg (IM) in the EDB muscle of 1 foot Placebo (IM) in the EDB muscle of the opposite foot Single dose	Eteplirsen: 0.5, 1, 2, 4, 10, or 20 mg/kg Once weekly IV infusion for 12 wks	Eteplirsen: 30 or 50 mg/kg, or Placebo* *Placebo: 24 wks, followed by 4 wks of eteplirsen at 30 or 50 mg/kg Once weekly IV infusion for 28 wks	Eteplirsen: 30 or 50 mg/kg Once weekly IV infusion for up to an additional 212 weeks after completing Study 201	Eteplirsen: 30 mg/kg Once weekly IV infusion up to 96 wks Untreated control: DMD patients not amenable to exon 51 skipping	Eteplirsen: 30 mg/kg Once weekly IV infusion up to 96 wks	Eteplirsen: 30 mg/kg Once weekly IV infusion up to 96 wks Untreated control: 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 [c])	NA	16 Apr 2015	NA
D120 Update: Data Cutoff Date				12 Aug 2015 (Week 208 [c])	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

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] ≥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 ±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 β-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 β-blocker) or QTc >450 ms.
6. Use of any aminoglycoside antibiotic within 12 weeks before the screening visit (visit 1) or need for use of an aminoglycoside antibiotic during the study (unless discussed and agreed with the Principal Investigator and Medical Monitor).

- 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	
Efficacy and Pharmacodynamics																											
PedsQL	X												X													X	
Muscle biopsy	X[s]													X[1,t]													X[2,t]
Functional tests [#]	X[v]				X				X				X[v]					X				X				X[v]	
9-Hole Peg Test	X																									X	
Pharmacokinetics																											
PK – blood		X[p]											X[q]													X[p]	
PK – urine													X[r]														
Safety Assessments																											
Physical exam	F[c]	B[de]	B[df]	B[df]	B[df]	B[df]	F[cf]	B[df]	B[df]	B[df]	B[df]	B[df]	F[cf]		B[df]	B[df]	B[df]	B[df]	B[df]	F[cf]	B[df]	B[df]	B[df]	B[df]	B[df]	F[cf]	
Vital signs[g]	X	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X
Weight[h]	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[k]													X[k]	
ECHO (EF, FS)	X	X[l]											X[k]													X[k]	
ELISPOT	X						X						X							X						X	
Safety lab tests[n]	X	X[w]	X		X		X		X		X		X[w]				X			X			X			X[w]	
AE questioning[o]	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[j]												X[j]													X[j]	

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	4
2	30 mg/kg/wk eteplirsen for 28 weeks 4	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 β-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[2].

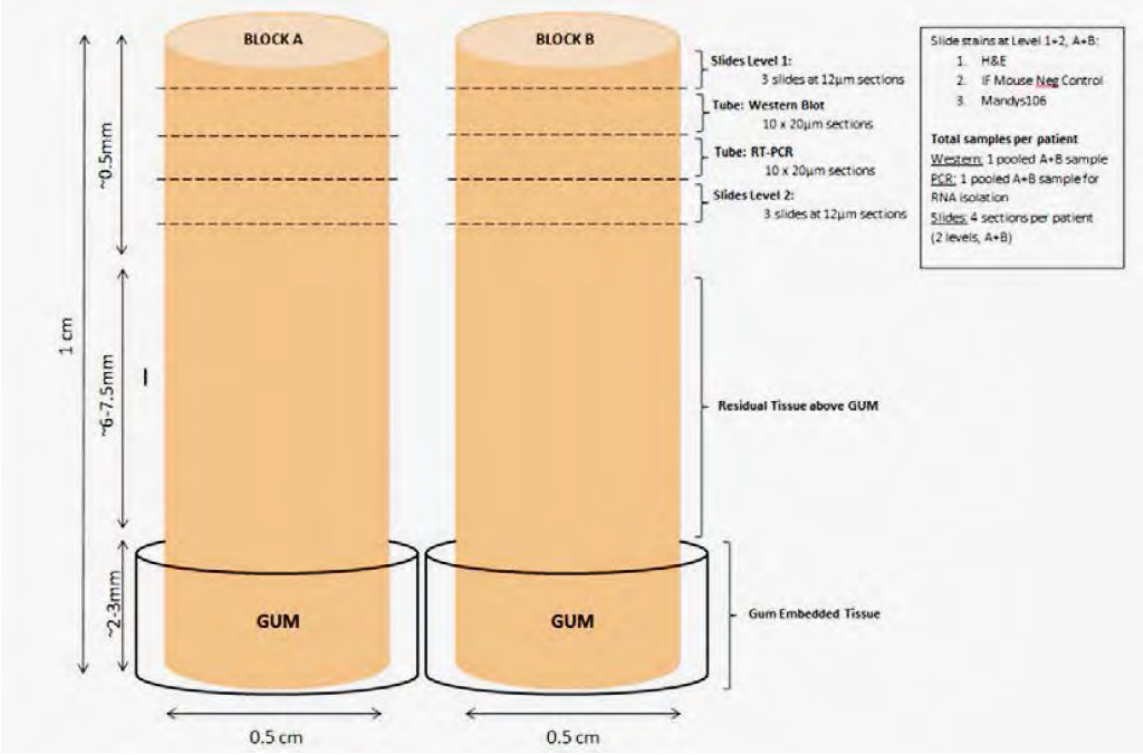
Methodology

During the **biopsy procedure,** sample tissue of approximately 5 mm^3 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.

[2] 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 2nd 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 2nd 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[3] (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) reaquisition 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.*

[3] 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.*

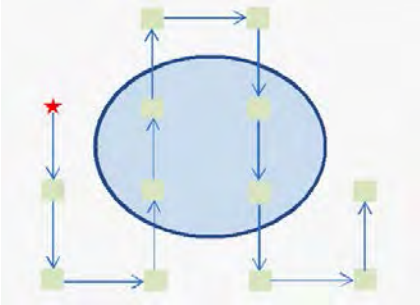
- *Although digital images were analyzed, they were not subjected to digital image analysis per se; they were analyzed by visual inspection of a single observer [the staff member] explained that the entire tissue section was first manually and virtually divided into 4 quadrants. One image was acquired 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 4th 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.

Figure 2 Method of Selecting Microscopic Fields for the 4th Biopsy



Source: SR-CR-15-006 p. 91 of 91

- Key Secondary Endpoints:

1. Changes from Baseline in CD3, CD4, and CD8 lymphocyte counts
   a. CD3, CD4 and CD8 positive T-cells
   b. Spot Forming T Cells were counted using an Immunospot Series 3B analyzer.
   c. The percent muscle fibers positive for MHC Class I or II expression
2. Changes from Baseline to Week 24 in the following clinical assessments:
   a. 6-Minute Walk Test
   b. Timed 4 Step Test
   c. Maximum voluntary isometric contraction test (MVICT) to measure elbow flexion and extension, knee flexion and extension, and grip strength.
   d. Timed 10-meter run from the North Star Ambulatory Assessment (NSAA).
   e. NSAA total score.

- Additional Endpoints:

3. Changes from Baseline in muscle biopsy levels of dystrophin intensity per fiber (determined by IHC)
   a. Methodology

Analysis was performed using Bioquant® Life Sciences software using Field Density analysis. This algorithm determined the fluorescent signal intensity that was within the defined region of interest, averaged across the entire image. Analysis was performed in the NML microscope room with room lights on. Revertant fibers were included and not left out of the analyses based on the threshold. Field Density value for the negative sample image (no primary antibody) was subtracted from Field Density values from each patient and positive control image. Percent Intensity was calculated from background subtracted values. Images were then normalized to this averaged normal control value and expressed as percent of 100%.

Reviewer's Analyses and Comments

The following passages were extracted from the Agency Inspection Report of the Nationwide Children's Hospital facility[3]:

- [redacted] *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.* [redacted] *that images were acquired by* [redacted] *During subsequent discussions with* [redacted] *stated that the 40x images were acquired from fields that were preferentially selected for their high fluorescence intensity to clearly show the increased intensity.* [redacted] *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 [redacted]) 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[3]:

- *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 ≥7years. 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 (Tmax) and value of maximum plasma concentration (Cmax), the apparent volume of distribution at steady state (Vss), the elimination half-life (t½), areas under the plasma concentration-curve (AUC), total clearance (CL), mean residence time (MRT), and renal (i.e., urinary) clearance (ClR).

- 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[4,5].

  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.

[4] 201 / 202 Clinical Study Report, 4658-us-201-e3-16-1-01, P 132 of 637
[5] 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 15th, 2011[6], so Amendments 5 and those after occurred while the treatment phase of the trial was underway.

Reviewer's Comment's

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.

[6] 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	• ***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.*** • Changed the overall duration of the study from 30 to 28 weeks. • ***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.*** • ***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*** • ***Changed the age range for patient enrollment from 5 to 15 years of age to 7 to 13 years of age.*** • Added requirement that patients be able to walk between 200 and 350 meters on 6MWT to entry criteria. • 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. • 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. • Added post-treatment muscle biopsies to the list of required assessments. • Specified that the primary efficacy endpoint would be dystrophin production (versus a general collection of data as per the original protocol)
2	25 May 2011	• Added the Timed 4-Step Test to the efficacy assessments. • ***Expanded the maximum distance on the 6MWT inclusion criterion from 350 to 400 meter.***
3	22 Jun 2011	• Clarified and added urine biomarker testing
4	10 Aug 2011	• Clarified that 6MWT would be administered twice during screening visit and that mean of 2 assessments ± 10% of the lower or upper limit (200 m, 400 m) would be value used to determine qualification. • 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 β-blockers and rescreened in 4 weeks or excluded from the study. • Added the DSMB to the protocol.
5	8 Sep 2011	• Clarified that MIP and MEP would be measured, not % predicted MIP and MEP. • ***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.***

Amendment	Date	Significant Changes in Conduct or Analysis of Study
6	04 Nov 2011	• ***Made the 6MWT a secondary endpoint.*** • Modified statistical method to Wilcoxon rank-sum test, because it was more appropriate for the sample size of this study. • 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. • Updated planned statistical analyses. • 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.
8	07 Jan 2012	• Extended the duration of the study from 24 to 28 weeks. • ***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.***
Mentioned as changed in the Clinical Study Report (Section 9.7.9.2); Date not specified.		• 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.
		• ***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.***
		• ***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.***

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)

			Eteplirsen			
Parameter		**Placebo N = 4**	**30 mg/kg/wk N = 4**	**50 mg/kg/wk N = 4**	**All Eteplirsen N = 8**	**All Patients N = 12**
Gender n(%)	Male	4 (100)	4 (100)	4 (100)	8 (100)	12 (100)
Age, years	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
Height, cm	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
Weight, kg	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
BMI, kg/m^2	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
Race, n(%)	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		Placebo N = 4	Eteplirsen 30 mg/kg/wk N = 4	Eteplirsen 50 mg/kg/wk N = 4	Eteplirsen All Eteplirsen N = 8	All Patients N = 12
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[a], 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	
$\%FEV_1$ (%)	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).
[a] 6MWT results are maximum observed value of 2 tests administered on 2 consecutive days at screening.
Abbreviations: 6MWT = 6-Minute Walk Test; %FEV1 = 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

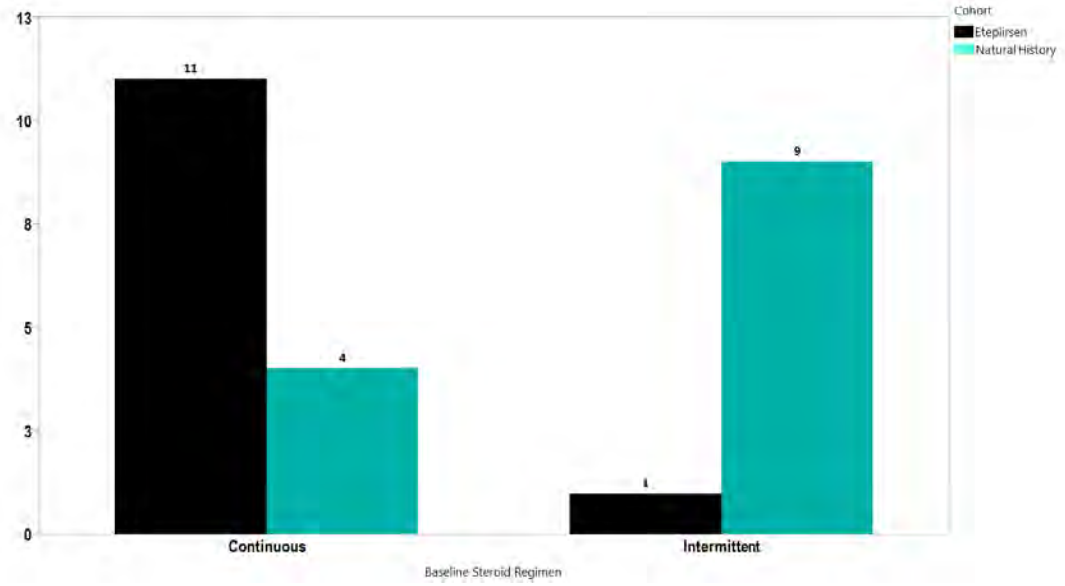


Figure 4 Baseline Steroid Use NSAA Exon 51 Amenable Population

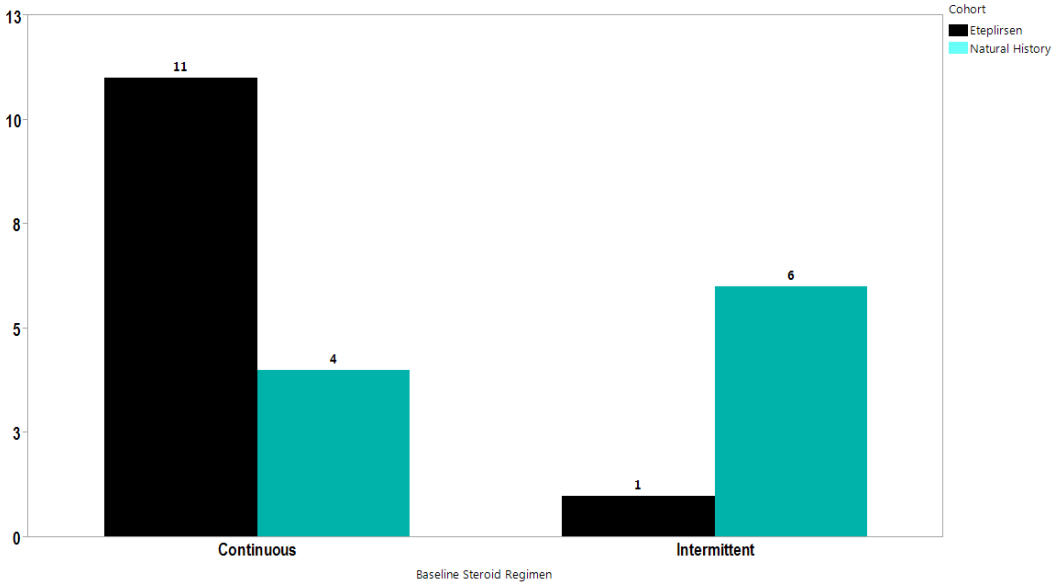
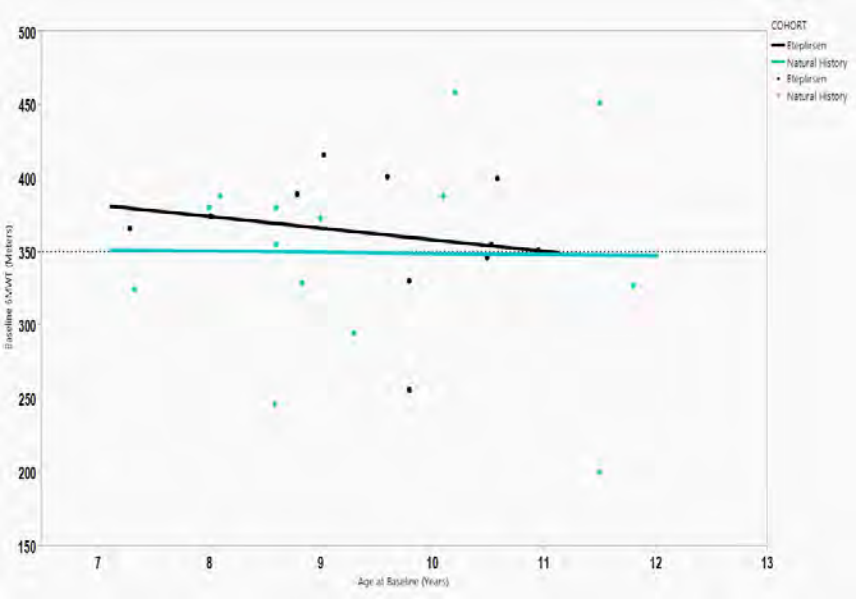
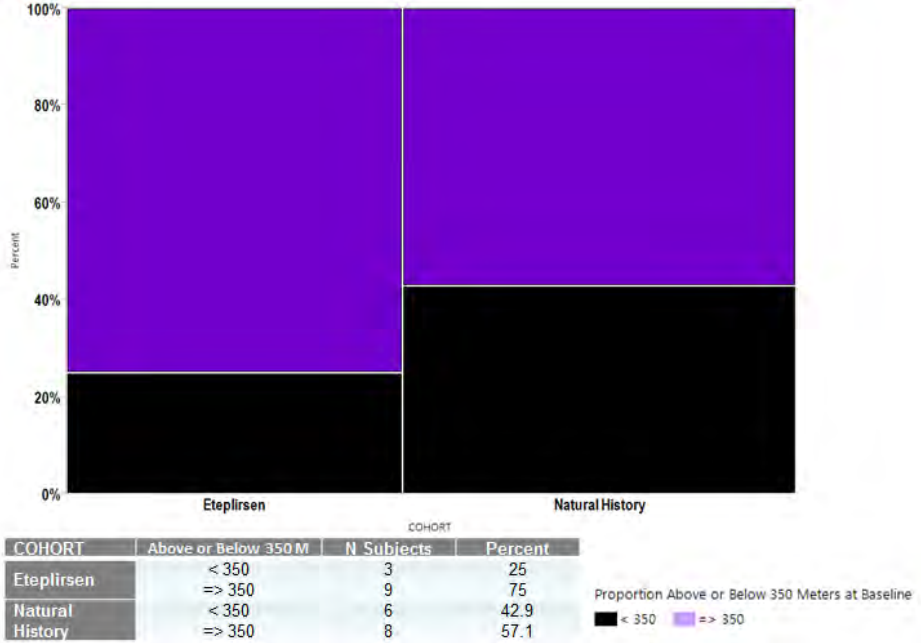


Figure 5 Baseline Six Minute Walk Test by Age and Treatment Group



Source: Medical Reviewer Analysis of 6MWTDER

Figure 6 Proportion of Subjects with Baseline Six Minute Walk Above and Below 350 Meters



COHORT	Above or Below 350 M	N Subjects	Percent
Eteplirsen	< 350	3	25
	=> 350	9	75
Natural History	< 350	6	42.9
	=> 350	8	57.1

Source: Medical Reviewer Analysis of 6MWTDER

Figure 7 Baseline NSAA by Age and Treatment Cohort

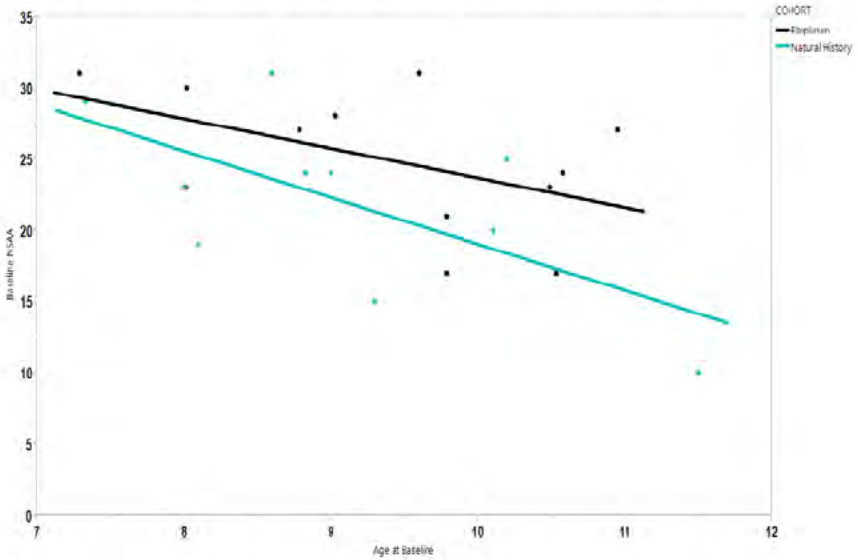
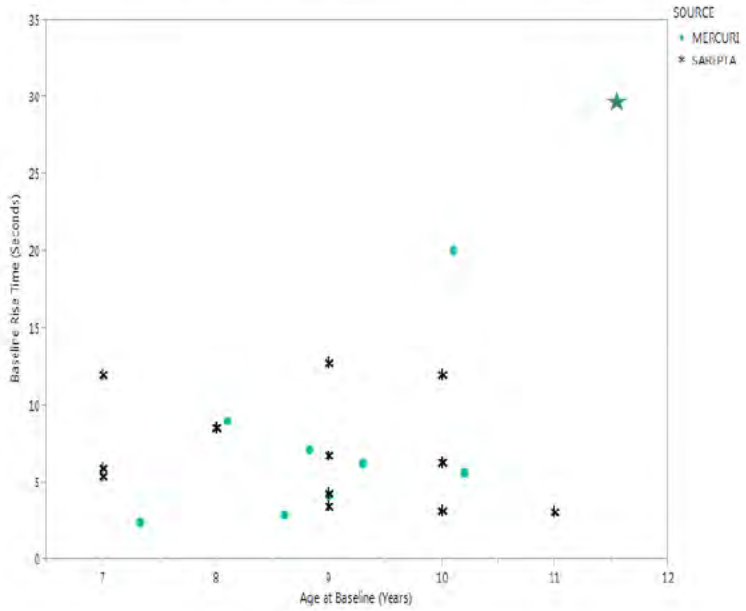


Figure 8 Baseline Rise Time by Age and Treatment Cohort



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 3rd Biopsy

This section describes the results from the evaluation of biomarker data up to and incuding the third biopsy (Week 48 on treament; 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[7]. In this first section of results, I will discuss the biomarker data from the 201 Study (up to the 3rd 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)[a]

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

[7] 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[b]	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[c]	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
[a]Results expressed as a percentage of total fibers counted.
[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, 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[8] 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.

[8] The datasets were (ADRB) reporting the 1st through 3rd biopsy data in the original NDA submission and from the dataset (FIBERS2) with the 1st through 4th biopsy data received September 16, 2015

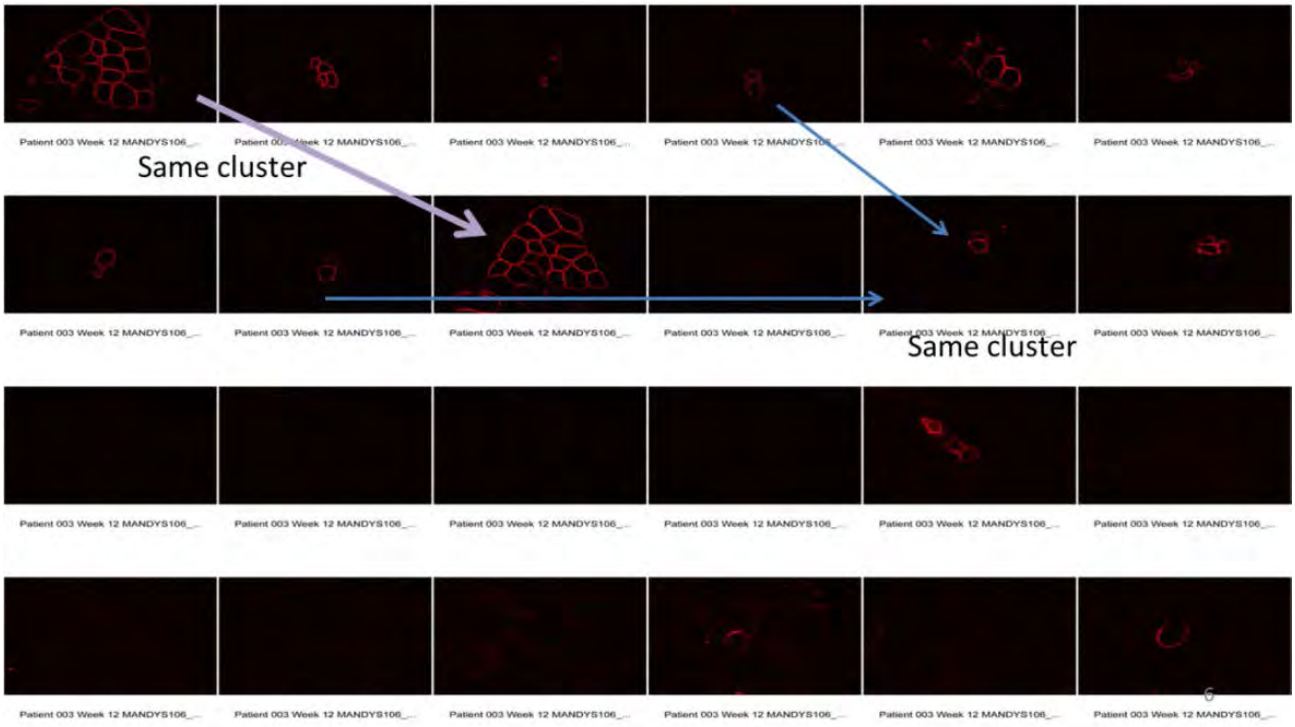
- Different muscle groups are used for different biopsies.

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 aquistion 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

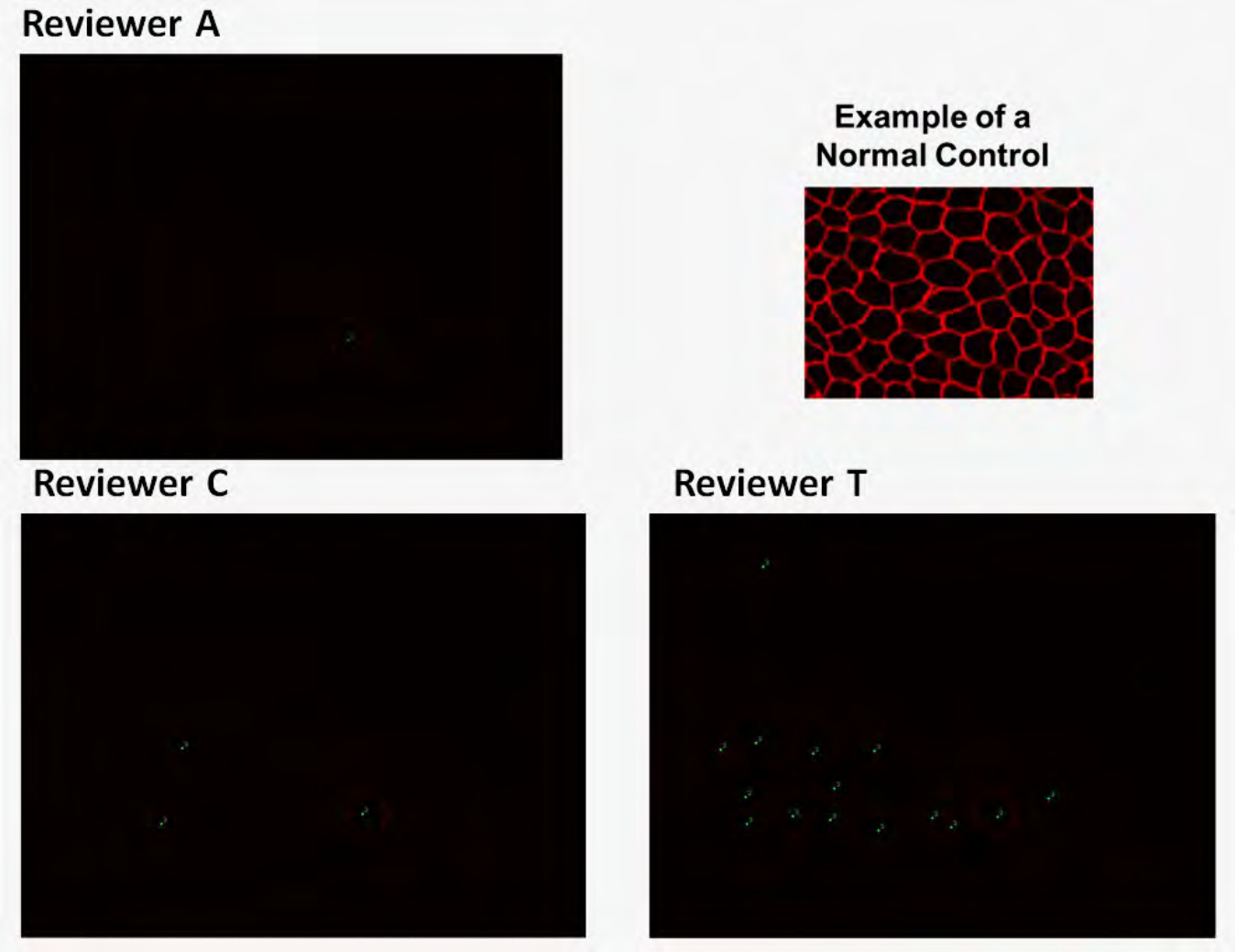


Source: Images from Study 201, Patient 003, Week 12

- The fiber count analysis gives the erroneous impression that the treatment reconstituted dystrophin expression (or at least dystropin –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).

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)

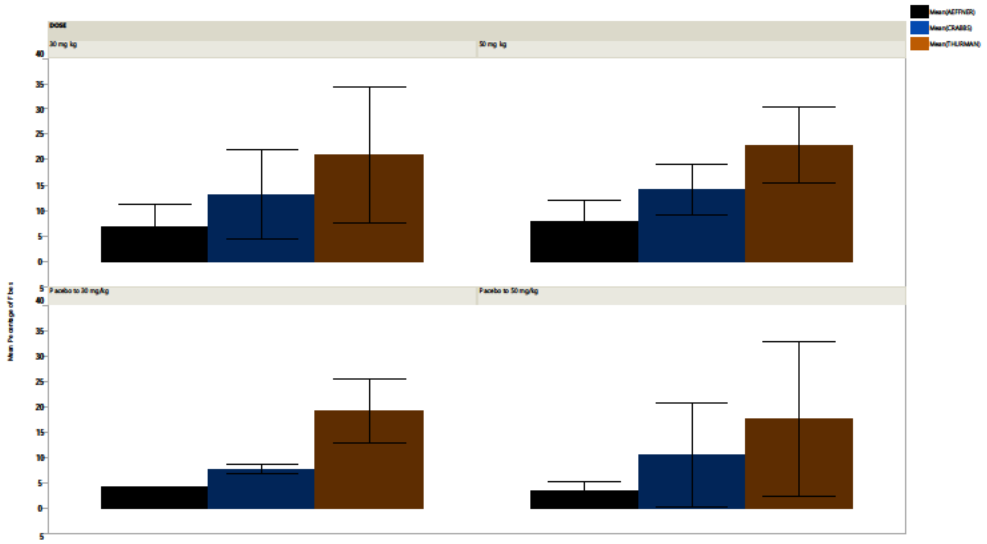


Source: Images for Reviewers A, C, T: 1005_ V001_ BL3im4; Image for Normal Control: 01006_30TT_48_MD_AL2im2.tif

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.

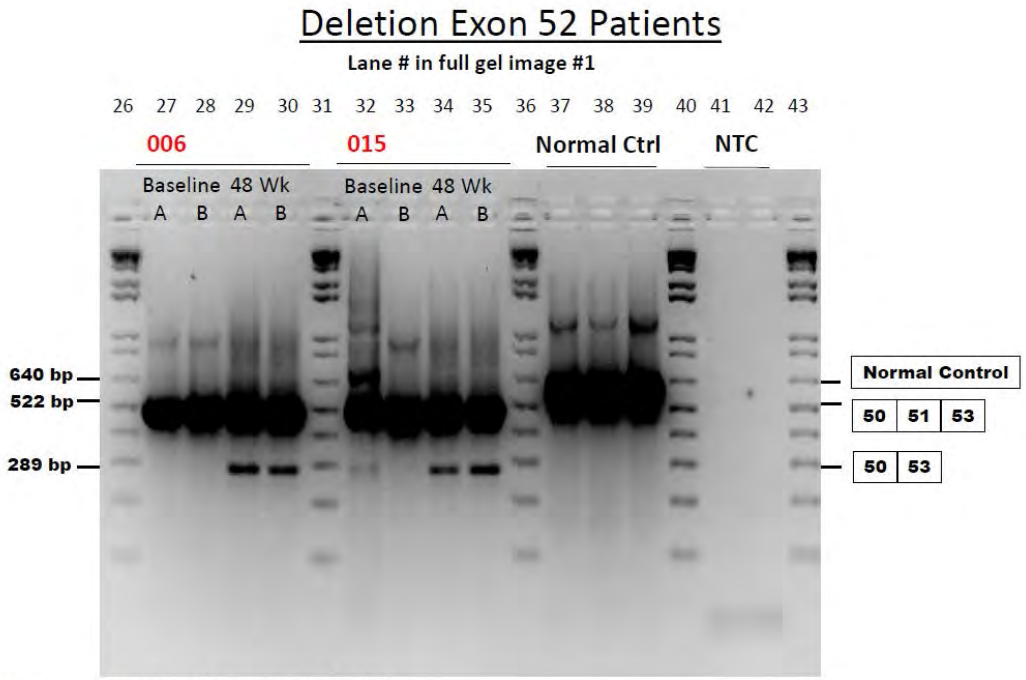
This variance and the level of discordance in the Pathologist's assessment is further analyzed in the discussion of the 4th Biopsy.

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



Lanes 26, 31, 36, 40, 43: Invitrogen 1kb+ ladder
Lanes 37, 38, 39: Normal Controls
Lanes 41, 42: No template Controls

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[b]	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
[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 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

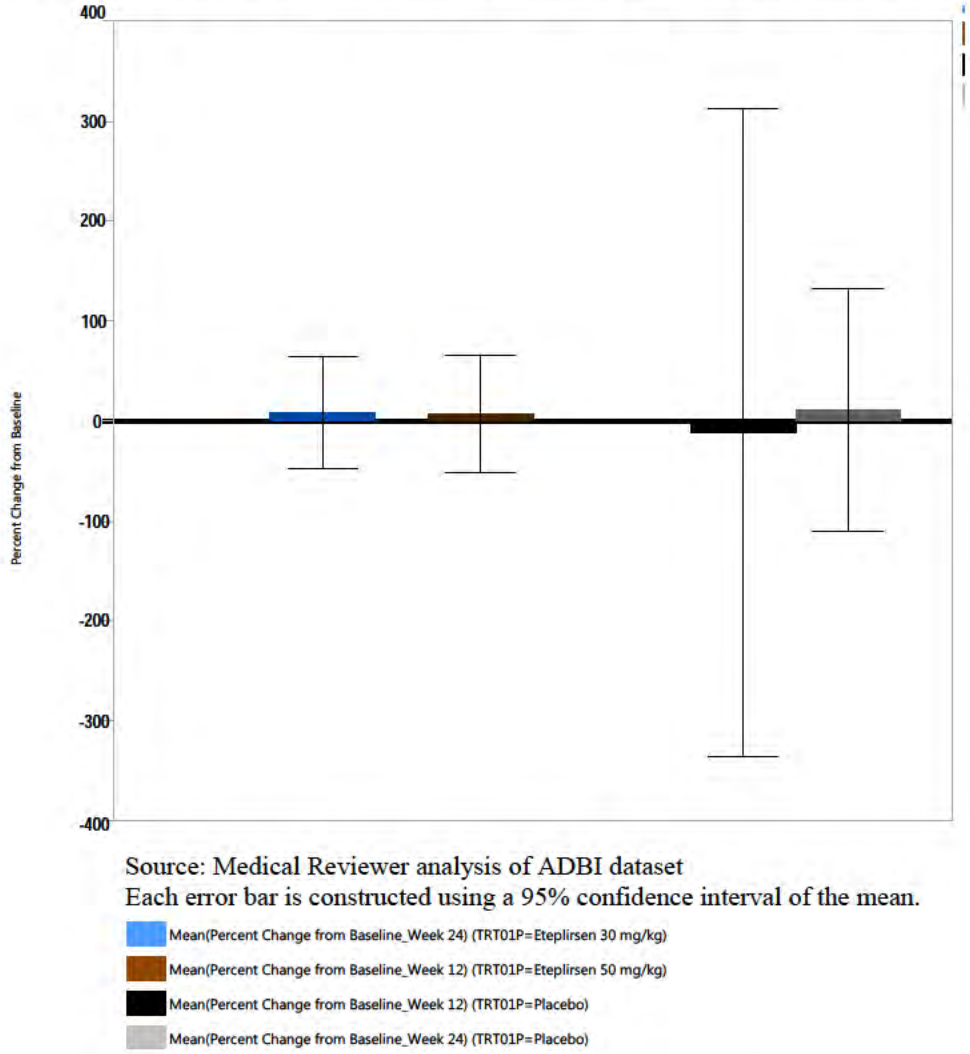
- The Applicant chose to disregard the results taken at 20X for Bioquant, which were negative.

[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



- 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 4th 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 bans, 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[b]	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.

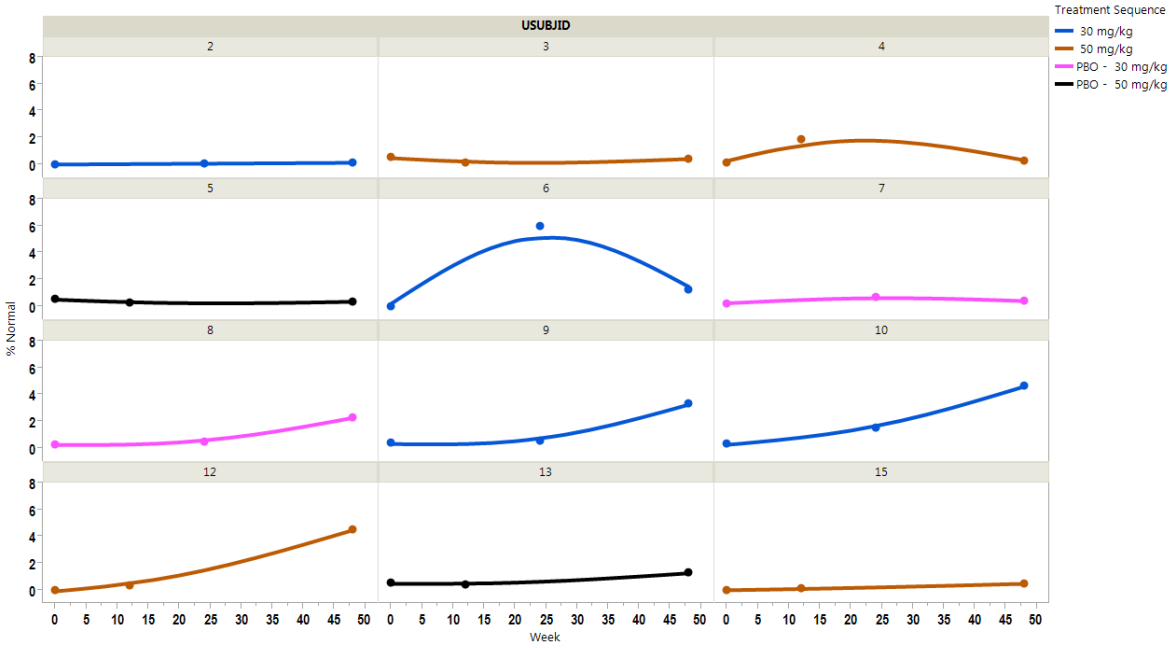
Figure 14 shows the reported percent of normal expression of MANDYS106-immunoreactivity in Western Blots from Biopsies 1 to 3 in Study 201/202. In these Western blots, the quantification was not done using a serial dilution so the actual percentages are not certain. The applicant appears to have compared 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

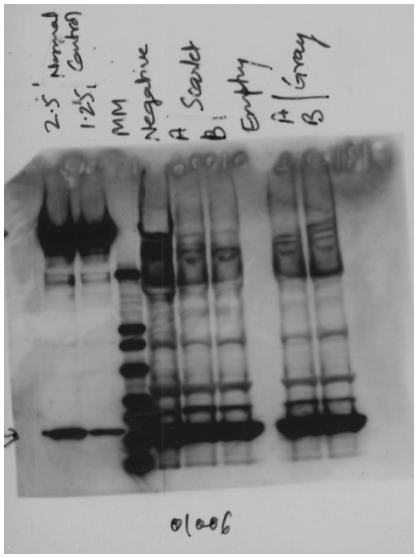
Treatment Sequence	USUBJID	Week 0	Week 12	Week 24	Week 48
	004	0.1	1.84	NA	0.25
	012	0	0.31	NA	4.52
	015	0	0.08	NA	0.48
[Placebo] - [30 mg/kg]	007	0.22	NA	0.67	0.38
	008	0.28	NA	0.46	2.31
[Placebo] - [50 mg/kg]	005	0.52	0.26	NA	0.35
	013	0.51	0.42	NA	1.28

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

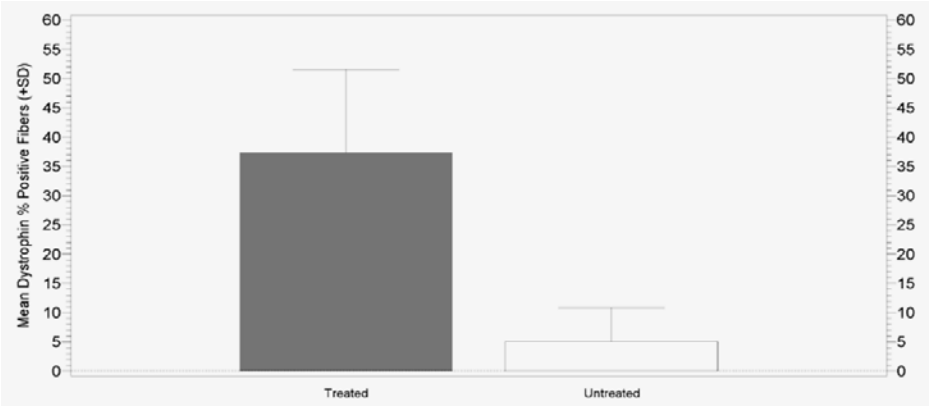
4th Biopsy Data

[AR] The methodologies used by the Applicant were relatively improved for the 4th 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 4th 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 4th 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 4th 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-immnoreactive Fibers in Eteplirsen treated Subjects to Untreated Controls



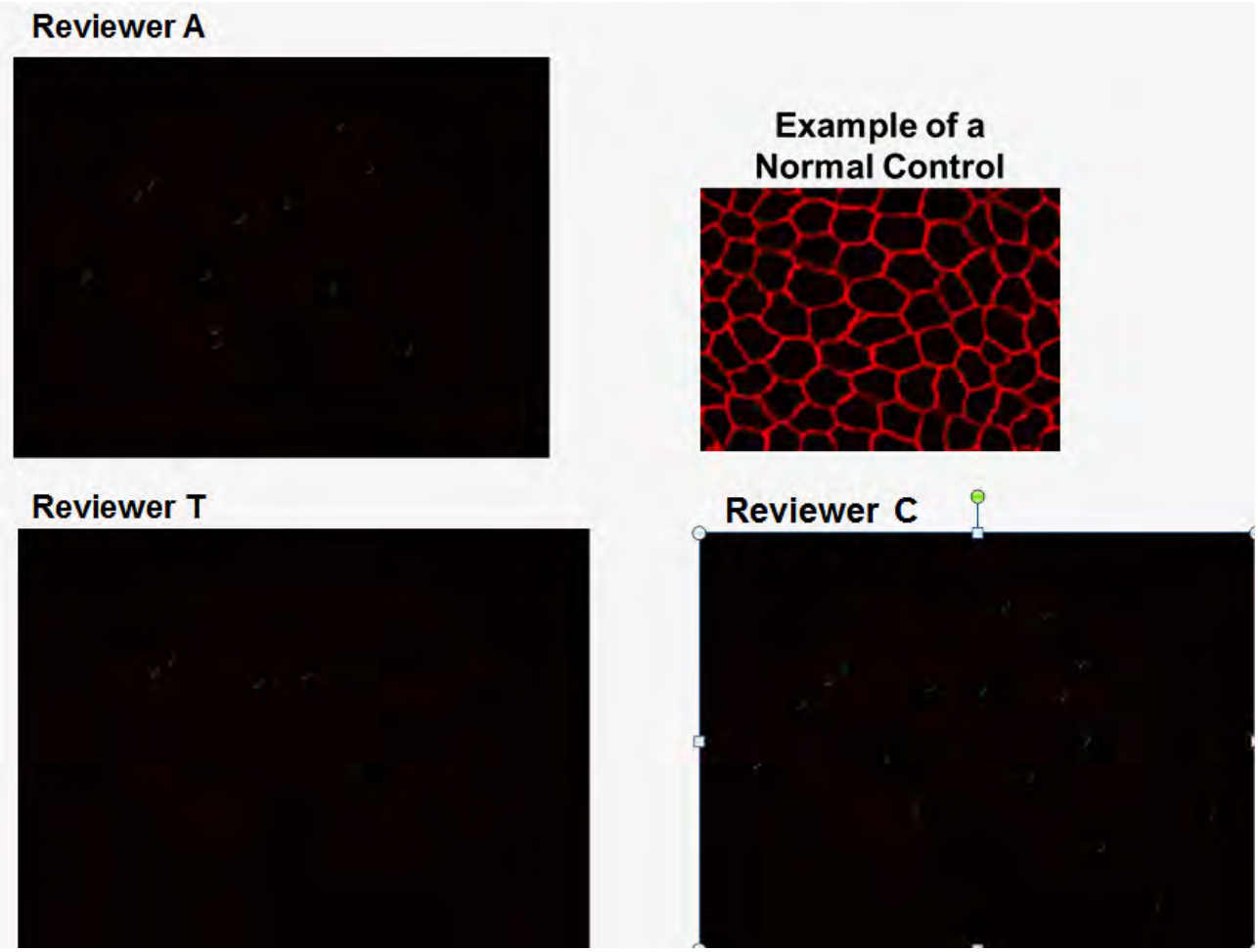
Source: Report 4658-us-cr-15-008

Medical Reviewer Comments and Analyses Specific to the 4th 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 dytrophin immunostaining was very faint in the 4th 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 4th biopsy (**Figure 17**).

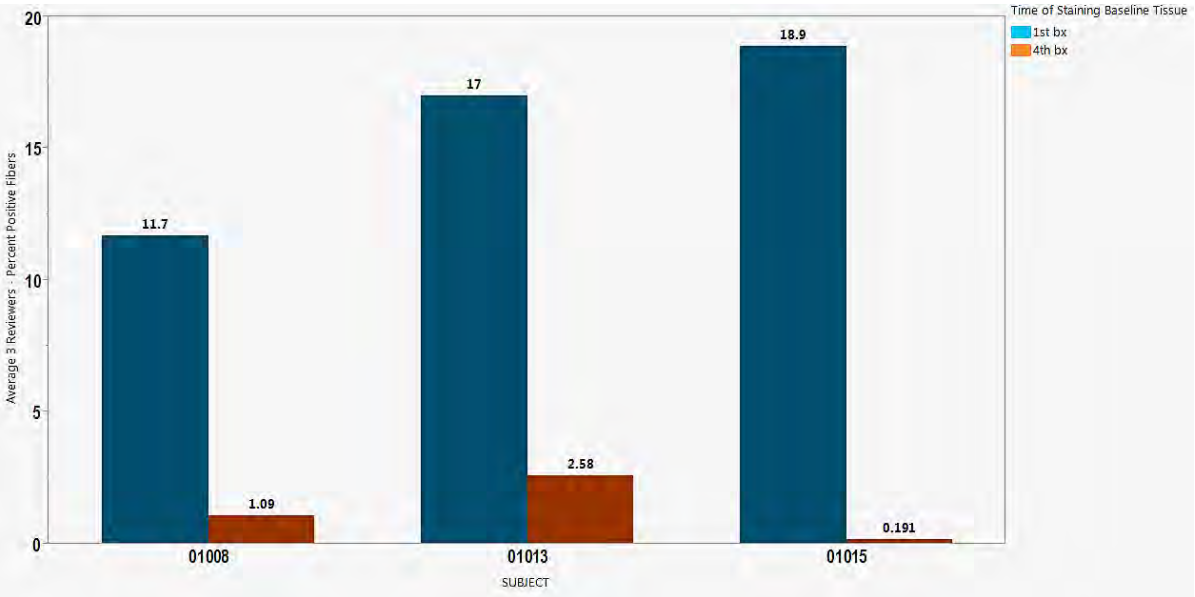
Figure 17 Images of MANDYS106-immunoreactive fiber counts from the Flagship Biosciences CRO from Biopsy 4 (Study 201/202 ITT Population)



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 – 4th 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 – 1st 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.

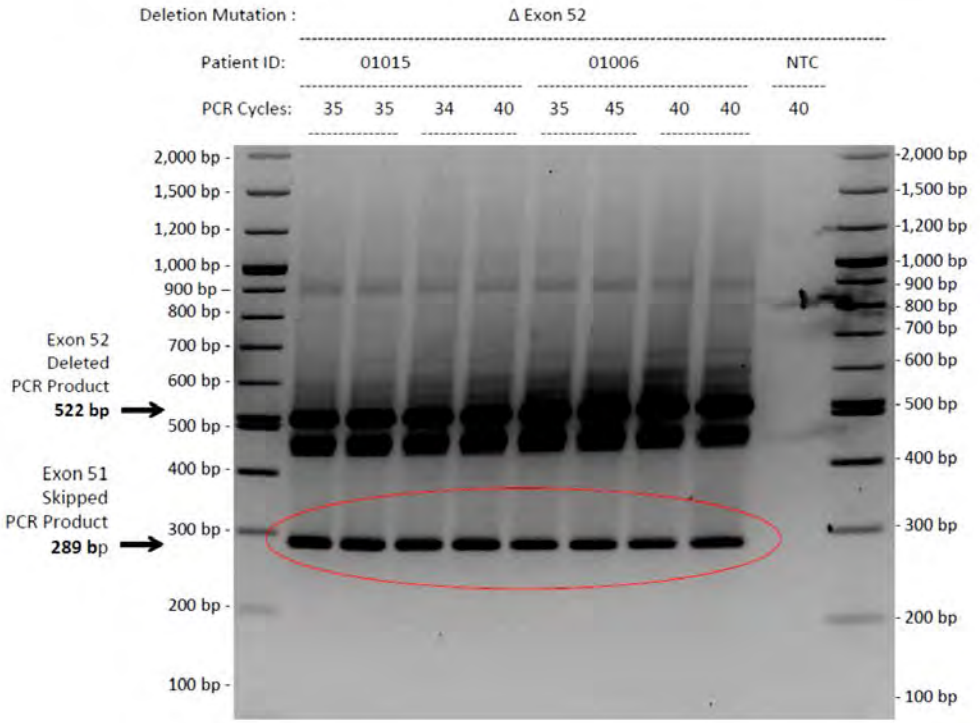
Source: Medical Reviewer's analysis of FIBERS2 dataset

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 4th biopsy.

Figure 19 Exon Skipping in Subjects 006, 015 and a Normal Control from Biopsy 4 in Study 201 / 202

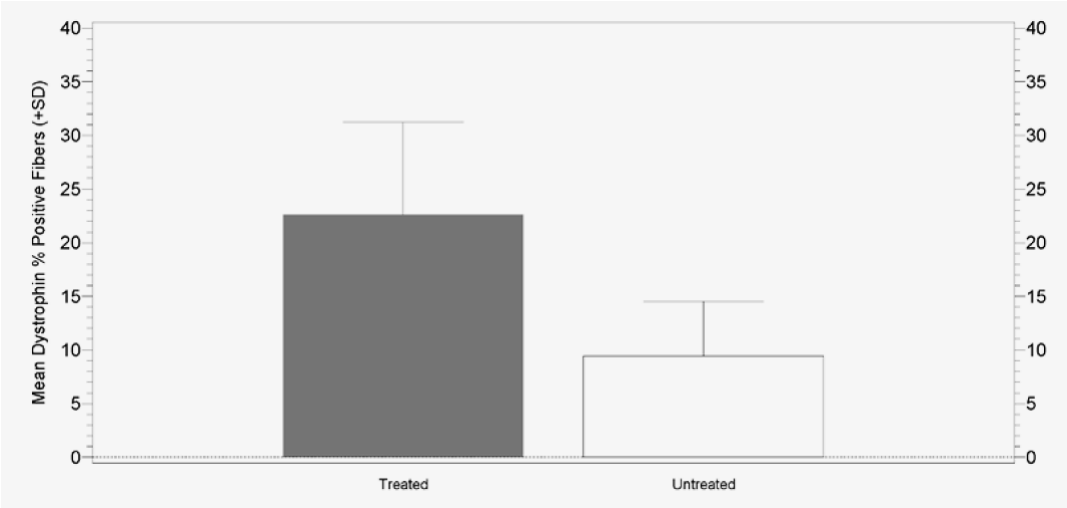


As with study 201/201, the applicant confirmed that the product was an exon 51-skipped product based on a sequencing result.

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 4th 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.

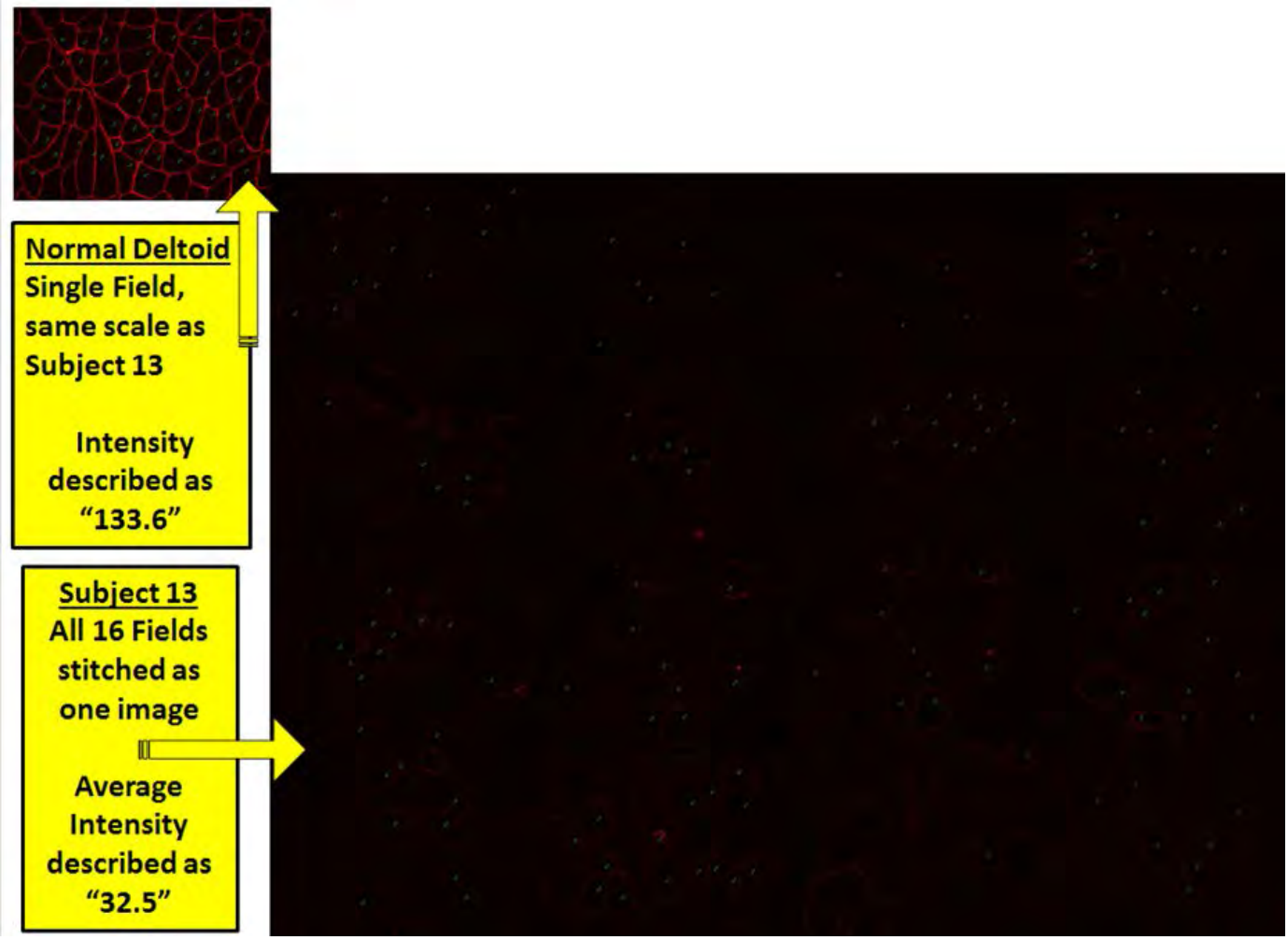
- Controls Used For Analysis Of The Fourth Biopsy

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

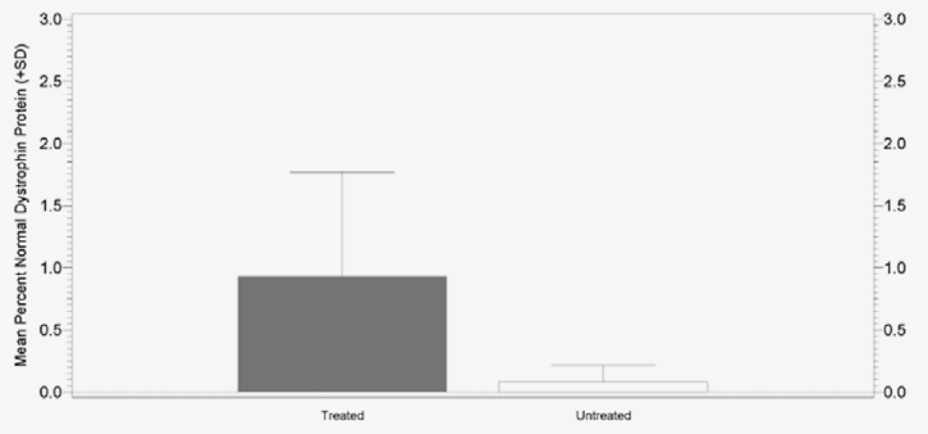


Source: Dataset from Study 202 images

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 4th biopsy.

Treatment at Week 180	Subject	4th Biopsy Gel #1	4th Biopsy Gel #2	Average for 4th Biopsy; BLOQ set to 0	Average for 4th 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[9]	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

[9] No Western blot was available for the 4th biopsy of Subject 005

Western blots using DYS1 were also done for control subjects selected by the Applicant for the 4th 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

Subject Number	Reported Value (per Protocol) – (Does not include levels below that of the serial dilution curve)	Average of Gel #1 and #2 (Includes levels below the serial dilution curve)
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 4th biopsy with baselines 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 4th biopsy.

Immunofluoroscence data from the mdx mouse model suggests that deltoid have 27% dystrophin-positive fibers compared to 45% in biceps and semitendonous 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 4th biospy 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.

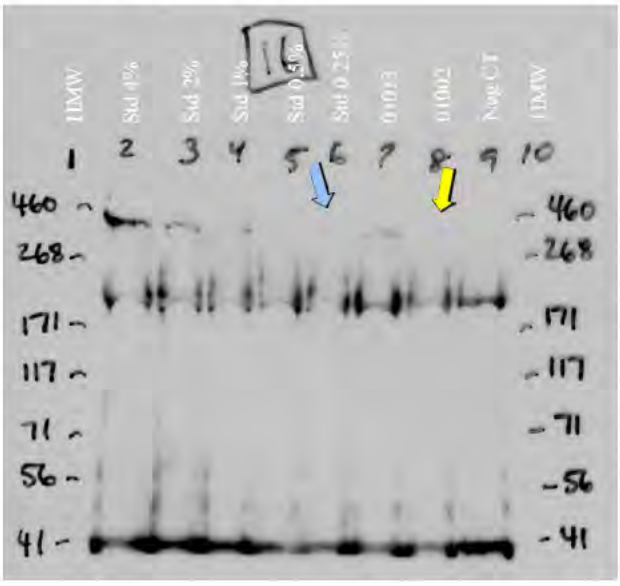
2. The quality of the standard dilution series affected their accurate quantitation

Data associated with an individual gel was considered acceptable if the standard curve R2 value was ≥ 0.90. Individual gels were graded as pass/fail based on this R2 criteria. All five (5) data points of the standard curve must be incorporated into the R2 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.

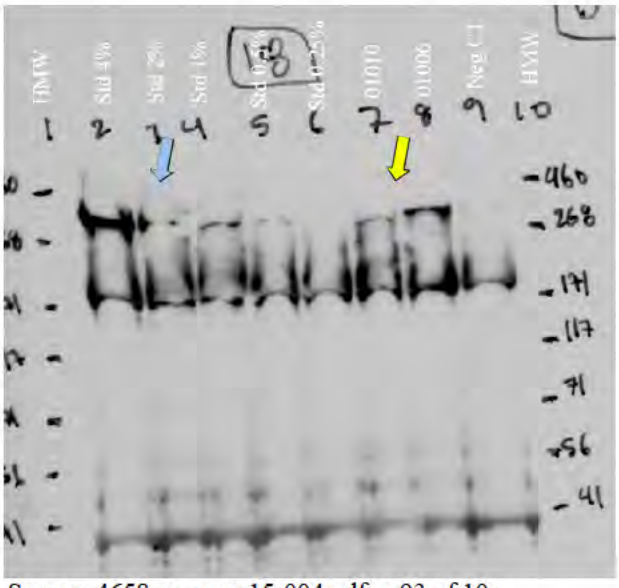
It is noteworthy that when levels below 0.25% were encountered, the applicant reported it as 0 (Below LOQ) and when levels above 4% were found, the samples were diluted to obtain quantitation within 0.25-4% and a dilution factor was then applied to the result. This would bias the results so that more 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, 4th 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, 4th biopsy



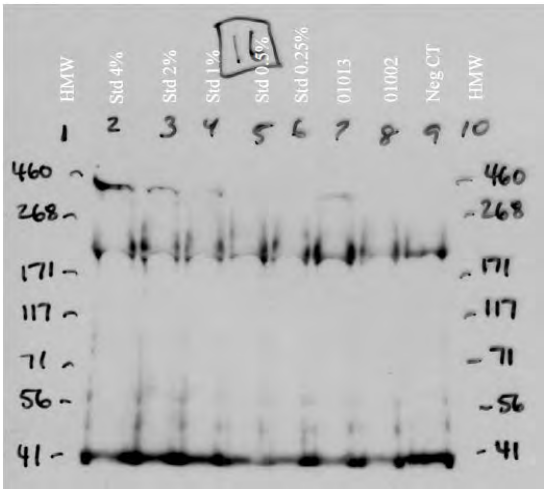
Source: 4658-us-sr-cr-15-004.pdf, p 93 of 10; data from p. 80 of the CSR.

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

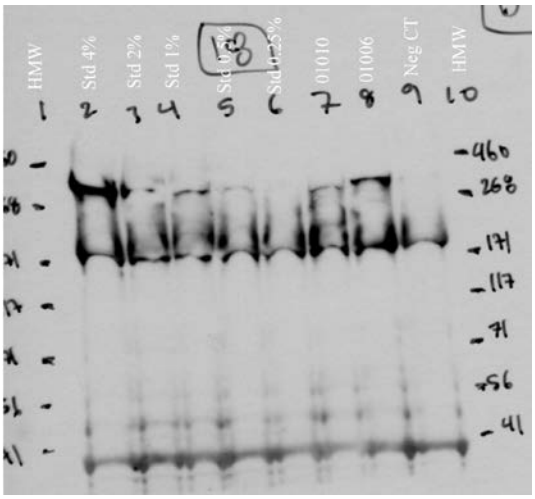
4. Certain DYS1-IR bands do not seem to correspond to the levels reported

Figure 25 Subject 2 reported as 0.28%



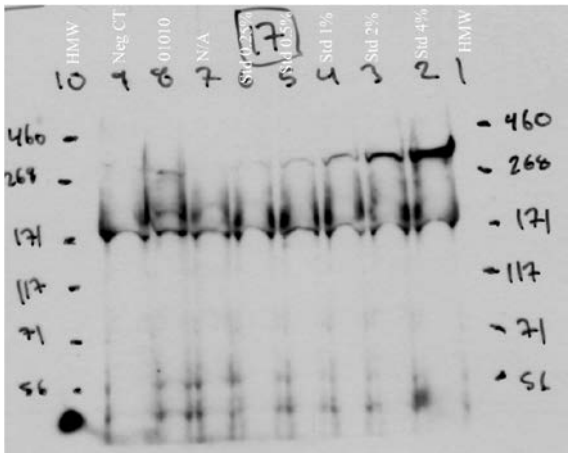
Source: 4658-us-sr-cr-15-004.pdf, p.88 of 101; Lane 7 – Subject 002 sample, Lane 6 – 0.25% standard

Figure 26 Subject 10 reported as 1.78%



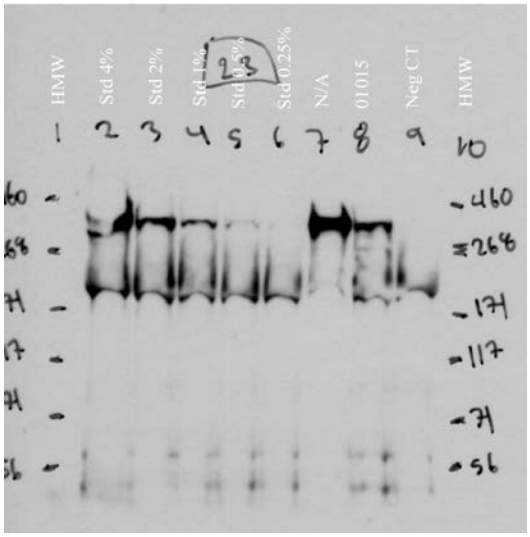
Source: 4658-us-sr-cr-15-004.pdf, p 93 of 101; Lane 7 – Subject 101 sample, Lane 5 – 0.5% standard, Lane 4 – 1.0% standard, Lane 3 – 2% standard

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

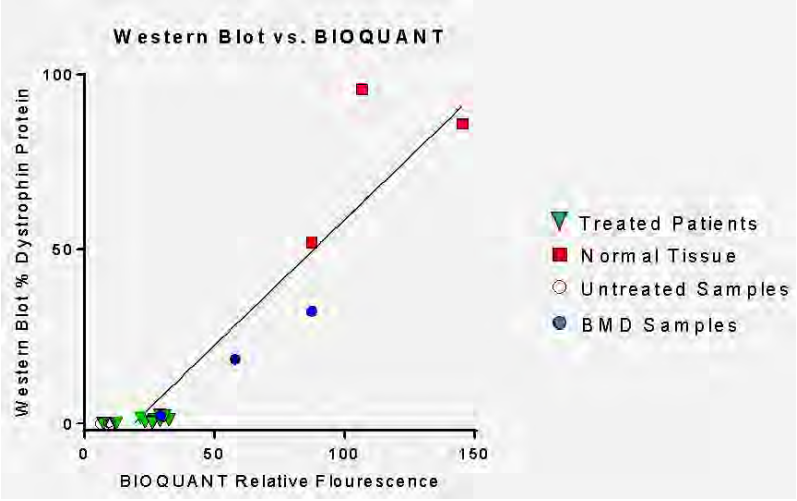
Such differences take on increased meaning given the few subjects in this "bridge" and in the original sample.

[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.

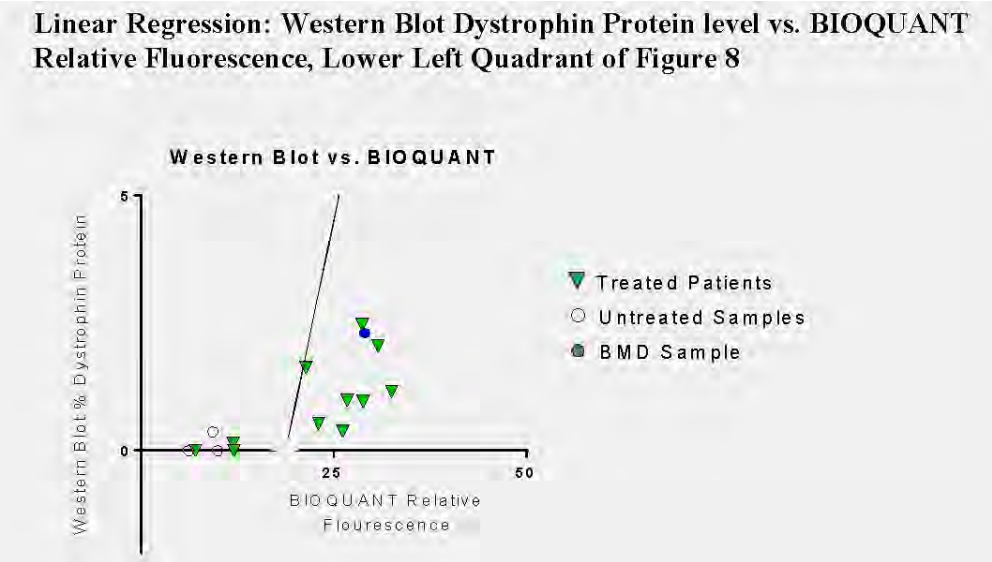
Based on their week-180 data with DMD, BMD, and healthy samples, the applicant claims that the r-square value of the western blotting and Bioquant data is 0.8741 (below from study report SR-CR-15-002). Much of the applicant's data has very low dystrophin; hence they provided a second graph enlarging 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)

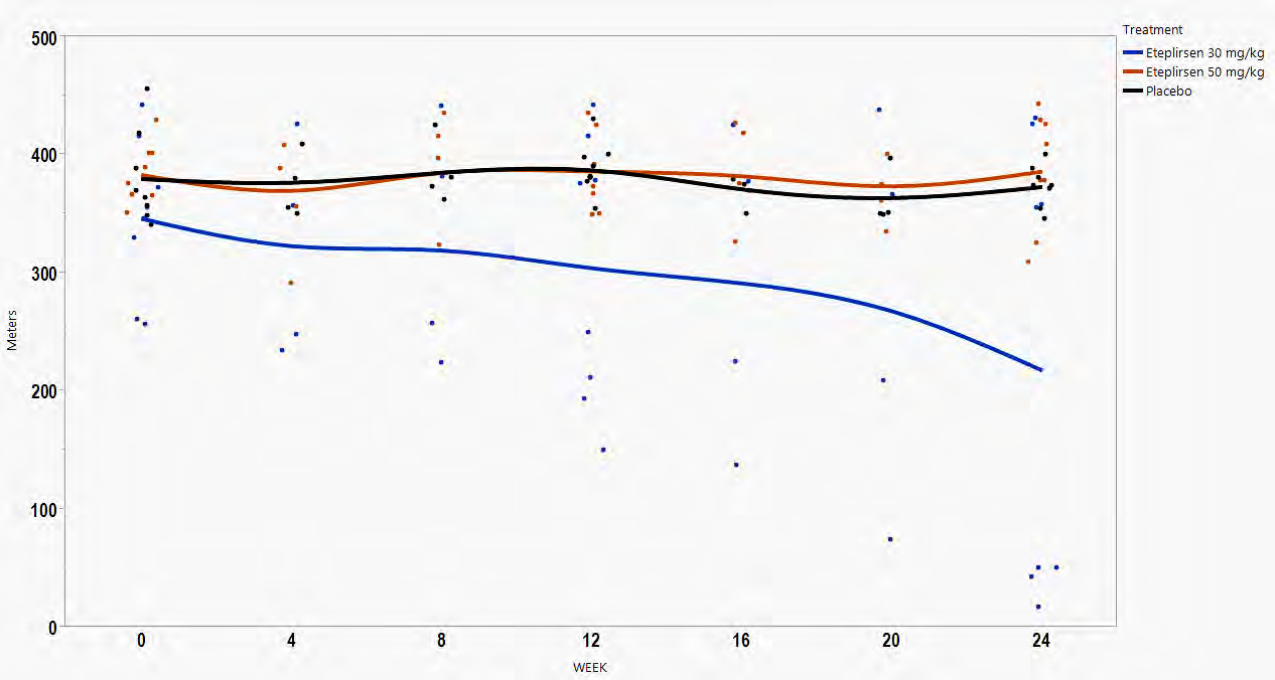
Treatment	Model Adjusted Chang from Baseline	P Value for treatment vs PBO	Estimated Treatment Effect	95% CI
Analysis of Change from Baseline for 6 Minute Walk Test Using Ranked Data				
Pbo	6.4			
30 mg	4.3	.425	-2.2	(-8.2, 3.9)
50 mg	8.8	.378	2.3	(-3.5, 8.2)
Pbo vs. All AVI-4658	6.4 / 6.6	0.939	0.2	(-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)

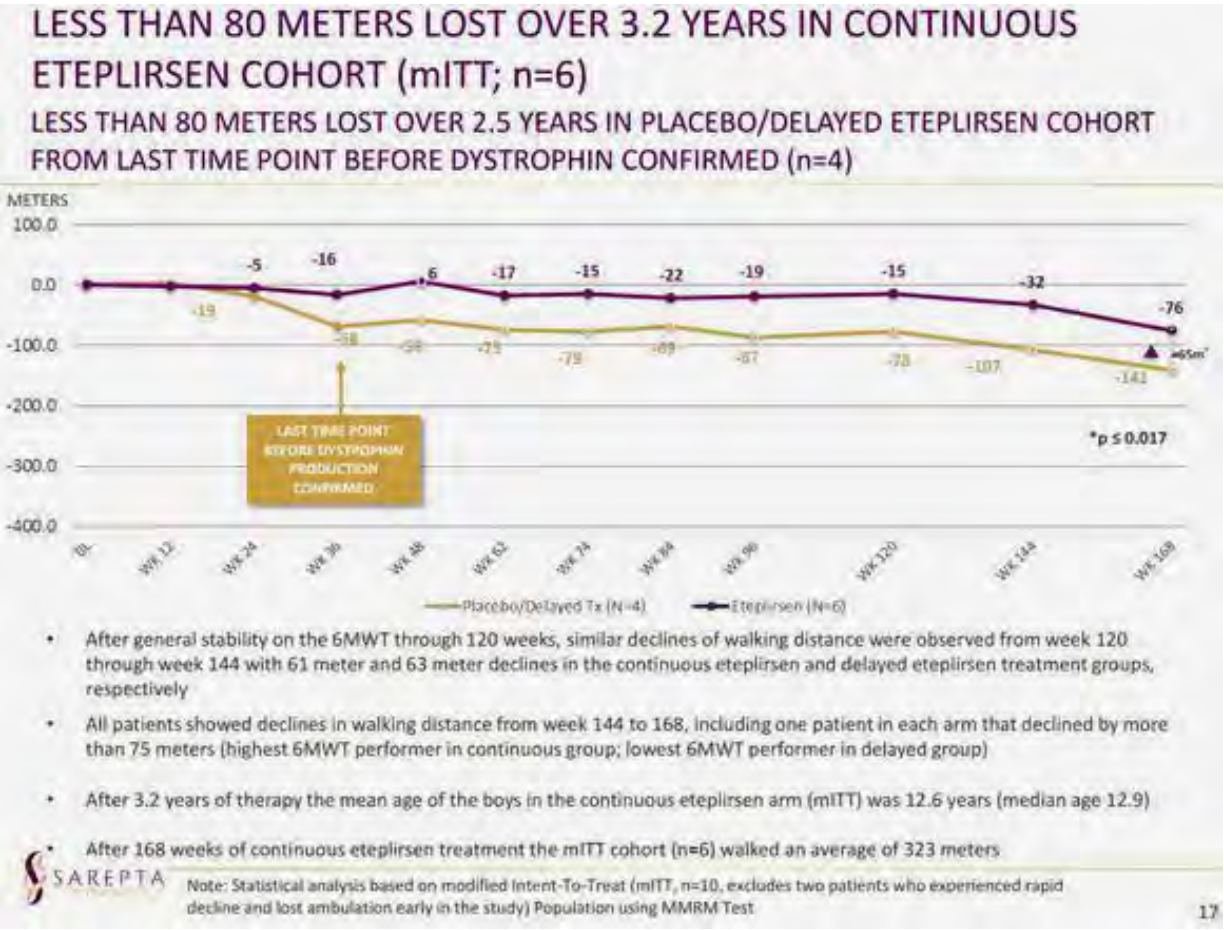


Source: Medical Reviewer Analysis of ADSMW.XPT

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**)[10].

[10] 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[11]

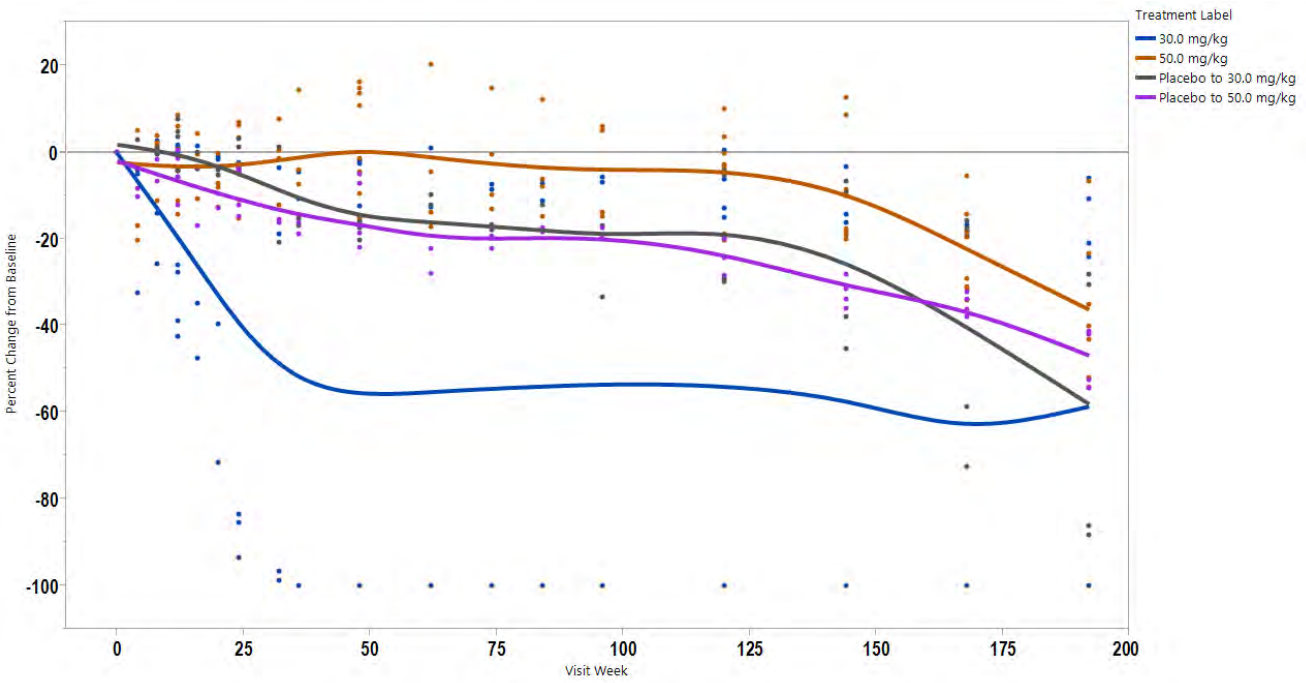


In this analysis, Sarepta has combined the patients actively treated in 2 dose groups from the beginning into a single group, which is different from the prespecified analysis, and then omitted the two subjects who declined in performance. They have also combined the two placebo sequence groups, although 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.

[11] 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)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[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
Week 24[c]				
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
Change at Week 24				
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

[a] mITT excludes patients 009 and 010; [b] Baseline is the last non-missing value before first dose; [c] 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

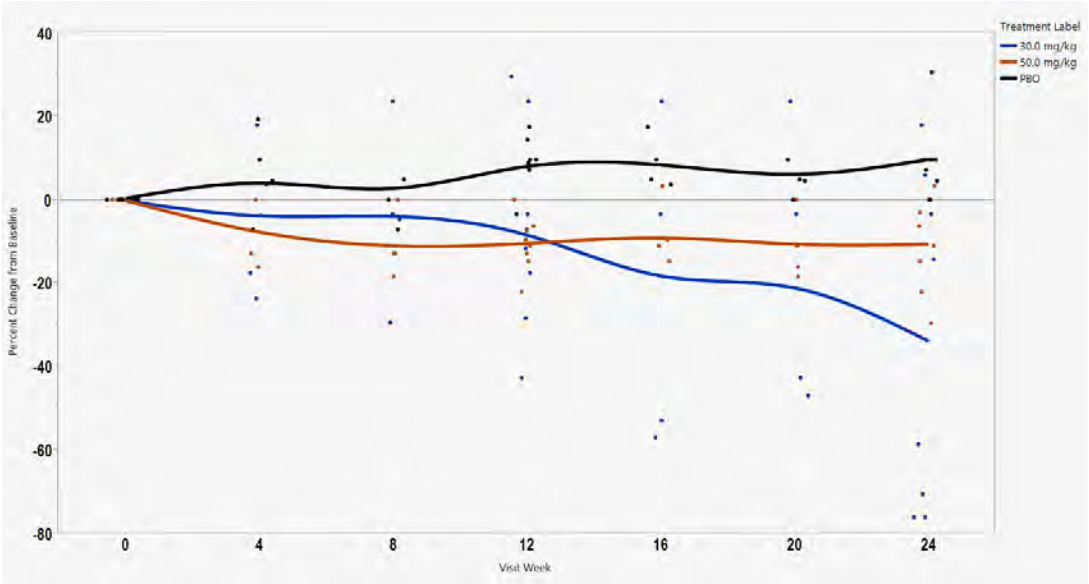
With respect to the treatment effects demonstrated in the placebo controlled portion of Study 201 / 202, there was a significant difference between the percent change from baseline for the contrast between placebo and 30 mg/kg group at 24 weeks in favor of *placebo* (**Table 17** and **Figure 34**).

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.[12]" According to the 201/202 study report, no statistically significant differences between the treatment groups were detected (**Table 18**).

[12] 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)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[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
Week 24[c]				
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
Change at Week 24				
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

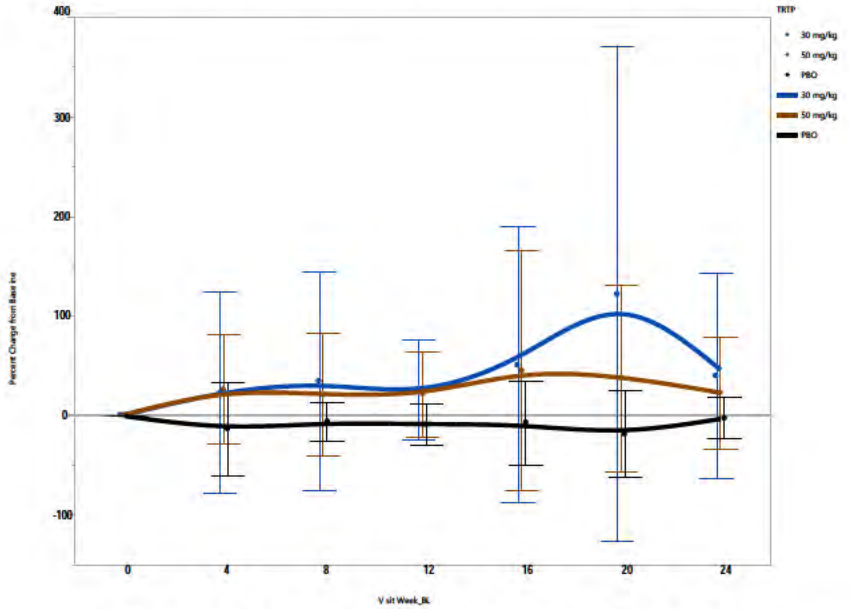
Source: Table 14.2.2.1, Table A.14.2.2.1
[a] mITT excludes patients 009 and 010.
[b] Baseline is the last non-missing value before first dose.
[c] Week 24 is the best time achieved on days 1 and 2 of that visit.
Abbreviations: max = maximum; min = minimum; mITT = modified intent to treat population; 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.

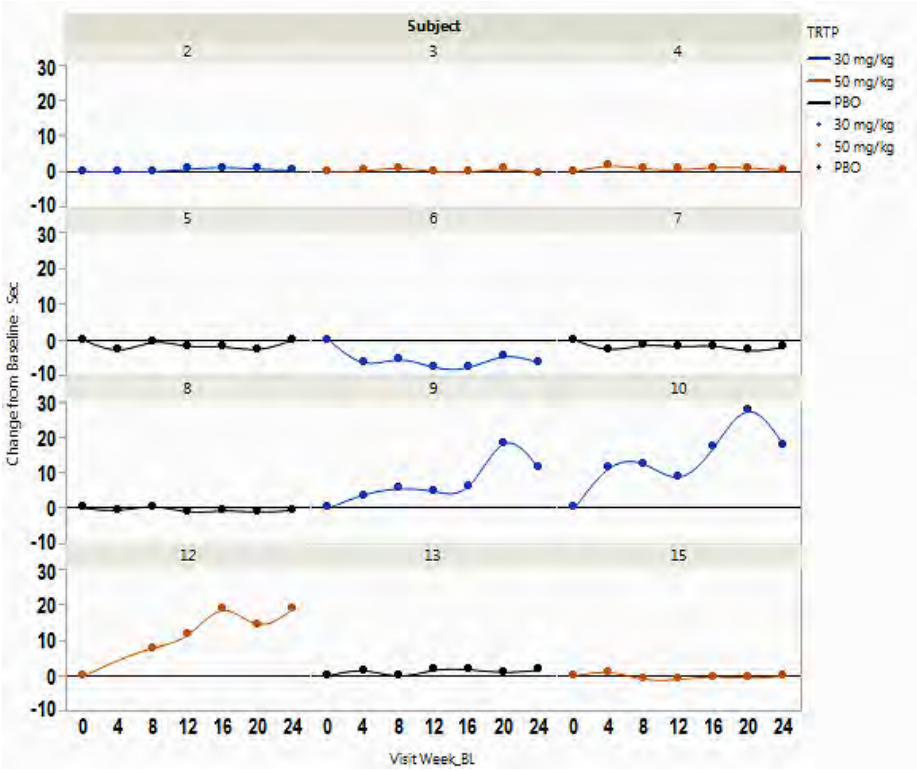
Figure 35 Percent Change from Baseline in Rise Time (95% CI) by Visit and Treatment during the Placebo Controlled Portion of the 201/202 Trial (ITT Population)



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[13]" (**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**).

[13] 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)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[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
Week 24[c]				
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
Change at Week 24				
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

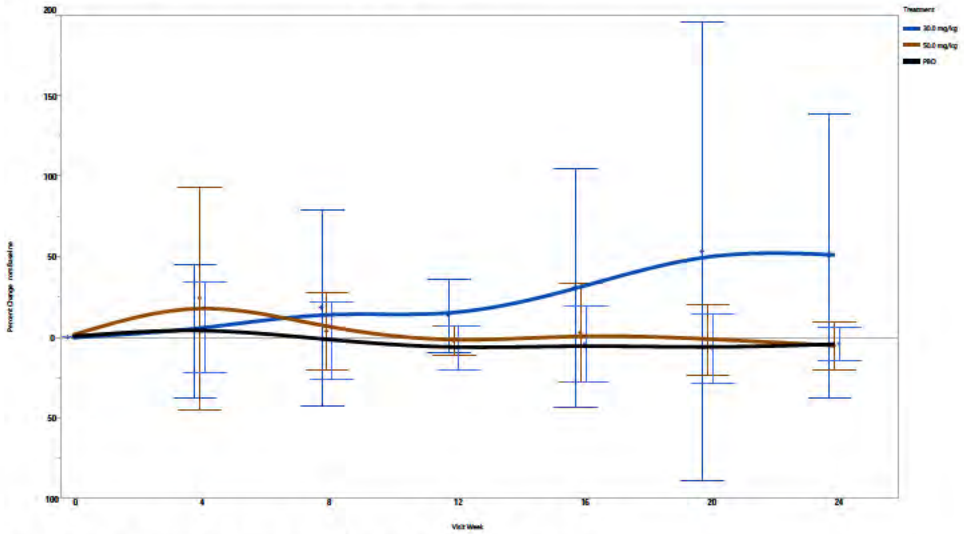
Source: Table 14.2.2.1, Table A.14.2.2.1
[a] mITT excludes patients 009 and 010.
[b] Baseline is the last non-missing value before first dose.
[c] Week 24 is the best time achieved on days 1 and 2 of that visit.
Abbreviations: max = maximum; min = minimum; mITT = modified intent to treat population; 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 diffeence between teatment 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 the10-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**).

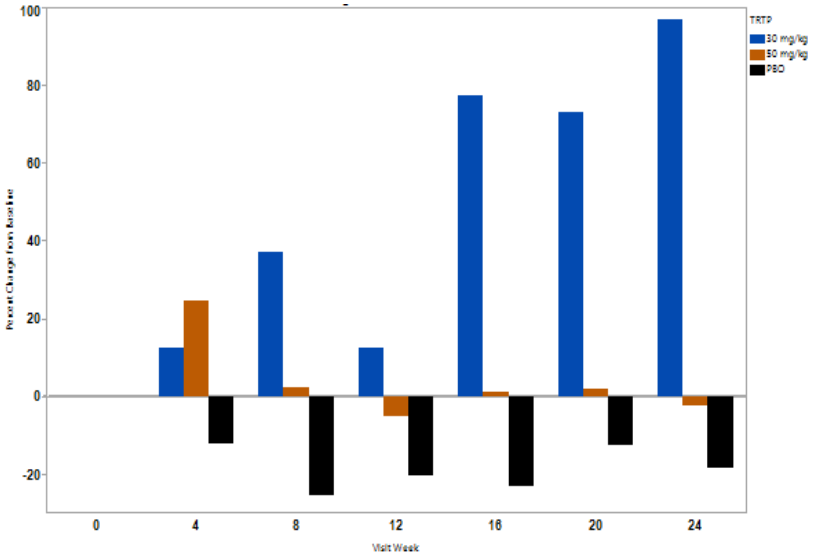
Table 20 Timed Four Step Test in the Placebo-Controlled Portion of Study 201/202 (ITT and mITT population)

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[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
Week 24[c]				
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
Change at Week 24				
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
[a] mITT excludes patients 009 and 010.
[b] Baseline is the last non-missing value before first dose.
[c] 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

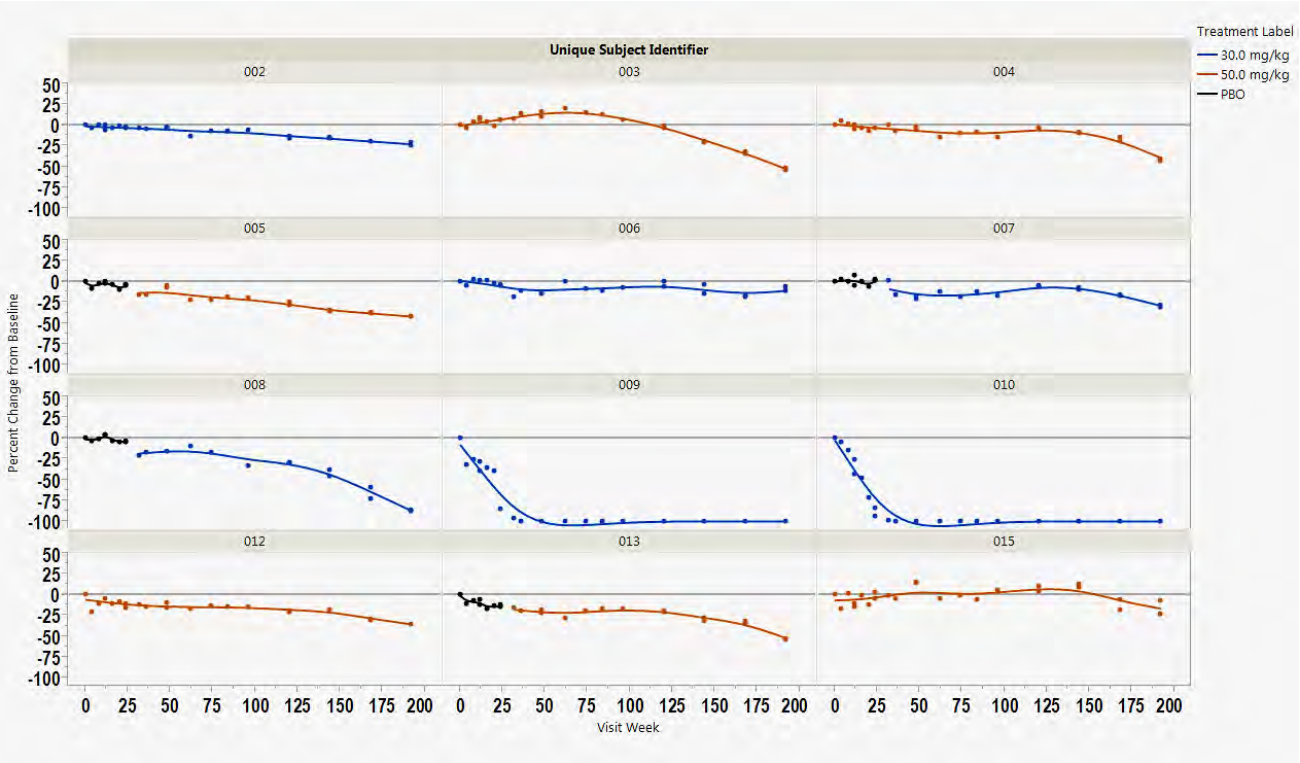
The Applicant has made claims that subjects originally randomized to placebo were notably stabilized after switching to eteplirsen by the 36 week visit (**Figure 32**). However, **Figure 57** (in long-term data description of next section) suggests that there is no clear improvementafter subjects switch 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

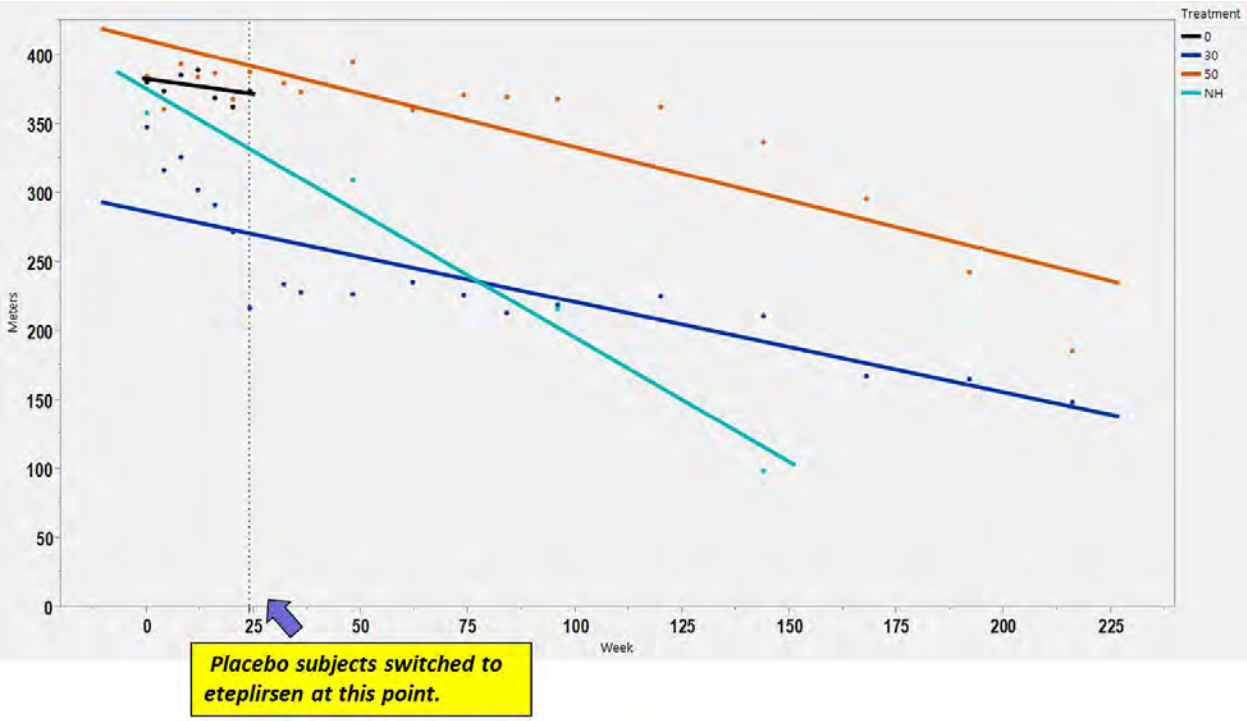
Reviewer's Comments

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.

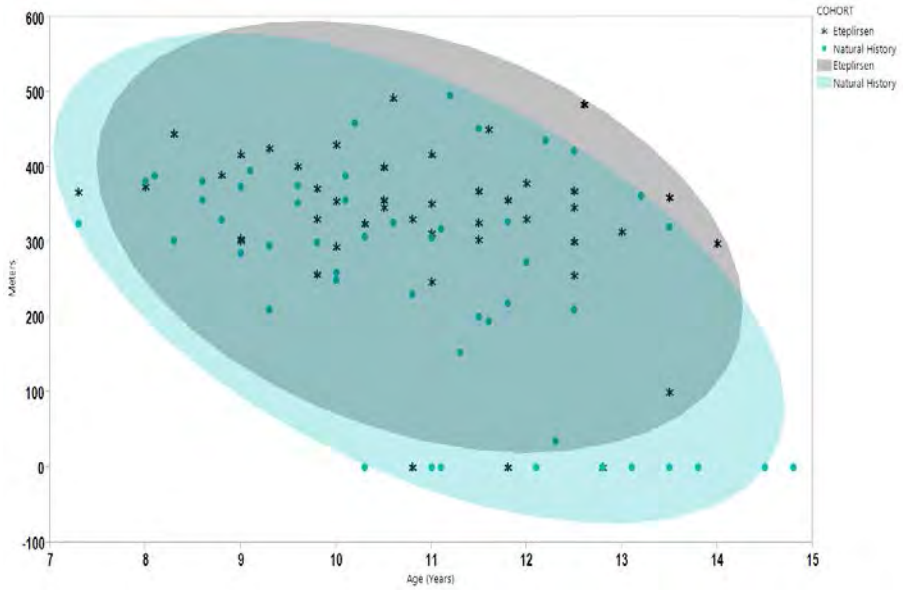
Figure 40 Performance on the Six Minute Walk by Treatment Group and Week (ITT Population)



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

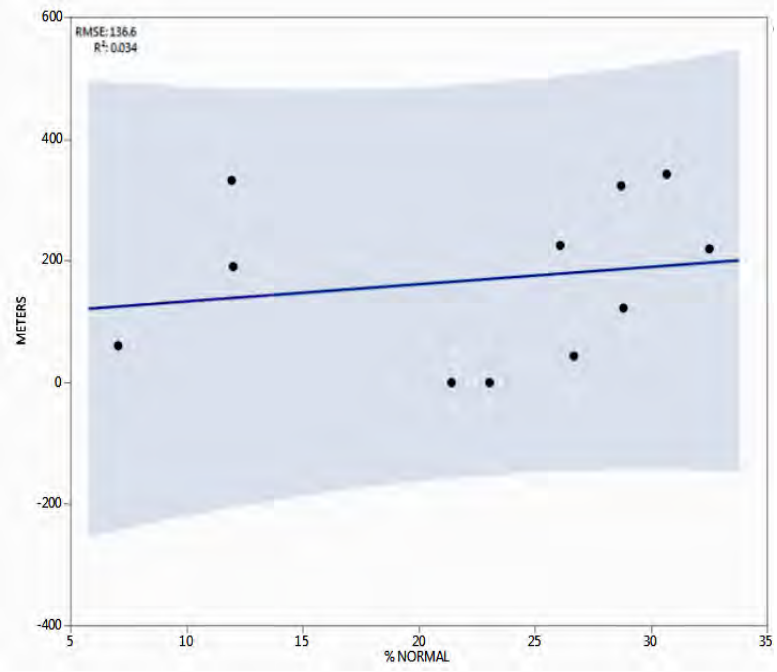


Source: Medical Reviewer Analysis of SWTDER dataset; the ellipses represent 95% normal density

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

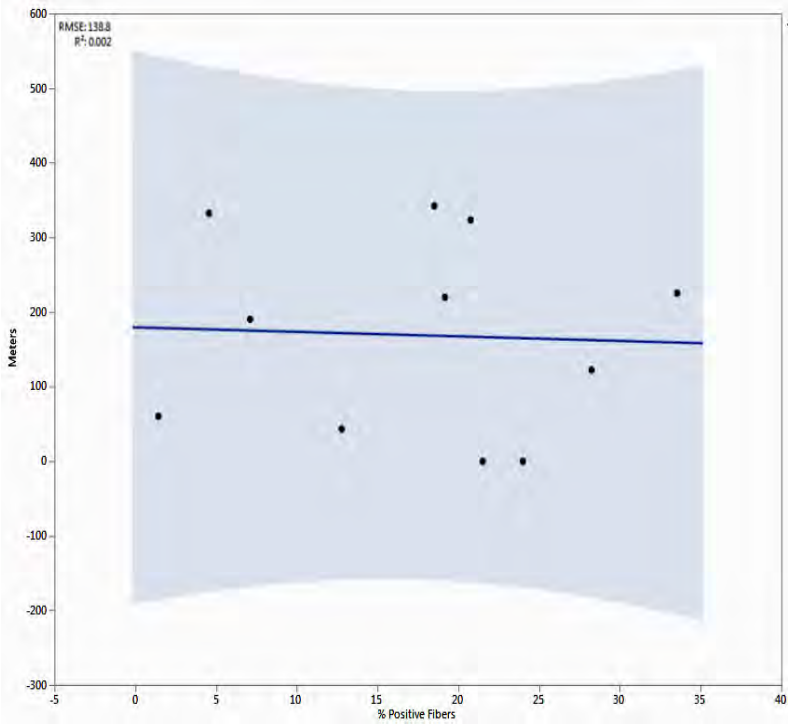
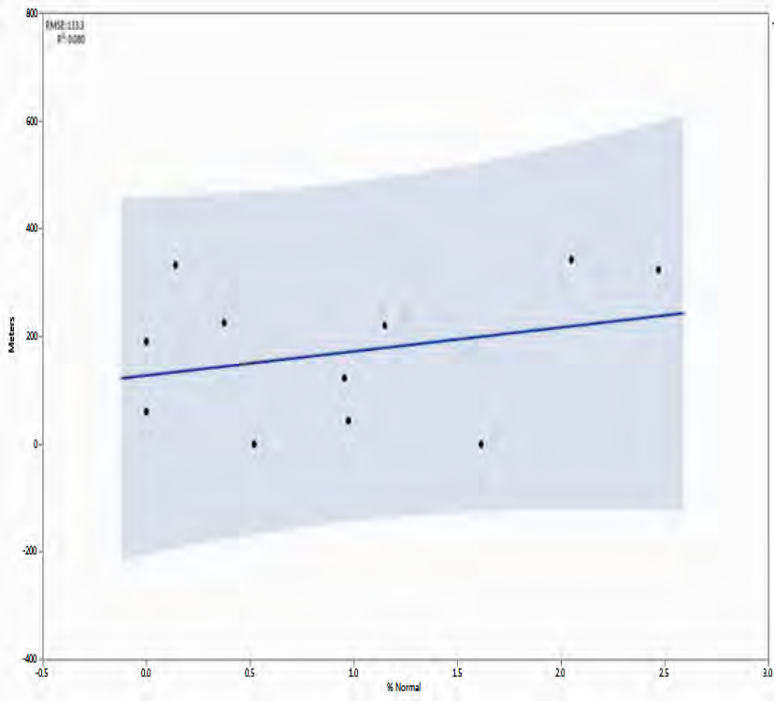


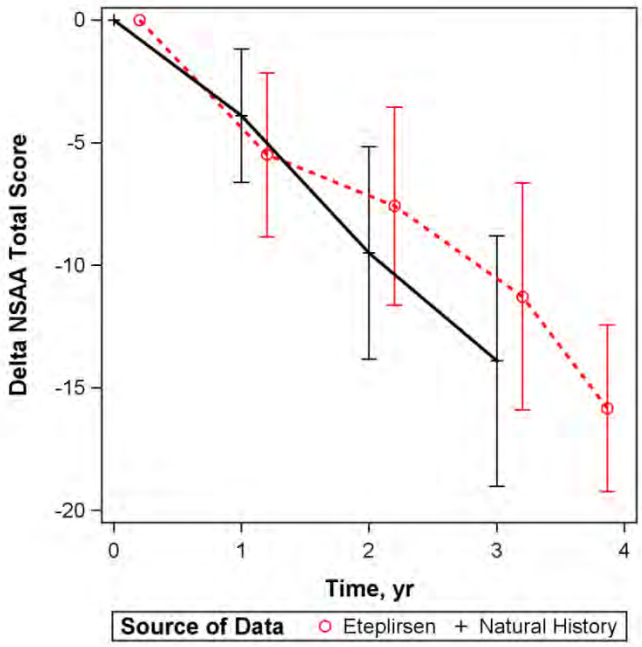
Figure 44 6MWT Distance vs WB % Normal for eteplirsen treated Subjects at 4 years



NSAA Total Score

Performance of the combined eteplirsen and natural history cohorts is depicted below (**Figure 45**).

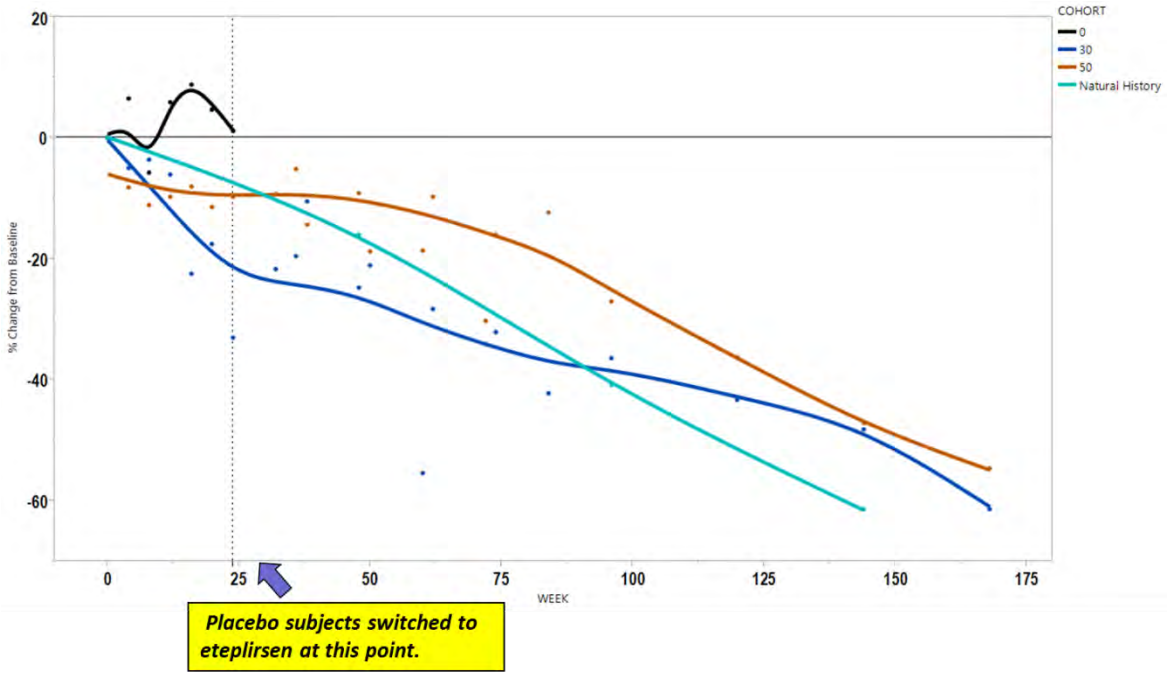
Figure 45 Change in the NSAA Total Score (95%CI) by Treatment Cohort



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 preforms inferiorly to the placebo subjects from the 201 trial.

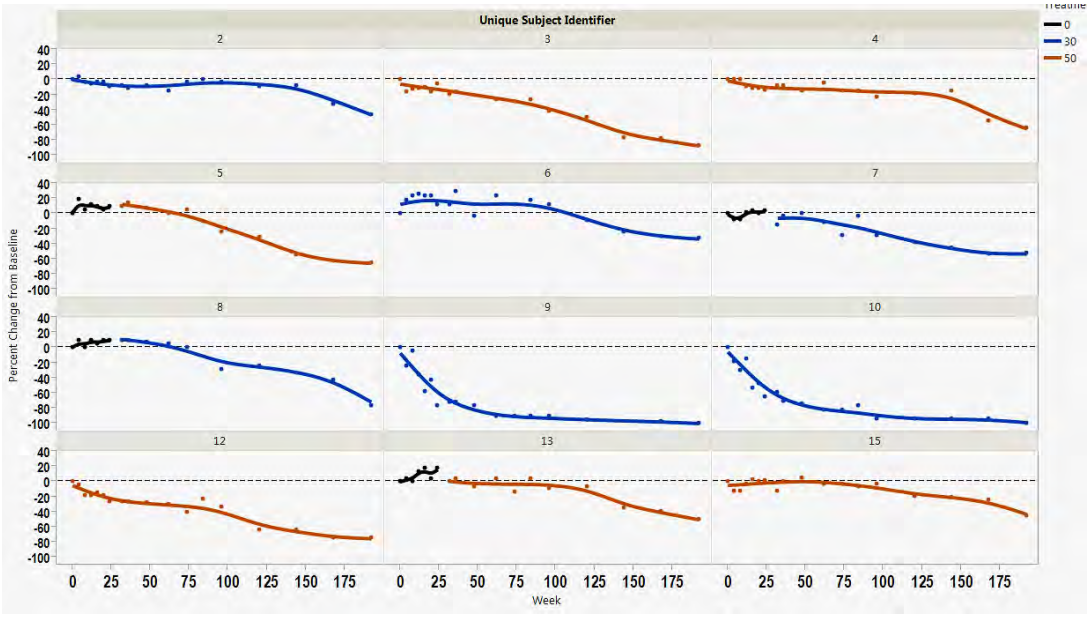
Figure 46 Percent Change in NSAA Score by Week and Treatment in Study 201 / 202 (ISS Safety Population)



Source: Medical Reviewer analysis of the t-nstar-csv.txt dataset

Figure 47 shows the change by subject.

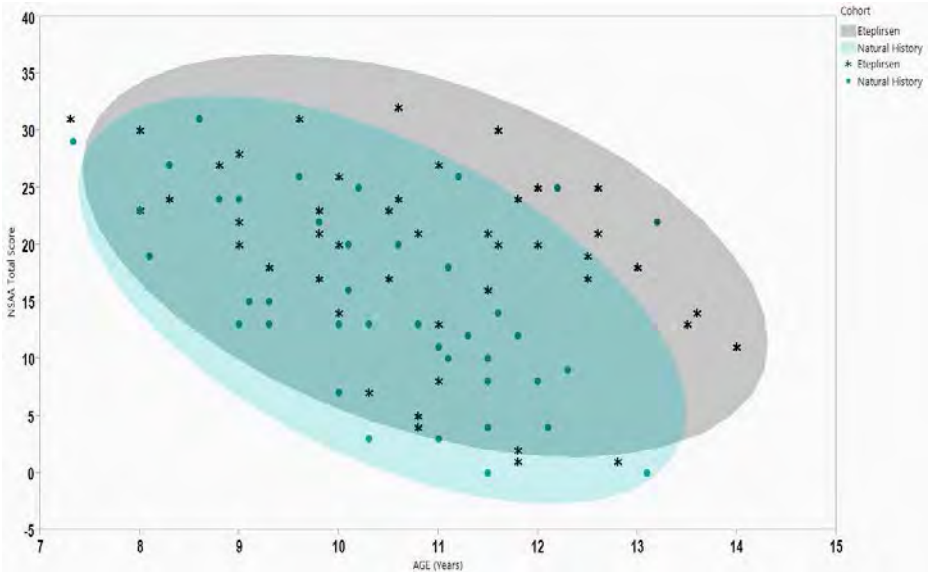
Figure 47 Percent Change in NSAA Total Score by Subject, Treatment and Visit Week in Study 201 / 202 (ITT Population)



Source: Medical Reviewer analysis of the t-nstar-csv.txt dataset

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

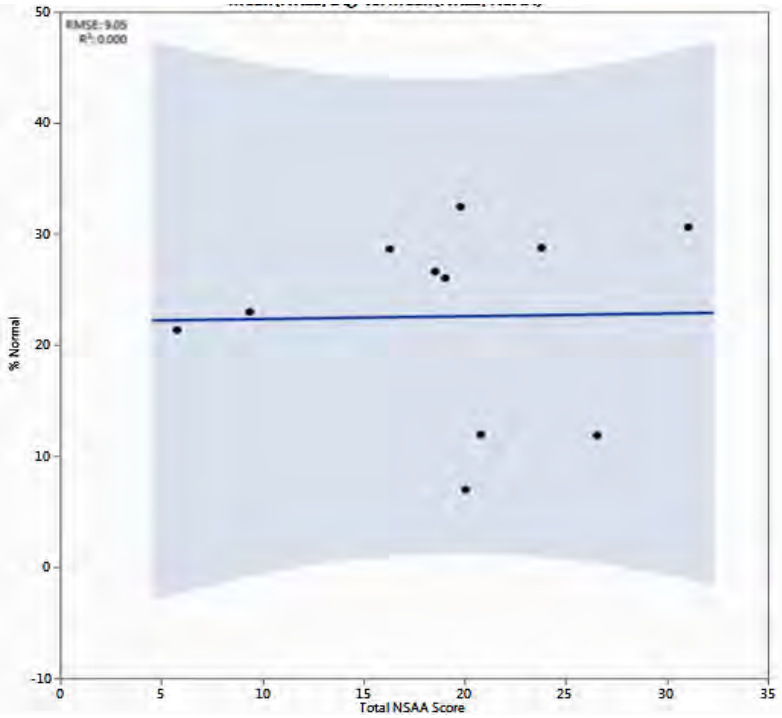


Figure 50 Total NSAA Score vs PPF % Normal

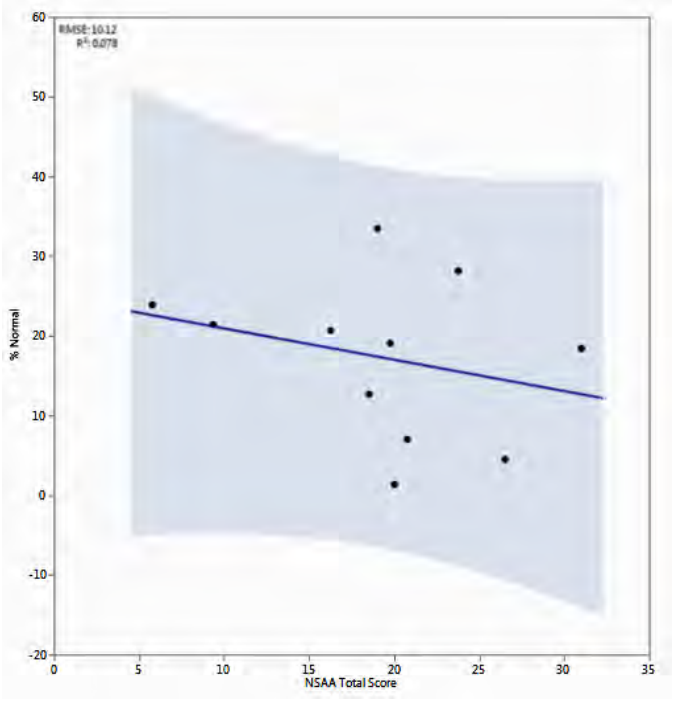
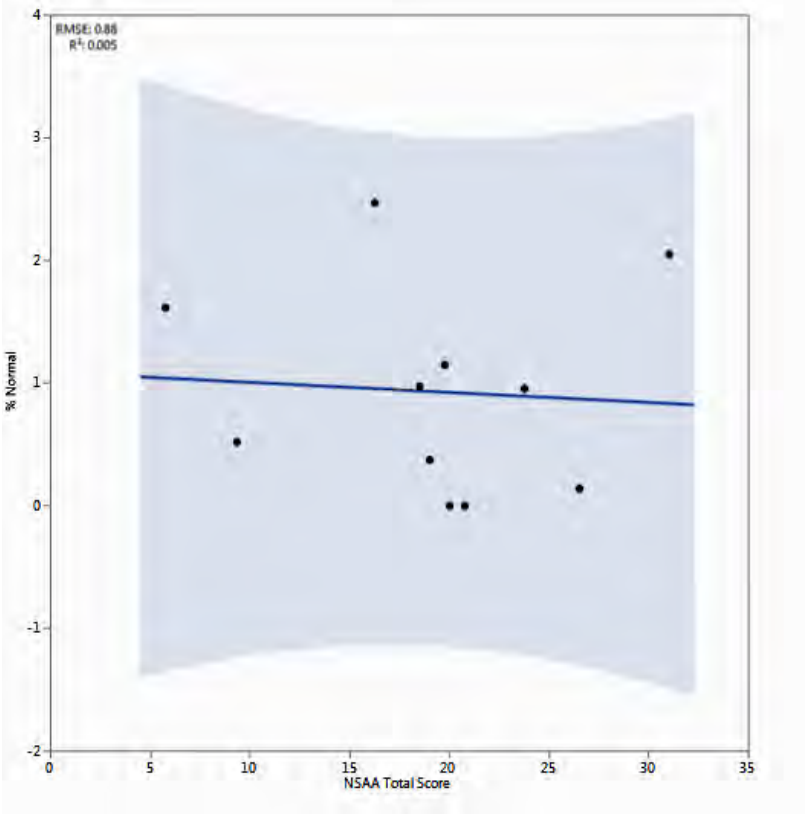


Figure 51 Total NSAA Score vs WB % Normal



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.

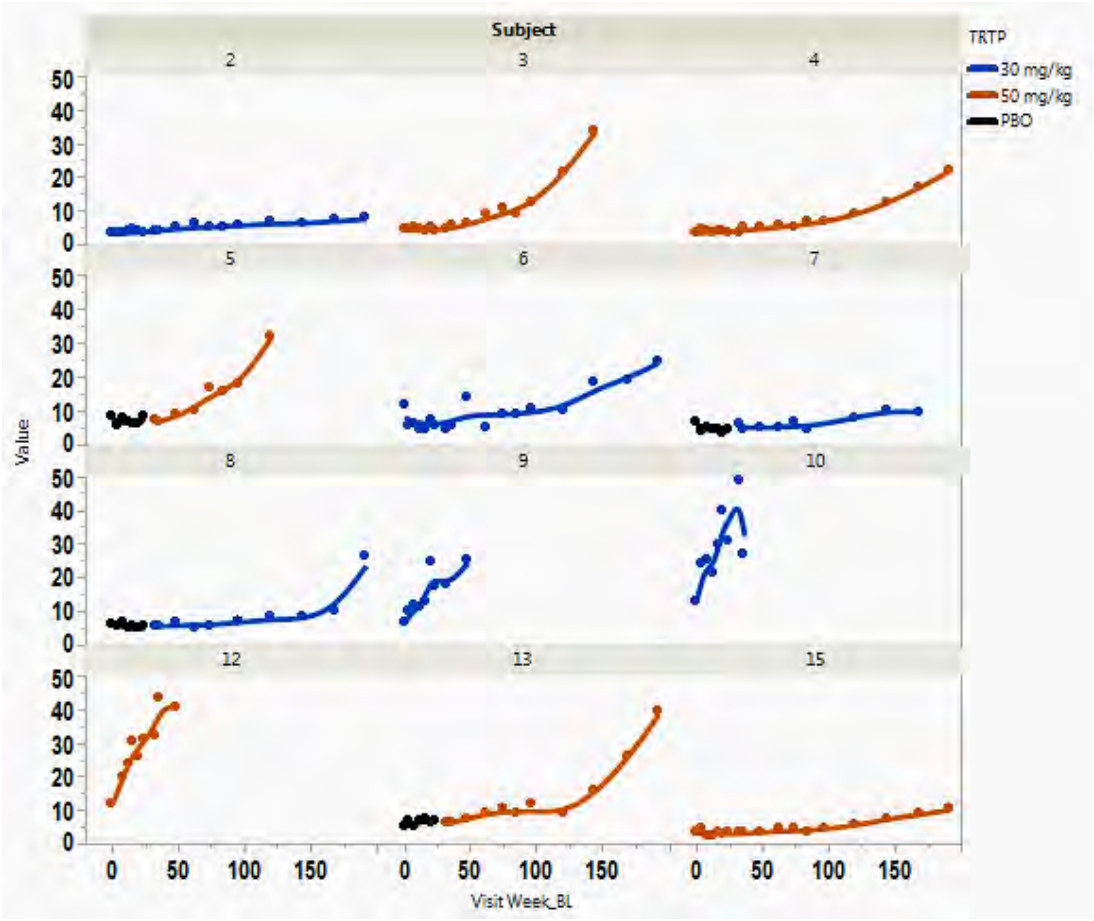
Table 21 Subjects with 'Missing' Rise Time data in the 201/202 study (ITT Population)

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

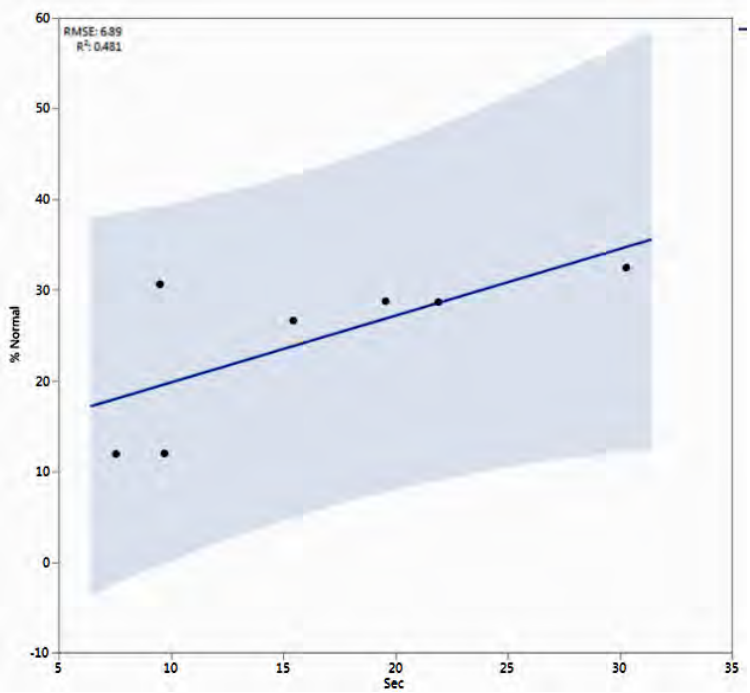


Figure 54 Rise Time Sec vs PPF % Normal for eteplirsen treated Subjects at 4 years

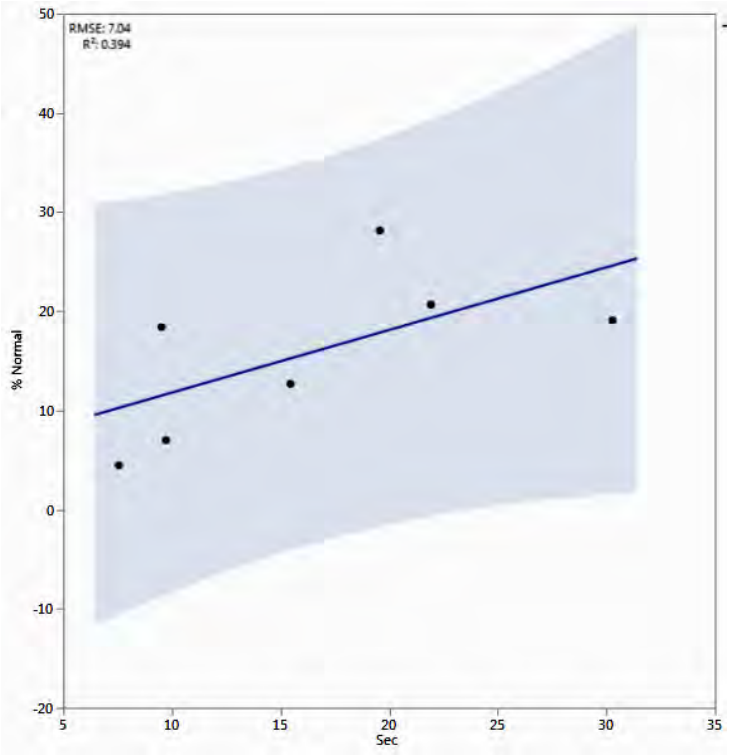
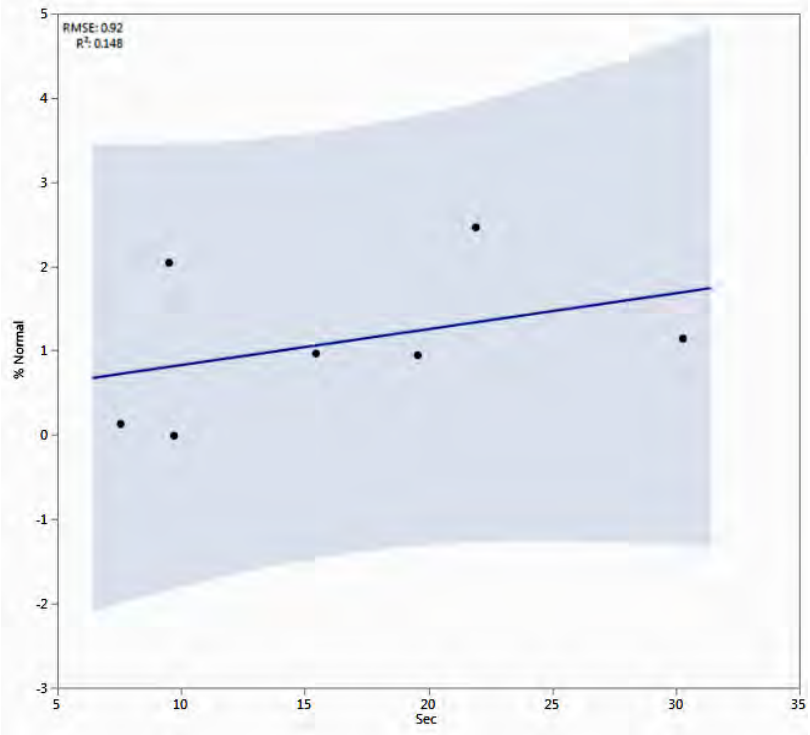


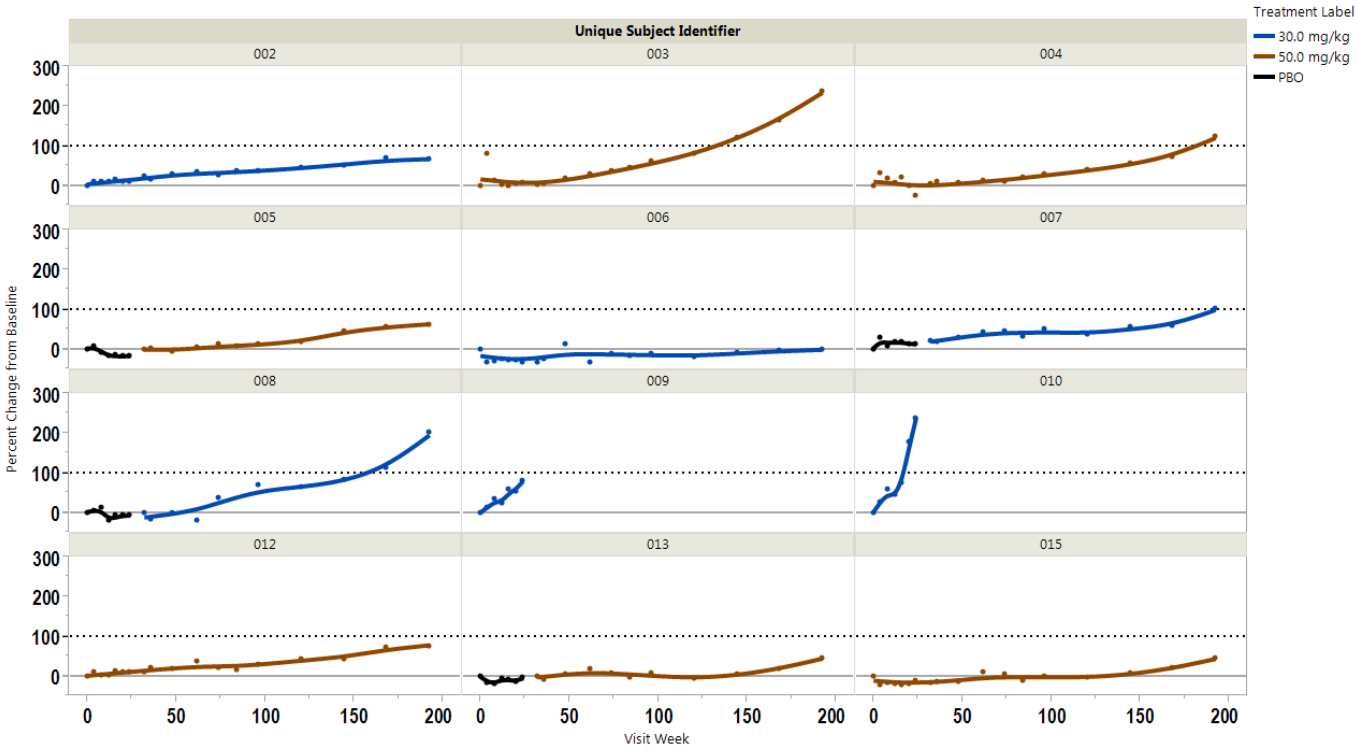
Figure 55 Rise Time Sec vs WB % Normal for eteplirsen treated Subjects at 4 years



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.

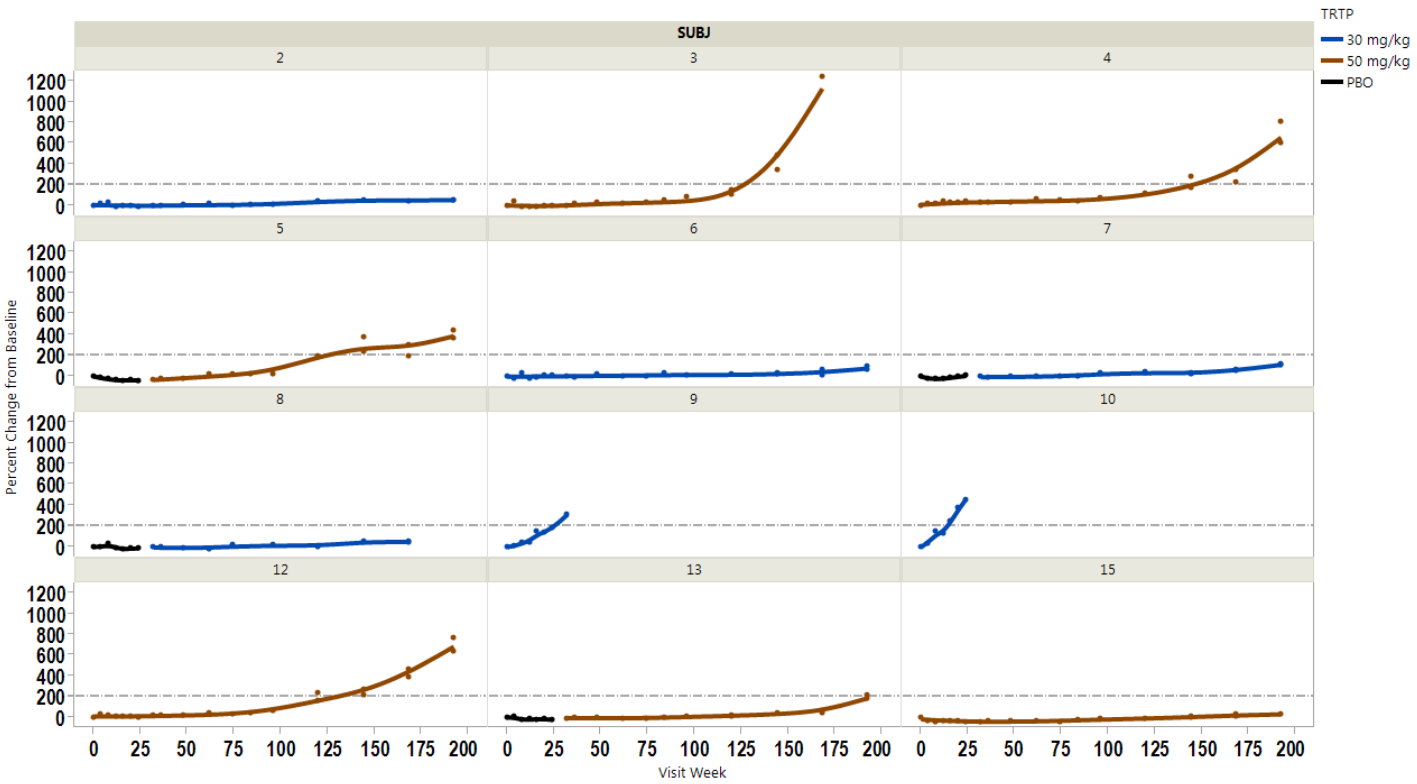
Table 22 Subjects with Missing Four Step test data in the 201/202 study

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 (≥ 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%*[14]

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

	Eteplirsen-treated Subjects (Study 201 / 202)	**Khirani et al.**	**Mayer et al.**
Age	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)*
Steroid Regimen	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.*

[14] Source: summary-clin-efficacy, p. 57 of 85

	Eteplirsen-treated Subjects (Study 201 / 202)	**Khirani et al.**	**Mayer et al.**
Other Key Factors	• None had scoliosis surgery prior to the study • All subjects ambulatory at the start of the study; subjects 009 and 010 could not complete the 10M run by week 192	*…Of the 48 remaining patients [screened,]25 had spinal surgery to correct scoliosis*	*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*

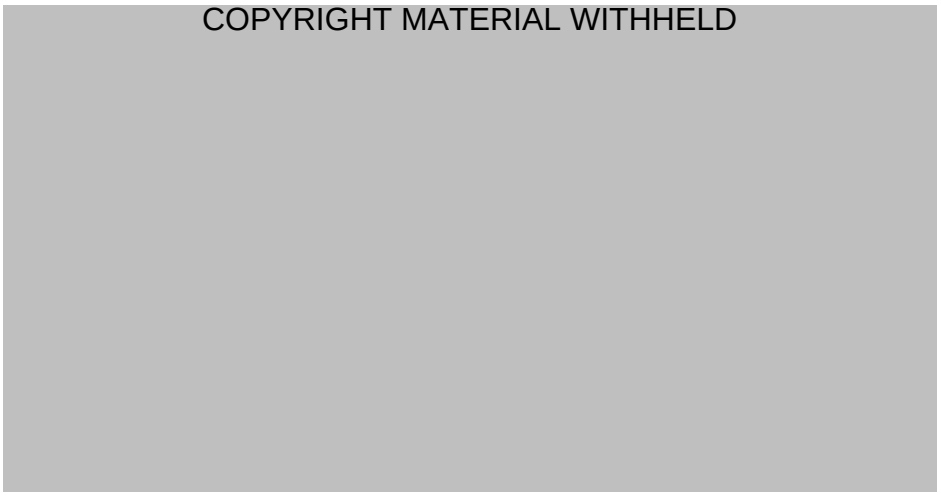
– Changes from Baseline in CD3, CD4, and CD8 lymphocyte counts in muscle biopsy tissue at Week 12 for groups 1 and 3a and at Week 24 for groups 2 and 3b.

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[15]



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

[15] 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) ≥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 Cockroft and Gault Formula.
5. 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**
Parameter																		
Genetic Analysis	X[18]																	
Study Drug Administration			X	X	X	X	X	X	X	X	X	X	X	X				
Magnetic Resonance Imaging (MRI)	X[9]																	
Safety Assessments																		
Laboratory Assessments	X	X	X		X			X						X[7]	X			X
AE and SAE Assessment[17]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Electrocardiogram (ECG)	X[3]		X[4]					X[4]						X[4]				X
Echocardiography (ECHO)	X[3]													X				X[8]
Pharmacodynamic Assessments																		
Pulmonary Function Tests (PFTs)	X[3]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Muscle Function Assessments[12]		X	X					X						X		X	X	X
Muscle Biopsy	X[10]														X[15]			
In Vitro Dystrophin Assessment	X																	
SAM Download[5]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacokinetic Assessments																		
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 β-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.

<u>Dystrophin-related endpoints</u>

- 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 ≥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 (α–actinin) bands were calculated. Then the values for the Dys and α–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.

<u>Functional endpoints included:</u>

- 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

<u>Analysis Populations</u>

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 Cmax, Tmax, and AUC0-24 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 (SaO2)
  - 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 (FEV1)
  - Percent predicted FEV1 (FEV1%)
  - FEV1/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)

	N (%) at the Eteplirsen dose (mg/kg)						
Disposition	**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[a]	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 Withdrawa	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)
Analysis Data Set	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Safety Population	4 (100)	2 (100)	2 (100)	3 (100)	4 (100)	4 (100)	19 (100)
Per Protocol Populatic	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						
Duration of dz (mean years)	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)

	eteplirsen dose (mg/kg/wk)						
Timepoint Statistic	**0.5 (n=3)**	**1.0 (n=2)**	**2.0 (n=2)**	**4.0 (n=2)**	**10 (n=4)**	**20 (n=4)**	**Total (n=17)**
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)

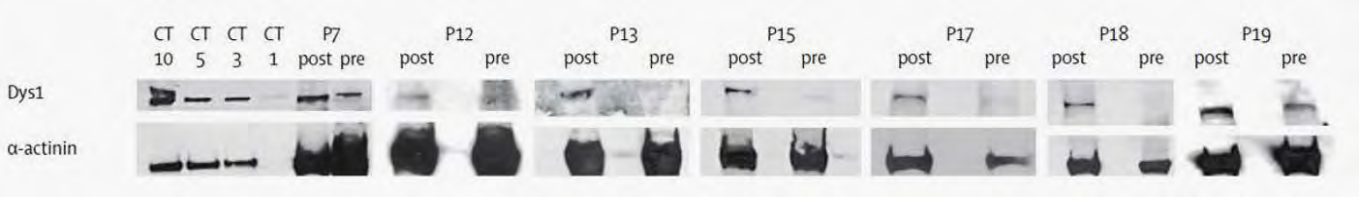
	eteplirsen dose (mg/kg/wk)						
Timepoint Statistic	0.5 (n=3)	1.0 (n=2)	2.0 (n=2)	4.0 (n=2)	10 (n=4)	20 (n=4)	Total (n=17)
Screening/Baseline Actual Value							
Mean (SD)	5.0 (0.00)	6.0 (2.83)	6.0 (1.41)	8.5 (0.71)	9.8 (0.96)	9.8 (0.96)	7.9 (2.29)
Median	5	6	6	9	10	10	9
Min, Max	5, 5	4, 8	5, 7	8, 9	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.

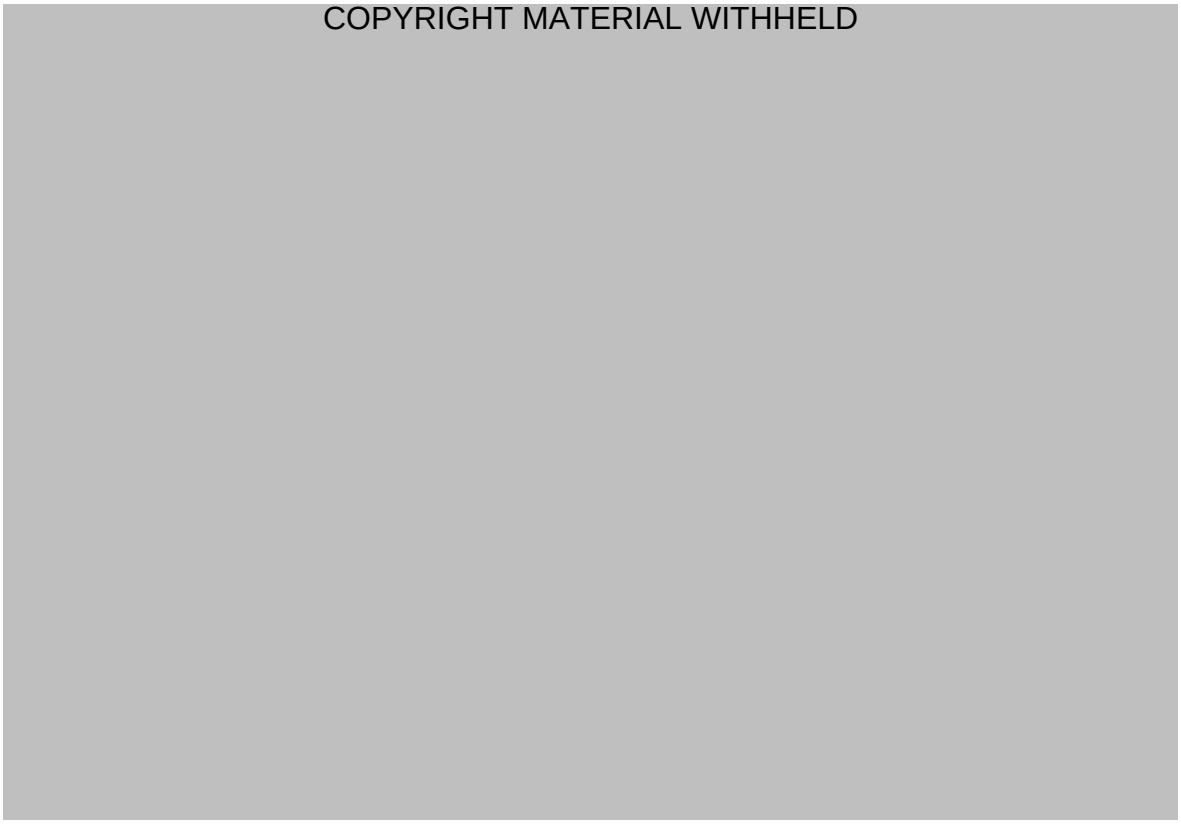
Table 30 Patient Level Biomarker Data for Study 28 (Safety Population)

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
Percent "Positive" Fibers"																			
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
Mean Fluorescence /Fiber (% Normal)																			
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
Western Blot Analysis with DYS-1 Antibody (percentage of normal controls)																			
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 α-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.

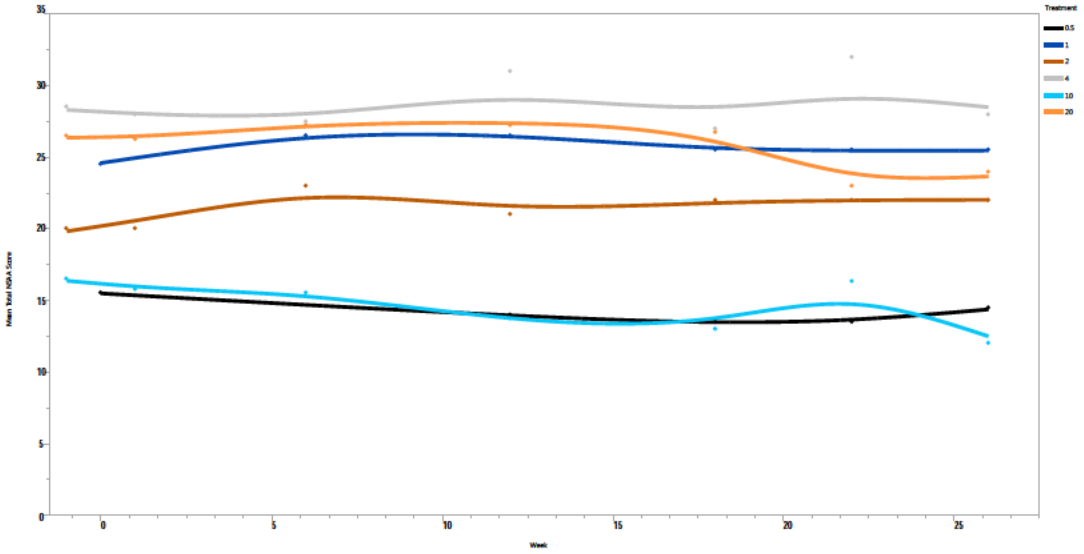
Clinical Tests of Function

The Applicant did not analyze and report the results of the 6MWT and NSAA Total Score, so I have analyzed then 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)

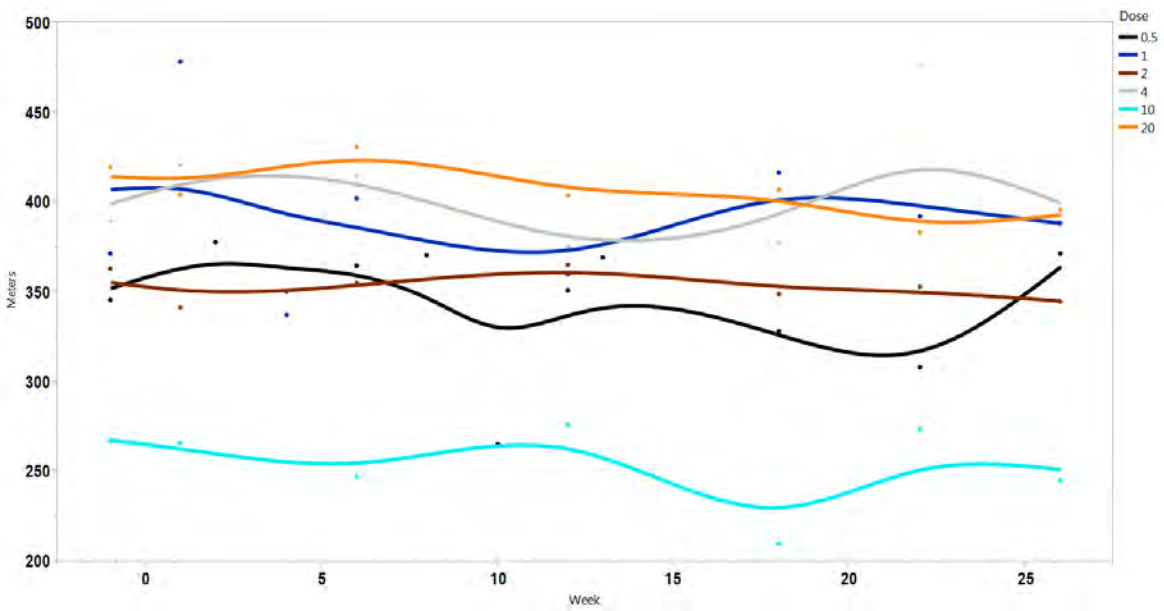


Source: Medical Reviewer's analysis of the Study 28 WA dataset

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[a]					Placebo[a]				
	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[b]	9	100	900	0.90	0	9	100	900	0
	0.225[c]	4	225	900	0.90	0	4	225	900	0

[a] 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.
[b] 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 ≥12 years to ≥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 α-sarcoglycan and β-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

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 4th 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 4th biopsy testing. Hence, the data using the 4th 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 week180 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

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)

			Eteplirsen							
	Placebo (N=4)	Untreated (N=15)	0.09 & 0.9 mg IM (N=7)	≤4 mg/kg IV (N=11)	10 mg/kg IV (N=4)	20 mg/kg IV (N=4)	30 mg/kg IV (N=82)	50 mg/kg IV (N=6)	All IV (N=107)	All Eteplirsen (N=114)
Days on Study Drug [a]										
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
Number of Infusions										
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
Number of Weeks Category										
<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%)
Patient Years at Actual Dose [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**

[a] For the untreated patient population, Days on Study Drug equals post-baseline Days on Study.
[b] 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	Severi	Prior dose	Date Onset / Resolved
33-01-006	eteplirsen 0.9 mg) Single dose	*Wound infection*	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.	*Vomiting*	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.	*Ankle fracture*	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,	*Femur fracture*	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:

Original term	**New Preferred Term**
Cataract Subcapsular	*Cataract*
Abdominal Pain Upper	*Abdominal Pain*
Abdominal Pain Upper	*Abdominal Pain*
Abdominal Pain Upper	*Abdominal Pain*
Abdominal Pain Upper	*Abdominal Pain*
Abdominal Pain Upper	*Abdominal Pain*
Abdominal Pain Upper	*Abdominal Pain*
Abdominal Pain Upper	*Abdominal Pain*
Abdominal Pain Upper	*Abdominal Pain*
Thrombosis In Device	*Device Occlusion*
Thrombosis In Device	*Device Occlusion*
Thrombosis In Device	*Device Occlusion*
Non-Cardiac Chest Pain	*Chest Pain*
Rhinitis	*Nasopharyngitis*
Rhinitis	*Nasopharyngitis*
Rhinitis	*Nasopharyngitis*
Respiratory Disorder	*Upper Respiratory Infection*
Viral Upper Respiratory Tract Infection	*Upper Respiratory Tract Infection*
Femur Fracture	*Fracture*
Foot Fracture	*Fracture*
Foot Fracture	*Fracture*
Lower Limb Fracture	*Fracture*
Radius Fracture	*Fracture*
Post Procedural Haematoma	*Haematoma*
Bone Pain	*Pain In Extremity*

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)

System Organ Class	Preferred term	N Total Events	N (30 mg/kg)	N (50 mg/kg)	N (Placebo)
Gastrointestinal disorders	*Abdominal pain*	4	0	3	1
	Diarrhoea	2	1	1	0
	Vomiting	4	2	2	0
General disorders and administration site conditions	*Catheter site pain*	4	2	2	0
	Device occlusion	3	2	1	0
	Infusion site extravasation	3	1	2	0
	Oedema peripheral	2	2	0	0
Infections and infestations	*Upper respiratory tract infection*	4	1	3	0
Injury, poisoning and procedural complications	*Contusion*	3	2	1	0
	Excoriation	2	1	1	0
	fracture	2	1	1	0
	Joint injury	2	2	0	0
	Muscle strain	2	1	1	0
	Procedural pain	10	3	4	3
Investigations	*Activated partial thromboplastin time prolonged*	2	0	2	0
	Blood creatine phosphokinase increased	2	2	0	0
	C-reactive protein increased	2	0	2	0
Metabolism and nutrition disorders	*Obesity*	2	2	0	0
	Vitamin D deficiency	2	2	0	0
Musculoskeletal and connective tissue disorders	*Arthralgia*	3	1	2	0
	Back pain	6	3	1	2
Nervous system disorders	*Balance disorder*	4	2	2	0
	Headache	7	2	3	2
Renal and urinary disorders	*Proteinuria*	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	*Nasal congestion*	5	3	1	1
	Pharyngeal erythema	2	1	1	0
	Upper respiratory tract congestion	2	2	0	0
Skin and subcutaneous tissue disorders	*Dermatitis contact*	3	2	1	0
	Erythema	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[11]. The most common preferred terms were *Headache, Upper respiratory tract infec*tion (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][a]					
		0 [0]	1 [1 to 2]	2 [3 to 5]	3 [6 to 14	4 [>14]	ND/NR
0.09 mg (N=2)	1	--	--	2 (100%)	--	--	--
	2	--	1 (50%)	--	1 (50%)	--	--
	3	1 (50%)	--	1 (50%)	--	--	--
	14-28[b]	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[b]	5 (100%)	--	--	--	--	--
	120	4 (80%)	1 (20%)	--	--	--	--

ND/NR = not done or not reported
[a] 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).
[b] 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) [11]. 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 ≥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

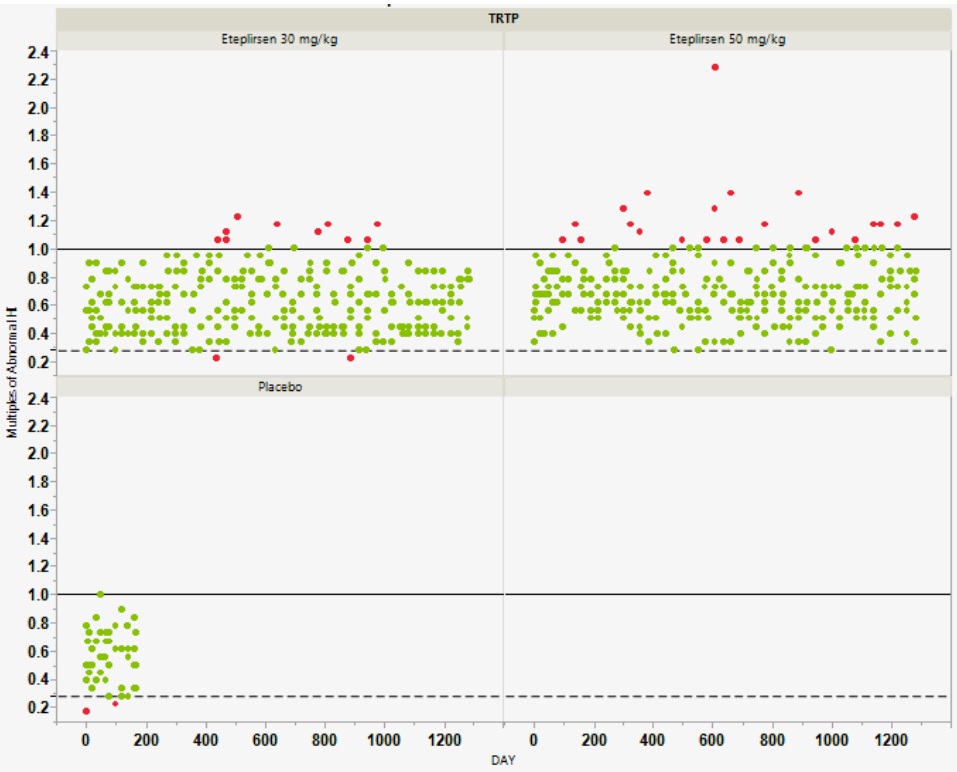
Medical Reviewer's Analyses and Comments

Laboratories in the 201 Study were conducted at the National Children's Hospital for the first 28 weeks and by the CRO (b) (4) 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.

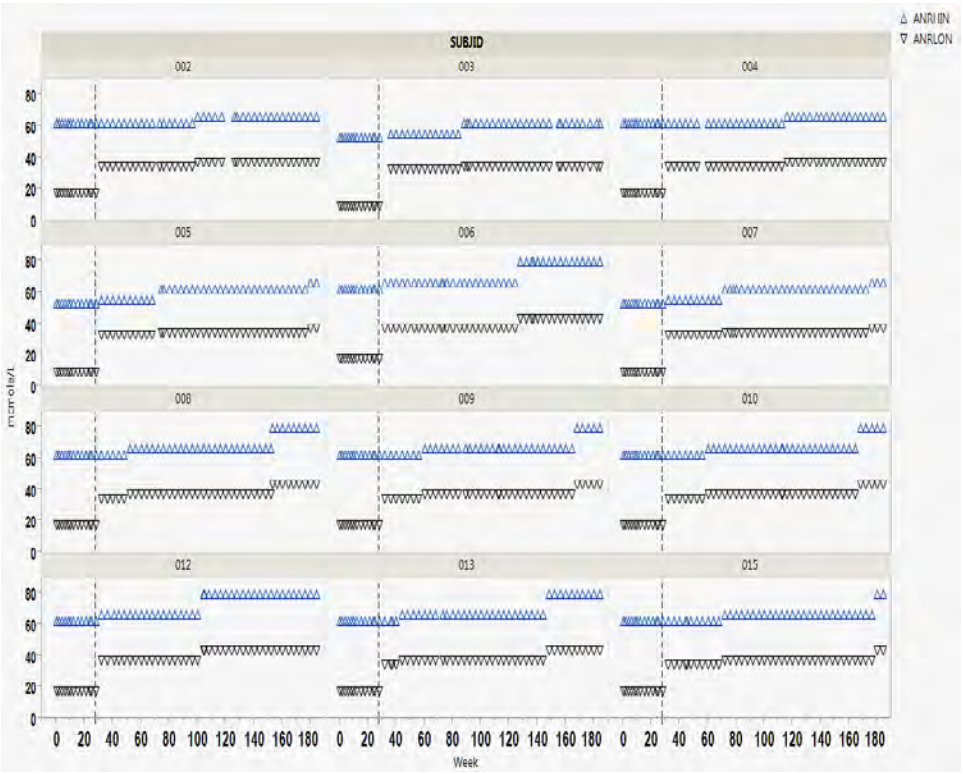
Table 37 Mean Multiples of the Abnormal HI Limit in Subjects with Abnormal BUN (201/202 Safety Population)

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

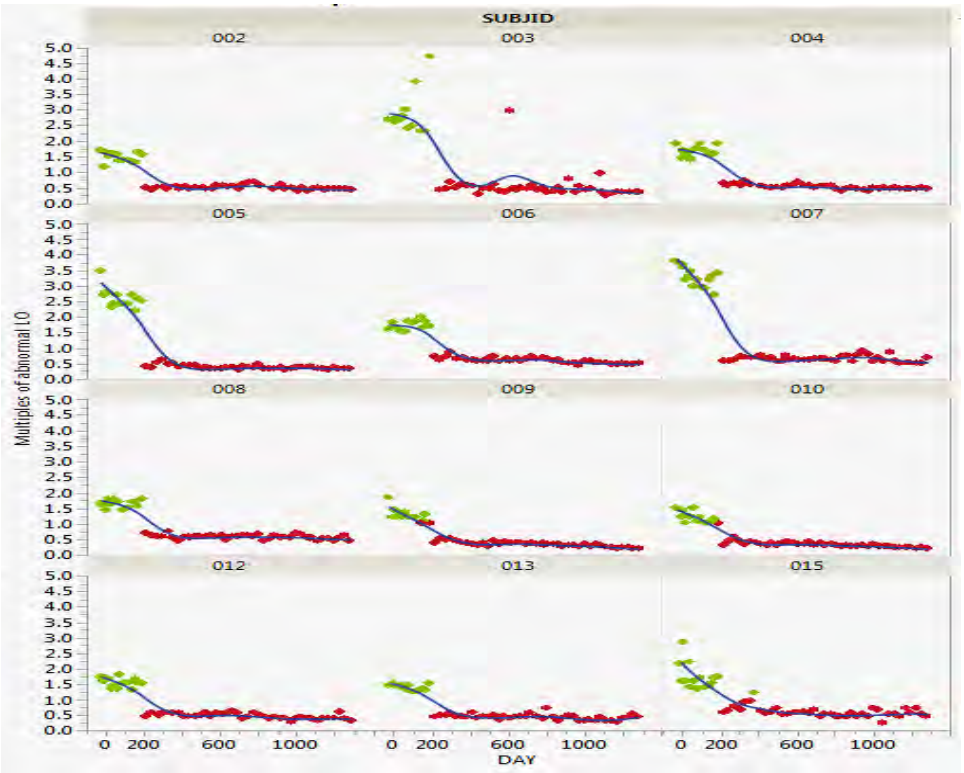
Figure 65 demonstrates the appearance of the Creatinine lab values plotted as the log function to allow better visualization, since there is a floor-effect of abnormal low values between multiples of 1 and 0 versus 1 and no actual limit for HI values)

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 eosinopils 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)

Subjec	Treatment	Multiples of abnormal HI	Value (10^9/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

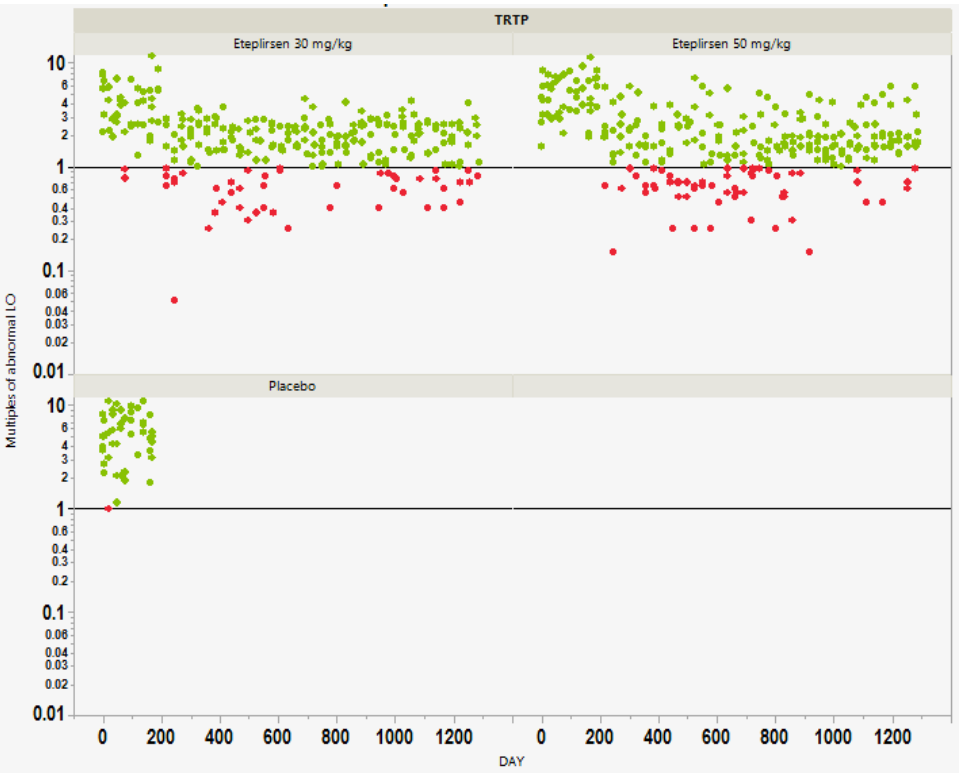
Table 40 Number of Values of the Different Normal Reference Limits for Lymphocytes (201/202 Safety Population)

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**).

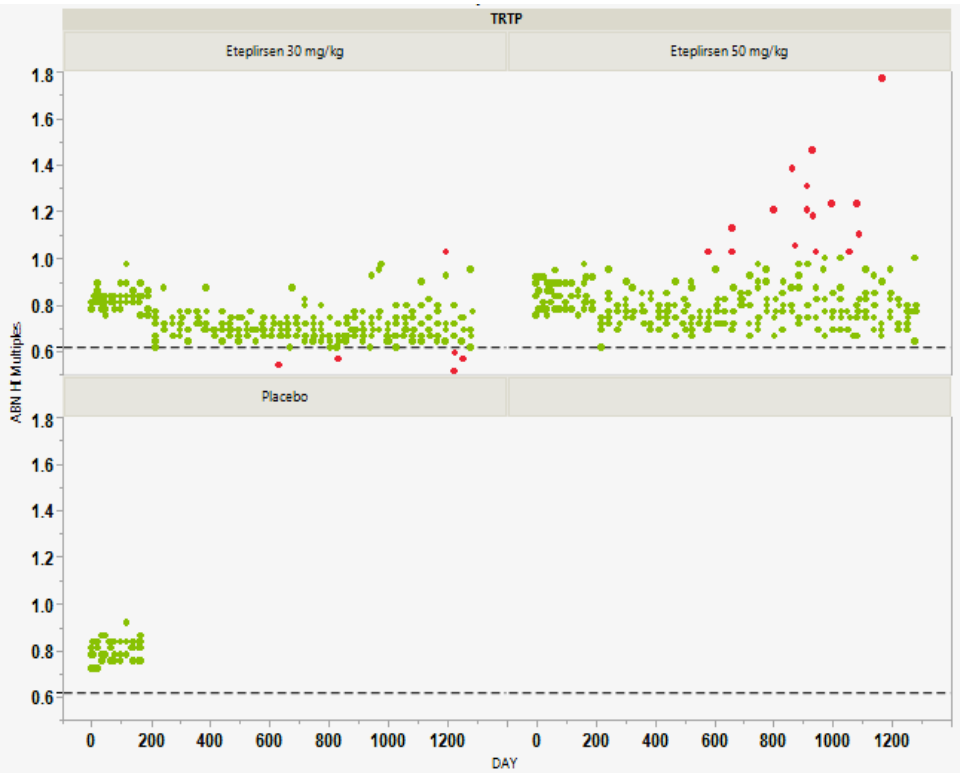
Figure 68 Multiples of the Abnormal HI Reference Limit for Neutrophil Values Versus Time by Treatment (Days) (201/202 Safety Population)

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 RBCvalues

- 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 ± 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)

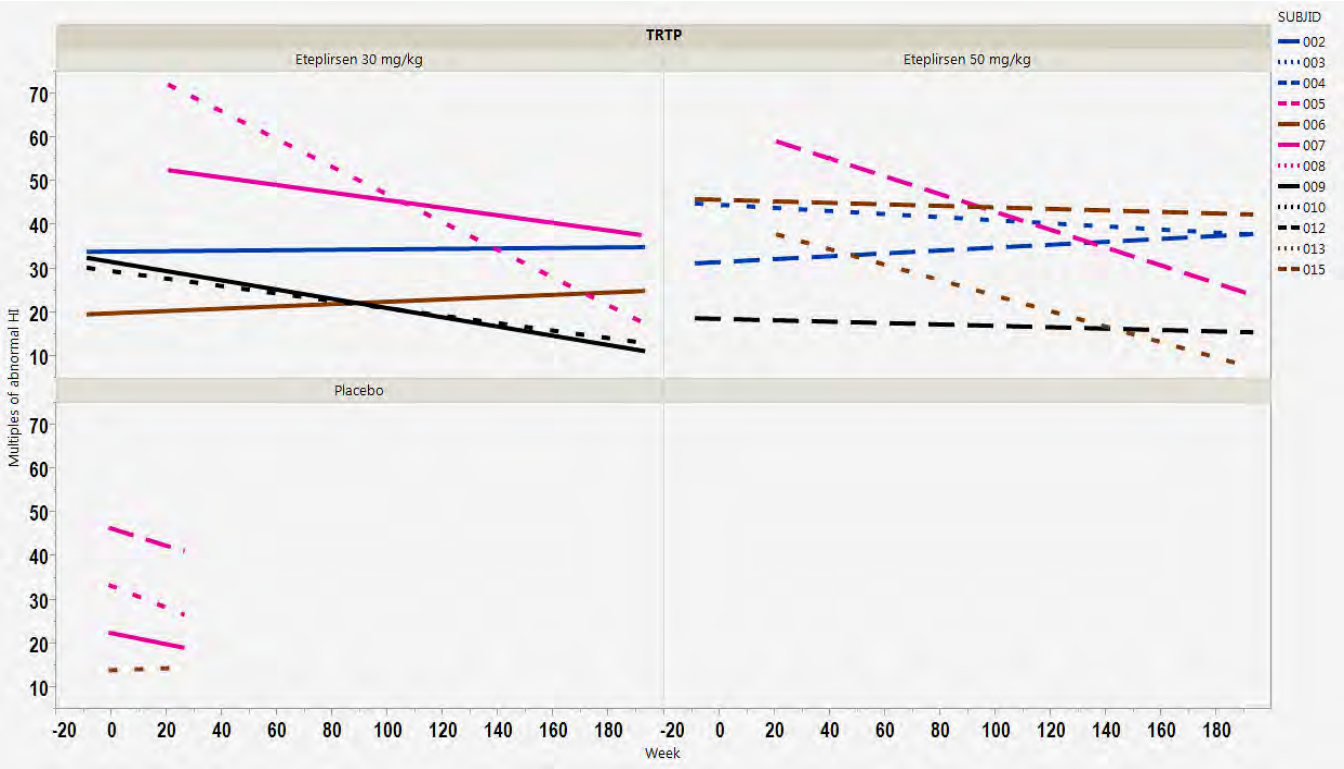
Abnormal LO Reference Limit	Abnormal HI Reference Limit	Number of values assessed based on this limit per Period	
		Period 1	Period 2
0	3	148	4
0.9	1.3	322	159

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)



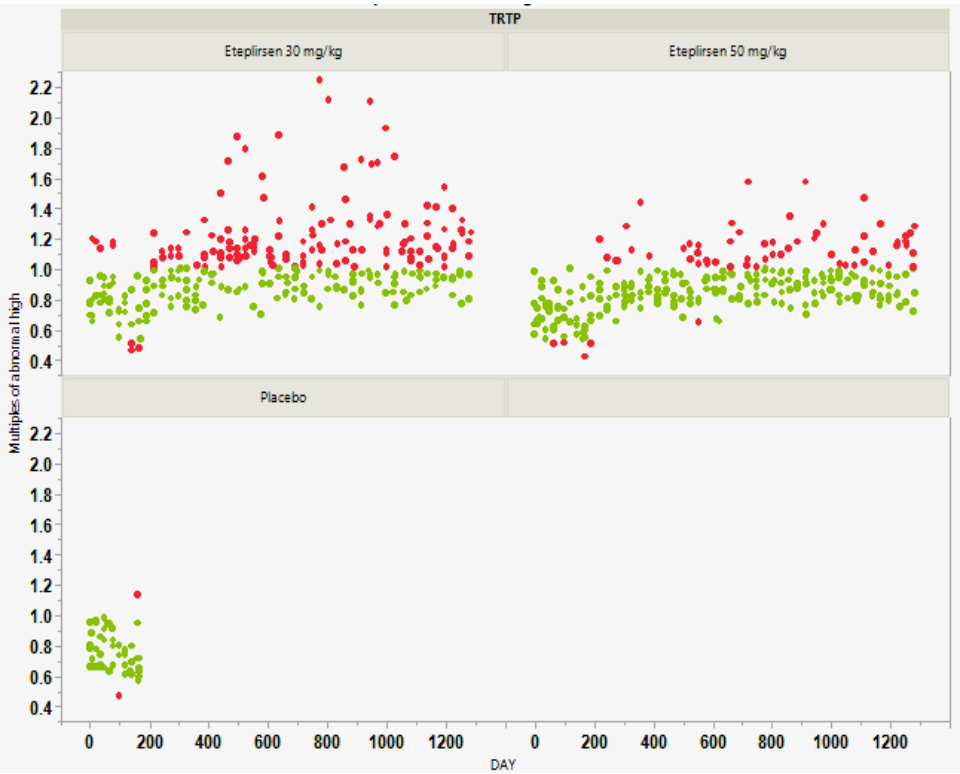
Source: Medical reviewer analysis of ADLB.XPT

- **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)



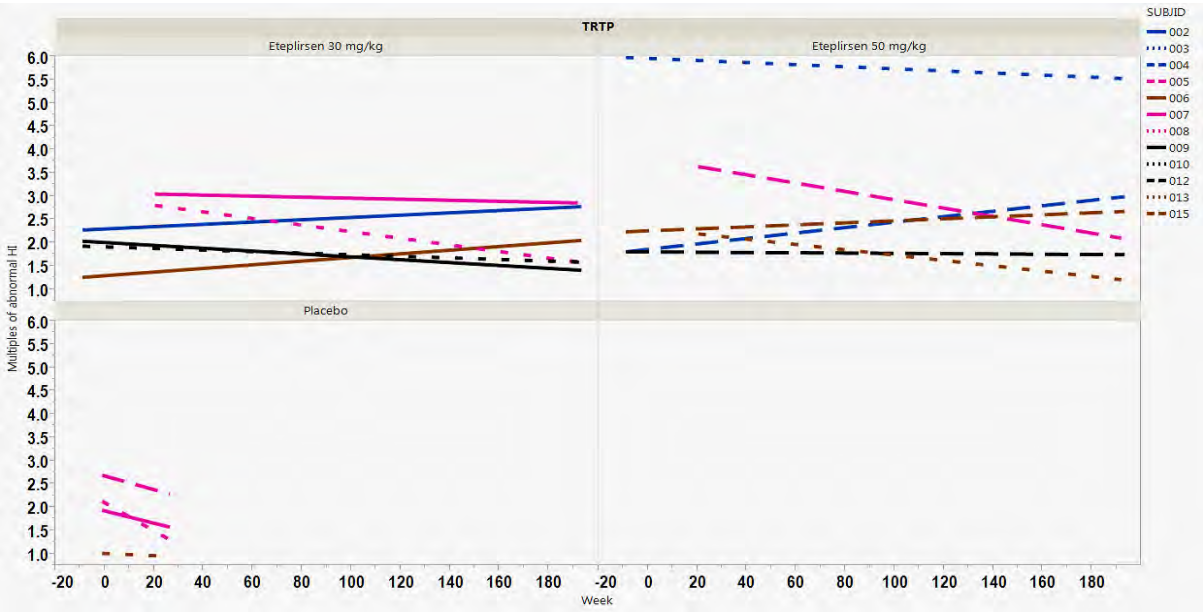
Source: Medical reviewer analysis of ADLB.XPT
Each dot represents a different lab value; green dots are normal and red abnormal

- **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**

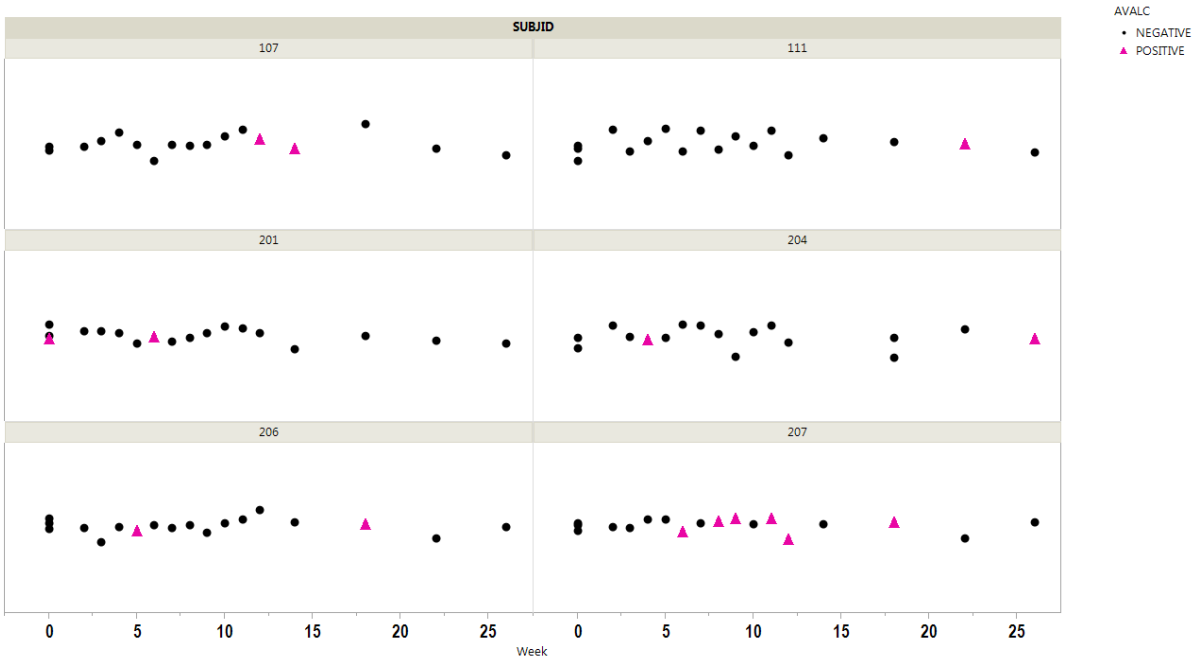
The event of being "positive" for urine myoglobin occurred in 6 subjects in Study 28 who were negative at baseline (**Table 44** and **Figure 74**). Notably, this lab was not performed in other studies.

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
	012	91	42	148	Visit 23
		93		610	Week 60
	013	92	53	1082	Week 127

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.

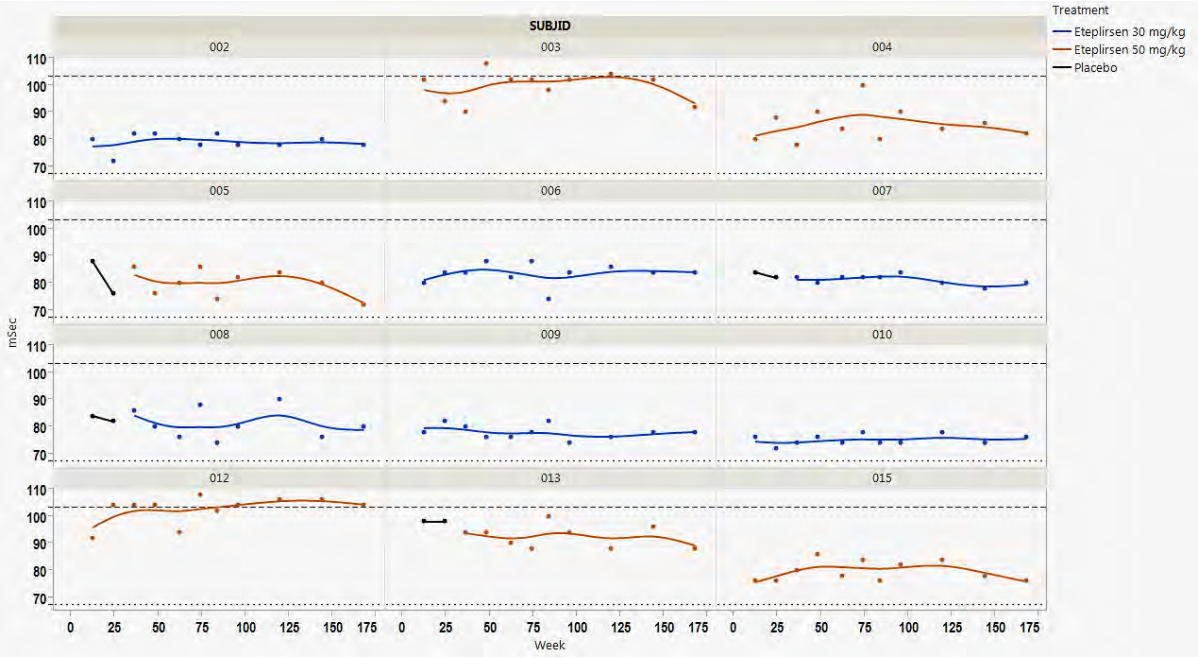
Electrocardiograms – Electrocardiograms (ECGs) in this population were evaluated by this reviewer using age appropriate limits as suggested by [Rijnbeek *et al.* 2001]♦.

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.

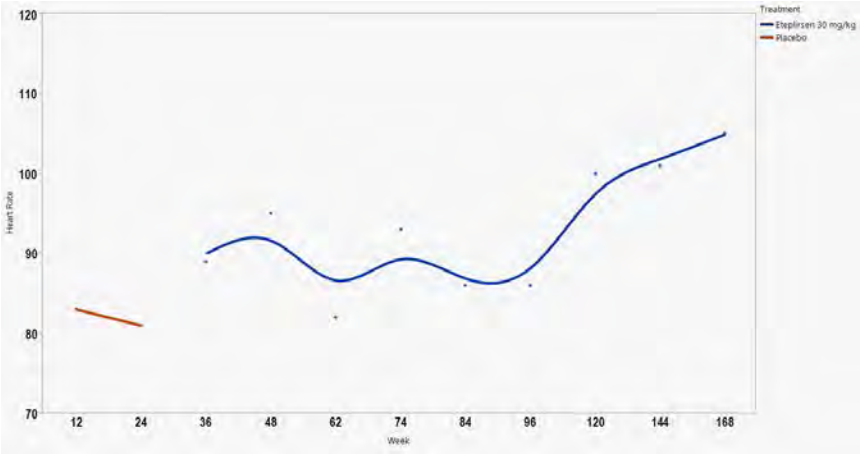
Table 47 Changes from Normal EKG Interpretation to Ventricular Hypertrophy in Study 201 / 202

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	**007** (30 mg/kg)
(N)	(LVH)	6	2	2	2	**005** (Placebo + 50 mg/KG **007** (30 mg/kg)
(N)	(RVH)	8	5	2	1	**006** (30 mg/kg); **007** PBO+30 mg/kg; **012** (50 mg/kg)
(N)	SHORT PR QINF/LAT	1	0	0	1	**005** (Placebo)
(RVH)	(BVH)	9	0	9	0	**003** (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[16]
  - 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

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

[16] 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

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)

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

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	• 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. • 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.
20151217	0028	• 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: o - Height and weight o - Rise time (labeled as Gowers in xlsx)
20151214	0025	• Results for select Week 216 (4.5 year) functional assessments in study 201/202 are presented in tabular format
20151210	0024	• Responses to information requests o Study 201/202 Week 180 western blot study and validation reports: Controls, RSD acceptance criteria, Gel images Quantitation o Start and resolution dates of 9 severe adverse events in Summary of Clinical Safety o Analysis of DMD control sample BLOQ values in Study 201/202 Week 180 western blot report o Study 28 western blot pre-treatment values o Prior dose dates for ankle fracture and femur fracture SAEs; Study 33 myoglobinuria criteria • Anti-dystrophin antibody and urine myoglobin assessments • Study 28 annotated western blot images; PCR product sequencing • Study 33 myoglobinuria criteria; Study 28 anti-dystrophin antibody dataset • Analysis of treated sample BLOQ values in Study 201/202 Week 180 western blot report

Date of Submission	Sequence Number	Content per the Applicant
20151207	0023	• 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 • Sarepta report SR-CR-010, entitled Pulmonary Function Measurements in Eteplirsen-Treated Patients Over 168 Weeks: Comparison to External Control Data and Scientific Literature • 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: o height and weight o - supportive care (physical therapy, orthoses, corrective surgery) o - Rise Time (labeled as Gowers maneuver in xlsx) o - 10-meter run/walk • A newly obtained dataset which contains patient-level supportive care data for the 12 patients participating in Study 4658-us-202
20151102	0021	• newly obtained datasets for subjects in the Italian DMD Registry (i.e. Professor Eugenio Mercuri's registry): o Baseline rise time for all subjects o Additional steroid treatment information for 6 subjects with genotypes amenable to exon skipping therapy o Prior steroid treatment duration and baseline height for 10 subjects with genotypes amenable to exon 51 skipping
20151026	0019	• Response to clinical Information Requests o upper and lower limits of quantitation o orthoses or other devices
20151023	0018	• 120 Day Module 2.7.4 —Summary of Clinical Safety • Datasets, Data Documentation, Programs, and Tables Figures Listings for: o Study 4658-us-202 o Study 4658-203 o Study 4658-204 o Study 4658-us-301 o Integrated Summary of Safety
20151013	0016	• 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.
20151001	0013	• Marked images from immunohistochemistry

Date of Submission	Sequence Number	Content per the Applicant
20150916	0011	• Response to Clinical Information request containing: o Week 180 4th Biopsy datasets
20150828	0009	• Datasets for the fourth biopsy (Tabulation and Analysis) are provided in this amendment. • SAS codes and programs • Clarification in labset variables • Biomarker information from requested by Dr. Rao
20150820	0008	• Response to 20150806 Information Request o Revised define files o Initial clarification of 'supportive care' in the 201/202 study o Promise for marked images o Multiple revised clinical data tables o PK datasets o Information of images used in publications
20150730	0004	• Reports on the 4th biopsy assays o Western blot assessment of dystrophin protein levels o RT-PCR assessment of DMD patient mRNA o BIOQUANT® assessment of dystrophin signal intensity o Scoring of immunofluorescence images for the presence of dystrophin positive muscle fibers
20150724	0003	• Week 192 (4 year) functional assessments in study 201 /202
20150626	0001	• Final submission of Rolling NDA o the complete clinical content contained in Modules 2 and 5
20150520	0000	• Nonclinical and chemistry, manufacturing and controls content submission for original NDA

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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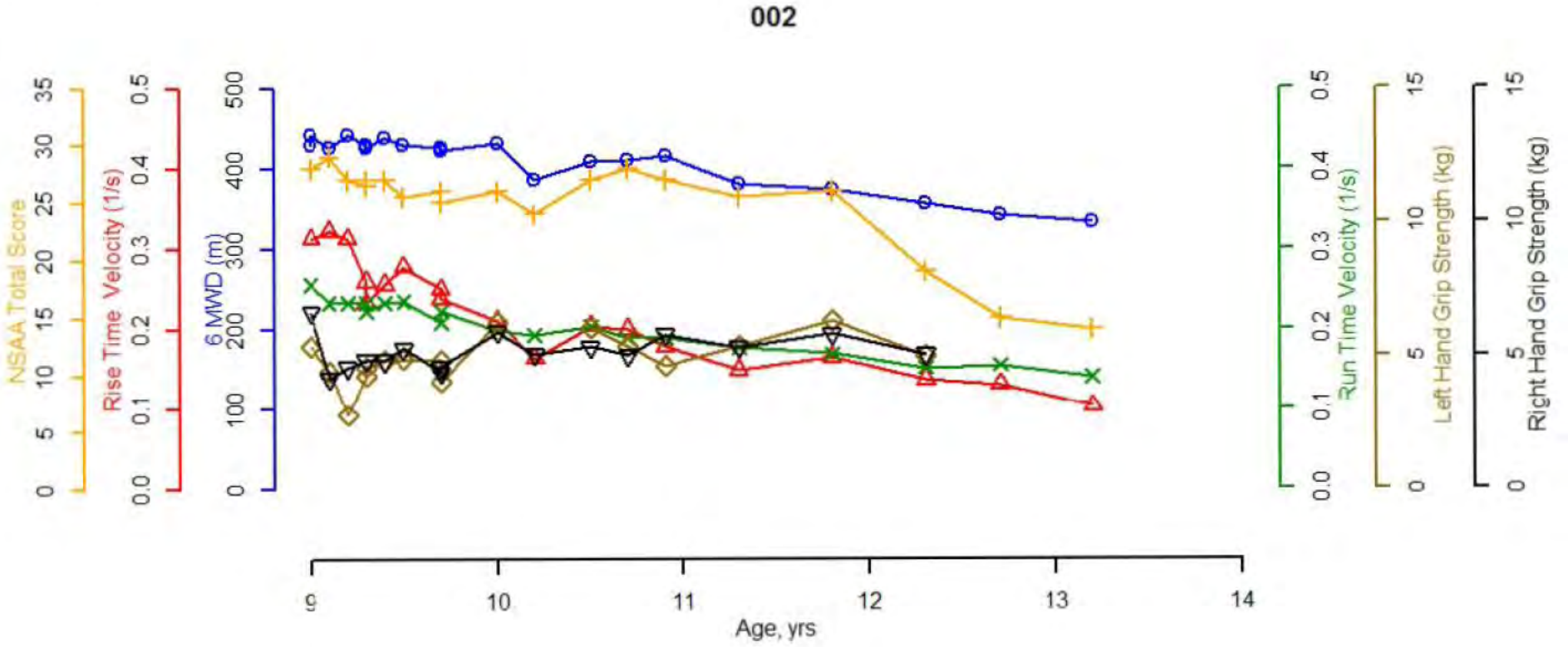

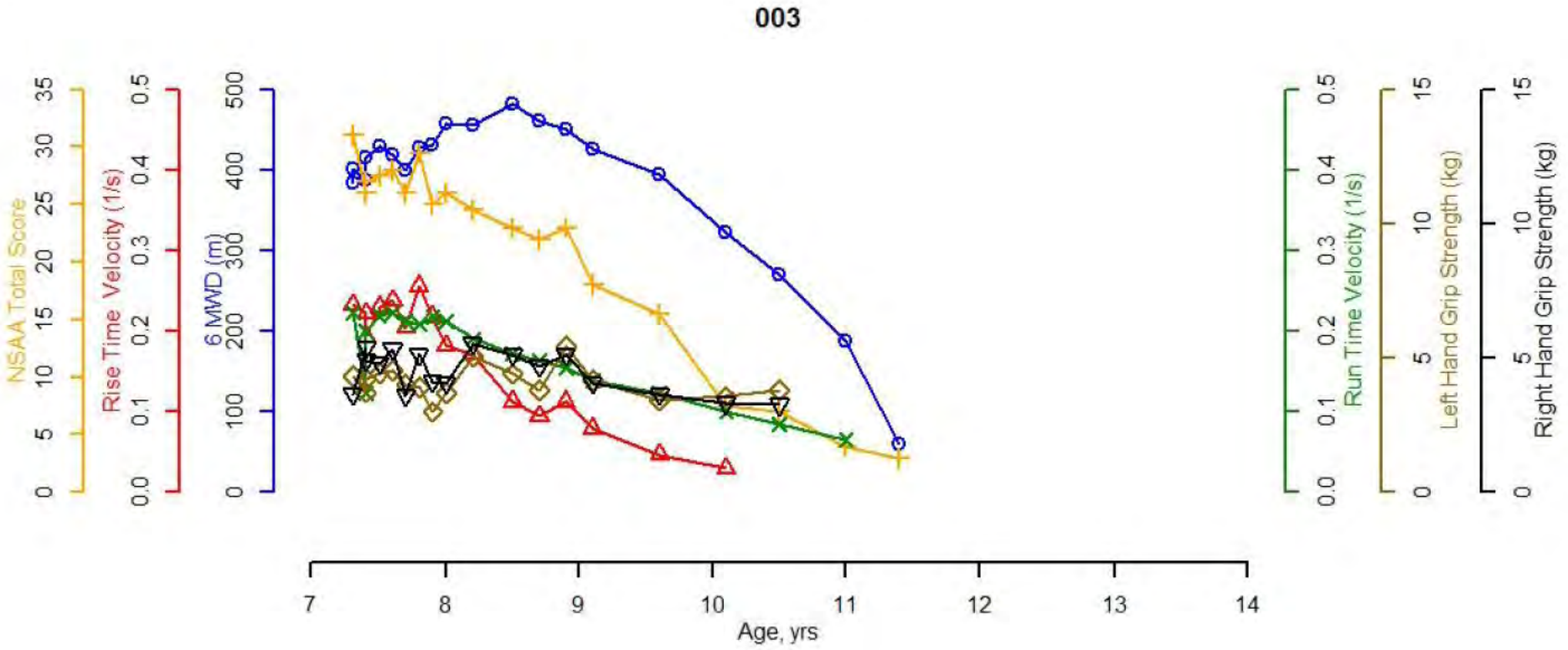

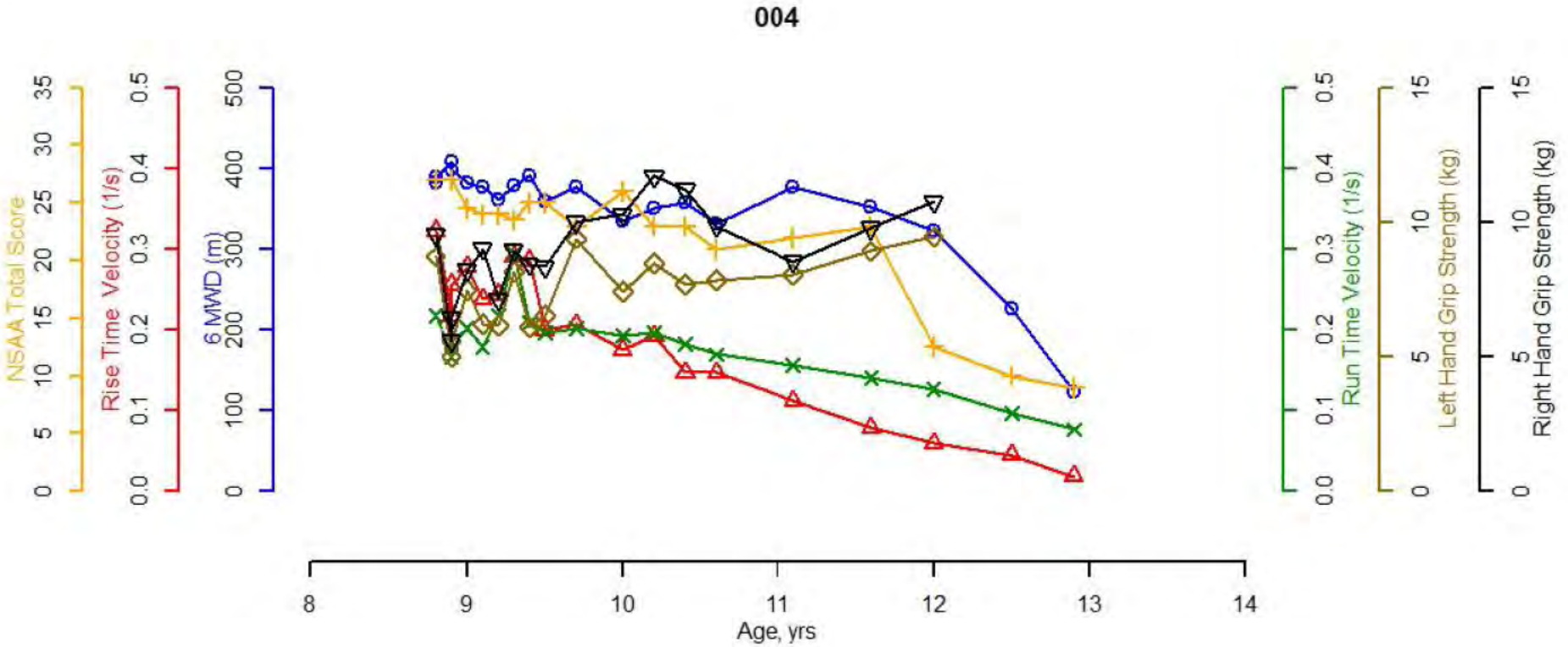

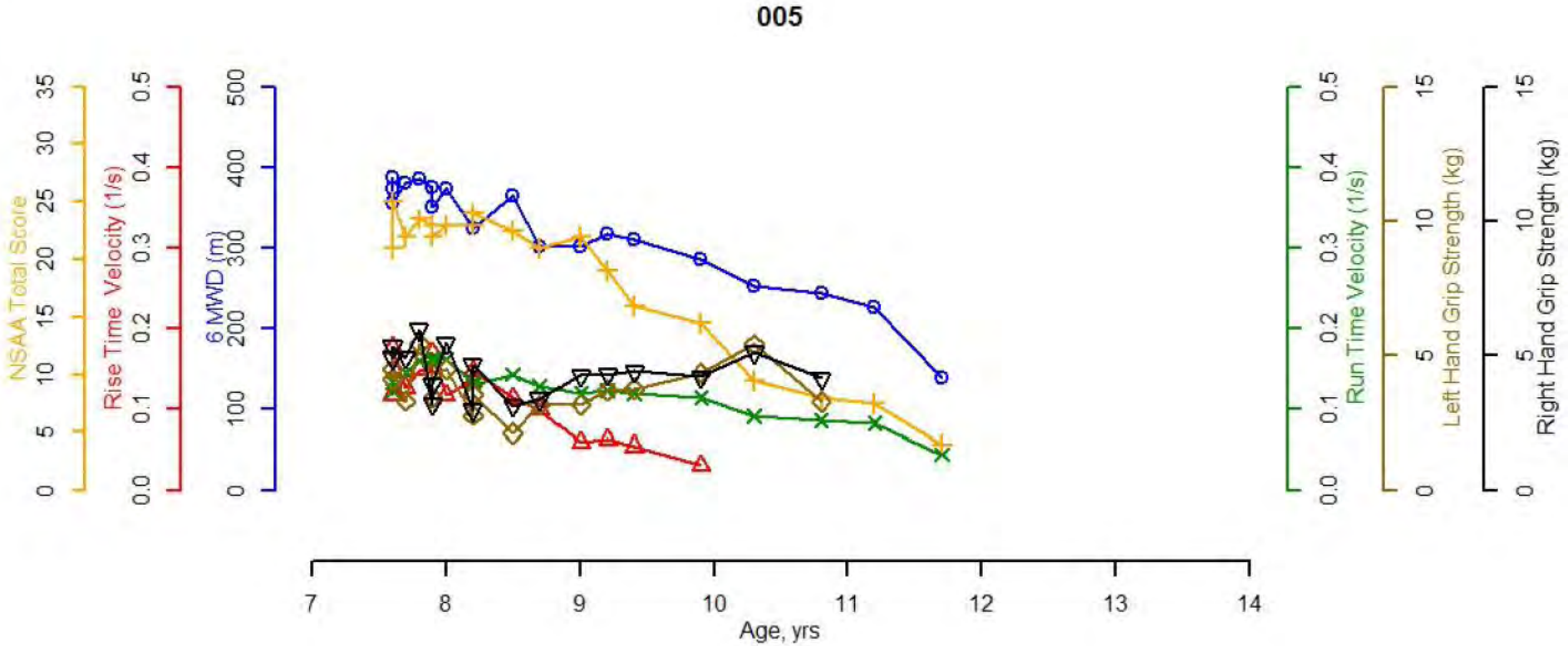

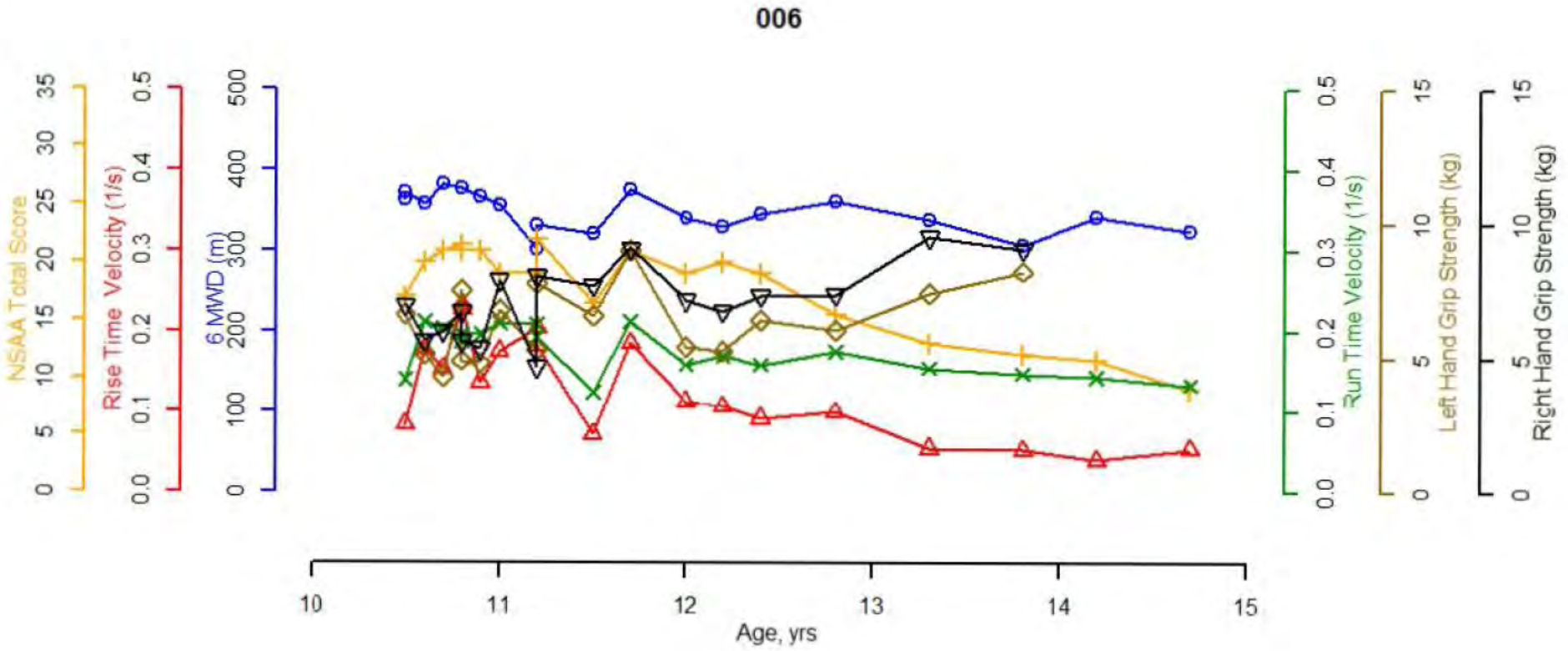

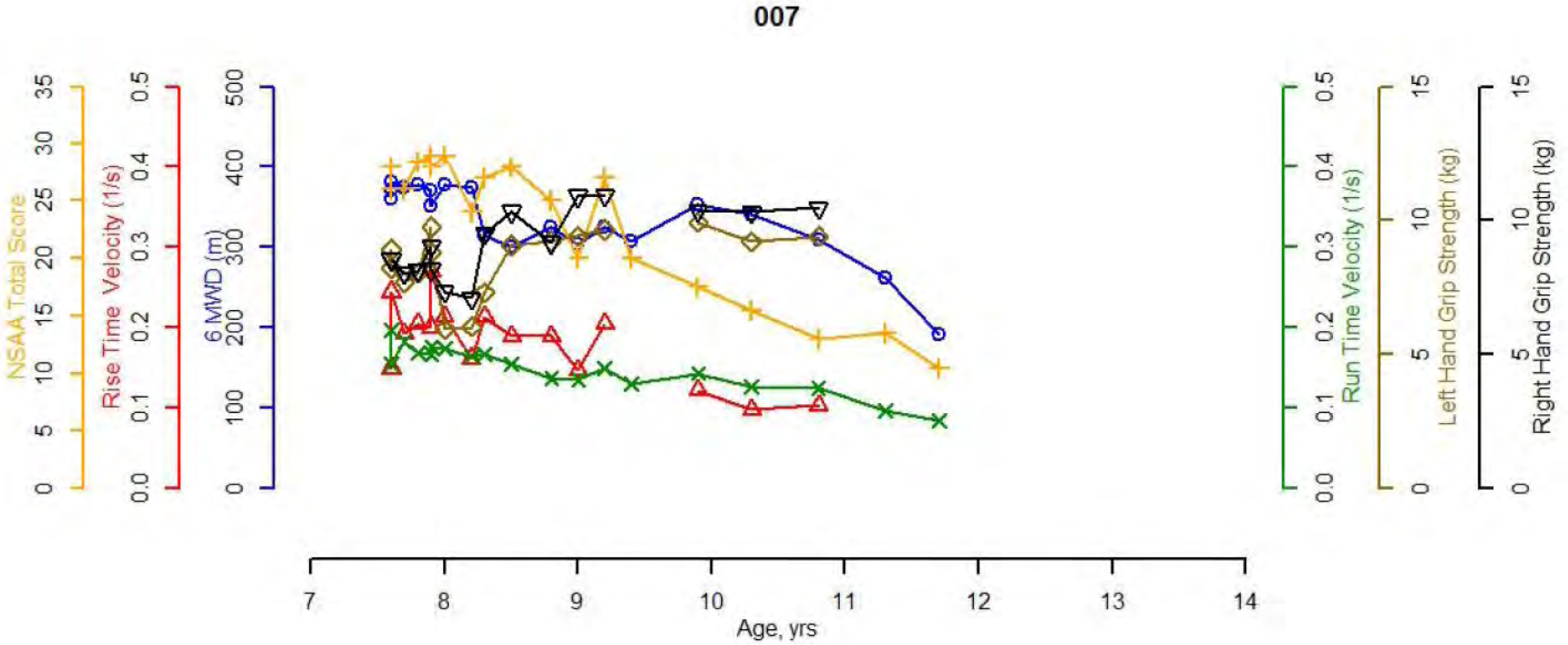

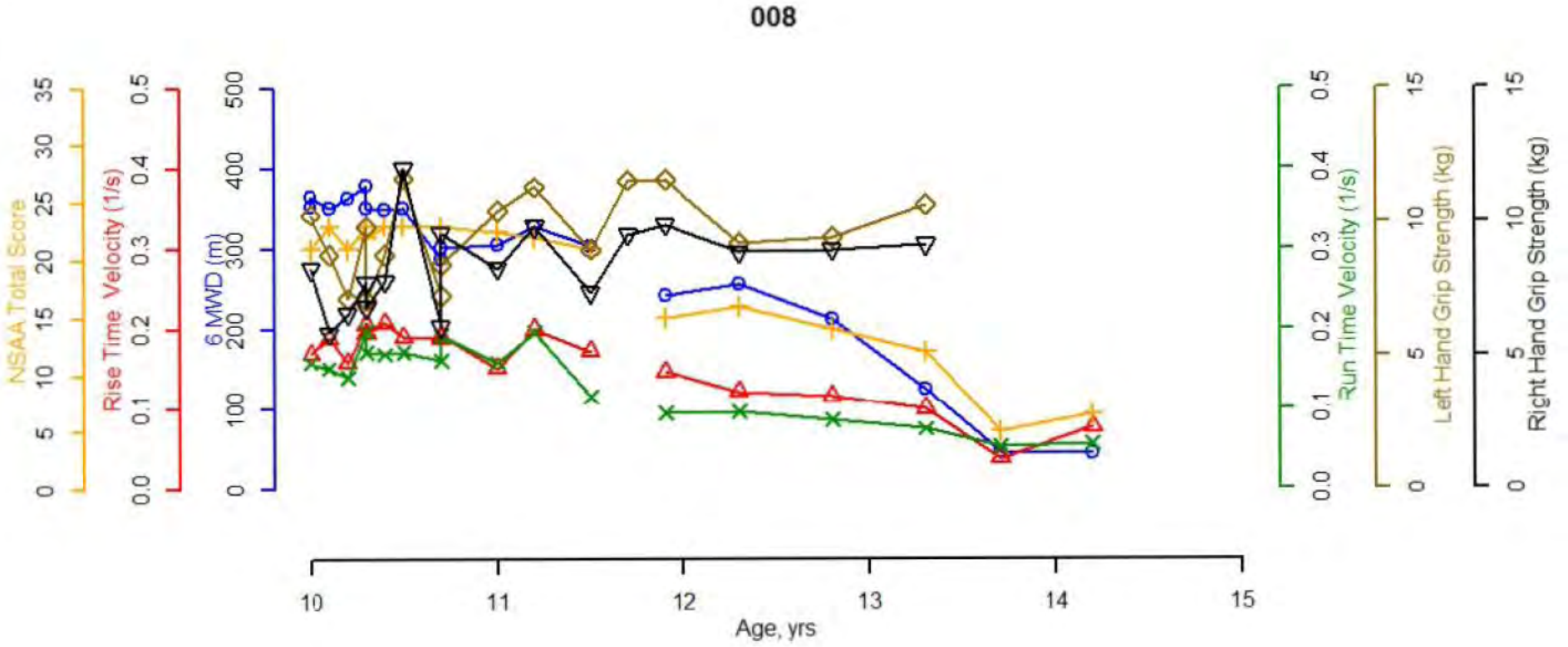

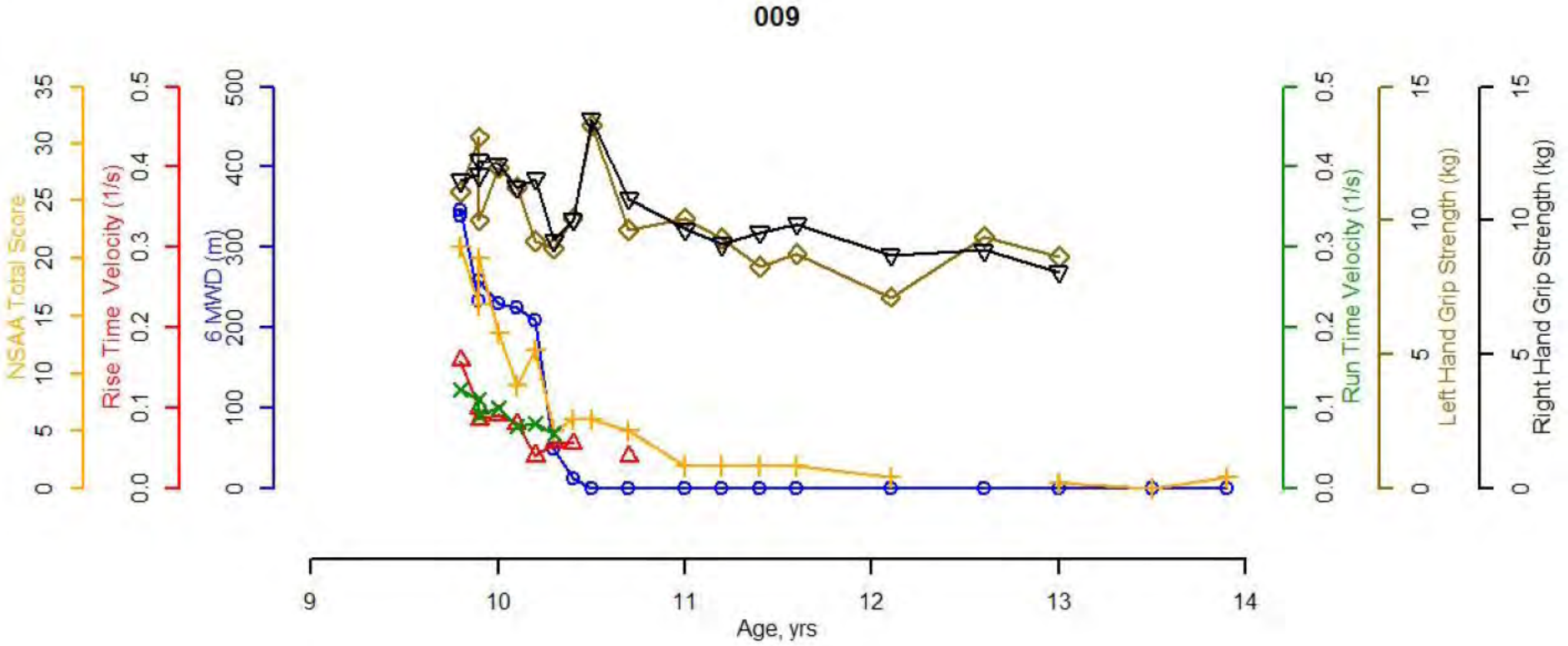

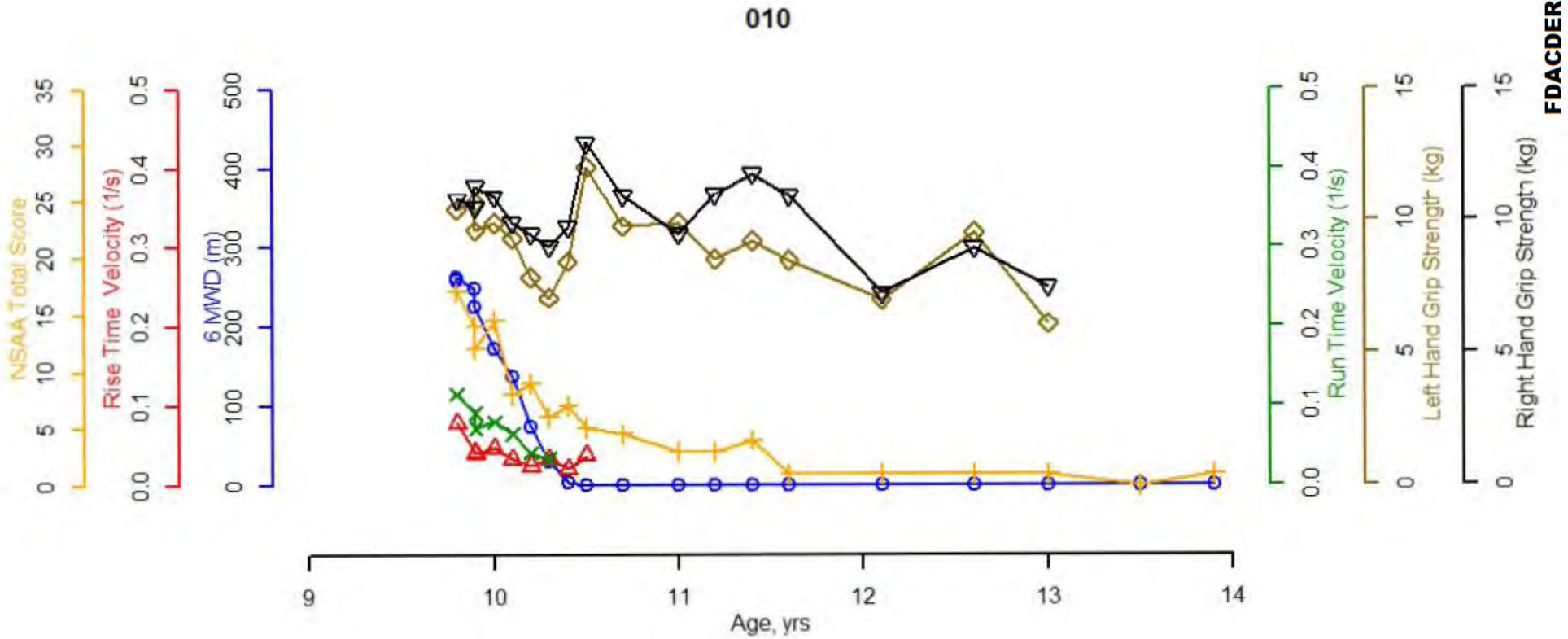

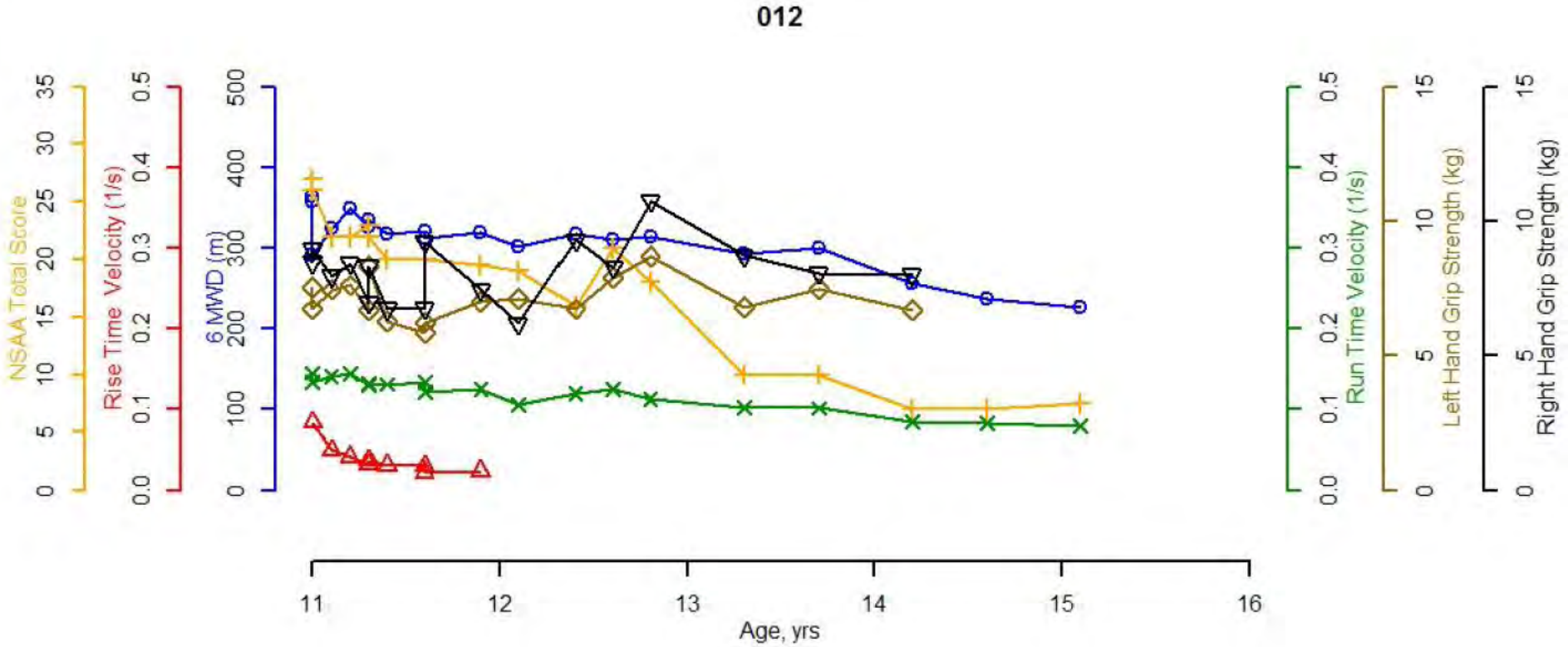

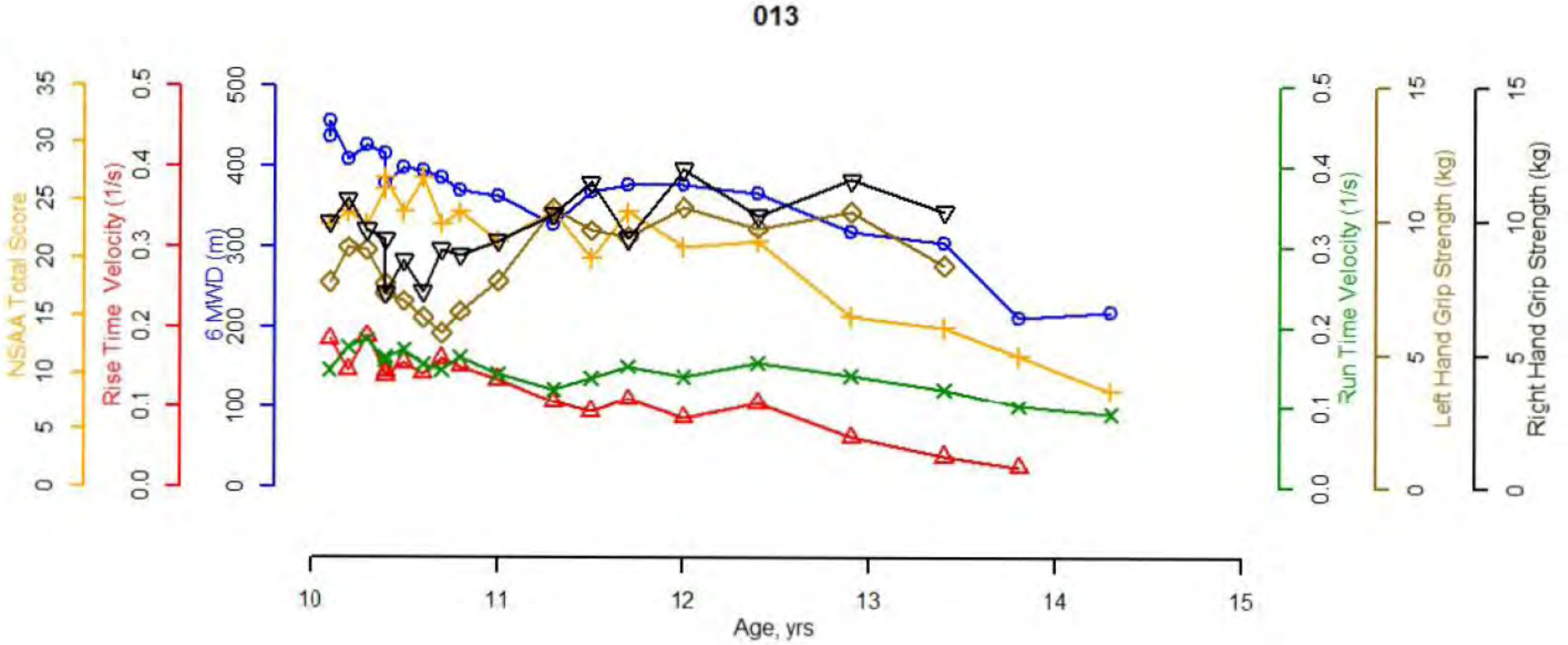

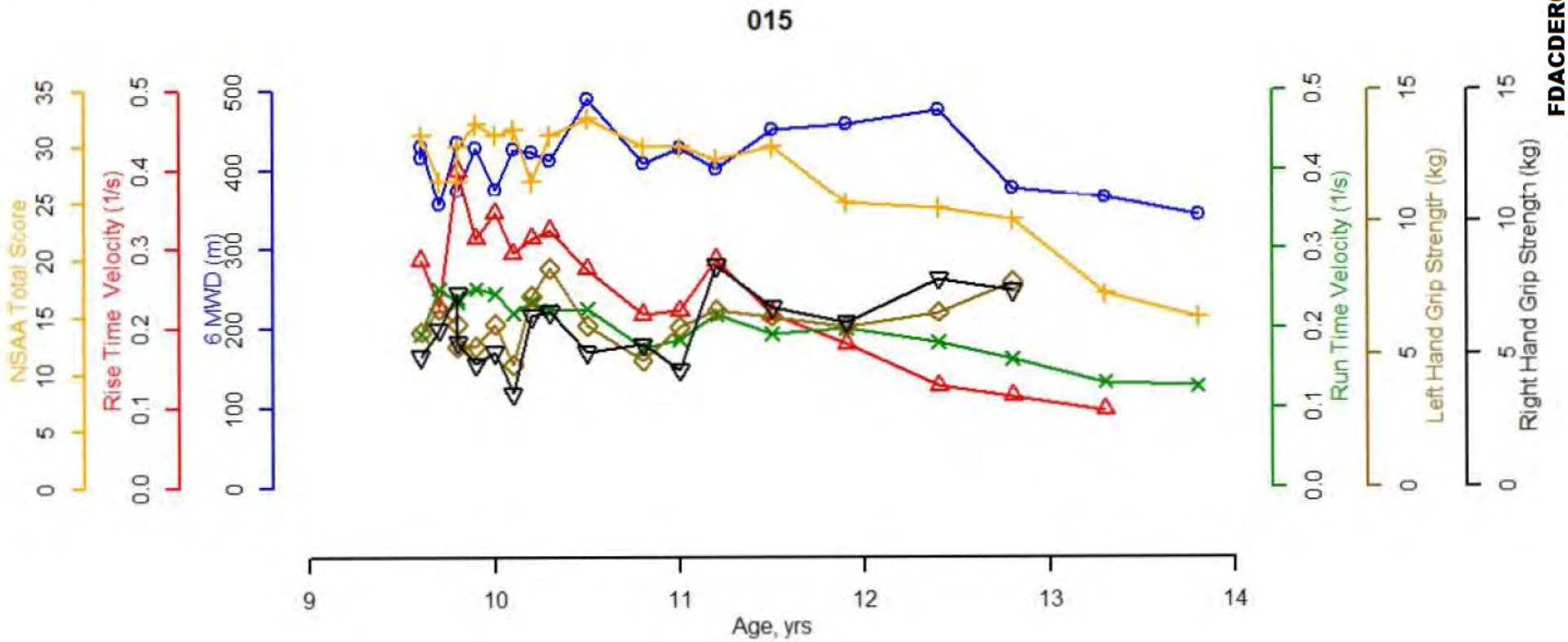

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/s/

CHRISTOPHER D BREDER
05/09/2016

V ASHUTOSH RAO
05/09/2016

RONALD H FARKAS
05/09/2016

Division Director Summary Review for Regulatory Action

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

Material Reviewed/Consulted OND Action Package, including:	**Names of discipline reviewers**
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

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• 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. • 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. • 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,	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.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	muscles of the heart and diaphragm progressively weaken during adolescence, and patients often die from cardiac or respiratory failure in their early 20s.	
Current Treatment Options	• 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.	There is a substantial unmet need for therapies in DMD.
Benefit	• 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). • Study 201 was negative. • 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	The applicant has not provided substantial evidence of efficacy from adequate and well controlled trials to support "conventional" approval. 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.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	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. • 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%)	

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	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.	
Risk	• The clinical safety database for eteplirsen is small: 114 total patients exposed, with only 36 exposed for ≥24 weeks and 12 exposed for ≥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.	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.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• 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.	
Risk Management	• 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.	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 (≈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) (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

Tissue	*Average % Exon 51 Splicing ± 1 SD*				
	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[1] 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)

[1] 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.

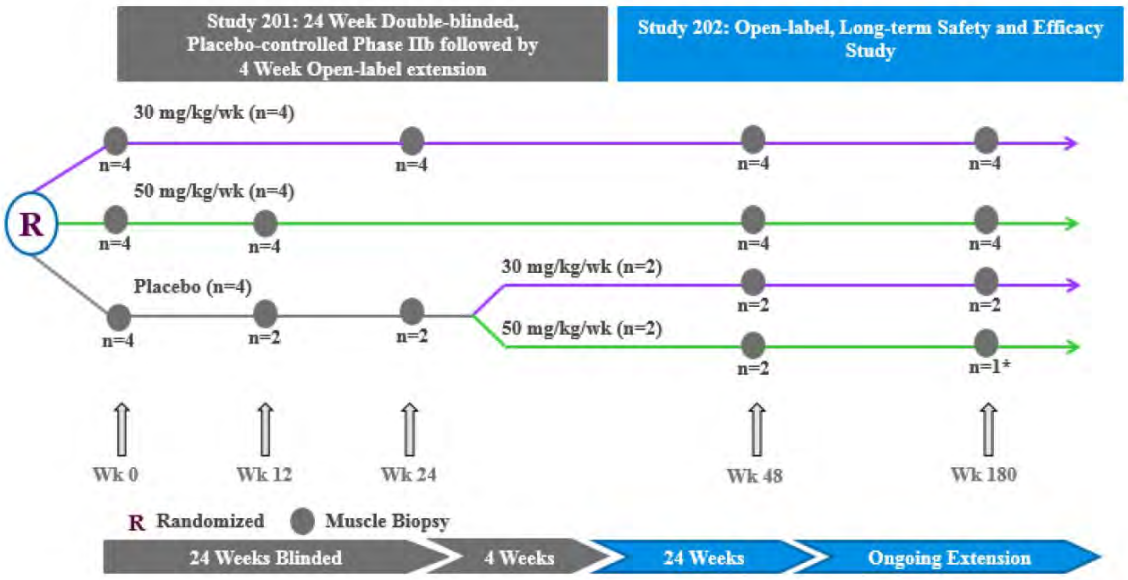
Study 33 was an exploratory study in which small doses of eteplirsen (up to 0.9 mg) were injected directly into a foot muscle in seven patients with DMD. The study showed a clear dose-response in mRNA expression and dystrophin production, with no effect at the initial dose tested, strongly supporting 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[2].
- 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.

[2] 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,[3] which stated the "*…percentage of dystrophin-positive fibers was increased to 23% of normal; no increases were detected in placebo-treated patients (p≤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

[3] *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.[4]

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.

[4] 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.[3] 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 fiver 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[5]"), 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

[5] 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
			P = 0.041	

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
			P = 0.023	

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
			P = 0.008	

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		
		SR-CR-16-003_GEL#2_DYS1_30MIN.TIF	2	FORD-22559	PASS	0.11			
	Week 48	SR-CR-16-003_GEL#1_DYS1_30MIN.TIF	1	CHEVY-22559	PASS	0.22	0.26	0.13	1.96
		SR-CR-16-003_GEL#2_DYS1_30MIN.TIF	2	CHEVY-22559	PASS	0.29			
301-02	Baseline	SR-CR-16-003_GEL#3_DYS1_20MIN.TIF	3	CHEVY-27336	PASS	0.35	0.35		
		SR-CR-16-003_GEL#4_DYS1_30MIN.TIF	4	CHEVY-27336	FAIL	0.26			
	Week 48	SR-CR-16-003_GEL#3_DYS1_20MIN.TIF	3	FORD-27336	PASS	0.36	0.36	0.01	1.03
		SR-CR-16-003_GEL#4_DYS1_30MIN.TIF	4	FORD-27336	FAIL	0.12			
301-03	Baseline	SR-CR-16-003_GEL#5_DYS1_30MIN.TIF	5	FORD-24422	PASS	0.06	0.06		
		SR-CR-16-003_GEL#6_DYS1_30MIN.TIF	6	FORD-24422	PASS	0.06			
	Week 48	SR-CR-16-003_GEL#5_DYS1_30MIN.TIF	5	CHEVY-24422	PASS	0.50	0.37	0.31	6.17
		SR-CR-16-003_GEL#6_DYS1_30MIN.TIF	6	CHEVY-24422	PASS	0.24			
301-04	Baseline	SR-CR-16-003_GEL#7_DYS1_30MIN.TIF	7	FORD-27138	PASS	0.04	0.04		
		SR-CR-16-003_GEL#8_DYS1_30MIN.TIF	8	FORD-27138	FAIL	0.06			
	Week 48	SR-CR-16-003_GEL#7_DYS1_30MIN.TIF	7	CHEVY-27138	PASS	0.10	0.10	0.06	2.50
		SR-CR-16-003_GEL#8_DYS1_30MIN.TIF	8	CHEVY-27138	FAIL	0.19			
301-05	Baseline	SR-CR-16-003_GEL#9_DYS1_30MIN.TIF	9	FORD-28500	FAIL	0.10	0.17		
		SR-CR-16-003_GEL#10_DYS1_30MIN.TIF	10	FORD-28500	PASS	0.17			
	Week 48	SR-CR-16-003_GEL#9_DYS1_30MIN.TIF	9	CHEVY-28500	FAIL	0.92	1.02	0.85	6.00
		SR-CR-16-003_GEL#10_DYS1_30MIN.TIF	10	CHEVY-28500	PASS	1.02			
301-06	Baseline	SR-CR-16-003_GEL#11_DYS1_20MIN.TIF	11	CHEVY-24986	PASS	0.37	0.37		
		SR-CR-16-003_GEL#12_DYS1_30MIN.TIF	12	CHEVY-24986	FAIL	0.46			
	Week 48	SR-CR-16-003_GEL#11_DYS1_20MIN.TIF	11	FORD-24986	PASS	0.30	0.30	-0.07	0.81
		SR-CR-16-003_GEL#12_DYS1_30MIN.TIF	12	FORD-24986	FAIL	0.29			
301-07	Baseline	SR-CR-16-003_GEL#13_DYS1_15MIN.TIF	13	FORD-20841	FAIL	0.04	0.17		
		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.
Source: P:\Sarepta\DMD\301\Programs\Analysis\Production\Listings\l_wb.sas, Date/time of run: 24JUN2016:10:59

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			
		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	1.57	1.33	6.54
		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 15.MIN.TIF	20	FORD-29648	FAIL	0.05			
	Week 48	SR-CR-16-003_GEL#19_DYS1_15.MIN.TIF	19	CHEVY-29648	PASS	0.12	0.12	0.01	1.09
		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	0.47	0.43	10.44
		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	0.09	0.07	4.50
		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	0.21	0.03	1.17
		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.
Source: P:\Sarepta\DMD\301\Programs\Analysis\Production\Listings\l_wb.sas, Date/time of run: 24JUN2016:18:59

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 methodoligical 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 estmates 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%¥	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%)

¥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[6] 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 ≥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

[6] End-of-Phase 2 meeting of March 13, 2013.

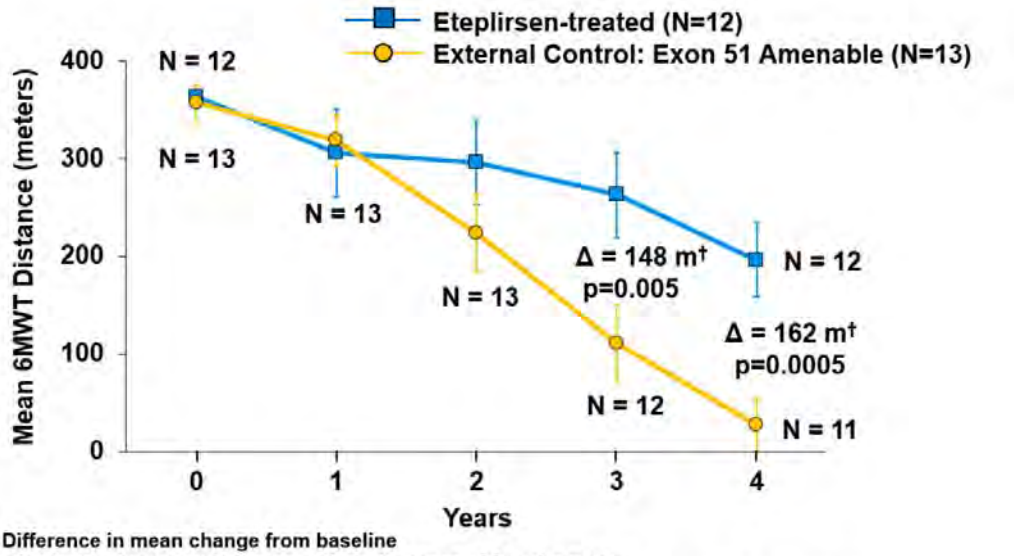
and Related Issues in Clinical Trials" – ICH E10 [2000]). FDA identified several issues related to the use of an external natural history for the applicant to address in the NDA. FDA asked the applicant to establish that treatment modalities, including the physical therapy programs and steroid regimens 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)



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)

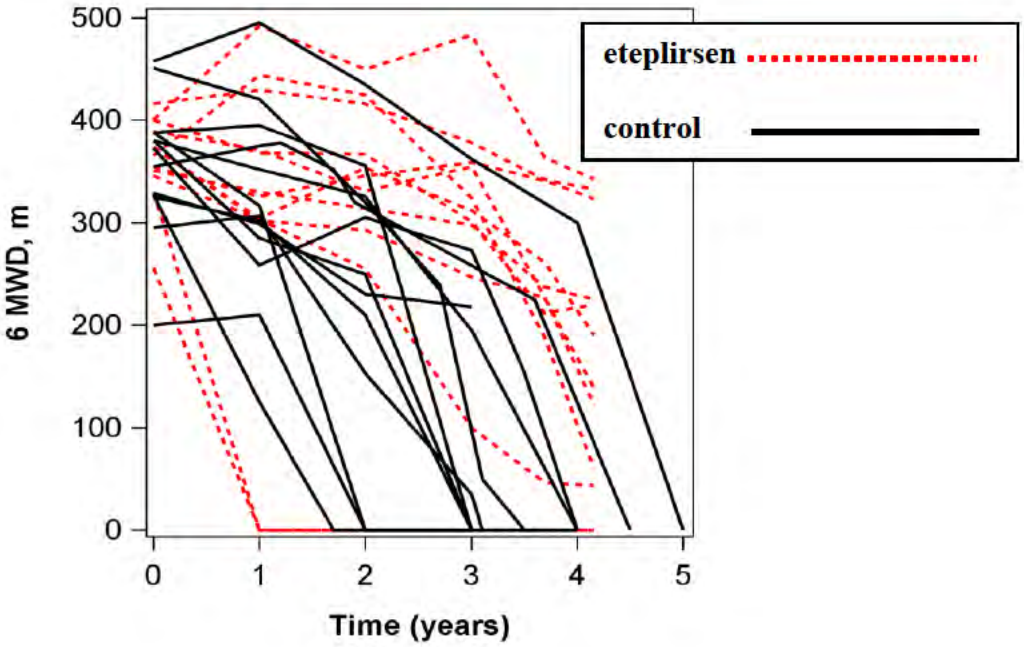
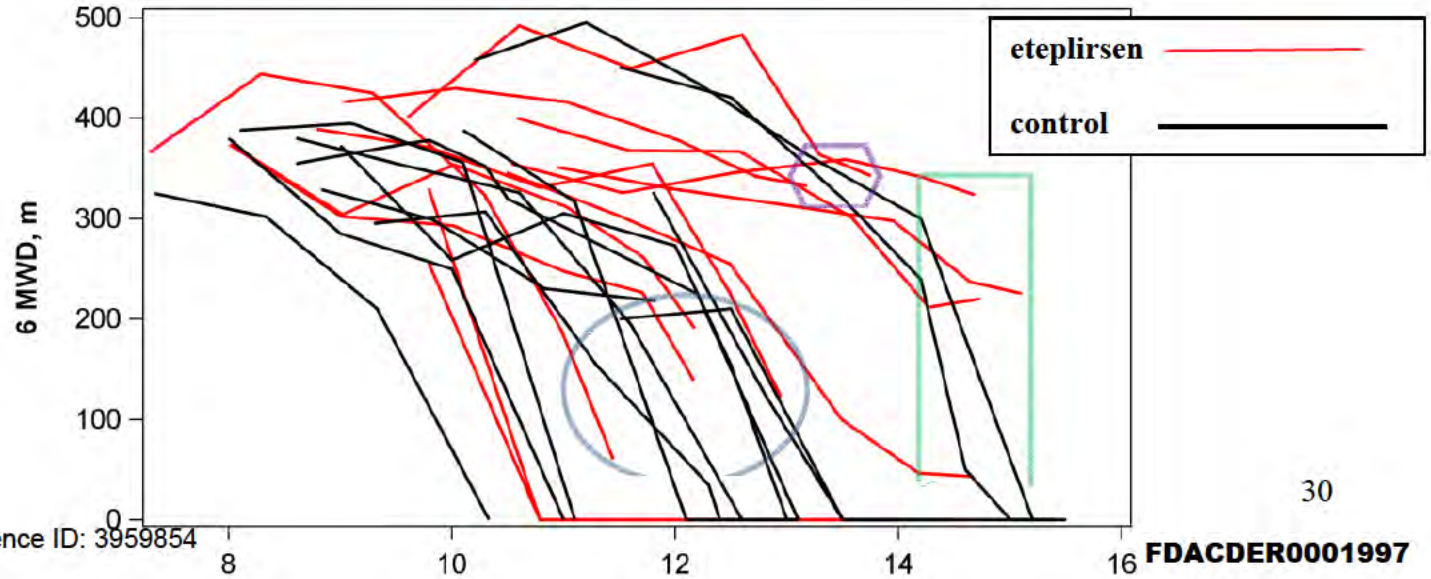


Figure 4: 6MWT distance vs. age in eteplirsen-treated patients in Study 201/202 and external control from the "Italian DMD Registry" and the "Leuven Neuromuscular Reference Center" registry (adapted from Dr. Farkas' memo)



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[7]. 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,

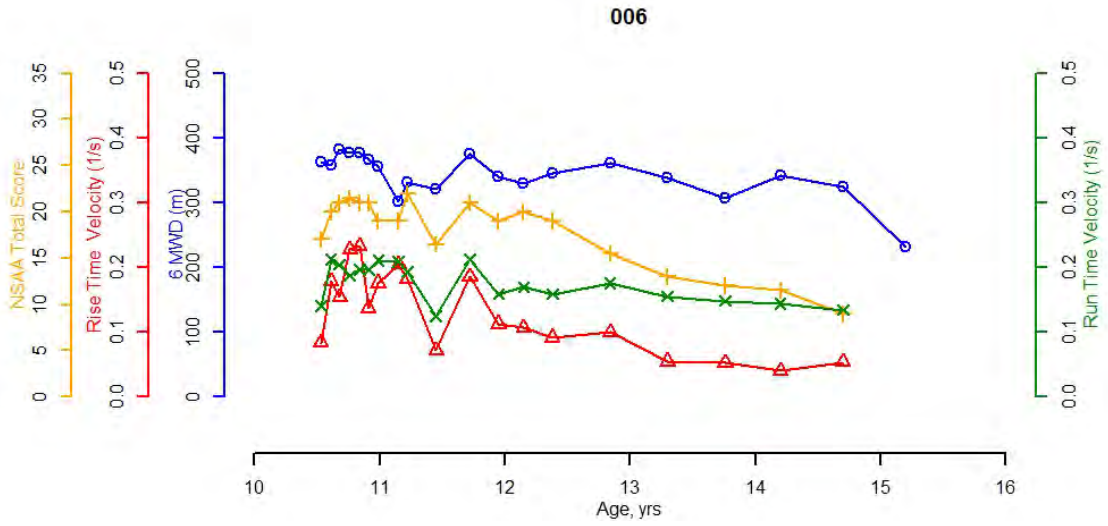
[7] Week 214 assessment, submitted to the NDA in December 2015, is the last one for which we have a complete set of clinical outcome measures. The applicant sent in May 2016 data from the Week 240 assessment limited to the 6MWT. These are presented in individual patient profiles, and have not been incorporated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

natural history data from the CINRG[8] 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[9] (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.

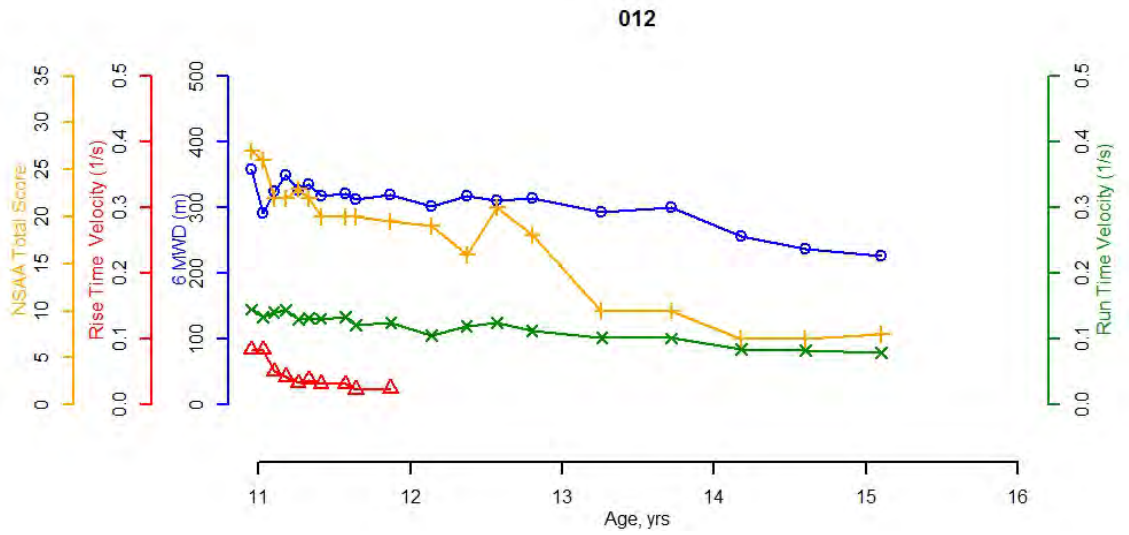
Figure 5: Clinical profile of Patient 006



[8] CINRG: Cooperative International Neuromuscular Research Group http://www.cinrgresearch.org/
[9] 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:

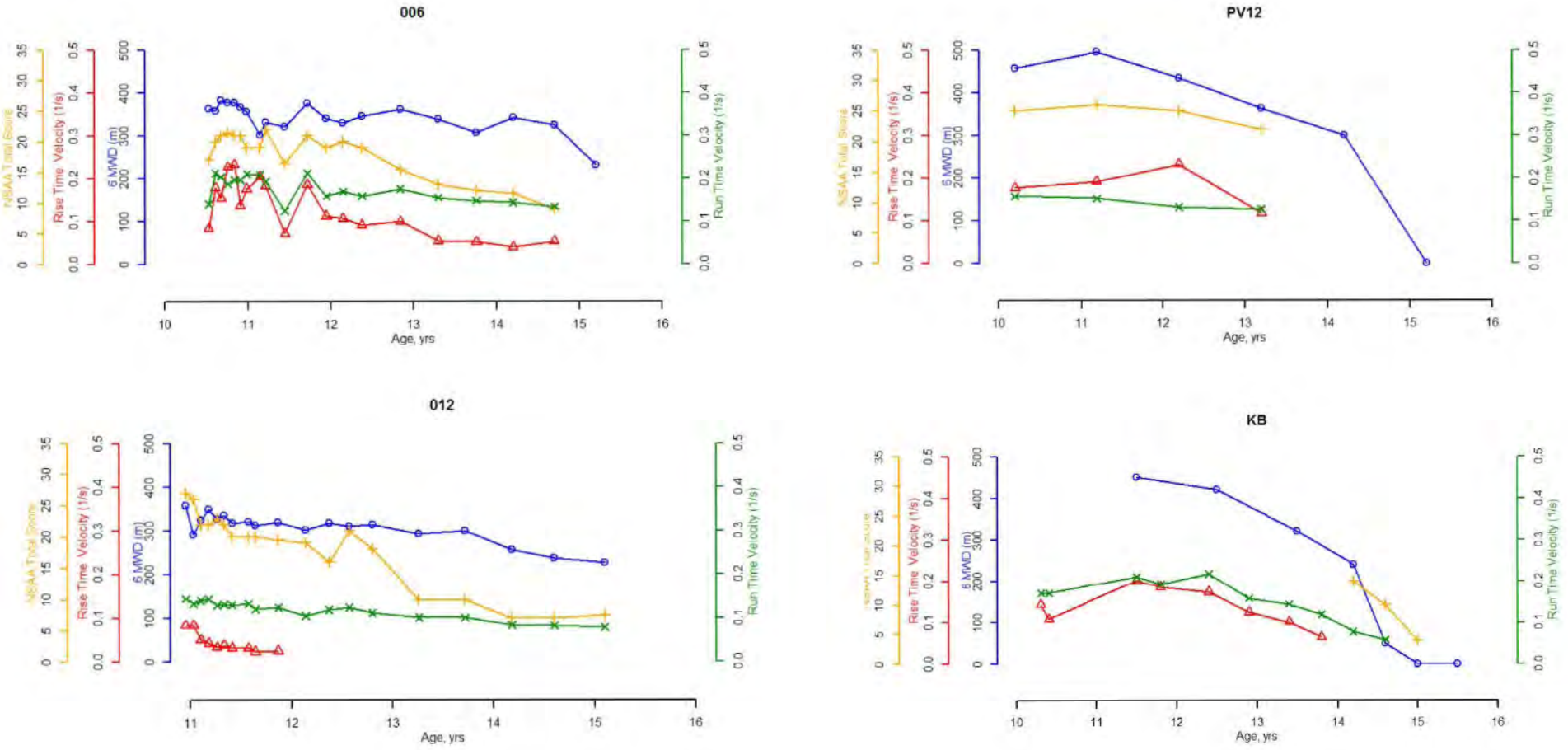
- At age 11 years, both patients had similar 6MWT distance, NSAA, rise time velocity and run time velocity.

- 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.

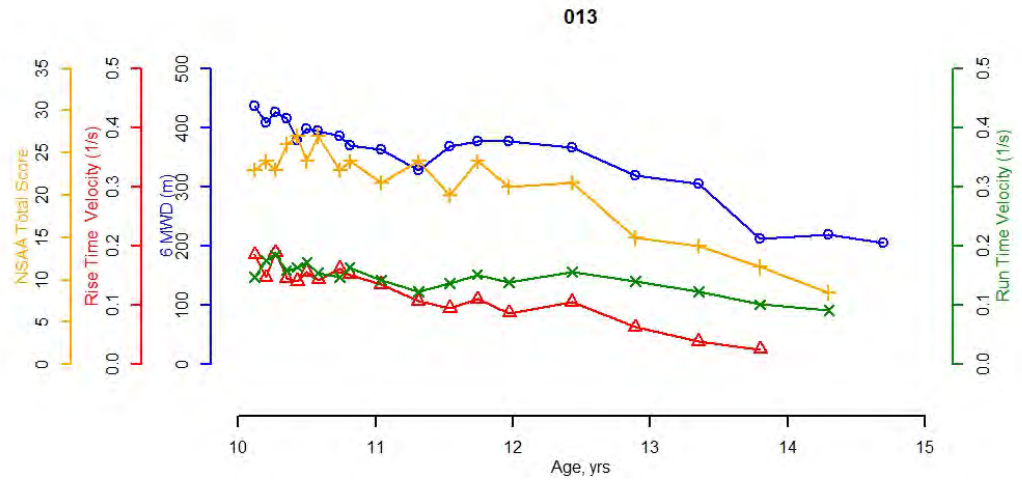
A similar observation can be made in a comparison between Patient 006 and Patient KB (Figure 7). Both patients had similar run time, rise time, and 6MWT around age 11. By age 14, they had similar 6MWT, NSAA, rise time and run time, indicating a similar disease course over a 3-year period of time. At 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



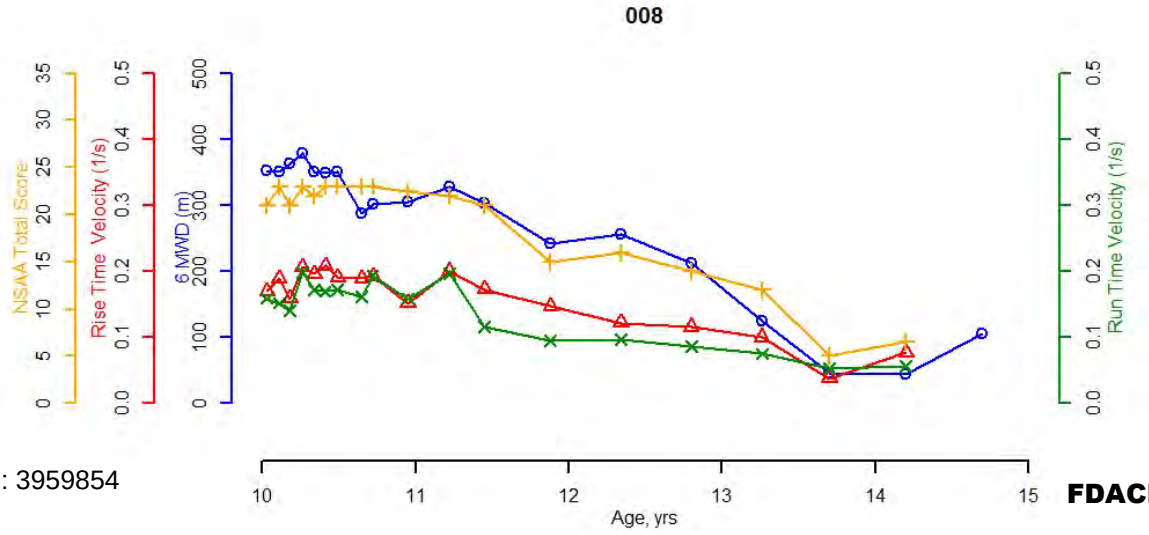
Patient 013 (Figure 8), who received placebo during Study 201, and was later switched to eteplirsen 50mg/kg, had the third highest 6MWT distance after age 14 years. Patient 013 is also showing a marked decline on most outcome measures, including rise time velocity from age 10.5, and a decline in NSAA 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.

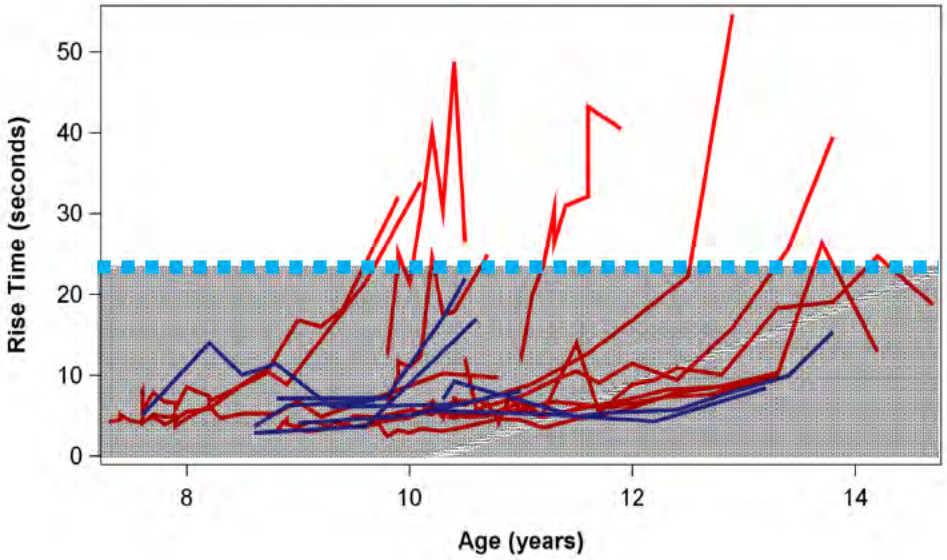
Figure 9: Clinical profile of Patient 008



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

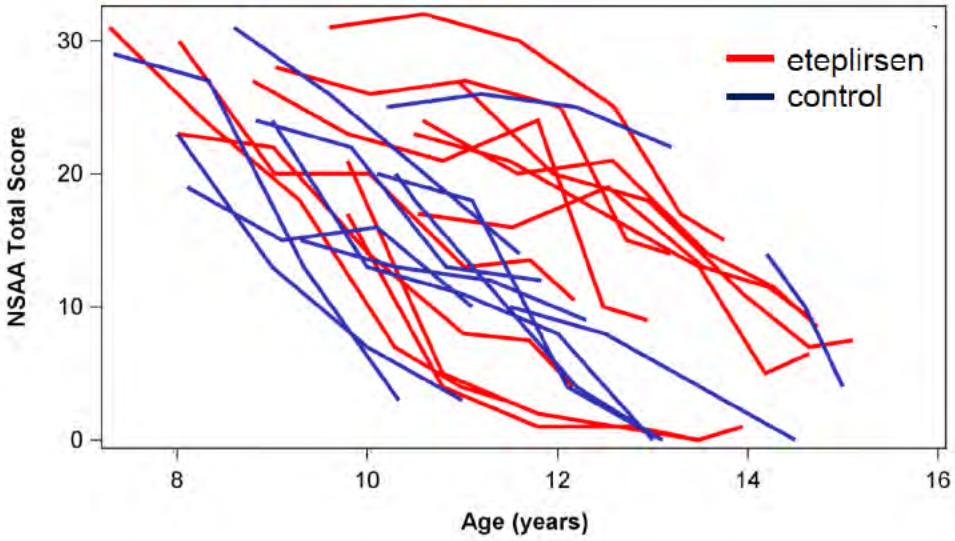
good work!," The Manual for Study 202 also stated that if the patient fell or could not rise from the floor, the test was over and time and distance should be recorded. On the other hand, the protocols for the historical control studies were very scant (see Appendix 2: Protocol of the Leuven Neuromuscular 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

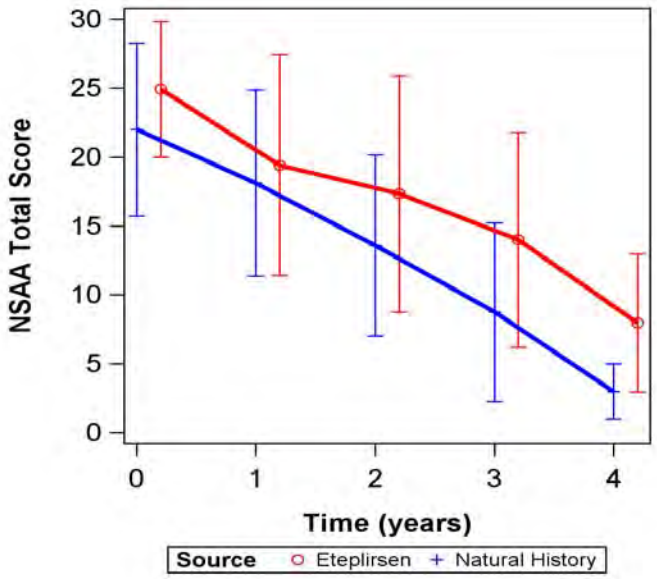
running. The NSAA is a comprehensive outcome measure, and arguably more fully reflects the functional abilities of DMD patients than the 6MWT. The NSAA remains, however, dependent on subject effort, and is not immune to possible bias. All eteplirsen-treated patients showed progressive declines in NSAA 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.

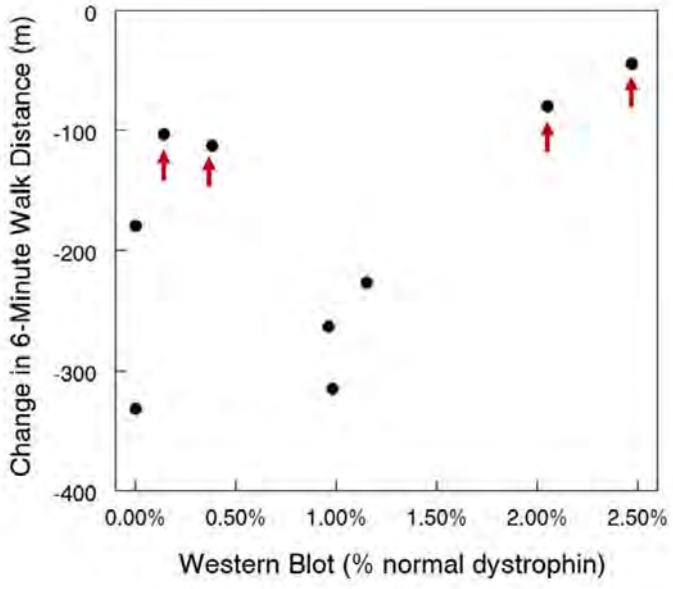
Figure 12: Mean NSAA scores over time



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, <u>in favor of placebo</u>, 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 it 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[10] 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

[10] Cooperative International Neuromuscular Research Group http://www.cinrgresearch.org/

have had an impact on study outcomes, as if 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.[11] 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.

[11] 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[12] 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[13] 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

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

[13] 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.

[14] 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.

[15] 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.

[16] 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% ± 0.84% of normal (mean ± 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

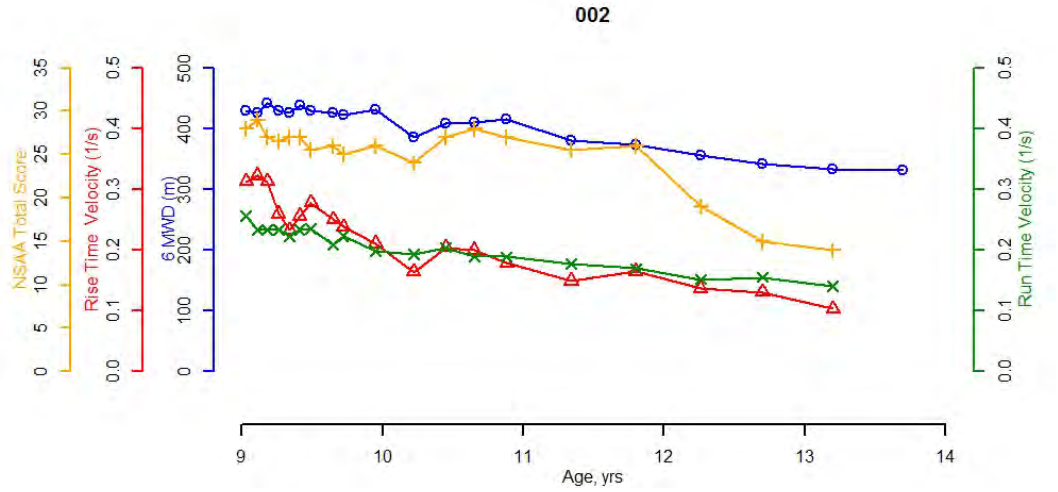
A Postmarketing Risk Evaluation and Mitigation Strategy is not needed for this product.

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.

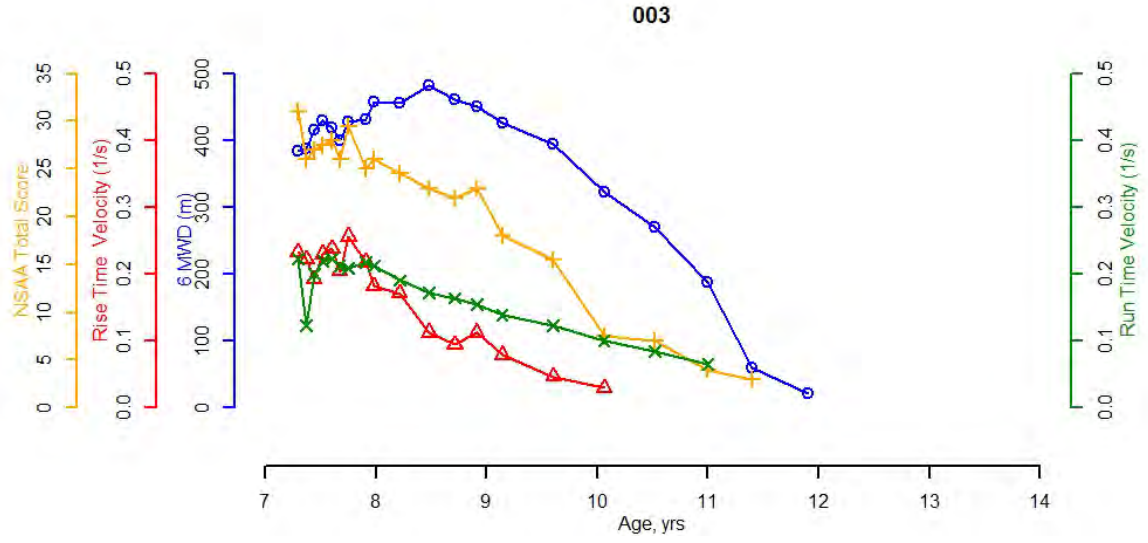
Appendix 1: Patient profiles

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.

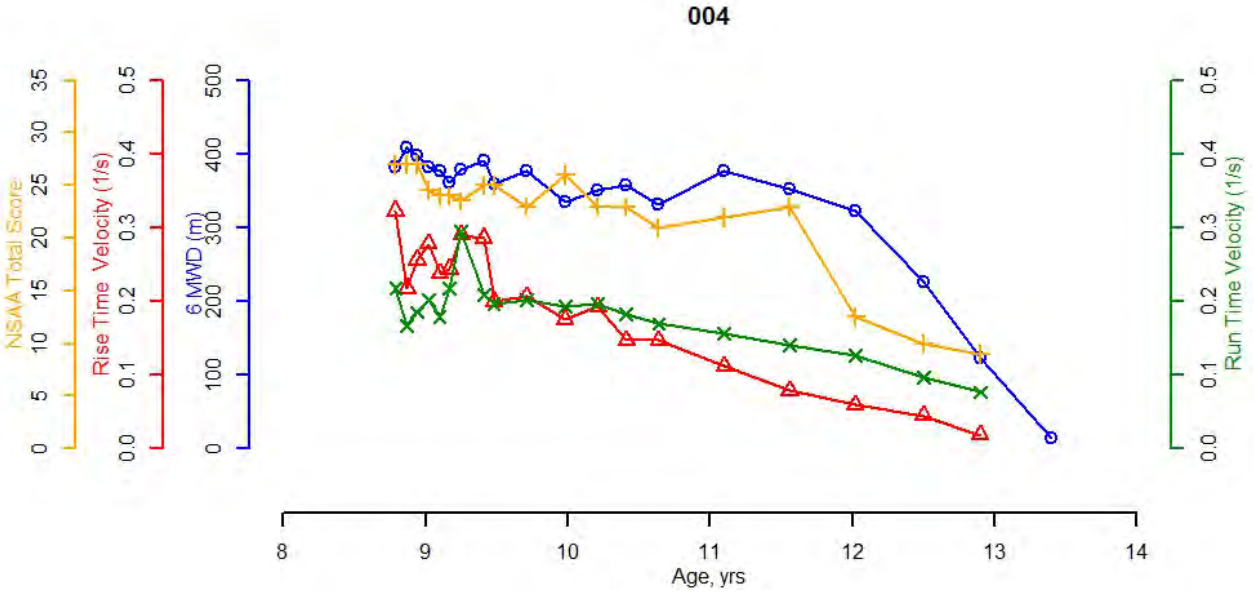
Patient 002 (eteplirsen 30 mg/kg) had a relatively mild course. Patient 002 has 0.14% dystrophin at Week 180, indication that eteplirsen is not likely to have contributed to the course of the disease in this patient.



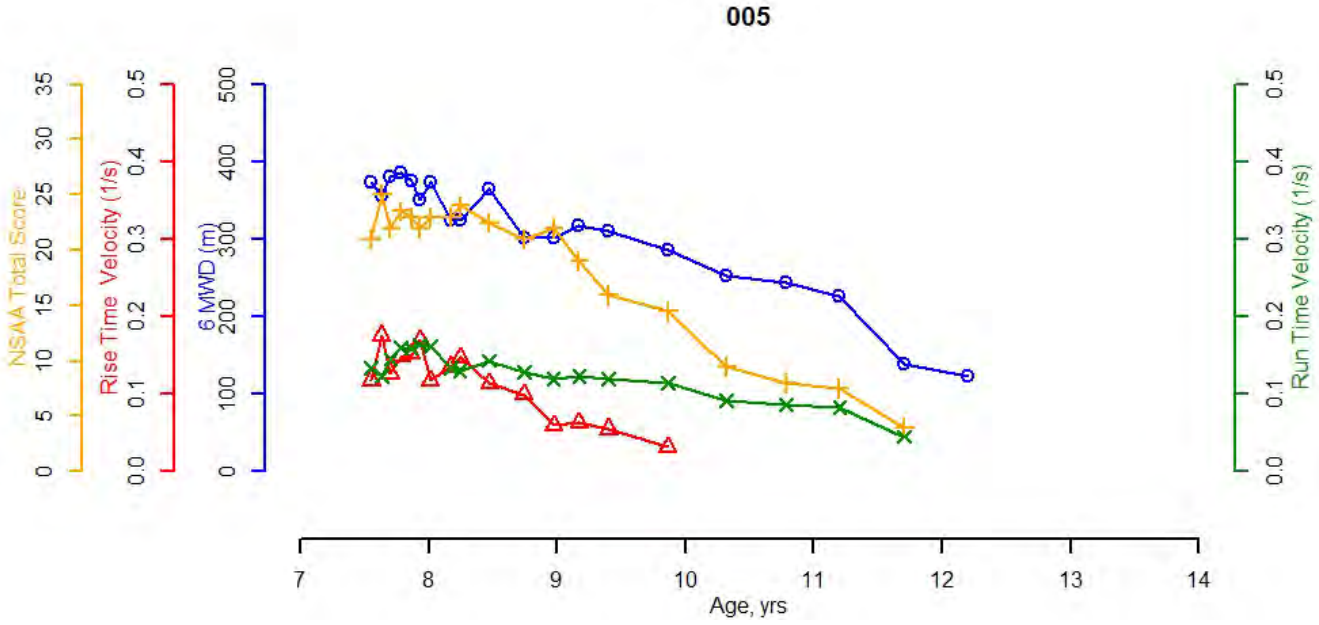
Patient 003 (eteplirsen 50 mg/kg) had a rapid decline in all outcome scales. Patient 003 had 0% dystrophin at Week 180.



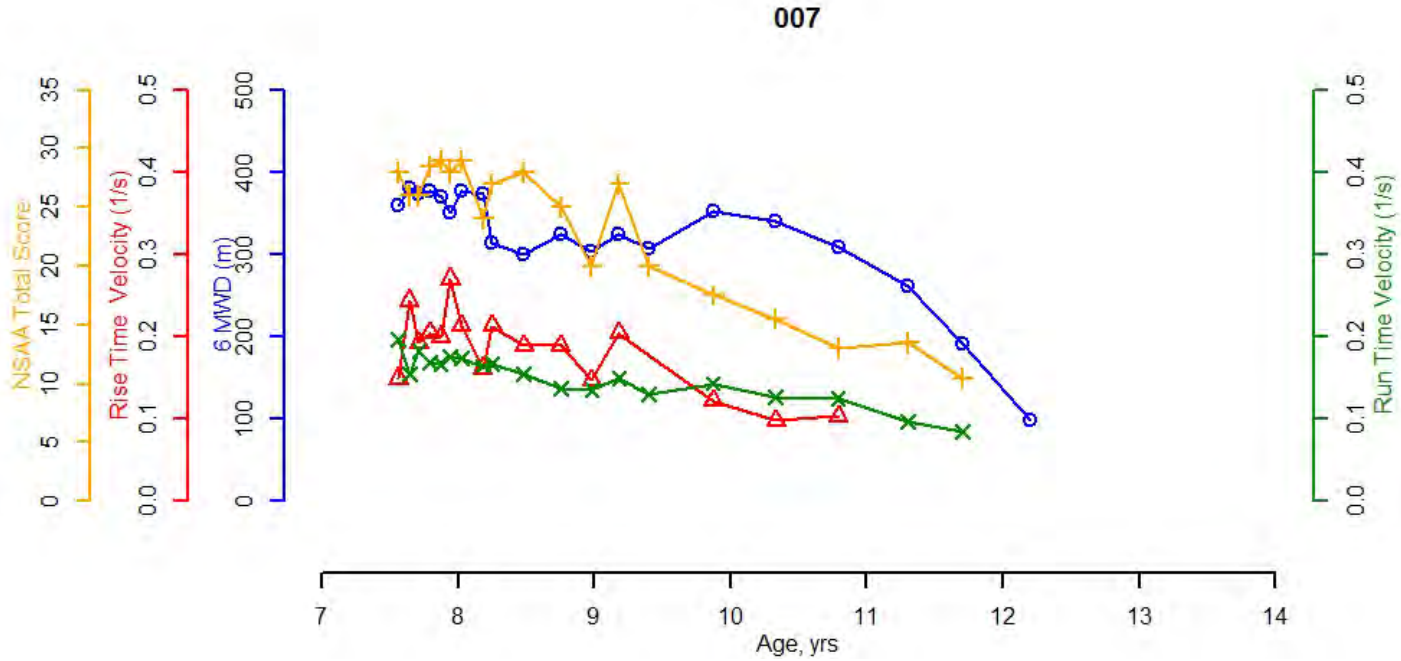
Patient 004 (eteplirsen 50 mg/kg) had relative stability up to age 11 and a half, and then rapidly declined in all outcome scales. Patient 004 had 0.96% dystrophin at Week 180.



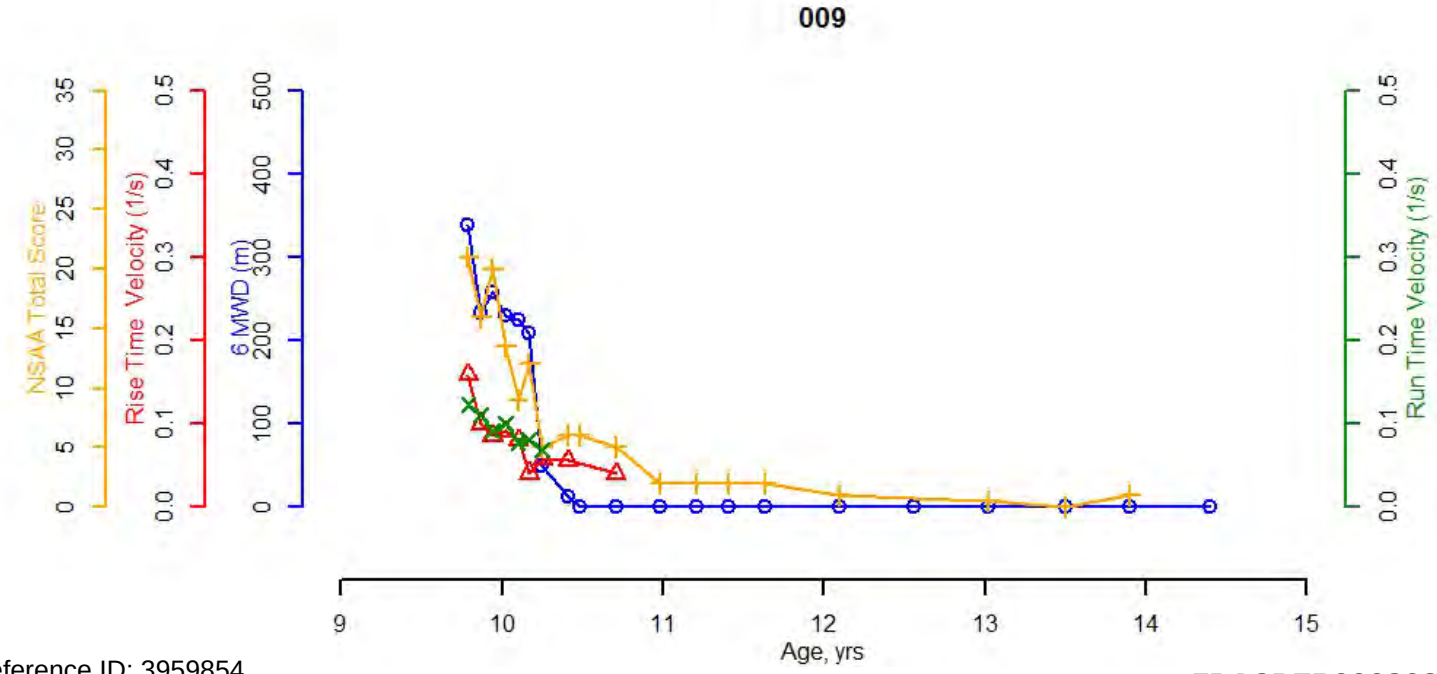
Patient 005 (eteplirsen 50 mg/kg preceded by placebo in Study 201) had a rapid decline in all outcome scales. Patient 005 lost the ability to rise at age 10 years. Patient 005 had no biopsy at Week 180.



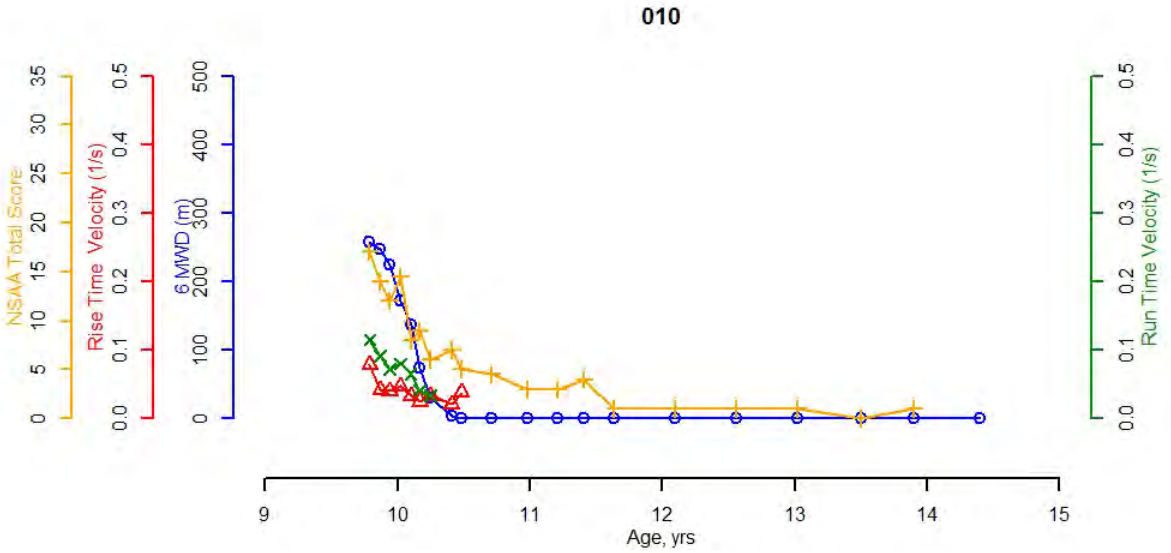
Patient 007 (eteplirsen 30 mg/kg preceded by placebo in Study 201) was relatively stable up to age 11 years, then had a steady decline in all outcome scales. Patient 007 had 0% dystrophin at Week 180.



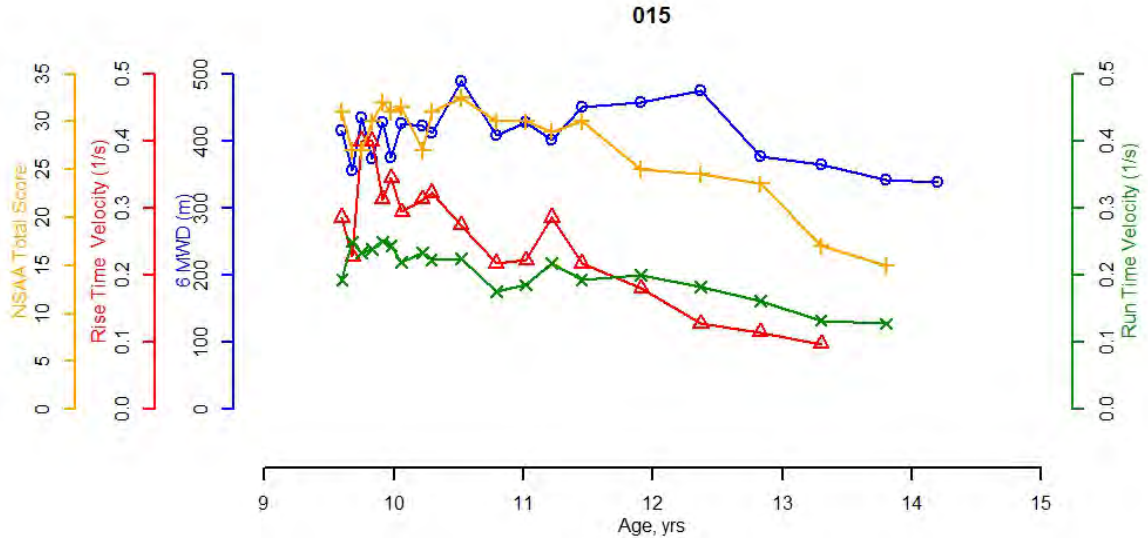
Patient 009 (eteplirsen 30 mg/kg) had a rapid decline in all scales from age 9.5 years. Patient 009 had 0.52% dystrophin at Week 180.



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 datacut 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" colom 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 fysio, no steroids)*

9="GSK968"

9.5="Stop GSK968"

Appendix 3: Protocol of the Italian DMD Registry

Protocol GUP07009/ GUP09010/ PP

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 physioterapist 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 physioterapist/neurologist from each partecipating 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 subgroupsThese 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.

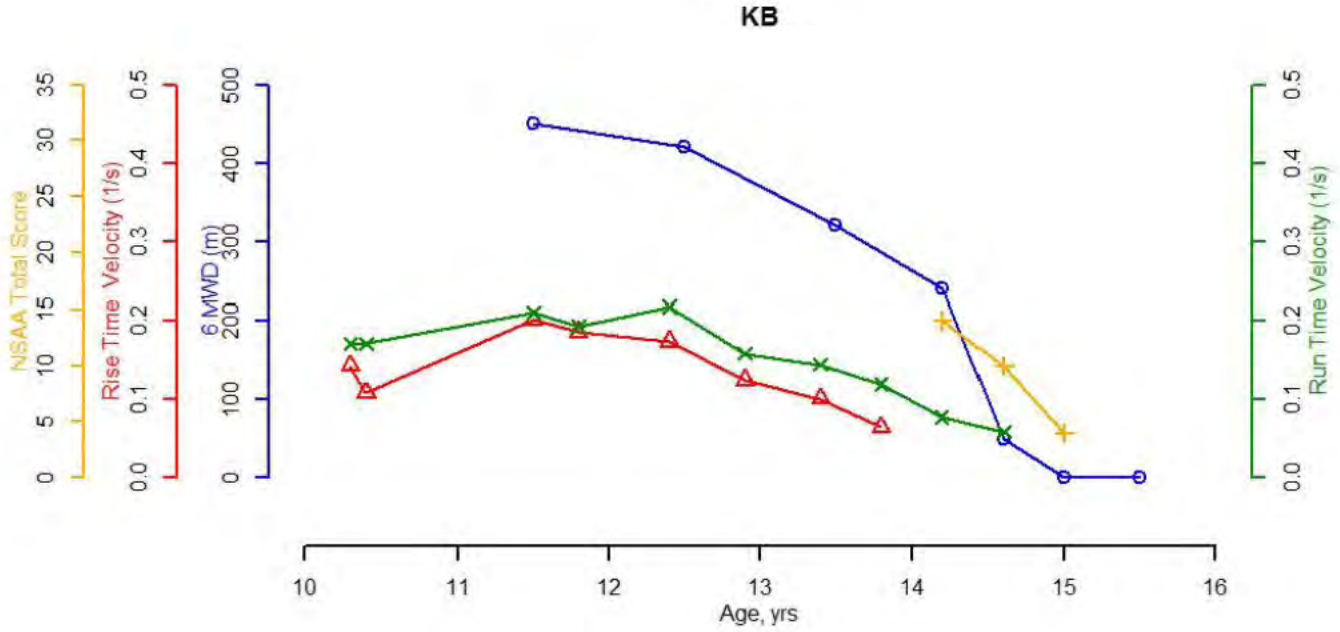
The patient CRF will be filled in by a part–time fellow in each centre. The CRF of each patient will be maintained on site under the responsibility of the P.I. in each institution. The CRF data of all centres will be then collected and entered in a dedicated database by the dedicated research fellow 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.

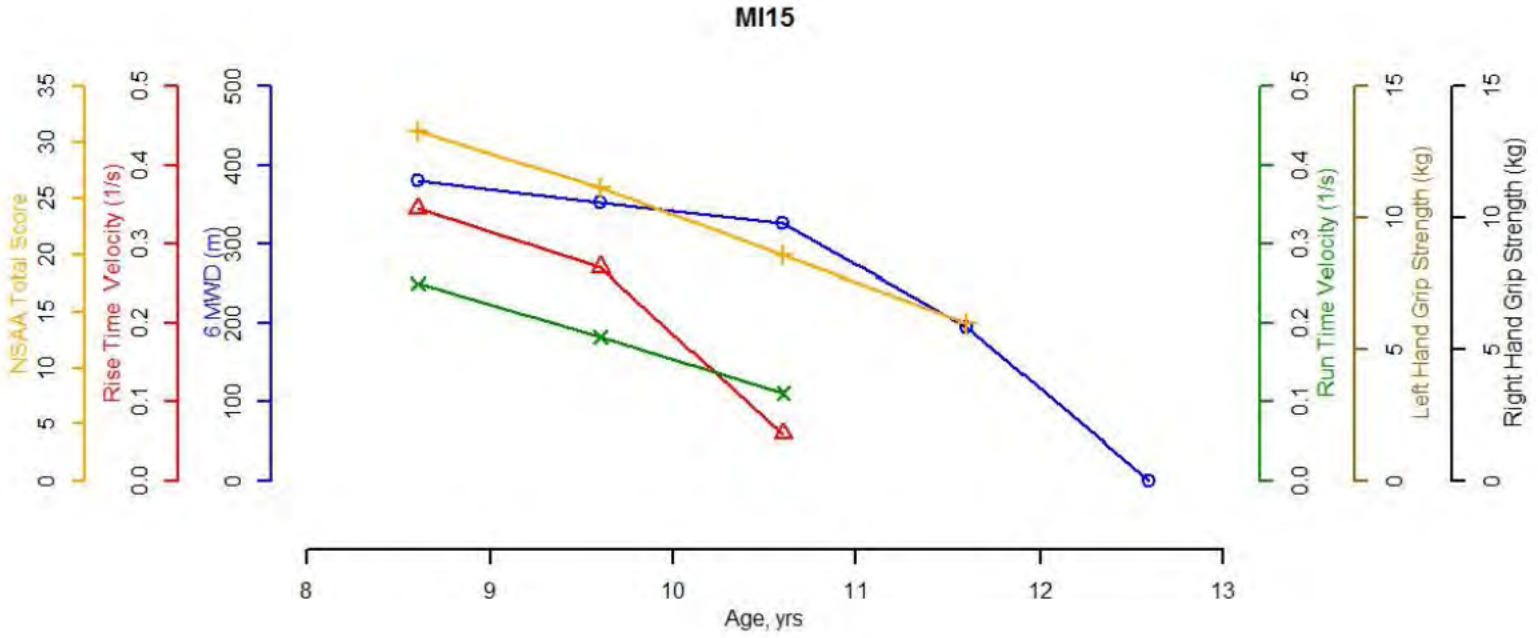
Maintenance of the study database will be the responsibility of the coordinator unit. The data will be stored and analyzed in accordance with Italian legislation. Only aggregate results will be disseminated.

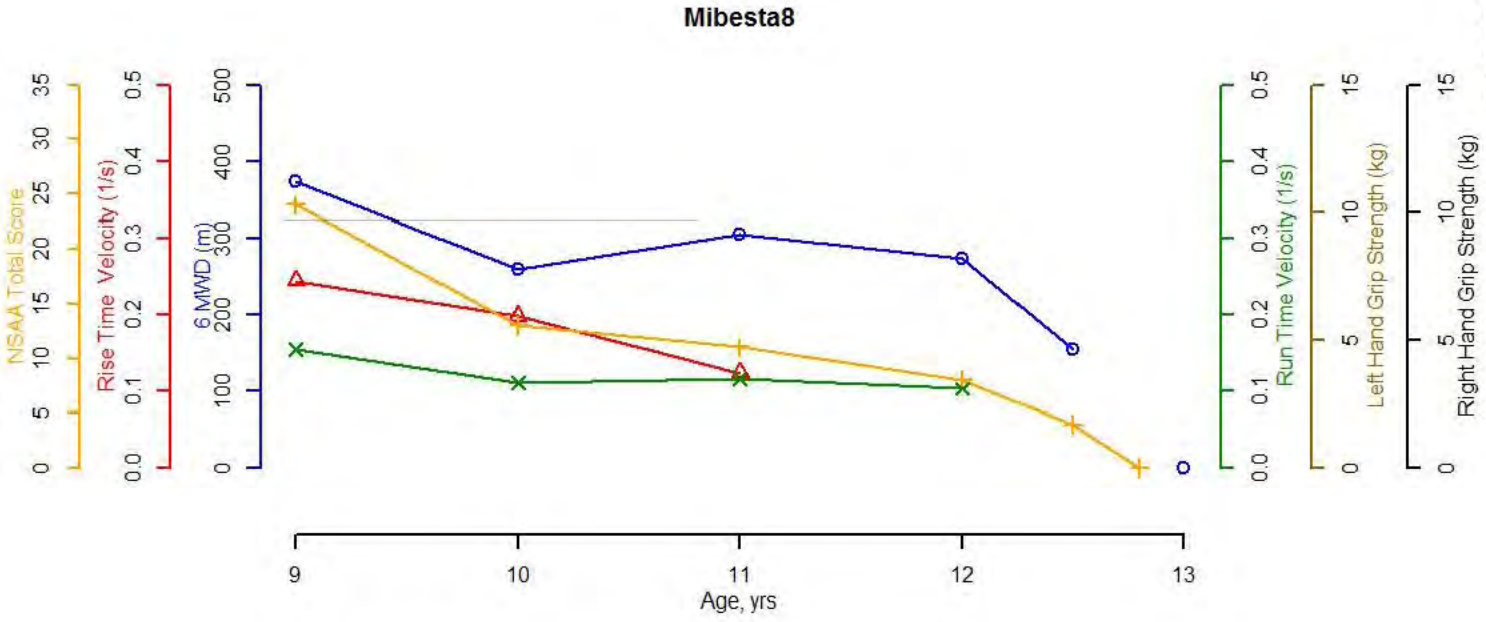
PLAN FOR STATISTICAL ANALYSIS:The distribution of each of the outcome variables (6MWT, NSSA, timed items) will be assessed. Descriptive analysis will be carried out by computing means and medians of continuous variables (as appropriate according to the type of distribution, i.e. whether approximately 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.

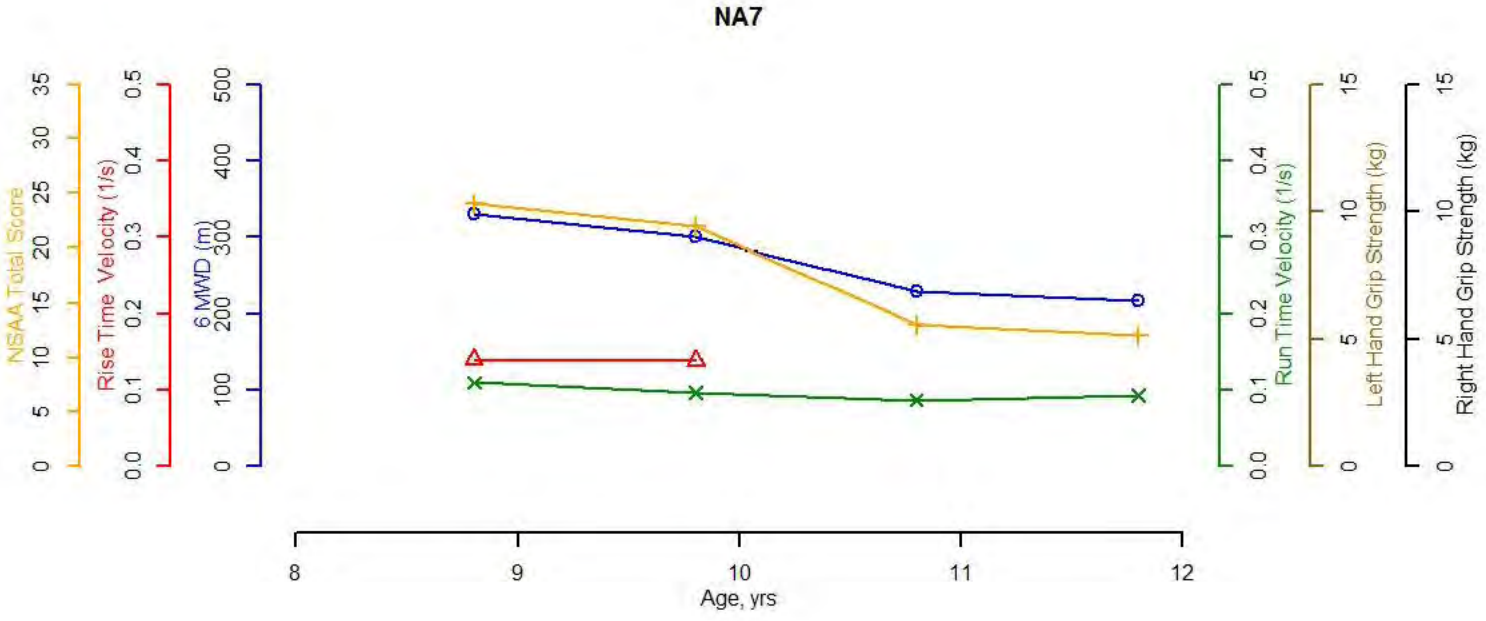
Verified: Date: 24 NOV 2015
Name: Prof E. Mercuri
Signature:

Appendix 4: Patient profiles from the Belgium and Italian Registries






Mibesta8
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs


NA7
NSAA Total Score
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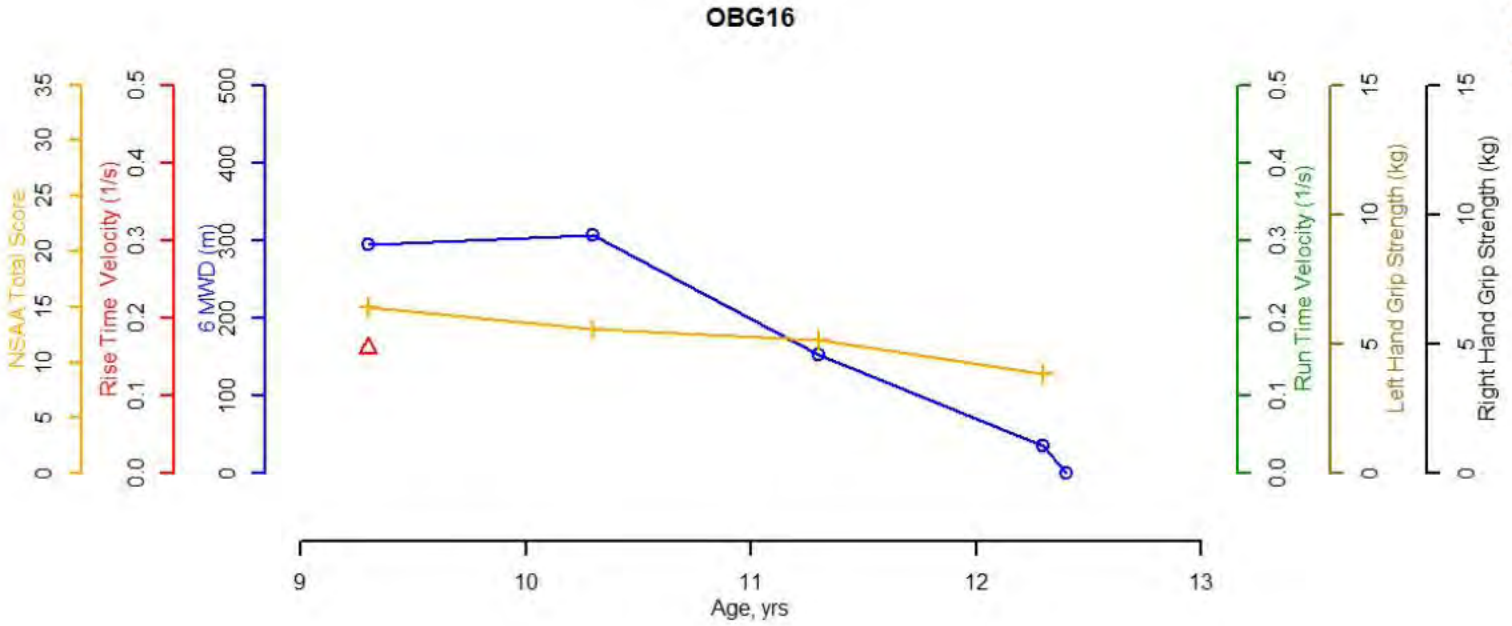
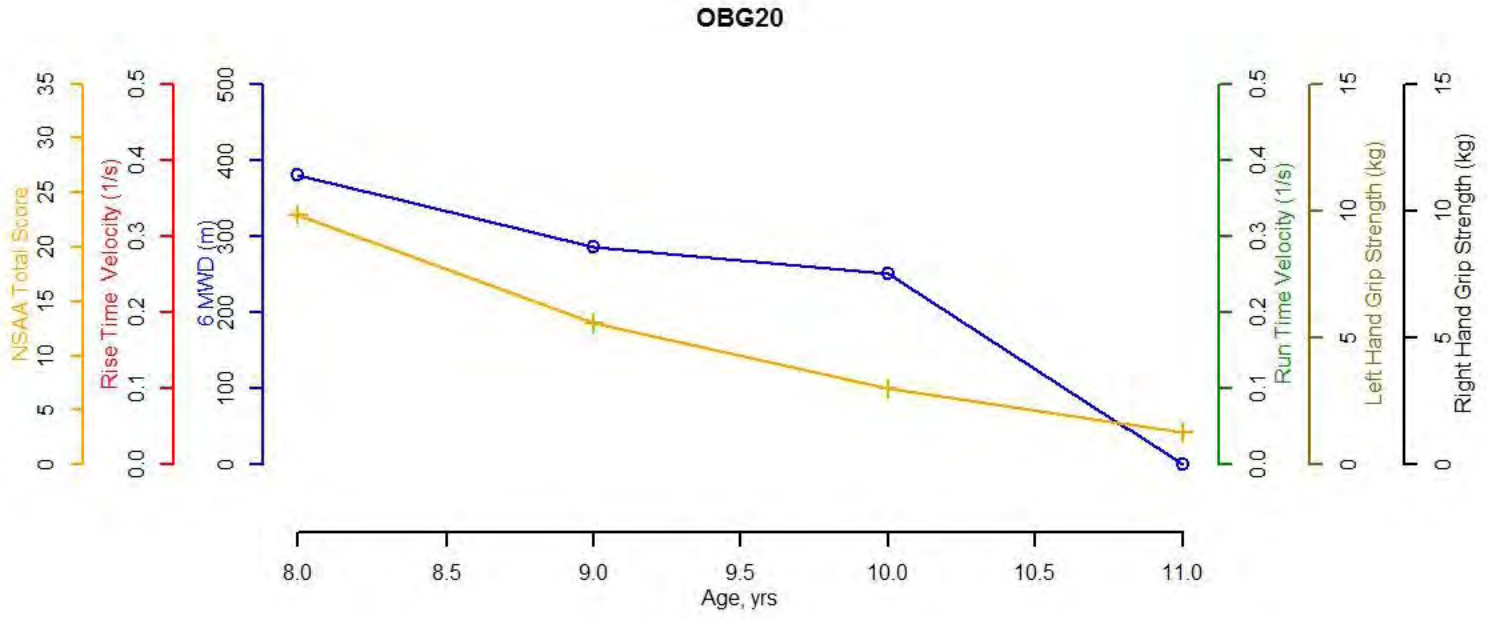
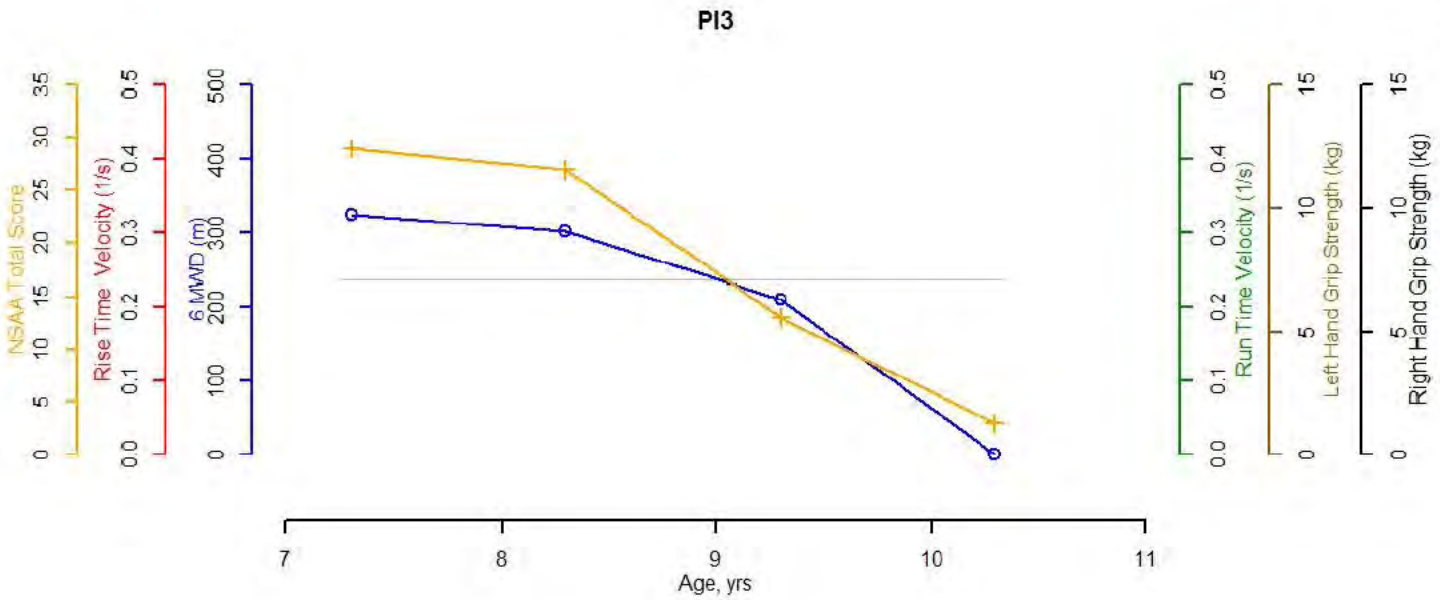
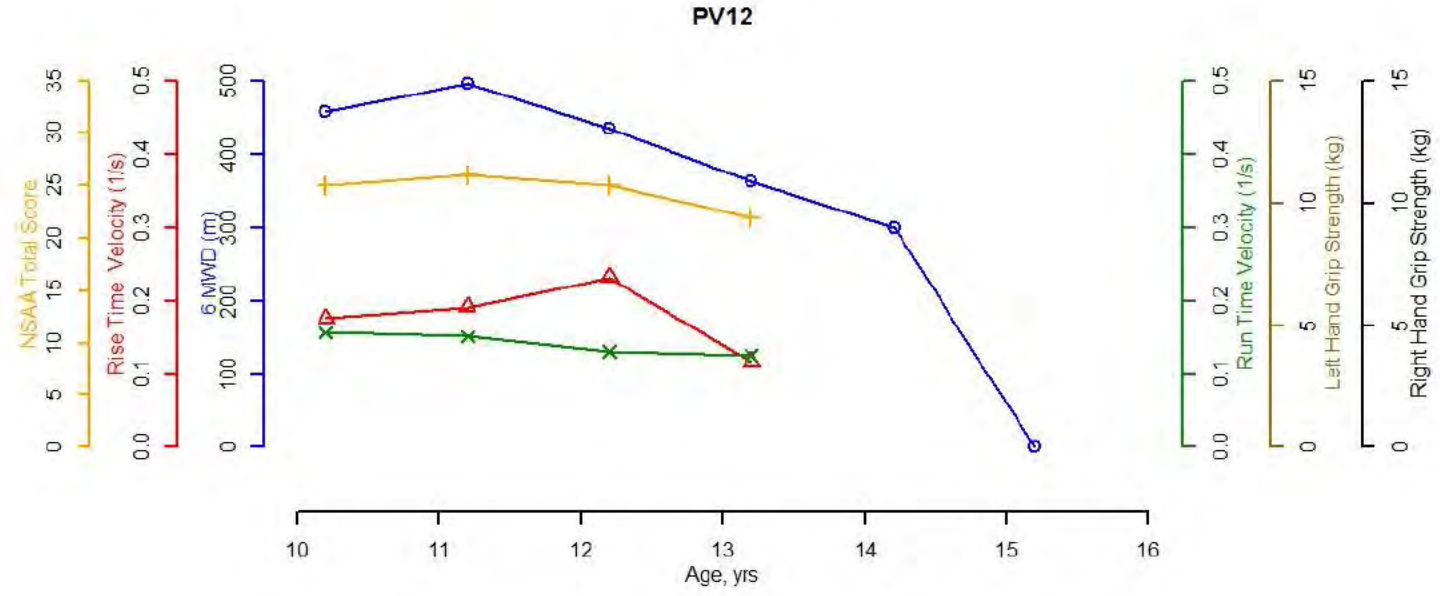
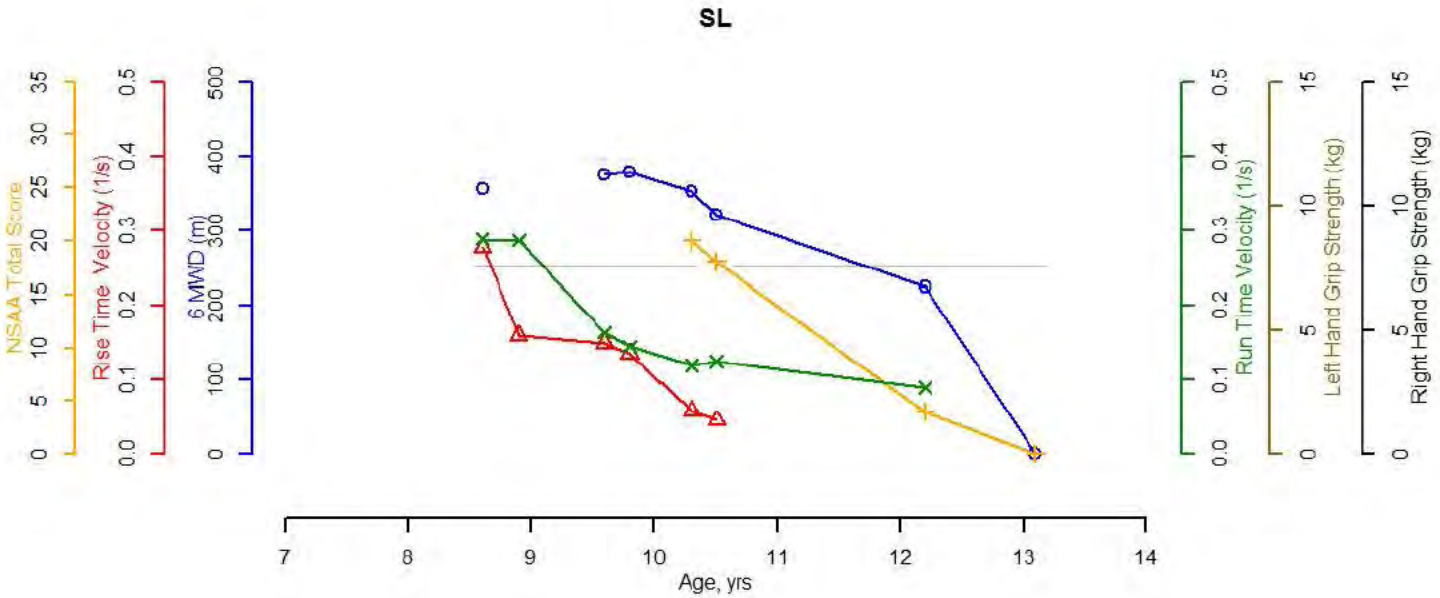
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs

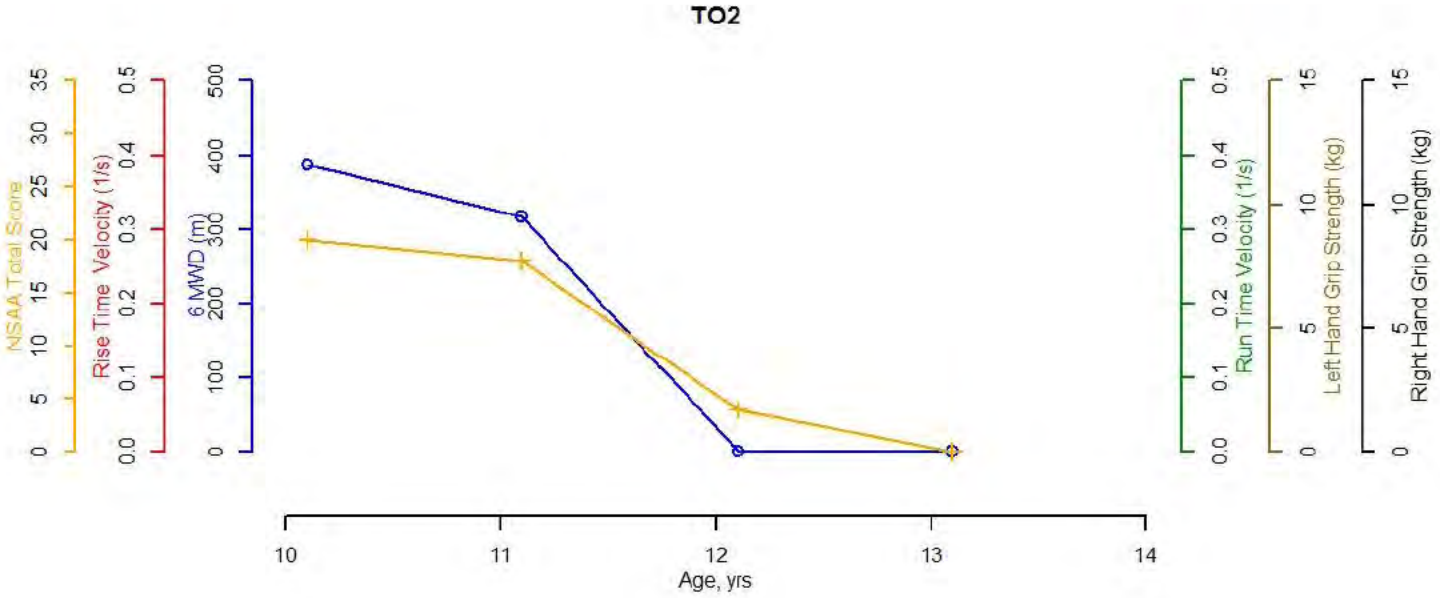


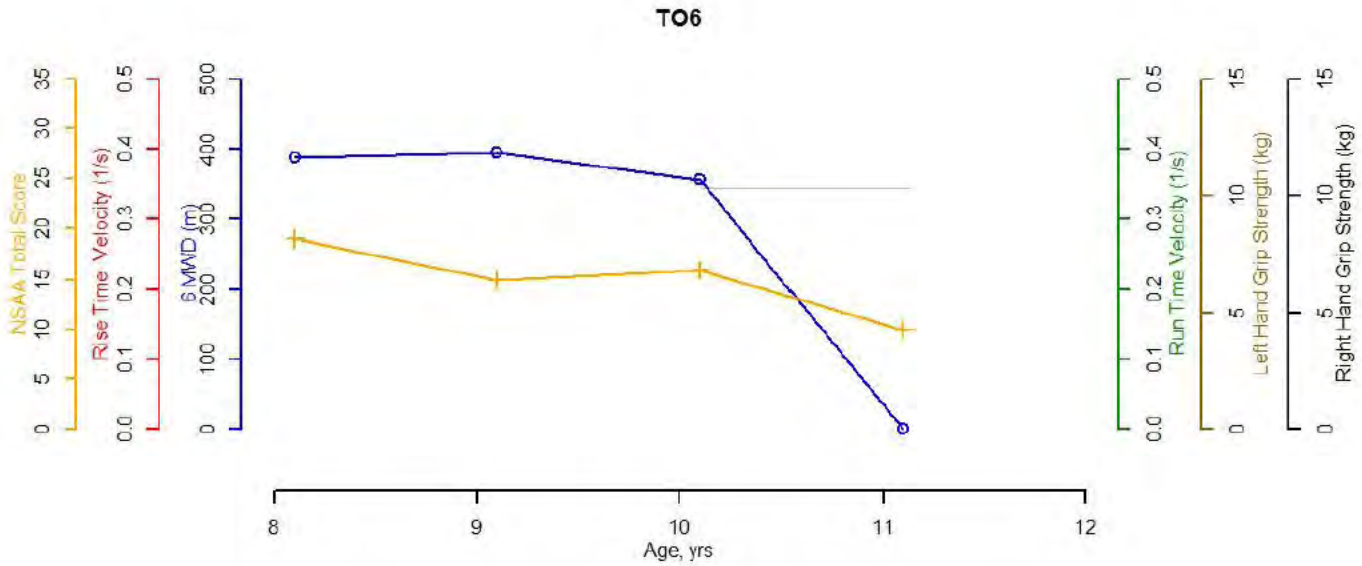


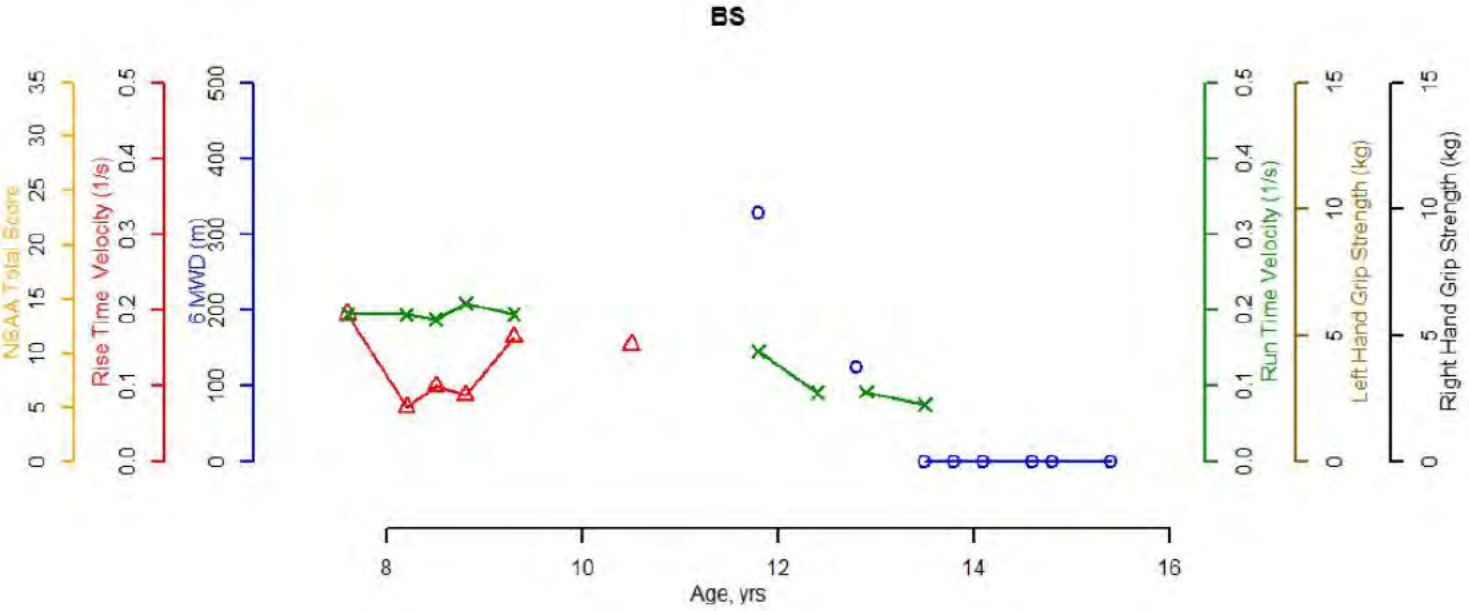

PI3
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs

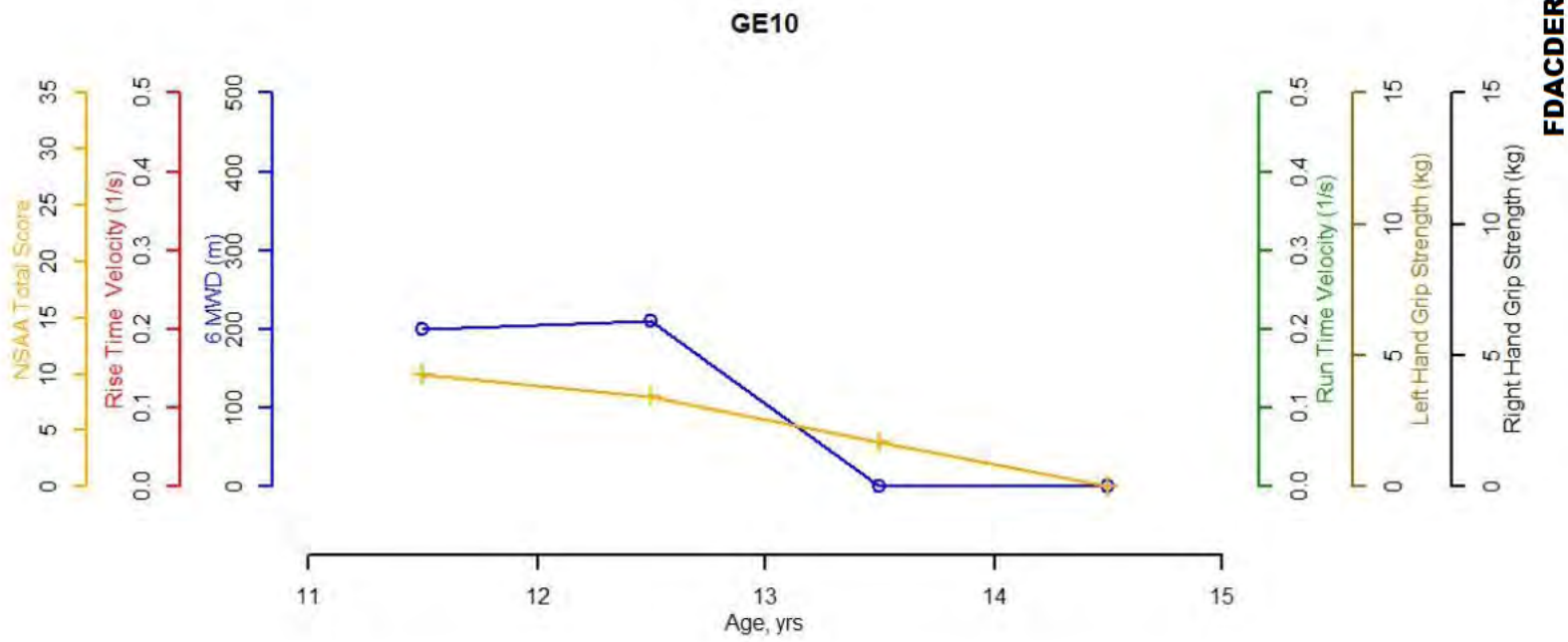

PV12
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs


SL
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs


TO2
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs






GE10
NSAA Total Score
Rise Time Velocity (1/s)
6 MWD (m)
Run Time Velocity (1/s)
Left Hand Grip Strength (kg)
Right Hand Grip Strength (kg)
Age, yrs

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) 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
Nationwide Children's Hospital		
(b) (4)	Laboratory Director	Protocol and final report review
	Laboratory Coordinator	Tissue allocation
	Histology technician	Assigned blinding codes to samples
Sarepta Therapeutics, Inc.		
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

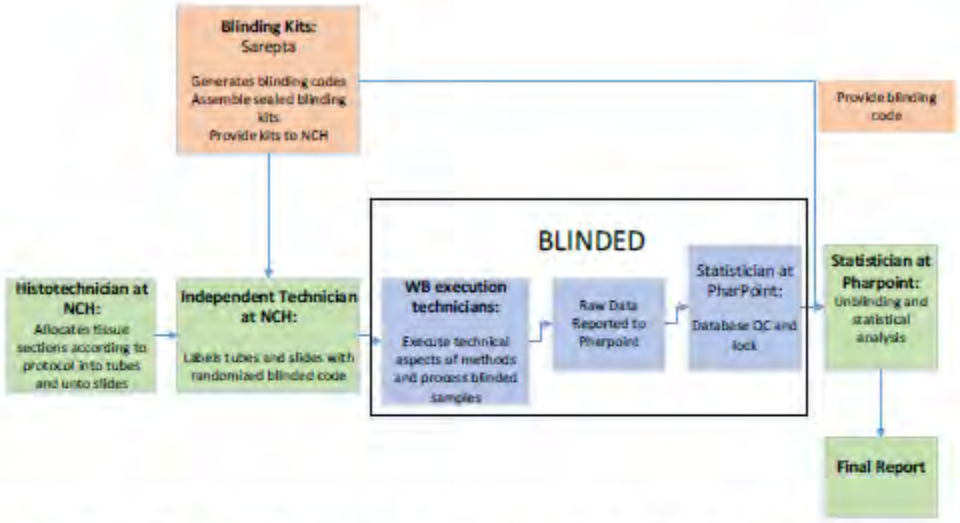


Figure 1: Flowchart Depicting Key Stages of Sample Blinding, Analysis and Unblinding

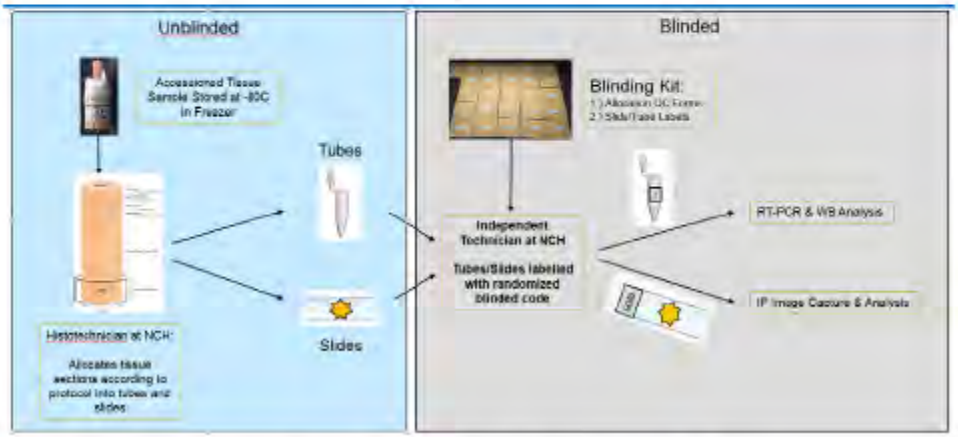


Figure 2: Blinding for Tissue Allocation for Western Blot Analysis

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This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ERIC P BASTINGS
07/15/2016

From: Rosenberg, Amy
To: Woodcock, Janet
Subject: FW: Publication highlights release of muscular dystrophy action plan
Date: Thursday, April 07, 2016 4:21:45 PM

Was not aware of this group!

From: National Institutes of Health (NIH) [mailto:nih.ocpl@service.govdelivery.com]
Sent: Thursday, April 07, 2016 1:33 PM
To: Rosenberg, Amy
Subject: Publication highlights release of muscular dystrophy action plan

Publication highlights release of muscular dystrophy action plan

04/07/2016 12:30 PM EDT

Plan provides strategies and opportunities for muscular dystrophy research.

From: Peterson, Jayne E
To: Woodcock, Janet
Cc: Ligon, Sharnell (CDER)
Subject: Sarepta AC meeting Background Package
Date: Tuesday, March 29, 2016 4:32:56 PM
Attachments: eteplirsen-n206488-pcnsdac-briefing-book.pdf

Hi Dr. Woodcock,

As I mentioned, we've received the Sarepta AC meeting background package for the April 25th PCNS AC meeting. I've attached.

Will send the Review Division's background package as soon as we receive.

Jayne

File could not be opened because it is not a supported or damaged.

From: Dunn, Billy
To: Woodcock, Janet
Subject: sarepta update
Date: Wednesday, March 15, 2017 3:53:01 PM
Attachments: Sarepta.pdf
eff-info-amend.pdf
IND77429_cmts_IHC protocols.pdf
Study of SRP-4045 and SRP-4053 in DMD Patients (ESSENCE) - NCT02500381.msg

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Janet,

Attached is our letter (1/17/17) with comments on their draft protocol and their subsequent response to our comments, which is with the review team. I've also included our 3/13/17 letter with comments on their bioassay protocols. We also have a draft protocol for exon 45 and 53 and a draft protocol for immunogenicity assessment under review. The last attachment is a note from Skip Nelson describing the UCLA referral for 21 CFR 50.54 panel review, which I think you're aware of directly from Skip.

I think that covers the bases for things that might come up if you speak with them.

Great chatting with you this morning. Talk to you soon.

Billy

14 pages have been withheld as b(4) (CCI/TS) immediately following this page

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

IND 077429

ADVICE/INFORMATION REQUEST

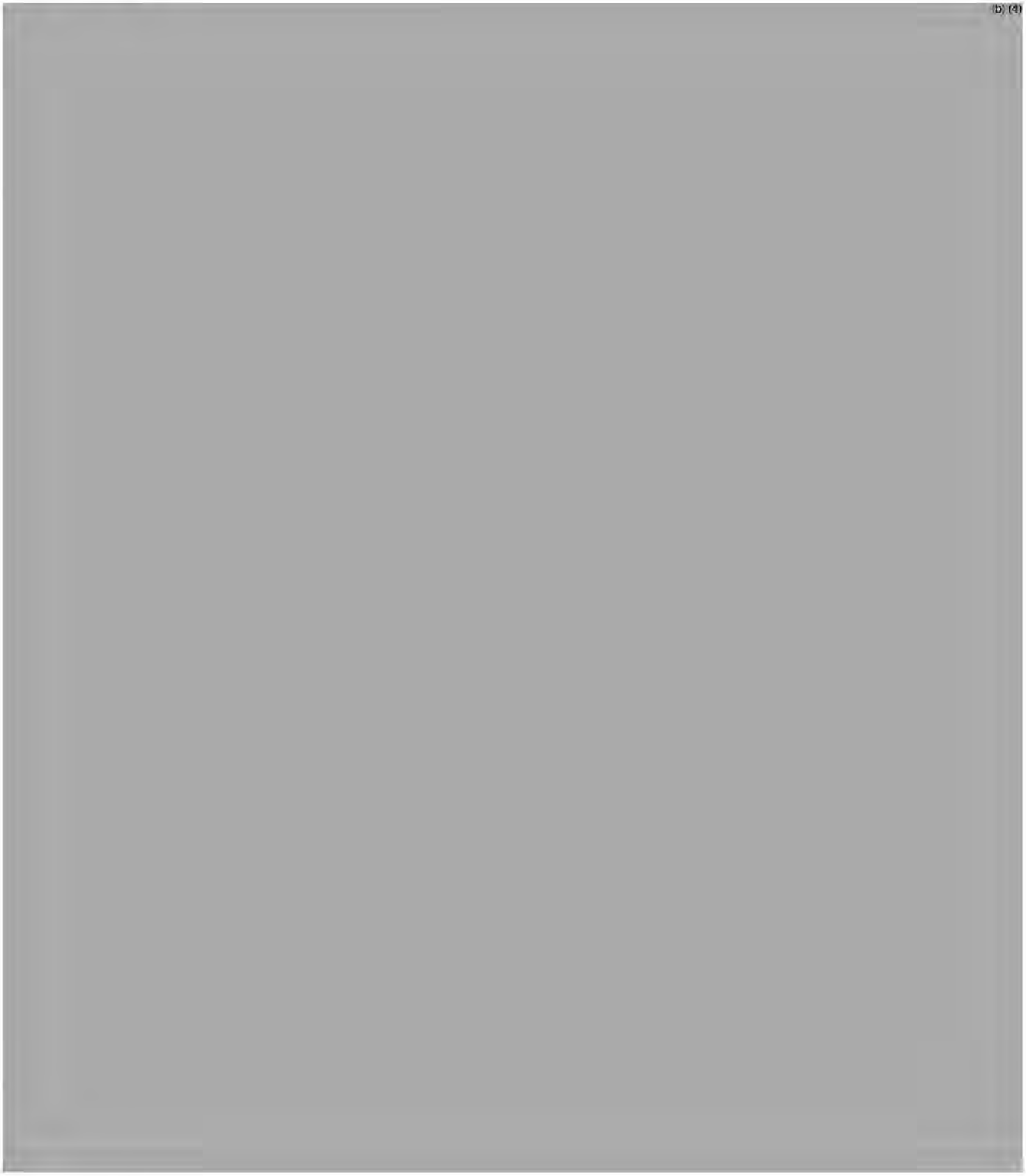
Sarepta Therapeutics, Inc.
Attention: Shamim Ruff, MSc.
Sr. Vice President, Regulatory Affairs and Quality
215 First Street, Suite 415
Cambridge, MA 02142

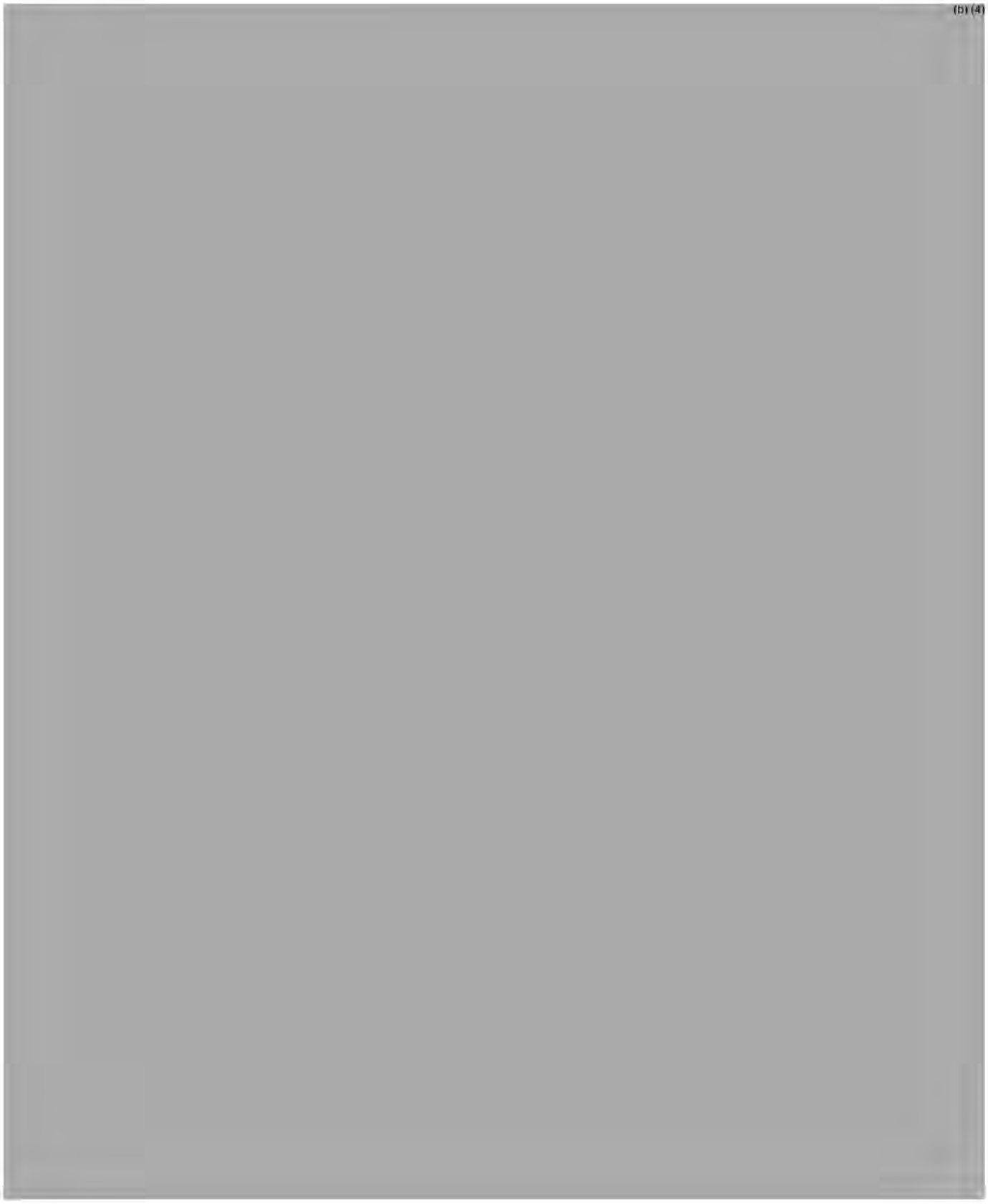
Dear Ms. Ruff:

Please refer to your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act for eteplirsen.

We also refer to the draft clinical protocol for Study (b) (4)

(b) (4)





(b) (4)

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If you have any questions, contact Fannie Choy, Regulatory Project Manager, at (301) 796-2899 or fannie.choy@fda.hhs.gov.

Sincerely,

{See appended electronic signature page}

Eric Bastings, M.D.
Deputy Director
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ERIC P BASTINGS
03/13/2017

From: Nelson, Robert "Skip"
To: Dunn, Billy
Cc: Bastings, Eric; Kozauer, Nicholas; Breder, Christopher D; Choy, Fannie (Yuet); Brill, Marieann; McCune, Susan
Subject: Study of SRP-4045 and SRP-4053 in DMD Patients (ESSENCE) - NCT02500381
Date: Thursday, March 02, 2017 12:44:59 PM
Attachments: image002.png

Dear Billy,

Based on e-mail and telephone communications over the past few days, I expect that there will be a referral from the UCLA IRB for a federal panel review of the protocol "Study of SRP-4045 and SRP-4053 in DMD Patients (ESSENCE)" (NCT02500381 - https://clinicaltrials.gov/ct2/show/study/NCT02500381) under 21 CFR 50.54. Chris Breder and I spoke today, and I understand that Sarepta has submitted several protocols with different approaches to the inclusion of a placebo group. The protocol that will be referred, as I understand it, is the (b) (4) placebo group with the ethical question being the appropriateness of allowing the use of an indwelling infusion port (recognizing that some boys would then have a port surgically placed with no prospect of direct benefit as they may be on placebo). I am told there are boys out there who are struggling with intravenous access, and at least one who was removed from the protocol because of access difficulties. I am told as well by the UCLA IRB that Sarepta is aware of this referral and is working with them in a supportive manner.

At the outset, let me reassure you that the work of putting together the panel review under 21 CFR 50.54 falls on our office. Of course, we welcome and need your input as we put the meeting (and the background materials) together. The general process of how FDA handles referrals under 21 CFR 50.54 is described in a final guidance found at https://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm127605.pdf. The Pediatric Advisory Committee is specifically chartered to advise the FDA Commissioner on protocols referred under 21 CFR 50.54. My suggestion is that once the referral is in hand, we can set up a meeting with you (DNP) to go over the process and answer any questions.

We have been criticized in the past for being slow in responding to these referrals in a timely manner. In order to have that not be the case here, we have already reserved a portion of the WO Great Room for either Thursday May 18 or Friday May 19 (this will be a one day AC meeting). We have polled our members of the Pediatric Ethics Subcommittee (PES) and the Pediatric Advisory Committee (PAC) about their availability for those two dates, and know we will have enough SGEs to be able to hold the meeting. Once the referral is in hand, we will also reach out to other appropriate SGE experts to supplement the PES/PAC membership. I do not anticipate the need for a presentation from DNP, but would want someone to be available at the table to answer any questions the AC may have.

Please note: What we do need from DNP by the Wednesday of next week is the list of competing products so that we may start the COI screening process. I am copying Fannie, as I assume she is the point-of-contact for obtaining this information. Marieann Brill is the PAC DFO (also copied on this e-mail).

I am giving you the early "heads up" as I need the competing products list before the official referral has been received. Given the controversy over the accelerated approval of Eteplirsen, I will inform Ellis Unger and Janet Woodcock of the protocol referral once I have received it. I will also inform Stephen Ostroff, as he will be the one to make the final determination unless we have a new FDA Commissioner by that time. Please let me know if there are others you believe I should keep apprised of the situation.

Again, I will set up a meeting once we have the referral in hand. Please let me know if you have any questions. Thanks.
Skip

Robert "Skip" Nelson, M.D., Ph.D.
Deputy Director and Senior Pediatric Ethicist

Office of Pediatric Therapeutics (OPT)
Office of Special Medical Programs (OSMP)
Office of the Commissioner (OC)
U.S. Food and Drug Administration
Tel: 301-796-8665; Mobile: (240) 328-7146; Fax: (301) 847-8619
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DOCUMENT INFORMATION PAGE

This page is for FDA internal use only. **Do NOT send this page with the letter.**

Application #(s):	IND 077429
Communication Type:	Correspondence
Communication Group:	IND Information Request or Advice
Communication Name:	Advice/Information Request
Communication ID:	COR-INDAD-02
Drafted by:	Choy
Clearance History:	Ling/Jin (Stat) 12/6/16; Chelliah/Heimann (CMC) 12/14/16; J Patro (Micro) 12/14/16; Breder 12/15/16; Bhattaram (OCP) 12/16/16; Hallett (OBP/immunogenicity) 12/22/16; Ash Rao (OBP/bioassay) 12/30/16; Kozauer 1/9/17; Bastings 1/12/17, 1/13/17; Unger 1/12/17
Finalized:	FC 1/13/17
Filename:	M:\INDs\IND077429 AVI-4658_eteplirsen(DMD)\PMR-PMC\Subpart H\IND077429_eteplirsen_FINALcmts-PMR protocol.doc
Signatory Authority:	Division Director, Deputy, or Chem TL. Person who is covering for the signatory authority can sign on their behalf (i.e, the signature block on the letter will not change).
Use Statement:	Use to send the IND sponsor either advice or a request for additional information for issues that would not potentially lead to a clinical hold and when the reviews are completed.
Notes:	NOTE-- IF THERE ARE SAFETY ISSUES THAT MAY LEAD TO A CLINICAL HOLD, USE "DEFICIENCY - POTENTIAL HOLD ISSUES (COR-INDAD-03)"

Version: 06/13/2016

END OF DOCUMENT INFORMATION PAGE

The letter begins on the next page.

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Sarepta Therapeutics, Inc.
Attention: Shamim Ruff, MSc.
Sr. Vice President, Regulatory Affairs and Quality
215 First Street, Suite 415
Cambridge, MA 02142

Dear Ms. Ruff:

Please refer to your Investigational New Drug Application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act for eteplirsen.

We also refer to your amendment dated October 28, 2016, containing the draft protocol for Study

(b) (4)

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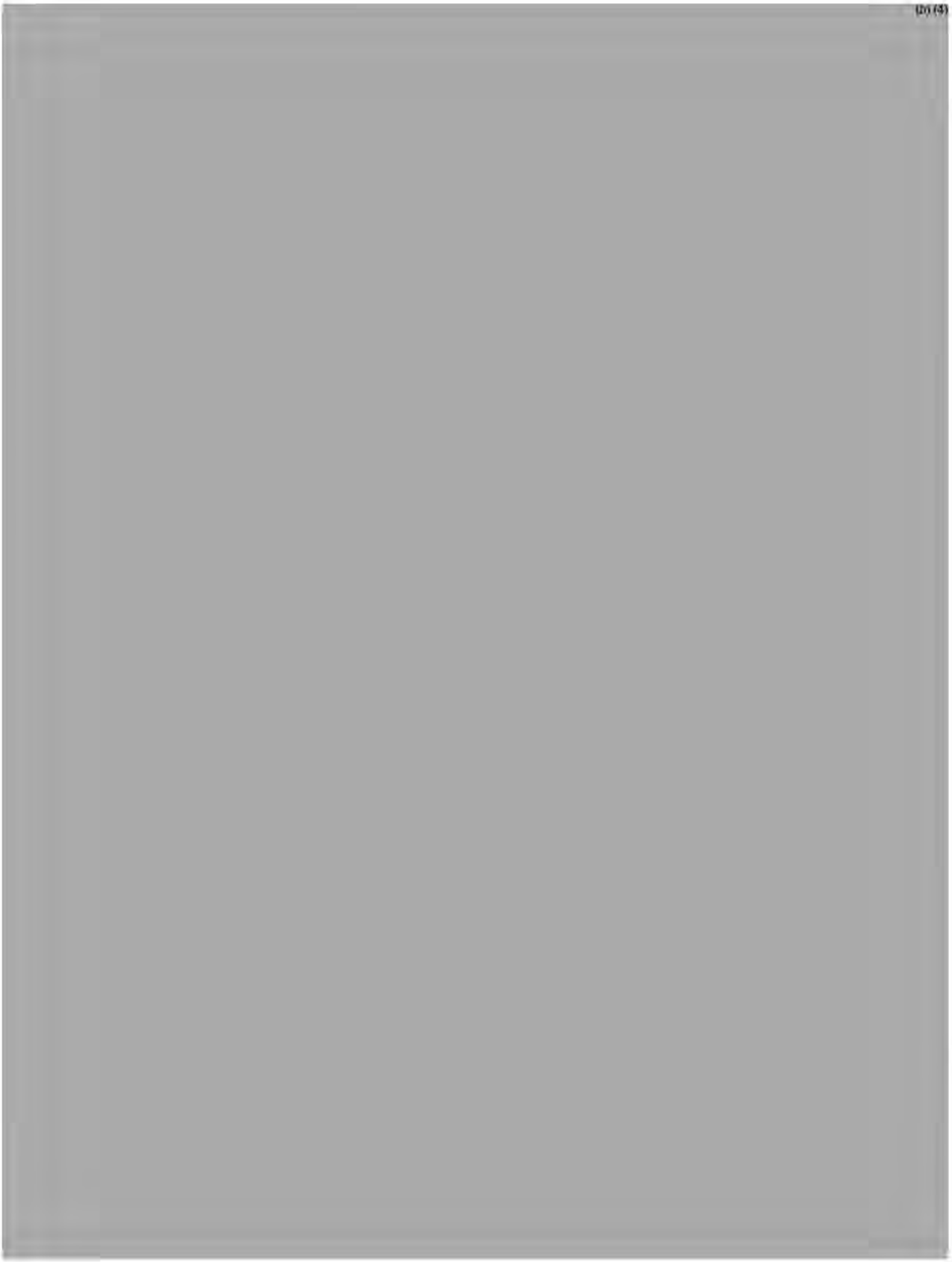

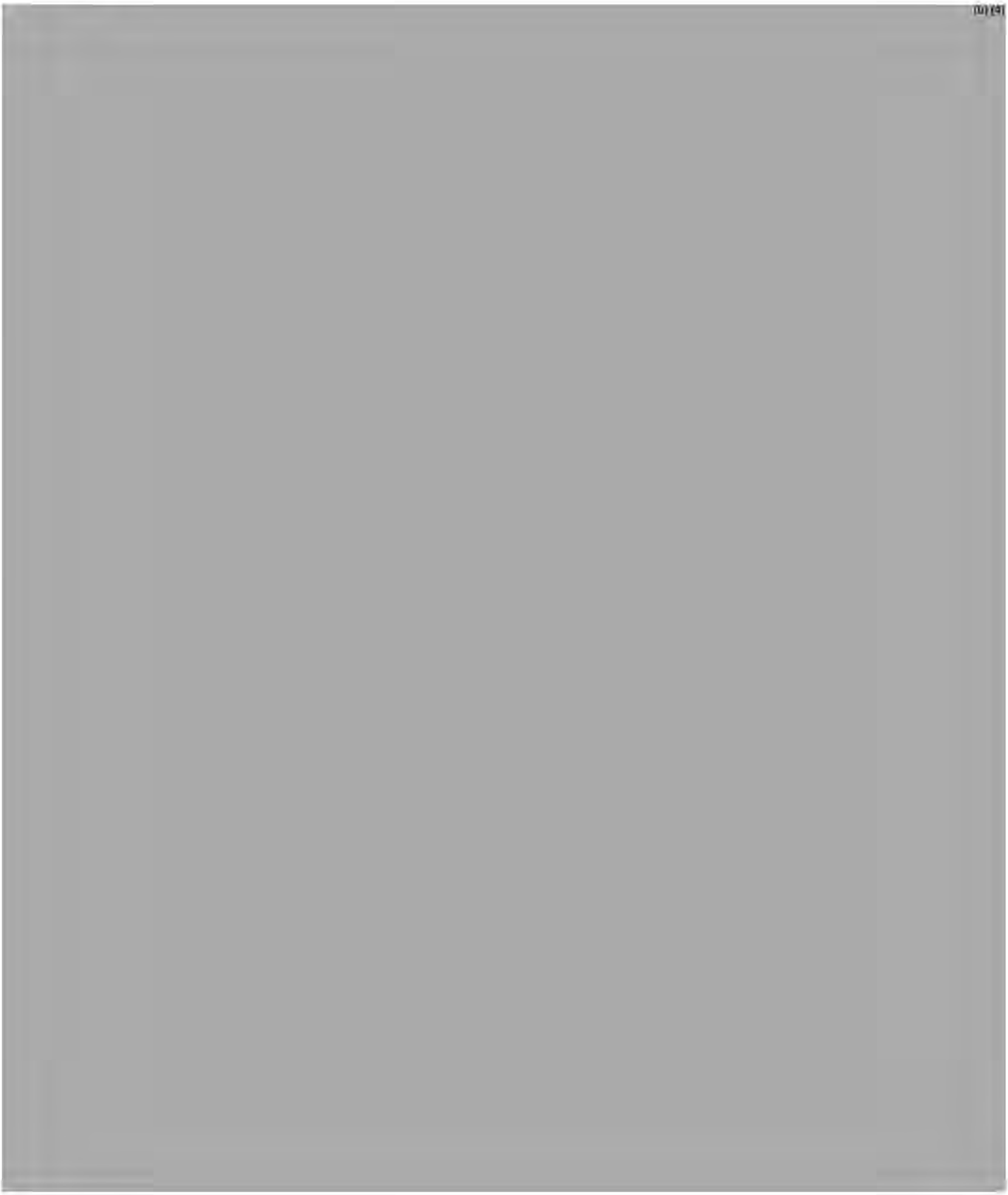

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If you have any questions, contact Fannie Choy, Regulatory Project Manager, at (301) 796-2899 or fannie.choy@fda.hhs.gov.

Sincerely,

{See appended electronic signature page}

Billy Dunn, M.D.
Director
Division of Neurology Products
Office of Drug Evaluation I
Center for Drug Evaluation and Research

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WILLIAM H Dunn
01/17/2017

CENTER DIRECTOR DECISIONAL MEMO

NDA#	**206488**
Drug Name	**EXONDYS 51 (eteplirsen)**
Indication	**Duchenne Muscular Dystrophy (DMD)**
Sponsor	**Sarepta**
Author	**Janet Woodcock, M.D.** **Director, Center for Drug Evaluation and Research (CDER),** **Food and Drug Administration**

(b) (5)

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13 pages of draft language have been withheld as b(5) immediately following this page

CENTER DIRECTOR DECISIONAL MEMO

NDA#	**206488**
Drug Name	**EXONDYS 51 (eteplirsen)**
Indication	**Duchenne Muscular Dystrophy (DMD)**
Sponsor	**Sarepta**
Author	**Janet Woodcock, M.D.** **Director, Center for Drug Evaluation and Research (CDER),** **Food and Drug Administration**

SUMMARY

This memorandum explains the CDER's final decision on the above application. I have read the reviews and recommendations by Drs. Unger (Office level), Bastings (Division level), Farkas (Cross-Discipline Team Lead), Breder and Rao (Clinical Reviewers), Ling (Statistical Reviewer), and Bhattaram, Wu, and Rogers (Clinical Pharmacology Reviewers). In addition to the review memoranda, I have also reviewed the Advisory Committee briefing materials, pertinent portions of the sponsor's submission, and multiple scientific statements submitted by the public, including a letter from a large number of DMD experts.

The review team has done an exemplary job in performing a detailed evaluation of the data submitted with the application. Nevertheless, I disagree with certain of their findings and come to a different conclusion, as discussed below.

I find that the data contained in NDA 206488 meet the standard for accelerated approval under 21 CFR 314. 510 based on the surrogate endpoint of increased dystrophin protein production, a surrogate endpoint that I conclude is reasonably likely to predict clinical benefit.

DISCUSSION

Extensive analyses have been performed by the team on the clinical results of the long-term experience of 12 patients administered the drug, and I will not recapitulate these.

Approval under 314.510 is based, among other things, on adequate and well-controlled clinical trials establishing that the drug product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit. Below, I discuss how both of parts of this standard are met.

A. Are the Data on Dystrophin Protein Production From One or More Adequate and Well-Controlled Studies?

The characteristics of adequate and well-controlled studies are laid out in 21 CFR 314.126. Three lines of evidence are pertinent to the conclusion that eteplirsen results in increased dystrophin production.

- Production of an appropriate mRNA transcript
- Quantitative assessment of dystrophin content in muscle biopsies by Western blot
- Semi-quantitative assessment of dystrophin in muscle tissue by immunohistochemistry (IHC) techniques

The sponsor provided data demonstrating an increase in mRNA expression following treatment with eteplirsen. The drug's proposed mechanism of action is to bridge a section of the pre-RNA to result in a shorter mRNA with an open reading frame, e.g., "exon skipping." In this case, the production of an appropriate mRNA transcript has been documented by PCR and Sanger sequencing. Although this establishes proof of mechanism, it does not mean that there is increased protein production.

In the following, I discuss the assessments related to dystrophin protein production (2. and 3.) in some detail. Much of the controversy over the adequacy of these assessments relates to the fact that rigorously validated assays were not used to evaluate the initial 3 muscle biopsies, apparently resulting in overestimation of the various readouts and some irreproducibility of IHC and Western blot dystrophin assays. For these reasons, I do not discuss or rely upon the results of these earlier assays, or on re-reads of them. With FDA's assistance, the sponsor improved the design and conduct of the assays and performed repeat biopsies on 11 of 12 patients at week 180. The control samples for these week 180 biopsies were stored baseline tissue (in 3 of 11 subjects) and baseline biopsies from subjects with exon 51 amenable mutations enrolled in another trial by the sponsor. FDA reviewers had the following concerns about these controls, leading them to conclude that the studies were not adequate and well controlled.

1. Most of the baseline biopsies were not from the same subjects as the week 180 biopsies (as the original tissue had been used up for the previous assays). Given this, the control subjects could differ in unknown ways from the test subjects.
2. The biopsies taken at week 180 were from different muscles in the upper extremity than the baseline biopsies, including subjects with baseline tissue as well as for control samples. It is hypothesized that there may be differences in dystrophin protein content among various muscles in DMD patients.
3. The existing baseline biopsies for the three subjects with 180 week data had been stored frozen for several years and may have changed (apparent decrease in dystrophin protein content) over time.

In my judgment, these issues increase the uncertainty around the results, but do not necessarily render them an inadequate basis on which to draw a conclusion. The non-treated control subjects were very similar in age and dystrophin mutation site to the treated subjects (sponsor Appendix 10, AC briefing package). The single deltoid muscle biopsy in the untreated control group (subject 7, sponsor Appendix 14, AC briefing package) had replicate dystrophin levels of 0.3% and below the limit of quantification, averaging out at below 0.3%, and not different than biceps biopsy results in other patients, suggesting

that variations in upper extremity biopsy site (concern b above) did not result in large differences in the findings. There was little difference in the dystrophin protein content found in the stored baseline samples and the frozen samples, as discussed below.

The data submitted with the original application, supporting the finding that eteplirsen increases the production of dystrophin protein, come from the quantitative assessment of (internally truncated) dystrophin in muscle tissue by Western blot using the controls described above. Much of the controversy around this method relates to the fact that the apparently achieved dystrophin levels are very much lower than originally hoped (and previously claimed by the sponsor and investigators).

In the 180 week assessment, the three subjects with baseline biopsies available had baseline dystrophin levels (reported as % of normal) below the level of quantification of the assay used (0.25%). These results were similar in magnitude to the baselines of the six additional control biopsies drawn from subjects in another study (highest level 0.37%). At week 180, two treated subjects had (an average of replicate) dystrophin levels above 2%, two had over 1%, and two additional had about 1%. Of these individuals, two subjects having both baseline and week 180 samples had clearly increased levels at week 180 compared to baseline. (The third subject with a baseline sample did not consent to a week 180 biopsy). Unsurprisingly, some subjects had week 180 dystrophin levels similar to the overall baseline control levels. Not all individuals are expected to respond to a drug intervention. The issue is whether the dystrophin levels found at 180 weeks were within the variability expected for this assay in such patients and, thus, could have arisen by chance, or whether they could have been caused by differences from the controls or from sample storage as outlined above, or whether they reflected a drug effect, and, thus, whether these data could be seen as adequate and well-controlled. The following data are relevant to this issue.

Because the original data on the presence of dystrophin by Western blot suffered some difficulties in interpretation because of lack of availability of baseline samples from most patients, the sponsor of this application submitted, subsequent to the Advisory Committee meeting on this drug, additional Western blot data from 12 patients with baseline and 48 week eteplirsen exposure, using baseline and post-treatment muscle biopsies from the same patients and muscle groups. This experiment clearly shows, using adequate controls, that the drug increases dystrophin protein production in some of the patients. The mean baseline dystrophin values in this study were very similar to the mean baseline values in the 180 week study. The achieved levels of dystrophin in these patients are lower than those seen in the Western blots from the week 180 patients. Only 2 of 12 patients achieved a level over 1% of normal control. It is not known if this result is due to a shorter duration of drug exposure or to other factors. Putting together the 180 week data and the additional 48 week data, I conclude that there is substantial evidence from Western blot experiments of increased dystrophin protein production, albeit at a low level.

A finding of increased dystrophin was also seen in several IHC assays performed by the sponsor. Both assays were originally performed with baseline and several pre-180 week assays by the sponsor as a part of the clinical trial. The validity of the results of these assays were questioned by FDA because of methodological problems in their conduct, as documented in the primary clinical review and in the inspection report. Therefore, I will not further consider the results of these original assays. As discussed for the Western blot above, the sponsor responded by performing an additional 180 week biopsy and repeating the assays. Baseline tissue was available, as for Western blot, from recut samples

in only three cases. In one of these, the subject did not consent to a biopsy at 180 weeks. To supplement the three baseline samples the sponsor included six other untreated patients from a different trial, as discussed above for the Western blot. In both assays, greater staining or intensity was observed after drug exposure at week 180 compared to controls. The results are described in more detail below.

A Percent Dystrophin Positive Fibers analysis was a semi-automated evaluation performed at 180 weeks and compared to the controls used for the 180 week study as discussed above. The percentage of positive fibers was assessed using a blinded read by Nationwide Children's Hospital and by three independent pathologists through Flagship Biosciences. The technique used to assess percent positive fibers was modified from the original assay in the following ways:

1. A computer algorithm (MuscleMap from Flagship) that performs non-linear mapping of all fibers was used for consistent and automated analysis of low intensity values, in contrast to a manual and non-standardized fiber counting technique in the prior assay.
2. The images were inverted and amplified to score the total fibers (the denominator for the percent positive fiber scoring).
3. An isotype matched secondary antibody staining step was incorporated to confirm lack of non-specific staining and reduce background noise. The background signal was subtracted from test sample values in calculation of percent intensity.
4. 8% of the images for re-analysis were blinded, renamed, randomized, and rotated 180 degrees.
5. A rejection factor for the inter-rater analysis score of <4 was established.
6. The images were acquired in a more systematic and random fashion to minimize bias, with predefined rules for random sampling of fields and avoiding artifacts.

These changes were likely to result in a more conservative reading of Percent Dystrophin Positive Fibers, and indeed the results, including the new untreated baseline controls, were read at 1.1% positive fibers (in contrast to a higher result in the prior baseline using the original technique). The 180 week cohort had a score, using this technique, of 17.4% positive fibers, showing a statistically significant difference. Now, these results are subject to the same caveats as discussed for the Western blot (1-3 above), in that there were only two baseline to 180 week pairs, that the baseline samples had been frozen for years, and that the external controls might differ in some way. So, these results cannot stand alone.

Other reviewers have pointed out that the (much higher) baseline values for Percent Positive Fibers from the original experiment are not very different from the 180 week values in this new experiment. However, I would point out that experimental conditions changed quite a bit, and very low values for all the external controls, statistically comparable to the frozen baseline results, were obtained in this recent experiment, suggesting that it returned a more conservative result. I do not believe that comparison of the original baseline data, obtained under one set of experimental conditions, can be compared to the later 180 week results, done under different, more optimized conditions and yielding very different results for new (external control) baseline samples.

The sponsor also performed a Mean Relative Fluorescence Intensity assay for dystrophin. This assay is commonly performed by laboratories evaluating DMD patients and is intended to be a semi-quantitative evaluation of dystrophin content. Using the six external baseline samples and the three stored study patient baseline samples, the mean intensity approximately doubled from baseline to 180 weeks. The technique for this assay did not change significantly from the technique used in the assay done as part of

the original protocol, and the baseline means for the patient samples were roughly comparable to the baseline means obtained in the new experiment.

Although the IHC assays provide only semi-quantitative assessments of dystrophin content, they do support an effect of eteplirsen on the proposed surrogate endpoint (an increase of dystrophin production as a result of drug exposure). The accompanying microscopy images also demonstrate correct localization of the molecule within the muscle fibers, a very important factor in any translation to clinical benefit.

In summary, I conclude that there is evidence from adequate and well-controlled trials, and supportive evidence, that exposure to eteplirsen increases dystrophin protein production in muscle cells.

B. Is the Effect on the Surrogate Endpoint "Reasonably Likely to Predict Clinical Benefit"?

In this case, the standard for clinical benefit does not require "cure" or "conversion to Becker MD (BMD) phenotype." Clinical benefit encompasses improvements (including slowing of disease progression) in how an individual feels or functions, or an improvement in survival. There is no question that, for DMD patients and their families, small improvements in function or delays in loss of function are meaningful benefits. Therefore, the question is:

What amount of increase in dystrophin production is reasonably likely to predict clinical benefit (even small benefits)?

The usual way to address this question would be to rigorously evaluate what is known about the correlation between dystrophin levels in muscle and expression of disease. The following summarizes the existing scientific literature on this topic and the challenges in interpreting it.

1. The clinical classification of disease severity (i.e., phenotype) in the literature appears broad, variable, and somewhat subjective.

   Experts usually classify patients clinically as DMD (severely affected at a young age); intermediate MD (also called DMD/BMD); or BMD, which can range from severe BMD to asymptomatic individuals with biochemical abnormalities, usually increased creatine phosphokinase (CPK). There is clearly a wide spectrum of disease wherein the ends of the spectrum are easily distinguishable, but the zone of real interest for this discussion, between DMD and intermediate presentations, is not rigorously categorized. In part, this is because "intermediate muscular dystrophy" (IMD) is less common, due to the consequences of having either in-frame mutations with a truncated protein expressed (leading to BMD) or out-of-frame mutations with little-to-zero protein expressed (leading to DMD), as discussed below.

2. Much of the prior data reporting the relationship of dystrophin protein levels to phenotype have been from IHC studies using a variety of techniques and antibodies.

   Anthony, et al., (*Neurology*, 83, 2014) in a collaborative cross-laboratory study, investigated the variability of techniques used to quantify dystrophin in individuals with muscular dystrophy. Blinded tissue sections from three DMD and three BMD muscle biopsies were tested in five

different laboratories accustomed to performing dystrophin quantification. Estimates of dystrophin expression using a somewhat standardized IHC technique were about 20%, 11% and 10% of normal for the three DMD samples, on average among the laboratories. Corresponding estimates of dystrophin content by Western blot, using an actin antibody to normalize for loading, but not a serially diluted standard control, resulted in dystrophin estimates of about 11%, 0, and 0.4% respectively, with fairly high CV's. Therefore, in this small sample, repeated across five experienced laboratories, IHC estimates were about 10 percentage points higher than Western blot estimates.

Significantly higher estimates by IHC by fluorescence intensity (overall about 23% of normal) than by Western blot were also seen in the evaluation of week 180 muscle biopsies in the Sarepta trial. Because much of the historical data on protein content vs phenotype has been reported using IHC analysis, extrapolating these findings to the current trial data is challenging. Additionally, Anthony et al., found that the inter-laboratory variability was greatest for the low levels of dystrophin found in the DMD patients. Western blot data in the literature quantifying dystrophin and relating it to phenotype is often from experiments that were not designed to distinguish among dystrophin levels below 10% of normal. These may have been reported out as "less than 10%." From this sponsor's well-controlled studies, the analytically accurate dystrophin baseline for many DMD patients might be in the range of 0.02-0.35 % normal, hence previous estimates of 5-10% might be an over-estimation using non-standardized and semi-quantitative methods.

3. Both IHC analyses and WB results are influenced by the anti-dystrophin antibodies used, as well as other experimental conditions

Significantly, if the epitope recognized by the antibody is modified by the deletion, the dystrophin isoform may not be recognized and a result read out as zero. For this reason, recent studies use multiple antibodies against known regions. Additionally, muscle biopsies in patients with BMD and DMD may be quite variable in degree of fibrosis and fatty replacement; this may decrease the reproducibility and representativeness of muscle biopsy estimates of dystrophin content by Western blot. Additionally, imaging methods, choices for normalization, biopsy handling, background standing, and a multitude of other experimental conditions can influence results.

4. The phenotype is significantly influenced by *dystrophin isoform quality* as well as *dystrophin quantity*.

Dystrophin is a very large protein with multiple functional domains. Generally, DMD results from an out-of-frame mutation (often a deletion) that leads to an unstable or unreadable mRNA transcript. Thus, DMD patients usually have zero or very low levels of dystrophin, but the DMD phenotype can also result from in-frame mutations that result in a unstable transcript or dysfunctional dystrophin isoform. BMD usually results from an in-frame mutation (often an exon deletion) that affects the functional quality of the protein and also the quantity produced. It remains unclear what role protein function plays vs quantity in leading to the wide range of variability in BMD phenotypes. There are a vast number of mutations that can lead to each of these phenotypes (Tuffery-Giraud, et al., *Hum Mutat*, 30, 2009), all of which can have different effects on protein function as well as protein production. This micro-heterogeneity is common in genetic diseases and is highly germane to

evaluation of interventions targeting the gene, gene expression, or protein function. There are also non-dystrophin-related factors that can modulate phenotype.

5. The literature contains various findings on the relationship of dystrophin expression to clinical status, including the low levels of dystrophin protein of interest in this case.

I note that in the decades since 1988, much technical progress has been made in standardizing Western blot techniques, and the results from early studies may not be fully comparable to those from recent experiments.

a. The seminal 1988 paper on this subject (Hoffman et al., *NEJM*, 318(21)) found that the majority of patients with DMD had undetectable levels of dystrophin using their Western blot technique and that 35 of 38 had levels below 3% in their assay. They also reported that one of seven "intermediate" patients had dystrophin levels below 3% of normal, as did one of the 18 patients with a BMD phenotype.

b. Beggs et al., (*Am J Hum Genet*, 49, 1991) published one of the early studies on the correlation between the level of dystrophin on Western blot and clinical features of BMD. Western blot was performed using a polyclonal serum and had about a 20% variability between blots according to the authors. In this study a number of patients with BMD or intermediate phenotype (DMD/BMD) were found to have dystrophin contents that overlapped with those of the DMD patients. Of four patients included with DMD phenotype, two had less than 5% dystrophin, and two had 10%, by their assay. Of patients with BMD/DMD phenotypes, eight were found to have 10% of normal dystrophin, two had 15%, one had 50%, and one had 100%. Three BMD patients with dystrophin levels of 10% were found; two of these had relatively mild disease.

c. Nicholson et al., (*J Med Genet*, 30, 1993) studied patients across a wide range of DMD and BMD phenotypes. They used loss of ambulation as a criterion to establish five functional groups, grouped from one (most severe, LOA before age 9) to five (LOA past age 40) (pre-steroid era). *They found a linear relationship overall between dystrophin levels (Western blot with Dy4/6D3 antibody, using myosin for a loading control) and their five categories, with more dystrophin protein translating to better function. They found no significant difference between any two adjacent groups however, which they interpreted as showing considerable overlap, as reflected in their patient level data (Appendix 1), which showed a number of less severe patients (e.g., Group 2 or 3) registering no or very low dystrophin abundance on their Western blot assay.* Of note, they reported a higher average level of dystrophin protein in severe DMD patients than other investigators, partly resulting from 5 of their 21 severe patients reported to have dystrophin protein levels above 20.

d. Neri et al., (*Neuromuscular Disorder* 17, 2007) reported on families with X-linked Dilated Cardiomyopathy. In these families, mutations give rise to absent dystrophin in heart muscle, but only reduced levels of nearly normal dystrophin in muscle tissue. One patient in their series had a normal neurological exam at age 23, an elevated CPK, and 29% of normal dystrophin protein in skeletal muscle by Western blot. This example can contribute to understanding the role of abundance of dystrophin protein vs compromised function.

e. Anthony et al., (*JAMA Neurology*, 71, 2014) evaluated the correlation between phenotype and mRNA and protein expression in patients with both in-frame and out-of-frame mutations amenable to exon 44 or 45 skipping. Studying a group of patients with closely related deletions could diminish variability due to differences in function of the truncated protein. Five samples from patients with clinical "mild" BMD and in-frame mutations underwent Western blot analysis using the Dys-2 antibody. Their mean protein expression was 17% (normalized to actin) with a standard deviation of 7.5%. Two of the "mild" patients had dystrophin levels in this assay of around 10%. Based on comparisons of IHC experiments with various antibodies, the authors found "*no clear correlation between the level of dystrophin transcript or protein expression with clinical severity" in 13 patients with in-frame mutations leading to BMD.* The finding of Neri et al., above, along with this report, reinforce the concept that protein function (i.e., quality) is an important determinant of clinical severity and undermine the concept that 10% dystrophin protein content is a threshold, since these patients had "mild" BMD.

f. Van den Bergen et al., (*J Neurol Neurosurg Psychiatry*, 85, 2014) compared dystrophin levels by Western blot with clinical severity in 27 patients with a clinical diagnosis of BMD. Dystrophin expression ranged from 4-71% and 3-78%, depending on the antibody used. *The authors found no linear relationship between dystrophin expression by Western blot using newly acquired muscle biopsies and clinical severity, muscle strength, or fatty infiltration on MRI.* Although this was the case for the majority of the patients, who had dystrophin levels above 20% of normal, four patients had levels at or below 10%. These patients generally had a more severe phenotype: one patient with a dystrophin level of 10% was wheelchair dependent at 45 years; one patient with a level of 7% developed trouble with stair walking at age 21; one patient with a level of 4% had a DMD phenotype with wheelchair dependency at age 10, one patient with a level of 3% had wheelchair dependency at age 25.

g. Anthony et al., (*Brain*, 134, 2011) studied 17 BMD patients with exon 51 or 53 skipping-amenable mutations by IHC methods. These patients primarily had very mild or asymptomatic disease; the one patient classified as severe was ambulatory at age 25 but unable to run. *There was a statistically significant difference in dystrophin expression by IHC when patients classified as mild disease were compared to asymptomatic patients.*

h. Bello et al., (*Neurology* 87, 2016) published a detailed study of loss of ambulation in DMD patients with particular exon deletions, using the CINRG-DNHS, a prospective natural history study. They found patients with exon 44 amenable mutations to have a two-year delay in loss of ambulation compared to the overall comparison group. This finding had previously been reported by another group (van den Bergen, et al., *J Neuromuscul Dis*, 1, 2014). The mutations studied (primarily single-exon deletion of exon 45) are known to undergo spontaneous skipping with production of some dystrophin. According to the Bello report, of six patients previously tested by IHC, three showed traces of dystrophin production and 0/four (possibly other patients) had dystrophin detectable by Western blot. These authors suggest that the observed differences in loss of ambulation (LOA) could be due to small amounts of spontaneously induced dystrophin that slightly ameliorate the ordinary DMD phenotype.

i. Cirak et al., (*Lancet*, 378, 2011) published a study (AVI-4658) using intravenously administered eteplirsen that showed a detectable increase in dystrophin protein levels using both Western blot and immunofluorescence in 3/19 patients. The authors reported that the functional properties of restored dystrophin were confirmed by assessing increased levels and co-localization of neuronal nitric oxide synthase (nNOS) and sarcoglycan with dystrophin. Such a protein assembly is suggested to be indicative of functional restoration of the dystrophin-associated glycoprotein complex in muscle fibers (Molza et al., *JBC*, 290, 2015; Wells KE et al., *Neuromuscul Disord*, 2003). Cirak et al., reported that the restoration was more so in patients with exon 49-50 deletions than in those with 45-50 deletions, which is consistent with a previous observation that nNOS binding domain is located in dystrophin exons 42-45 (Lai Y et al., *J Clin Invest*, 2009). These studies suggest that important functional domains are included in the dystrophin protein induced by eteplirsen.

To summarize what is known about the association between dystrophin levels and phenotype, dystrophin content above about 10% on Western blot is usually associated with a BMD phenotype, except in patients with higher levels of dystrophin (including above 50%) who potentially have functionally deficient protein leading to a DMD phenotype. Within the BMD phenotype, a proportional inverse relationship between disease severity and protein expression has not generally been demonstrated (i.e., between 10-100%), although there may be a broad association, as seen in the Anthony study (*Brain*, 134, 2011). This may be due to the fact that protein quality, rather than quantity, plays a key role in determining phenotype in BMD. Patients with DMD are usually found to have no detectable, or very low levels of, dystrophin. Dystrophin content in the 3-10% range has been associated with DMD, DMD/BMD, and BMD phenotypes. I find no evidence of a threshold value for protein content and expression of a DMD phenotype, although the majority of DMD patients reported in the literature have dystrophin that is undetectable by the Western blot assays used. Generally, the divide between DMD and BMD, in terms of protein, is the result of the consequences of an OOF or an in-frame mutation, respectively. I believe that the conventional threshold, at or below 10% protein, was derived from the IHC data that seem to estimate low-level protein content about 10% percentage points higher on IHC than on Western blot, so that the majority of DMD patients would read out at 10% of normal dystrophin on IHC. I believe that evidence from Western blot and other experiments discussed above show that protein in the range between undetectable and 10% of normal is likely to be very important for clinical presentation, all other things being equal, i.e., mutation status and non-dystrophin-related factors affecting phenotype.

These findings are germane to the determination of "reasonably likely to predict clinical benefit." The broad phenotypic distinctions made in the clinic (e.g., DMD vs IMD vs BMD) are different from the prediction of benefit to an individual patient who has a specific baseline dystrophin level and whose mutation and external factors do not change pre- and post-drug. For example, extending ambulation by six months to a year would not normally move a patient from one to another of these categories, but could be very important to quality of life (e.g., as suggested in the Bello study). This is also true for other functional improvements.

For these reasons, incorporating the analysis of dystrophin content discussed above, I conclude that the biochemical data strongly support the idea that low-level increases in dystrophin production are reasonably likely to predict clinical benefit.

Additional support for "reasonably likely" comes from the long-term experience with the drug. The sponsor's comparison of the experience of the treated cohort to natural history data does not reach the level of substantiation required for traditional approval based on the clinical data. However, it is highly suggestive of improvement in some parameters, in some patients, over natural history. My conclusion is informed by all the caveats expressed in the reviews about the pitfalls of non-randomized comparisons. Given that the two exon 52 deletion patients in the study had fairly good long-term results in terms of rate of disease progression, the question arises as to whether exon 52 is a prognostic factor that could have skewed the results.

Several facts militate against this conclusion. First, one of the exon 52 deletion trial subjects (subject 6) had a fairly low score on the 6MWT at entry and a very low score on the NSAA, compared to other subject around his age. He also was the only subject in the trial noted to be unable to rise without external support at baseline. Additionally, the Italian external cohort had exon 52 deletion representation.

Questions have been raised about the correlation of dystrophin levels from Western blot with clinical outcomes. The 6 Minute Walk Test does not show a strong correlation. I evaluated the NSAA in children who could still walk (because the NSAA primarily scores activities related to walking) and who also had a dystrophin result at 180 weeks (Table 1). I did this because the NSAA includes multiple measures and therefore might have some noise averaged out. I looked at the absolute decline in NSAA in patients since study initiation, and did not correct for the initial time some patients spent on placebo. I only evaluated patients who were ambulatory. There was a positive (inverse) correlation between dystrophin by Western blot and rate of decline in NSAA score, . (Figure 1) This adds additional support to the idea that dystrophin production is "reasonably likely to predict clinical benefit." In totality, I find that the comparative disease course data provide additional support for the use of the surrogate endpoint of an increase in dystrophin expression as "reasonably likely to predict clinical benefit."

Therefore, both the biochemical data and the clinical data lead me to conclude that an "increase in dystrophin production" is reasonably likely to predict clinical benefit in DMD.

CONFIRMATORY TRIALS

The sponsor is currently conducting a nonrandomized, concurrently controlled trial in patients with mutations amenable to exon 51 skipping compared to untreated DMD patients with other exon deletions. Because of the relatively low level of protein induced, additional doses should be aggressively pursued and, if successful, a dose-comparison trial could be confirmatory. The sponsor has also planned to initiate a randomized trial with a related compound in other exons. The clinical results from these trials can inform the predictive value of the surrogate endpoint.

EXPLORATION OF ADDITIONAL DOSES, REGIMENS, AND DRUG-MUTATION INTERACTION

The dystrophin levels achieved in this development program are well below those initially hoped for. I agree with Dr. Farkas and other reviewers that the sponsor should aggressively explore higher doses or more frequent administration of eteplirsen. It appears that this is possible given the toxicology data and the clinical safety profile observed to date.

Because patients in the Sarepta 180 week cohort had a range of deletions in the dystrophin gene, variability in the pharmacodynamic response among deletions is of great interest. The two patients with over 2% dystrophin in the 180 week Western blot both had exon 52 deletions. These patients also fared fairly well, clinically. This raises the question of whether patients with this exon deletion naturally produce more dystrophin. One of these subjects had a baseline sample available. It was found to be below the limit of quantitation. There was an exon 52 subject included in the added baseline controls. This subject's assay had replicate results of 0.3% and below the limit of quantification, respectively, as discussed above. This suggests that baseline dystrophin levels are not higher in exon 52 deletion subjects and that there may be a drug-deletion interaction, wherein subjects with this deletion may have a more robust pharmacodynamic response to the drug. There were a number of apparent non-responders to the drug. It will be important to find out if this is mutation specific. It is likely that more detailed knowledge about each patient's specific mutation will have to be generated to study this in detail.

COMMENTS ON THE DEVELOPMENT PROGRAM AND REVIEW

The development program for eteplirsen was seriously deficient in a number of respects that may have led to delay in broad access and certainly led to difficulties in regulatory review. In my assessment, the most egregious flaw was the lack of robust and high-quality assays early in the development program. Inaccurate conclusions from the assays used led to a flawed development program. Additionally, the entire drug development field must recognize that there is no such thing as an "exploratory study" for a serious, life-threatening illness without therapeutic options. Randomization should be performed very early in the development program, and open-label studies should be avoided. When possible, seamless adaptive dose-finding and early efficacy studies should be carried out with the goal of most efficiently generating the data needed to demonstrate safety and effectiveness.

The flaws in the eteplirsen development program led to severe challenges in regulatory review. 21 CFR 312.80, concerning drugs intended to treat life-threatening or severely-debilitating illness, states that FDA has determined "that it is appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness…Physicians and patients are generally willing to accept greater risks or side effects from products that treat life-threatening and severely-debilitating illnesses than they would accept from products that treat less serious illnesses." I note that the acceptable risks include greater uncertainty about the effects of the drug. The Peripheral and Central Nervous System Drugs Advisory Committee met on this application on April 25, 2016. There was a split vote (7 against, 6 for) on the question of accelerated approval for this drug, reflecting the greater than usual uncertainty about the application. This vote was taken before the additional data on protein expression were submitted.

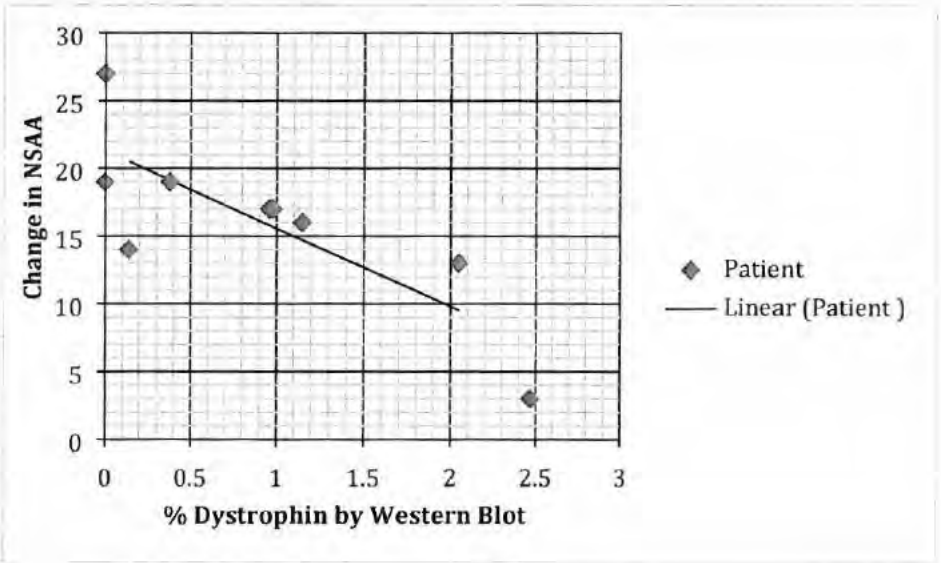
To conclude, the studies used in this analysis to support the effect of eteplirsen on dystrophin were adequate and well-controlled as specified in 314.126. In addition, the surrogate of increased dystrophin production is reasonably likely to predict clinical benefit. Given the deficiencies that have been identified in the development program, my conclusion to rely on the surrogate endpoint described above represents the greatest flexibility possible for FDA while remaining within its statutory framework. In this case, the flexibility is warranted because of several specific factors, including: the life-threatening nature of the disease; the lack of available therapy; the fact that the intended population is a small subset of an already rare disease; and the fact that this is a fatal disease in children. Of note, the therapy has been relatively safe in the clinic, although intravenous administration always carries risk. In addition, adequate confirmatory studies are underway and planned and are capable of further refining our understanding of the biomarker and providing evidence about the nature of the clinical benefit. The approval does not create any risk of compromising the confirmatory trials because of their nature. Therefore, I find that the probable benefits outweigh the foreseeable risks and that this application should be approved under 21 CFR 314.510.

Table 1 Patient Data on Change from Baseline in 6MWT and NSAA

Subject	Baseline WB	180 Week WB	Fiber Intensity	PDPF	Δ 6MW	Δ NSAA
					180 Weeks Change from Baseline	
002	N/A	0.14	MD	4.54	-67	-14
003	N/A	0	7	1.42	-174	-27
004	N/A	0.96	28	29	-168	-17
005	0	N/A	N/A	N/A	-231	-19
006	N/A	2.47	29	21	-23	-3
007	N/A	0	12	7	-197	-19
008	N/A	0.98	26	12	-291	-17
009	N/A	0.52	23	22	0	0
010	N/A	1.62	21	24	0	0
012	N/A	0.38	26	33	-114	-19
013	0	1.15	32	19	-170	-16
015	0	2.05	30	18	-1	-13

Data from Sarepta Therapeutics , Inc. PCNSD Advisory Committee Briefing Document, Appendix 5, p. 149 (6MW and NSAA0 Appendix 11, p. 155, (Percent Positive Dystrophin Fibers (PPDF), Appendix 12 p. 156 (fiber intensity) 14, p. 159. (Western blot),

Figure 1. Decline in NSAA by % Dystrophin on Western blot



This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JANET WOODCOCK
07/14/2016

Philips, Howard

From: Temple, Robert
Sent: Friday, September 20, 2013 2:36 PM
To: Jenkins, John K; Bastings, Eric; Woodcock, Janet; Unger, Ellis
Subject: RE: GSK - IND105284 - Phase III Pivotal Study Outcomes

(b) (4)

From: Jenkins, John K
Sent: Friday, September 20, 2013 2:29 PM
To: Bastings, Eric; Woodcock, Janet; Temple, Robert; Unger, Ellis
Subject: RE: GSK - IND105284 - Phase III Pivotal Study Outcomes

Eric

(b) (4)

John

From: Bastings, Eric
Sent: Friday, September 20, 2013 9:38 AM
To: Jenkins, John K; Woodcock, Janet; Temple, Robert; Unger, Ellis
Subject: FW: GSK - IND105284 - Phase III Pivotal Study Outcomes

(b) (4)

.

Eric

From: Sherman Alfors [mailto:sherman.n.alfors@gsk.com]
Sent: Friday, September 20, 2013 6:32 AM
To: Choy, Fannie (Yuet)
Subject: GSK - IND105284 - Phase III Pivotal Study Outcomes

Dear Fannie,

The purpose of this communication is to inform FDA of the outcome of our initial analysis of the results from our Phase III study DMD114044. The analysis showed no statistical or clinically meaningful difference in the primary endpoint, the 6 minute walking distance test, between drisapersen and placebo. Full evaluation of the benefit-to-risk profile of drisapersen treatment across all studies is anticipated to be completed by year end.

(b) (4)

If you have any questions regarding this information, please do not hesitate to call me.

Thank you,

Sherman

Sherman N. Alfors
Global Regulatory Affairs
GlaxoSmithKline
☏ 8-703-6030 / 919-483-6030 ✉ sherman.n.alfors@gsk.com

Philips, Howard

From: Temple, Robert
Sent: Wednesday, October 02, 2013 8:47 AM
To: Jenkins, John K; Unger, Ellis
Subject: RE: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

Note that making the ph 3 drug properly controlled (it starts prior to approval and availability) should cause NO delay if we are prepared to go with accelerated approval, which is certainly still on the table.

From: Jenkins, John K
Sent: Wednesday, October 02, 2013 8:42 AM
To: Unger, Ellis; Temple, Robert
Subject: RE: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

You can expect that she is coming to the meeting with strong ideas on what should be done, so this is not really a "briefing". If we do not agree with any of her points we will need to make a strong case or the train will leave the station with us on the platform watching. She has been taking a very aggressive tone on breakthrough drugs and will not look favorably on anything she see as delaying this moving forward. If at all possible it would be good to have the dystrophin data for the GSK drug by the time we meet.

John

From: Unger, Ellis
Sent: Wednesday, October 02, 2013 8:37 AM
To: Jenkins, John K
Cc: Bastings, Eric; Temple, Robert; Kweder, Sandra L; Locicero, Colleen L
Subject: RE: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

Ok. Thanks for the important heads up.

From: Jenkins, John K
Sent: Wednesday, October 02, 2013 8:36 AM
To: Bastings, Eric; Unger, Ellis; Temple, Robert; Kweder, Sandra L
Cc: Jenkins, John K
Subject: FW: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

As a heads up, the things Janet wants to talk about include:

1. Asking DSI to inspect the site that did the immunohistochemistry now, rather than waiting until the NDA is submitted
2. Asking the field to inspect the manufacturing facility now since they are in production
3. Review what we know about the GSK drug that failed, with particular attention to any dystrophin data and the dose that was used in the pivotal trials. Janet has heard from parents who think their sons were benefiting from the GSK drug and who think the drug failed in the Phase 3 trial because they had to titrate the dose down due to toxicity and may have gone to a dose below the minimally effective dose
4. Any update on the long-term follow up on the patients on the Sarepta drug, she has heard they now have the 96 week data and it is encouraging
5. Discussion of our request for new biopsies in the patients on the Sarepta drug. She says the community is "up in arms" about this, but may be willing to agree if these data are truly necessary

6. Discussion of the planned phase 3 trial, she is strongly opposed to the idea of a placebo controlled trial and says the community will not accept such a design

As you can see, Janet has been talking to many people in the community and will be coming into the "briefing" with strong ideas of the path forward for this drug. I wanted to share this information so you can start thinking about these issues in advance. Janet wants to meet with us BEFORE we meet with the sponsor. I would like to have an internal pre-meeting with the team before we meet with Janet.

John

From: Woodcock, Janet
Sent: Tuesday, October 01, 2013 3:20 PM
To: Jenkins, John K; Unger, Ellis
Cc: Ligon, Sharnell (CDER); Bechtel, Christine
Subject: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

Philips, Howard

From: Temple, Robert
Sent: Wednesday, October 02, 2013 8:50 AM
To: Jenkins, John K
Subject: RE: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

Don't worry, I will. But success here depends on the expected limited availability of the drug, which, so far as we know, persists. It's enormously to everyone's advantage to do that, again, if the amount of drug is limited. If it is not I agree4 that the study will not be doable.

From: Jenkins, John K
Sent: Wednesday, October 02, 2013 8:47 AM
To: Temple, Robert
Subject: RE: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

Bob

I told Janet of your thinking on this and her response was "I do not agree." She was loaded for bear when I met her yesterday on this and many other topics. It was NOT a pleasant meeting. So, you will need to put on your best arguments for why a placebo controlled trial is needed since he mind has been made up by her conversations with the parents.

John

From: Temple, Robert
Sent: Wednesday, October 02, 2013 8:45 AM
To: Jenkins, John K; Bastings, Eric; Unger, Ellis; Kweder, Sandra L
Subject: RE: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

Thanks. The GSK results make me more sure than ever that the phase 3 trial needs to be placebo-controlled and randomized, not an "externally controlled trial with the control being patients with a different deletion. Given the shortage of drug I don't understand the reason for objection. Once in the placebo grou[p there could be early crossover, etc when drug becomes available. But we'll talk.

From: Jenkins, John K
Sent: Wednesday, October 02, 2013 8:36 AM
To: Bastings, Eric; Unger, Ellis; Temple, Robert; Kweder, Sandra L
Cc: Jenkins, John K
Subject: FW: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

As a heads up, the things Janet wants to talk about include:

1. Asking DSI to inspect the site that did the immunohistochemistry now, rather than waiting until the NDA is submitted
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6. Discussion of the planned phase 3 trial, she is strongly opposed to the idea of a placebo controlled trial and says the community will not accept such a design

As you can see, Janet has been talking to many people in the community and will be coming into the “briefing” with strong ideas of the path forward for this drug. I wanted to share this information so you can start thinking about these issues in advance. Janet wants to meet with us BEFORE we meet with the sponsor. I would like to have an internal pre-meeting with the team before we meet with Janet.

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From: Woodcock, Janet
Sent: Tuesday, October 01, 2013 3:20 PM
To: Jenkins, John K; Unger, Ellis
Cc: Ligon, Sharnell (CDER); Bechtel, Christine
Subject: I'd like to have another briefing on the muscular dystrophy drug (not the GSK one) Thanks. jw

Philips, Howard

From: Temple, Robert
Sent: Tuesday, October 15, 2013 4:19 PM
To: Blount, Aprile
Subject: FW: *Mtg Pkg Link Update* : INTERNAL/Type C Mtg: IND077429 /eteplirsen (Sarepta) for DMD

I copied the e-room thing.

From: Choy, Fannie (Yuet)
Sent: Tuesday, October 15, 2013 10:06 AM
To: Jillapalli, Devanand; Wu, Ta-Chen; Siddiqui, Ohidul I; Zhang, Li; Rogers, Hobart
Cc: Temple, Robert; Unger, Ellis; Freed, Lois M; Wilcox, Barbara; Choy, Fannie (Yuet); Pacanowski, Michael A; Bhattaram, Atul; Men, Angela; Bastings, Eric; Farkas, Ronald; Jin, Kun
Subject: RE: *Mtg Pkg Link Update* : INTERNAL/Type C Mtg: IND077429 /eteplirsen (Sarepta) for DMD

All,

Sponsor has submitted additional info related to the Type C meeting package: briefing package correction, responses to IR and reference (links attached below). I'll also update the links in the meeting invite.

"…typo in the briefing document for this meeting. On page 19 of 51, Figure 1 entitled "Study Schematic for Design 1: Double-Blind Placebo-Control Design," the treatment arm of the proposed study is mislabeled as containing 40 treated patients. This number should actually be 80 treated patients. The correct number is given in the document text. The same figure is also included in Appendix 11.2, page 41 of 51".

eRoom: http://eroom.fda.gov/eRoom/CDER9/DivisionofNeurologicalProducts/0_1964e
Reference in EDR: \\Cdsesub4\NONECTD\IND077429\5389915
SAS and CSV data files for Study 4658-us-201/202 functional assessments through Week 96:
\\CDSESUB4\NONECTD\IND077429\5391476

Thanks
Fannie

-----Original Appointment-----
Subject: *Mtg Pkg Link* : INTERNAL/Type C Mtg: IND077429 /eteplirsen (Sarepta) for DMD
When: Thursday, October 31, 2013 12:00 PM-1:00 PM (GMT-05:00) Eastern Time (US & Canada).
Where: CDER WO 4270 conf rm Bldg22

10/9/13 Update:

- Add Mtg Pkg Link | eRoom: http://eroom.fda.gov/eRoom/CDER9/DivisionofNeurologicalProducts/0_1964e
- Add Preliminary Cmts Template; Desk copies have been distributed
  << File: IND077429 eteplirsen_DRAFT Prelim Cmts.doc >>

INTERNAL Meeting: to prep for Type C Meeting/TC
IND: 077429
Product/Sponsor: eteplirsen / Sarepta Therapeutics
Indication: Duchenne muscular dystrophy

Purpose:

- To discuss confirmatory postmarketing clinical study design of eteplirsen, in anticipation of NDA filing based on existing data

Internal: ~~**11/25/13 10:00 am**~~ **10/31/13 12:00 Noon**
Sponsor: ~~**12/9/13 3:00 pm**~~ **11/8/13 4:00 pm (Tcon)**

~scheduled by Fannie Choy on 9/18/13; Resch 9/27/13~ 301-796-2899~

Philips, Howard

From: Temple, Robert
Sent: Tuesday, October 15, 2013 11:05 AM
To: Bastings, Eric; Unger, Ellis; Woodcock, Janet
Subject: FW: Sarepta Confirmatory

I don't plan to respond at the moment. Perhaps after our Friday meeting.

From: (b) (6) [mailto: (b) (6)
Sent: Saturday, October 12, 2013 4:44 PM
To: Temple, Robert
Subject: Sarepta Confirmatory

Hello Dr. Temple,
I'm sure that you have heard that (b) (6) drisapersen study failed. Although I don't consider it a failure so much as an experiment in sub-therapeutic dosing, with all of the expected variation between patients and an inability to produce clear benefit in a short timeframe across the entire population.

I wanted to let you know that I had an opportunity to go to the World Muscle Society meeting last weekend, and I spent about an hour and a half with the Vice President in charge of orphan drug development for GSK. He assured me that GSK will share granular data on all three of their placebo-controlled studies. What this means is that we will have data from over 100 boys with the same genotypes targeted by eteplirsen who were treated on a placebo arm for anywhere between 24-28 weeks. I think it's laughable to suggest that little boys on a drisapersen placebo arm would perform any differently than in a potential eteplirsen placebo arm. So in essence, the data for the placebo arm of Sarepta's confirmatory study has already been captured; it's just been done by GSK.

It is also starting to sound like Sarepta's manufacturing ramp-up has been successful enough that they will be able to dose all US boys who will fit the inclusion criteria for the study. The stability of the 96 week data lends more and more credence to the idea that these 12 boys are the luckiest boys with Duchenne who ever walked the planet. As the top DMD clinician in the world, Dr. Muntoni, said about the eteplirsen 96 week results, "without [eteplirsen] this is not what happens in my clinic."

Given these facts, the inclusion of a placebo arm in Sarepta's confirmatory, putting boys on a saline placebo and cutting them open multiple times, feels like nothing more than sadism hidden behind a mask of scientific integrity.

I would love a chance to discuss this further if you think it would be valuable. Please do just let me know.

Thanks very much,
(b) (6)
(b) (6)

Philips, Howard

From: Temple, Robert
Sent: Tuesday, October 22, 2013 3:04 PM
To: Blount, Aprile
Subject: FW: Available slot for a f/u meeting with Dr. Woodcock on eteplirsen

Are we following this? I need to be there.

From: Unger, Ellis
Sent: Tuesday, October 22, 2013 2:25 PM
To: Lu, Wei; Temple, Robert
Cc: Shekitka, Barbara; Blount, Aprile
Subject: RE: Available slot for a f/u meeting with Dr. Woodcock on eteplirsen

I am tied up Thursday from 9-11. I can't get free.
Friday – both times are open
Monday – I could get open.

From: Lu, Wei
Sent: Tuesday, October 22, 2013 8:35 AM
To: Temple, Robert; Unger, Ellis
Cc: Shekitka, Barbara; Blount, Aprile
Subject: Available slot for a f/u meeting with Dr. Woodcock on eteplirsen
Importance: High

Good morning Dr. Temple, Dr. Unger,

I need your help to find an opening slot for the follow up meeting with Dr. Woodcock on eteplirsen. Barbara provided me with your availabilities based on the few choices that we have (see the list below):

Thursday, 10/24, 9-11am
Friday, 10/25, 10-11am or 2-3pm
Monday, 10/28, 9-10am

However, Dr. Jenkins is on leave on Friday (10/25), and he could do Thursday 10-11am. Perhaps, I could go back to ask for the Monday slot again. But I wanted to check with you if any of your meetings on Thursday at 10-11am could be tabled. Please let me know asap, since these slots are a hot commodity, it won't be long on the market.

Thanks bunch!
Wei

_____
Wei Lu, RN, MS
CDER/OEP/DEO
Bldg 51, Room 6174
Office: 301-796-3448
Fax: 301-847-8753
Email: Wei.Lu@fda.hhs.gov

Philips, Howard

From: Temple, Robert
Sent: Thursday, October 31, 2013 5:28 PM
To: Blount, Aprile; Choy, Fannie (Yuet); Unger, Ellis
Cc: Shekitka, Barbara
Subject: RE: (Checking Availability) Sponsor TC/Type C Mtg: IND077429 /eteplirsen (Sarepta) for DMD

I could if I have to, I guess.

From: Blount, Aprile
Sent: Thursday, October 31, 2013 3:28 PM
To: Choy, Fannie (Yuet); Unger, Ellis; Temple, Robert
Cc: Shekitka, Barbara
Subject: RE: (Checking Availability) Sponsor TC/Type C Mtg: IND077429 /eteplirsen (Sarepta) for DMD

RT could probably miss his 3pm mtg. I'll double check with him.

From: Choy, Fannie (Yuet)
Sent: Thursday, October 31, 2013 2:45 PM
To: Unger, Ellis; Temple, Robert
Cc: Choy, Fannie (Yuet); Shekitka, Barbara; Blount, Aprile
Subject: RE: (Checking Availability) Sponsor TC/Type C Mtg: IND077429 /eteplirsen (Sarepta) for DMD

Hi,

As discussed in today's meeting, we'll extend the duration to 2 hrs. Are you available to start the meeting at 3:30 pm?

Thanks
Fannie

-----Original Appointment-----
From: Choy, Fannie (Yuet)
Sent: Wednesday, September 18, 2013 6:30 PM
To: Choy, Fannie (Yuet); Unger, Ellis; Bastings, Eric; Farkas, Ronald; Jillapalli, Devanand; Men, Angela; Wu, Ta-Chen; Jin, Kun; Siddiqui, Ohidul I; Zhang, Li; Bhattaram, Atul; Rogers, Hobart; Pacanowski, Michael A; Pariser, Anne; Rao, Ashutosh; Guidos, Robert
Cc: Temple, Robert; Freed, Lois M; Wilcox, Barbara; Bauer, Larry J; Ware, Jacqueline H; CDER 120 Calendar; Jenkins, John K; Dunn, Billy; Woodcock, Janet; Fritsch, Jeff; Kambhampati, Rao V; Heimann, Martha R; Stephens, Olen; Lapteva, Larissa
Subject: Reschedule: Sponsor TC/Type C Mtg: IND077429 /eteplirsen (Sarepta) for DMD
When: Friday, November 08, 2013 4:00 PM-5:00 PM (GMT-05:00) Eastern Time (US & Canada).
Where: CDER WO 4270 conf rm Bldg22

9/30/13 Update: Reschedule Meeting - New date/time Friday 11/8/13 4 pm
Thank you!

9/27/13 Update: Reschedule Meeting

SPONSOR Telecon: Type C
IND: 077429
Product/Sponsor: eteplirsen / Sarepta Therapeutics
Indication: Duchenne muscular dystrophy

Purpose:

- To discuss confirmatory postmarketing clinical study design of eteplirsen, in anticipation of NDA filing based on existing data

Internal: ~~**11/25/13 10:00 am**~~ **10/31/13 12:00 Noon**
Sponsor: ~~**12/9/13 3:00 pm**~~ **11/8/13 4:00 pm (Tcon)**

Mtg Request: << File: 077429-130910-0059-type-c-tcon-rqst.pdf >>

~scheduled by Fannie Choy on 9/18/13; Resch 9/27/13; 9/30/13~ 301-796-2899~

Philips, Howard

From: Temple, Robert
Sent: Tuesday, November 12, 2013 4:55 PM
To: Bastings, Eric; Unger, Ellis; Farkas, Ronald
Subject: RE: In the news

That's sort of amazing. We made it perfectly clear that the 6MW endpoint and patient pop'ns they studied were OK AND that they'd best get the study going ASAP.

From: Bastings, Eric
Sent: Tuesday, November 12, 2013 10:53 AM
To: Temple, Robert; Unger, Ellis; Farkas, Ronald
Subject: In the news

FDA raises doubts about trial of Sarepta lead drug, shares plunge

A view shows the U.S. Food and Drug Administration (FDA) logo at the lobby of its headquarters in Silver Spring, Maryland August 14, 2012. Picture taken August 14, 2012. REUTERS/Jason Reed

A view shows the U.S. Food and Drug Administration (FDA) logo at the lobby of its headquarters in Silver Spring, Maryland August 14, 2012. Picture taken August 14, 2012.

Credit: Reuters/Jason Reed

(Reuters) - Sarepta Therapeutics Inc said the U.S. Food and Drug Administration has expressed doubts that the design and goals for the trial of its lead drug were sufficient to support marketing approval, sending its shares down nearly 60 percent.

The FDA suggested that the drug to treat a rare muscle disorder be tested against a placebo in a new trial.

The regulator cited the recent failure of the trial of a competing drug from GlaxoSmithKline and Prosensa and new data on the disease.

Sarepta's drug, eteplirsen, is being developed to treat Duchenne muscular dystrophy (DMD) - a rare, degenerative disorder that mostly affects boys and hampers muscle movement.

The drug works by increasing the production of a protein called dystrophin, the lack of which is the chief cause of DMD.

Data from a mid-stage trial released in October last year showed that eteplirsen significantly improved walking ability in DMD patients.

However, GSK and Prosensa's drisapersen, which also works to restore dystrophin levels, failed to show a statistically significant improvement in the distance that DMD patients could walk in six minutes compared with placebo in a late-stage trial in September.

In remarks to Sarepta, the FDA said it now believed a placebo-controlled trial would better remove bias in walking ability that might be susceptible to individual effort or patient care.

"... It seems worthwhile to consider selection of other endpoints and/or populations for the next trial of eteplirsen," Sarepta said it was told by the FDA in a meeting last week.

Prosensa shares, which have lost about 85 percent of their value since it announced drisapersen's trial failure, were up 19 percent at $4.46 in early trading on Tuesday.

Sarepta said the FDA's request would delay the initiation of dosing in a confirmatory study to at least the second quarter of 2014.

A follow-up meeting with the FDA has been scheduled this month to discuss the confirmatory study design, the company said.

Philips, Howard

From: Temple, Robert
Sent: Wednesday, November 13, 2013 4:24 PM
To: Blount, Aprile
Subject: FW: CONFIRMED: Telecon/Sarepta: IND 77429 (eteplirsen) to discuss study design

Am I?

From: Choy, Fannie (Yuet)
Sent: Wednesday, November 13, 2013 10:33 AM
To: Blount, Aprile
Cc: Choy, Fannie (Yuet); Temple, Robert
Subject: RE: CONFIRMED: Telecon/Sarepta: IND 77429 (eteplirsen) to discuss study design

Hi, Aprile

Would you please confirm with Dr Temple if he is attending this meeting?

Thanks
Fannie

-----Original Appointment-----
From: Choy, Fannie (Yuet)
Sent: Friday, November 08, 2013 6:33 PM
To: Choy, Fannie (Yuet); Temple, Robert; Jenkins, John K; Unger, Ellis; Bastings, Eric; Farkas, Ronald; Jillapalli, Devanand
Cc: CDER 120 Calendar; Woodcock, Janet; Ware, Jacqueline H
Subject: CONFIRMED: Telecon/Sarepta: IND 77429 (eteplirsen) to discuss study design
When: Friday, November 15, 2013 1:00 PM-2:00 PM (GMT-05:00) Eastern Time (US & Canada).
Where: CDER WO 4311 conf rm Bldg22

11/12/13: CONFIRMED

SPONSOR Telecon: Type C
IND: 077429
Product/Sponsor: eteplirsen / Sarepta Therapeutics
Indication: Duchenne muscular dystrophy

Purpose:
Follow-up on the 11/8/13 meeting: To discuss the study design of a clinical trial for eteplirsen.

Preliminary Cmts
<< File: IND077429_Preliminary Cmts.pdf >>

~scheduled by Fannie Choy on 11/8/13; confirmed 11/12/13~ 301-796-2899~

Philips, Howard

From: Temple, Robert
Sent: Wednesday, November 13, 2013 4:25 PM
To: Blount, Aprile
Subject: FW: CONFIRMED: Telecon/Sarepta: IND 77429 (eteplirsen) to discuss study design

I see it's on my Friday calendar.

From: Choy, Fannie (Yuet)
Sent: Wednesday, November 13, 2013 10:33 AM
To: Blount, Aprile
Cc: Choy, Fannie (Yuet); Temple, Robert
Subject: RE: CONFIRMED: Telecon/Sarepta: IND 77429 (eteplirsen) to discuss study design

Hi, Aprile

Would you please confirm with Dr Temple if he is attending this meeting?

Thanks
Fannie

-----Original Appointment-----
From: Choy, Fannie (Yuet)
Sent: Friday, November 08, 2013 6:33 PM
To: Choy, Fannie (Yuet); Temple, Robert; Jenkins, John K; Unger, Ellis; Bastings, Eric; Farkas, Ronald; Jillapalli, Devanand
Cc: CDER 120 Calendar; Woodcock, Janet; Ware, Jacqueline H
Subject: CONFIRMED: Telecon/Sarepta: IND 77429 (eteplirsen) to discuss study design
When: Friday, November 15, 2013 1:00 PM-2:00 PM (GMT-05:00) Eastern Time (US & Canada).
Where: CDER WO 4311 conf rm Bldg22

11/12/13: CONFIRMED

SPONSOR Telecon: Type C
IND: 077429
Product/Sponsor: eteplirsen / Sarepta Therapeutics
Indication: Duchenne muscular dystrophy

Purpose:
Follow-up on the 11/8/13 meeting: To discuss the study design of a clinical trial for eteplirsen.

Preliminary Cmts
<< File: IND077429_Preliminary Cmts.pdf >>

~scheduled by Fannie Choy on 11/8/13; confirmed 11/12/13~ 301-796-2899~

Philips, Howard

From: Temple, Robert
Sent: Tuesday, January 14, 2014 7:09 PM
To: Woodcock, Janet; Jenkins, John K
Subject: RE: did you make any progress on the drisapersen

I need to look at it further, (b) (4) [redacted] But I'll look at it more.

From: Woodcock, Janet
Sent: Tuesday, January 14, 2014 2:16 PM
To: Temple, Robert; Jenkins, John K
Subject: did you make any progress on the drisapersen

Issue after I left? What do you think of Ron Farkas's idea? jw

Philips, Howard

From: Temple, Robert
Sent: Thursday, January 16, 2014 6:16 PM
To: Walton, Marc
Cc: Blount, Aprile
Subject: RE: Biomarker Qualification - dystrophin - letter to decline

You should have them by now. No change in principle, just language.

From: Walton, Marc
Sent: Thursday, January 16, 2014 8:17 AM
To: Temple, Robert
Subject: RE: Biomarker Qualification - dystrophin - letter to decline

Bob,

Had to send it to Janet to alert her without waiting further (you were copied on that email), but I am not sending it to the submitter until Monday. If you have any comments we can consider putting them in the letter before sending it out.
Marc

From: Temple, Robert
Sent: Wednesday, January 15, 2014 5:25 PM
To: Walton, Marc
Subject: RE: Biomarker Qualification - dystrophin - letter to decline

I don't expect to have much but as I said tomorrow AM.

From: Walton, Marc
Sent: Wednesday, January 15, 2014 8:22 AM
To: Unger, Ellis; Temple, Robert
Cc: Amur, Shashi; Noone, Marianne; Dunn, Billy; Bastings, Eric; Farkas, Ronald
Subject: RE: Biomarker Qualification - dystrophin - letter to decline

Thanks Ellis.
Turbo editing did pick up some flaws that turbo writing created. I altered the cumbersome and less than crystal phrasing that you noted.

I will send to Janet to offer her the opportunity to review, in light of her hands-on involvement in the topic.

Thanks
Marc

From: Unger, Ellis
Sent: Tuesday, January 14, 2014 9:49 PM
To: Walton, Marc; Temple, Robert
Cc: Amur, Shashi; Noone, Marianne; Dunn, Billy; Bastings, Eric; Farkas, Ronald
Subject: RE: Biomarker Qualification - dystrophin - letter to decline

Marc and Marianne,

Some turbo editing. I highlighted a question about a possible typo (in yellow) and I posed a question about a sentence (see balloon). Otherwise it seems fine. Quite good.

And this means that Ron, Billy, Eric and Ellis are finished with this. Thanks!

Ellis
<< File: DMD letter draft- Jan14_2013_unger.doc >>

From: Walton, Marc
Sent: Tuesday, January 14, 2014 4:02 PM
To: Farkas, Ronald; Dunn, Billy; Bastings, Eric; Unger, Ellis; Temple, Robert
Cc: Amur, Shashi; Noone, Marianne
Subject: Biomarker Qualification - dystrophin - letter to decline

Ron, Billy, Eric, Ellis, Bob,

As we discussed here is a letter that declines to take on the dystrophin quantitation project into the formal biomarker qualification program, advises them to engage in a community wide effort, and offers them an option of pursuing qualification for a less regulatory-critical context of use.

I do want to move this quickly, so please try to look at this soon (this week if possible) and let me know if this is OK with you, or if you need some edits to avoid saying or implying something. If there is something undesired but not obvious to us not centrally engaged in these drug programs, please add a balloon comment so that I can understand.

After we have all agreed upon the letter, we will send it out of OTS – Biomarker Qualification Program.

Because of what you said about Janet's detailed interest in this area, ShaAvhree would prefer if Janet sees the letter too. You can send it to her while you look at it, or if you prefer we can send it to her with explanation for comment (would copy you of course). Let me know which you prefer.
<< File: DMD letter draft- Jan14_2013.doc >>
Thanks
Marc

Philips, Howard

From: Temple, Robert
Sent: Tuesday, January 28, 2014 3:03 PM
To: Blount, Aprile
Subject: FW: HOLD: Update on DMD drugs study design (drisapersen-eteplirsen)

From: Choy, Fannie (Yuet)
Sent: Tuesday, January 28, 2014 11:13 AM
To: Temple, Robert; Unger, Ellis
Cc: Choy, Fannie (Yuet); Farkas, Ronald; Blount, Aprile; Shekitka, Barbara
Subject: RE: HOLD: Update on DMD drugs study design (drisapersen-eteplirsen)

Hi, Drs Temple & Unger,

I have tentatively scheduled this meeting for DNP to update Dr. Woodcock on the DMD study design and path forward. – Week of 2/10/14.
Dr Woodcock has accepted, but Dr Jenkins will be out on Mon 2/10 and Tue 2/11. Please let me know if the time will work for you.

Thanks
Fannie

-----Original Appointment-----
From: Choy, Fannie (Yuet)
Sent: Monday, January 06, 2014 12:06 PM
To: Choy, Fannie (Yuet); Woodcock, Janet; Jenkins, John K; Temple, Robert; Unger, Ellis; Dunn, Billy; Bastings, Eric; Farkas, Ronald; Tandon, Veneeta; Jillapalli, Devanand; Bhattaram, Atul; Jin, Kun; Siddiqui, Ohidul I; Krudys, Kevin
Cc: CDER 120 Calendar; Bradley, Nicole; Sinha, Vikram; Rao, Ashutosh; Lu, Wei; Ligon, Sharnell (CDER)
Subject: HOLD: Update on DMD drugs study design (drisapersen-eteplirsen)
When: Monday, February 10, 2014 4:00 PM-5:00 PM (GMT-05:00) Eastern Time (US & Canada).
Where:

Purpose: Update on DMD drugs study design (drisapersen-eteplirsen)

- to follow-up on previous discussion and path forward.

~scheduled by Fannie Choy on 1/27/14~ 301-796-2899

Philips, Howard

From: Temple, Robert
Sent: Wednesday, January 29, 2014 6:28 PM
To: Blount, Aprile
Subject: FW: Briefing to Dr. Woodcock on DMD drugs study design (drisapersen-eteplirsen)

Need to tell her.

From: Unger, Ellis
Sent: Wednesday, January 29, 2014 6:20 PM
To: Lu, Wei
Cc: Farkas, Ronald; Dunn, Billy; Bastings, Eric; Choy, Fannie (Yuet); Vail, Victor H; Shekitka, Barbara; Jenkins, John K; Temple, Robert
Subject: RE: Briefing to Dr. Woodcock on DMD drugs study design (drisapersen-eteplirsen)

I'm free for both time slots, 2/5 and 2/6.

From: Lu, Wei
Sent: Wednesday, January 29, 2014 5:06 PM
To: Jenkins, John K; Unger, Ellis; Temple, Robert
Cc: Farkas, Ronald; Dunn, Billy; Bastings, Eric; Choy, Fannie (Yuet); Vail, Victor H; Shekitka, Barbara
Subject: Briefing to Dr. Woodcock on DMD drugs study design (drisapersen-eteplirsen)

Good Evening Dr. Jenkins, Dr. Temple and Dr. Unger,

As you may have known that Dr. Woodcock is very interested to hear your thoughts on the drugs study design (drisapersen-eteplirsen) to treat DMD. We made two slots open for the briefing next week:

1. Wednesday, 1/5 at 9-10am;
2. Thursday, 1/6 at 4:30-5:30pm

Thank you so much for being flexible. Please let me know your preference between those two at your earliest convenience.

Best regards,
Wei

_____
Wei Lu, RN, MS
CDER/OEP/DEO
Bldg 51, Room 6174
Office: 301-796-3448
Fax: 301-847-8753
Email: Wei.Lu@fda.hhs.gov

Philips, Howard

From: Temple, Robert
Sent: Thursday, January 30, 2014 1:21 PM
To: Blount, Aprile
Subject: FW: Briefing to Dr. Woodcock on DMD drugs study design (drisapersen-eteplirsen)

One I will have to do.

From: Choy, Fannie (Yuet)
Sent: Thursday, January 30, 2014 1:14 PM
To: Shekitka, Barbara; Blount, Aprile
Cc: Temple, Robert; Unger, Ellis
Subject: FW: Briefing to Dr. Woodcock on DMD drugs study design (drisapersen-eteplirsen)

Hi, Aprile and Barbara

The briefing has been scheduled on 2/6 4:30-5:30 pm. We have also scheduled a pre-meeting discussion with DNP and Drs. Temple and Unger on 2/5 10-11 am.

Thank you,
Fannie

From: Unger, Ellis
Sent: Wednesday, January 29, 2014 6:20 PM
To: Lu, Wei
Cc: Farkas, Ronald; Dunn, Billy; Bastings, Eric; Choy, Fannie (Yuet); Vail, Victor H; Shekitka, Barbara; Jenkins, John K; Temple, Robert
Subject: RE: Briefing to Dr. Woodcock on DMD drugs study design (drisapersen-eteplirsen)

I'm free for both time slots, 2/5 and 2/6.

From: Lu, Wei
Sent: Wednesday, January 29, 2014 5:06 PM
To: Jenkins, John K; Unger, Ellis; Temple, Robert
Cc: Farkas, Ronald; Dunn, Billy; Bastings, Eric; Choy, Fannie (Yuet); Vail, Victor H; Shekitka, Barbara
Subject: Briefing to Dr. Woodcock on DMD drugs study design (drisapersen-eteplirsen)

Good Evening Dr. Jenkins, Dr. Temple and Dr. Unger,

As you may have known that Dr. Woodcock is very interested to hear your thoughts on the drugs study design (drisapersen-eteplirsen) to treat DMD. We made two slots open for the briefing next week:

1. Wednesday, 1/5 at 9-10am;
2. Thursday, 1/6 at 4:30-5:30pm

Thank you so much for being flexible. Please let me know your preference between those two at your earliest convenience.

Best regards,
Wei

_____
Wei Lu, RN, MS
CDER/OEP/DEO

Bldg 51, Room 6174
Office: 301-796-3448
Fax: 301-847-8753
Email: Wei.Lu@fda.hhs.gov

Philips, Howard

From: Temple, Robert
Sent: Wednesday, February 05, 2014 6:06 PM
To: Bhattaram, Atul
Subject: RE: Eteplirsen Sample Size

Try to arrange with Aprile tomorrow or Friday.

From: Bhattaram, Atul
Sent: Wednesday, February 05, 2014 11:27 AM
To: Temple, Robert
Subject: Eteplirsen Sample Size
Importance: High

Hello Dr Temple

I would like to go over the excel spread sheet used for determining sample size calculations in the planned confirmatory study for Eteplirsen. Will you have sometime today and I can stop by your office. Ron and others have already seen this excel spread sheet.

Please let me know.

Thank you

Atul

Venkatesh Atul Bhattaram
Pharmacometrics
Office of Clinical Pharmacology
US Food and Drug Administration

Philips, Howard

From: Dunn, Billy
Sent: Tuesday, February 11, 2014 10:12 AM
To: Jenkins, John K; Unger, Ellis; Temple, Robert
Cc: Farkas, Ronald
Subject: RE: What was the outcome of the call with Sarepta?

John,

We did not push them on their prior assertion that they did not have the data.

Billy

From: Jenkins, John K
Sent: Monday, February 10, 2014 8:49 PM
To: Unger, Ellis; Temple, Robert
Cc: Dunn, Billy; Farkas, Ronald
Subject: RE: What was the outcome of the call with Sarepta?

I'm glad to hear that the sponsor has been cooperative. How did they explain their prior report that they had no data for Western?

Also, I'm a bit concerned about the comment about us doing the analysis. We have a MaPP on clinical source data, we generally do not do adjudications ourselves, though we can take a look to understand how they did the analysis. If we were going to do this ourselves we would need to have a protocol for blinding, etc. I hope what you meant was the former and not the latter.

From: Unger, Ellis
Sent: Monday, February 10, 2014 7:01 PM
To: Jenkins, John K; Temple, Robert
Cc: Dunn, Billy; Farkas, Ronald
Subject: RE: What was the outcome of the call with Sarepta?

The division spoke to Serepta, and they promised to provide (Billy can correct me if I'm wrong):

Several thousand images from immunoflourescence-slides – to be sent by COB tomorrow. We'll have uploaded them and have access to images by end of week. Ash has been involved in discussions, and will be doing analyses.

Westerns and PCRs: They expect to have them here by end of week. Apparently Jerry Mendell has the data and will send to Serepta, who will send to FDA

Ellis

-----Original Message-----
From: Jenkins, John K
Sent: Monday, February 10, 2014 6:52 PM
To: Dunn, Billy; Farkas, Ronald; Unger, Ellis; Temple, Robert
Subject: What was the outcome of the call with Sarepta?

Re biomarker data?
John

Philips, Howard

From: Farkas, Ronald
Sent: Wednesday, February 19, 2014 8:55 AM
To: Jenkins, John K
Cc: Dunn, Billy; Unger, Ellis; Temple, Robert
Subject: FW: Sarepta Updated Timeline!! re: IND 77429

John,
Sarepta's latest estimate is the end of this week for the western blot data.
thanks
Ron

From: Choy, Fannie (Yuet)
Sent: Friday, February 14, 2014 5:20 PM
To: Dunn, Billy
Cc: Bastings, Eric; Farkas, Ronald; Choy, Fannie (Yuet); Rao, Ashutosh
Subject: FW: Sarepta Updated Timeline!! re: IND 77429

Billy – Sarepta has changed the submission timeline again, when I asked for confirmation that all data would be here by next Tuesday. Thanks Fannie

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From: Shamim Ruff [mailto:SRuff@Sarepta.com]
Sent: Friday, February 14, 2014 4:54 PM
To: Choy, Fannie (Yuet)
Cc: Matthew Rael
Subject: RE: FDA Information Request: re: IND 77429 - Follow up

Dear Fannie,

I can confirm that the RT-PCR data can be with you next by Tuesday morning.

However, the Western Blot and the exploratory antibody (Dys 2 and 3) IF images will take longer as there are thousands of files involved (DYS2/3: 7,500+ images translates to a submission to FDA with 450+ PDF files and Western Blot: 75+ images translating to a submission to FDA with ~25 PDF files). This last set could take until the end of next week to process.

Please accept my apologies for any inconvenience caused.
Regards,

Shamim

Shamim Ruff
Vice President, Head of Regulatory Affairs and Quality
p 857-242-3709 c (b) (6)
e sruff@sarepta.com

215 First Street, Suite 7, Cambridge, MA 02142

Philips, Howard

From: Farkas, Ronald
Sent: Sunday, February 23, 2014 12:35 PM
To: Jenkins, John K
Cc: Dunn, Billy; Unger, Ellis; Bastings, Eric; Jillapalli, Devanand; Rao, Ashutosh; Temple, Robert
Subject: RE: Update on eteplirsen - public presentations
Attachments: Kaye_AVI.pdf.html

John,

You asked about published western data, but for completeness of the response there is also western data that has been shown be Sarepta in public presentations - there are probably other presentations other than the one attached that we are not aware of (this presentation was posted on line).

I can't figure out from the full images of the western blots just sent to us where some of the bands Sarepta is showing came from (patient 6 and 10). Other images show artifacts that aren't shown in the slides (like apparently unequal loading or staining of lanes). At first glance, there seems to be reason for concern of misrepresentation of the data, even beyond that fact that it isn't clear what band represents dystrophin in the patient samples.

thanks

Ron

Below are the full gels.

Patient 2
The band identified as dystrophin at week 24 is at the location of a prominent band in the negative control (red box). All the bands in the week 24 lanes are darker than all the bands in the baseline lanes, suggesting that the result is due to unequal loading (yellow boxes, [not all bands are boxed])

Patient 6

this first blot below seemingly has 'assay sensitivity' as shown by staining of dystrophin in the normal controls. There is little to no staining in patient 6 pre- or post-treatment, despite much higher loading of protein. This seems like evidence that there is no increase in dystrophin in patient 6

Below seemingly is the Mandys106 blot that was in Kaye's slide presentation. It isn't clear to me what bands correspond to those shown in the slide presentation.

Patient 9
The band shown by Kaye (right image) for patient 9 is, at least, clearer on the gel - but again in a lane that appears to be darker for all bands, and it is not clear that the band they selected represents dystrophin

Patient 10
It isn't clear to me what bands were used in Kaye's presentation. whatever they selected seems like it must also have been heavily manipulated photographically - like deleting edges of the band that were darker than the central part.

From: Farkas, Ronald
Sent: Saturday, February 22, 2014 6:21 PM
To: Jenkins, John K
Cc: Farkas, Ronald; Dunn, Billy; Unger, Ellis; Bastings, Eric; Jillapalli, Devanand; Rao, Ashutosh; Temple, Robert; Rogers, Hobart
Subject: RE: Update on eteplirsen
Importance: High

John,

Important correction: In response to your question about published western blot data for eteplirsen, in addition to what I sent you Thursday there were two earlier published studies from the Muntoni lab (London) of eteplirsen when it was called AVI-4658. These studies haven't been a topic of recent discussion with Sarepta, but show immunohistochemistry and western blot data.

I don't think Sarepta sent the immunohistochemistry or western data from these studies, but will recheck (Ash or others - please respond if you are aware of us having the raw data from these studies).

To my eyes, and my understanding of Ashes initial impressions, the immunohistochemistry and western data recently sent by Sarepta is looking far less impressive than portrayed in their regulatory submissions and the Mendell paper - my initial impression is that we need to be concerned that the Mendell paper, at least, represents scientific misconduct through the omission and misrepresentation of results such that findings are not accurately portrayed.

I understand the problems caused by more time spent gathering data, but I'm thinking that we get the Muntoni data before we take the next step with Sarepta

The first study used direct injection of eteplirsen into the EDB muscle of 7 boys, and was reported in Kinali et al., 2009 (attached, including separate web appendix). Western blot for 5 patients was shown in the publication (figure below).

The second study was an IV dose-escalation study in 19 boys for 12 weeks (Cirak et al, 2011, attached). The highest dose was 20 mg/kg (lower than the 30 mg/kg and 50 mg/kg used in the Mendell study). Western blot for most of the 20 mg/kg boys was shown in the paper, as the figure below. Quantification of the westerns was reported as showing up to 18% of normal dystrophin. This

paper also presented evidence of decreased inflammation in treated boys, and increases in dystrophin-associated proteins by immunohistochemistry.

Tell me if you have any other questions.

Thanks

Ron

From: Farkas, Ronald
Sent: Thursday, February 20, 2014 1:32 PM
To: Jenkins, John K; Dunn, Billy; Unger, Ellis
Subject: RE: Update on eteplirsen

John,

At the meeting with Sarepta on July 23, 2013 the sponsor asserted that interpretable western blot data was available for only 1 patient, and that western blot data from the other patients was not interpretable due to use of a different, wrong antibody. The western data Mendell recently said he has appears to be the same data that Sarepta says is not interpretable due to use of the wrong antibody. A few weeks ago when we met with Janet and the review team a few hours after meeting with Mendell and the other experts and parents, it wasn't clear to us that Mendell was talking about the *same data* that we had previously dismissed as uninterpretable (sight unseen, based on Sarepta's assertions). When we more recently asked Sarepta to send us all the Western blot data they reinforced that they did not consider the data interpretable, and seemed to suggest that the data did not support increased dystrophin expression in eteplirsen-treated patients. We will, of course, soon have the data in hand (most recent estimate is late Friday), and be able to evaluate for ourselves.

I've attached what I think is the only publication containing western blot data for eteplirsen - the paper shows the single western blot that we were aware of at the July 23 meeting.

Tell me if that answers your questions.

Thanks

Ron

From: Jenkins, John K
Sent: Thursday, February 20, 2014 1:02 PM
To: Farkas, Ronald; Dunn, Billy; Unger, Ellis
Subject: RE: Update on eteplirsen

Ron

Thanks. Do you recall when we learned that Mendell had full Western data versus the one patient the sponsor told us they had? Some of the time course of events may have been different had we known of the full Western data earlier. The first I heard of full Western data was a couple of weeks ago in the meeting with parents where Mendell told us he had the full data. Do you know if he had published those data?

John

From: Farkas, Ronald
Sent: Thursday, February 20, 2014 8:39 AM
To: Jenkins, John K; Dunn, Billy; Unger, Ellis
Subject: RE: Update on eteplirsen

John,
The minutes are attached, and the part of the discussion about western blots is below (in bold). The sponsor considers the western blots that were assessed in all patients and that Dr. Mendell brought up in the meeting recently to have been performed with an antibody that is not well-suited for detecting dystrophin under the protein denaturing conditions that generally are used when performing a western blot. Western blot with a different antibody that the sponsor considers to be appropriate was conducted in only one patient. My understanding from Ash is that while there can be some differences in performance of different antibodies, in this case either antibody should have been suitable for western blot.
thanks
Ron

pre-minutes
d. Accurate quantification of the amount of truncated dystrophin produced by eteplirsen is critical for considering Subpart H approval. We agree that the immunofluorescence method has some advantages over western blot, in particular, permitting the subcellular localization of dystrophin. However, the immunofluorescence method does not incorporate the type of calibration necessary for reliable quantification. We have considered the concerns you raised regarding quantification of dystrophin by western blot (e.g., low expression level, large size of dystrophin, etc), but note that the method is commonly used in similar clinical studies, and that at least some western blot data were collected for eteplirsen-treated patients (e.g., figure 4-5, page 32 of your meeting package). We continue to believe that western blot data with appropriate calibration would be useful to quantify the dystrophin produced by eteplirsen, and will work closely with you to agree on a protocol for conducting these analyses.

***Meeting Discussion*:**
The sponsor stated that the western blot method was used in only one patient (Figure 4-5 in the meeting package), and was not assessed in all patients since the wrong antibody was used to identify dystrophin. The sponsor stated that they consider the immunohistochemistry method superior to western blot for the reasons described in the briefing book. However, the sponsor acknowledged that western blot data could be supportive and stated that the western blot method would be used to quantify dystrophin using the correct antibody in the new biopsies.

From: Jenkins, John K
Sent: Thursday, February 20, 2014 8:19 AM
To: Dunn, Billy; Unger, Ellis; Farkas, Ronald
Subject: RE: Update on eteplirsen

Am I remembering correctly that in one of our meetings with Sarepta last summer/fall the company told us they did not have data for Western blots because the samples and processes were not adequate? I'm still having trouble reconciling that statement, assuming I am correct in what I remember, with what Dr. Mendell told us a couple of weeks ago. Can someone take a look at the meeting minutes from the meetings last summer/fall and send me the ones that capture those conversations?

From: Dunn, Billy
Sent: Wednesday, February 19, 2014 2:12 PM
To: Jenkins, John K; Unger, Ellis; Farkas, Ronald
Subject: Re: Update on eteplirsen

Yes, that is the plan. The only wrinkle is Sarepta finishing their submission on time. We are on them to do so. We will arrange the meeting.

Billy

From: Jenkins, John K
Sent: Wednesday, February 19, 2014 02:03 PM
To: Dunn, Billy; Unger, Ellis; Farkas, Ronald
Cc: Jenkins, John K
Subject: FW: Update on eteplirsen

Can we plan to schedule an internal meeting for mid- to late-next week to review the biomarker data?

John

From: Woodcock, Janet
Sent: Wednesday, February 19, 2014 2:01 PM
To: Jenkins, John K
Subject: RE: Update on eteplirsen

Yes agree. Much depends on what we think of this biomarker data. jw

---

From: Jenkins, John K
Sent: Wednesday, February 19, 2014 12:43 PM
To: Woodcock, Janet
Cc: Jenkins, John K
Subject: FW: Update on eteplirsen

Janet

I have been away on leave for a few days so I asked for an update on eteplirsen. See below for when we expect to have the biomarker data from Sarepta. I don't know how long it will take Ash to do a high level look at the data, but it seems we should plan an internal meeting to review the data to inform any discussions with Sarepta about the path forward. I think it is likely that the internal meeting would have to be next week at the earliest.

Do you agree?

John

From: Unger, Ellis
Sent: Wednesday, February 19, 2014 12:09 PM
To: Dunn, Billy
Cc: Jenkins, John K; Temple, Robert; Farkas, Ronald
Subject: RE: Update on eteplirsen

Any idea how long Ash's review will take?

From: Dunn, Billy
Sent: Wednesday, February 19, 2014 9:59 AM
To: Jenkins, John K; Unger, Ellis; Temple, Robert; Farkas, Ronald
Subject: RE: Update on eteplirsen

John,

We've received 2 of 4 planned submissions. We have the bulk of the IF data and we have the PCR data. Submissions with some supplementary IF data along with the Western data are expected this week. Ash is beginning his review of the data. We will have an internal meeting promptly following his review to go over the data and prepare for the f/u brainstorming session with Sarepta.

Billy

From: Jenkins, John K
Sent: Wednesday, February 19, 2014 8:20 AM
To: Unger, Ellis; Temple, Robert; Dunn, Billy; Farkas, Ronald
Subject: Update on eteplirsen

Billy and others

Can you please give me an update on where things stand with getting the biomarker data from Sarepta? Do we have an internal meeting scheduled to go over the data?

John

Philips, Howard

From: Rao, Ashutosh
Sent: Friday, February 28, 2014 1:17 PM
To: Unger, Ellis; Farkas, Ronald; Bastings, Eric; Dunn, Billy; Temple, Robert
Cc: Choy, Fannie (Yuet)
Subject: RE: Sarepta dystrophin IHC update

Dear all,

Based on follow-up discussions, here are two updates on the assessment of Sarepta' s dystrophin raw data-

1. There are now two updated files in DNP's eRoom that includes the week 12 or week 24 IHC data with the baseline/week 48 data –same file names/file location. The key to the patient numbers and whether they had a week 12 or week 24 biopsy is pasted below. The sequence of images for each patient is - baseline, week 12 or 24, week 48. Please download the files to your computer (218 MB) so it can be eventually deleted from the eRoom. The week 12/24 data looks a little better than the week 48 data, this may be because the week 48 data was obtained from a different muscle sub-group than the baseline/weeks 12/24.
   http://eroom.fda.gov/eRoom/CDER9/DivisionofNeurologicalProducts/0_1adb2
2. I am still looking at Sarepta' s WB data with MandyS106. We are also assessing the antibody itself to better understand which band(s) might correspond to dystrophin by comparing it to our own data or from other publications. While the quality of Sarepta' s images is poor, understanding the antibody's expected performance might help perform a more realistic, albeit qualitative, assessment of the band labeled as 'Dys' on their MandyS106 WBs.

Please feel free to send questions or comments.

Ash

The table below shows the treatment cohort assignment and biopsy schedule for each c

Table 1: Treatment Cohort Assignment and Biopsy Schedule

Cohort	Patients	Biopsy Schedule
Group 1: Eteplirsen 50 mg/kg	003, 004, 012, 015	Baseline, Week 12, Weel
Group 2: Eteplirsen 30 mg/kg	002, 006, 009, 010	Baseline, Week 24, Weel
Group 3a: Placebo/delayed treatment 50 mg/kg	005, 013	Baseline, Week 12, Weel
Group 3b: Placebo/delayed treatment 30 mg/kg	007, 008	Baseline, Week 24, Weel

From: Rao, Ashutosh
Sent: Thursday, February 20, 2014 6:14 PM
To: Unger, Ellis; Bastings, Eric; Dunn, Billy; Temple, Robert

Cc: Farkas, Ronald
Subject: Sarepta dystrophin IHC update

Hi Dr. Unger,

This is an update on my preliminary assessment of the immunohistochemistry (IHC) raw data sent by Sarepta for etiplersen. After discussing with Ron, I wanted to run them by you and get your input before doing more.

Sarepta sent in raw image files for dystrophin IHCs from 12 patients at baseline, post treatment week 12, week 24, and week 48. There were 24 images per patient. I stitched together all 24 images from each set on to a single page. I also generated a PDF file that shows each patient's dystrophin at baseline or week 48 on individual pages. The images were not otherwise modified. Please see 2 PDF files located in the DNP e-room here – **http://eroom.fda.gov/eRoom/CDER9/DivisionofNeurologicalProducts/0_1adb2**

The file named *Sarepta IHC Stitched **White** Bk.pdf* has all images with a white background that shows the patient number and treatment (baseline followed by week-48 images). Positive and negative control images are on the last 2 pages, respectively. The file named *Sarepta IHC Stitched **Black** Bk.pdf* has the exact same images as above but with a black background to better visualize the fluorescent red dystrophin. Each page shows a collage of 24 image as labeled on the file with the white background. I found it helpful to see the black background PDF in full-screen mode and flip the pages forward to see any differences. Each patient's baseline image set is followed by that patient's week-48 image set. The data for patients 006 and 015 are clearer than the others.

- Is this format helpful to view the quality of the IHC data?
- Would you recommend doing the same with the week-12 and week-24 data?

Sarepta has also sent in RT-PCR data. All patients showed the skipped dystrophin transcript after treatment at week 48, and especially patients 006 and 015. We can also discuss more once the Western blot data are in and we've had a chance to review it. I'd be happy to clarify if there are any questions. Thanks.

Regards,
Ash

Philips, Howard

From: Dunn, Billy
Sent: Friday, March 21, 2014 6:34 PM
To: Jenkins, John K; Unger, Ellis; Temple, Robert; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard; Bastings, Eric; Farkas, Ronald; Choy, Fannie (Yuet)
Subject: RE: Sarepta Proposal for Eteplirsen Program

John,

We began working with Janet's office yesterday on scheduling the internal meeting to discuss the sponsor's proposal and our post-brainstorming meeting comments to the sponsor. That will be on the calendar shortly following Fannie's confirmation of availability. The seminar/workshop idea should work well. We have an independent upcoming CPIM with one of the scientific groups interested in quantitative dystrophin measurements, and we should be able to join the seminar/workshop with the CPIM effort. We will discuss this at the internal meeting.

Billy

From: Jenkins, John K
Sent: Friday, March 21, 2014 2:44 PM
To: Unger, Ellis; Temple, Robert; Dunn, Billy; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard
Cc: Jenkins, John K
Subject: FW: Sarepta Proposal for Eteplirsen Program

Billy

We will need to schedule an internal meeting to review the sponsor's response to the brainstorming meeting. I discussed this with Janet on Wednesday and I think it is critical that we devote time and resources to understanding the differing conclusions regarding the immunohistochemistry data, since that is a pivotal part of any planned NDA, as well as working together to develop better methods for dystrophin quantification going forward. Janet suggested that we may want to ask Ash and Rich to help lead an effort for a scientific seminar/workshop (non-public) where we invite in sponsors and experts to better understand available methods and ways to enhance them. This could be an agenda topic at the next internal meeting. I am on leave starting today until Wednesday April 2. It is OK to hold the internal meeting without me to keep this project moving forward.

John

From: Chris Garabedian [mailto:cgarabedian@sarepta.com]
Sent: Friday, March 21, 2014 12:44 PM
To: Woodcock, Janet; Temple, Robert; Jenkins, John K; Unger, Ellis; Dunn, Billy
Cc: Shamim Ruff; Ty Howton; Frank J. Sasinowski
Subject: Sarepta Proposal for Eteplirsen Program

Dear Drs. Woodcock, Temple, Jenkins, Unger, and Dunn:

Thank you for the informal brainstorming meeting and the constructive dialogue that took place on Wednesday, March 19th. We have considered the various kinds of input that you provided at this brainstorming session and we propose the following course of actvities for you to consider. Once we receive formal guidance from the Agency, we will begin to move forward on finalizing these study protocols and begin the process of IRB approvals so we can begin screening and dosing new patients as soon as possible.

1. **Clinical Studies**

Sarepta proposes the following clinical trials for further discussion with you:

Proposed Eteplirsen Studies

Study Type	Population	N	Endpoints
Open label with control	Ambulatory exon-51-amenable DMD patients ≥7 years; Concurrent control arm of DMD patients with same inclusion/exclusion criteria that are non-exon-51 amenable	Approx. 60:60	6MWT, dystrophin, safety, exploratory functional endpoints
Open label	Exon-51-amenable DMD patients 4-6 years	Approx. 20	Safety, dystrophin, exploratory functional endpoints
Open label	Nonambulatory exon-51-amenable DMD patients ≥7 years	Approx. 20	Safety, dystrophin, exploratory functional endpoints
Non-interventional natural history	Non-exon-51 amenable DMD patients; all comers	TBD	Safety

2. Dystrophin Methodology

Thank you for your generous offer for us to engage with FDA experts, and also for your additional offer to allow us access to consult experts from the FDA laboratories as well as access to/use of FDA laboratories to work on standardization and refinement of our dystrophin assay methodology.

In order to clarify the quantification methodology of the dystrophin data from the existing Phase 2 clinical trial, we will ask the pathologistat Nationwide Children's Hospital who generated the dystrophin-positive fiber data to make herself available to you. This may allow you to better understand how these data were generated and interpreted. We look forward to scheduling these meetings as soon as possible.

Sarepta will work closely with the Agency to reach agreement on our dystrophin quantification methods for use in our upcoming studies. We have a protocol to conduct immunofluorescence, western blot, and RT-PCR assessments at a minimum. If the timing of muscle biopsies are expected to occur before agreement on these methodologies are reached with the Agency, Sarepta will explore freezing the blocks for future analysis. Sarepta will also investigate the potential assessment of nNOS as a confirmatory marker of functional dystrophin and use of new dystrophin detection and quantification methods, such as mass spectrometry, which may supplement but not initially replace our existing immunofluorescence-based assay.

3. NDA Submission

From the views we heard expressed by the Agency, it appears the FDA may be open to an NDA filing based on the clinical outcomes data from the existing Phase 2 study. Sarepta is prepared to submit the NDA this summer, with an understanding that the chances of a positive review would be bolstered by supplemental data, such as 144-week clinical data (e.g., 6MWT, Pulmonary Function tests), early safety data from additional eteplirsen exposed patients in the upcoming studies, and/or a possible fourth muscle biopsy from the ongoing Phase 2 study.

4. Follow-On Exon Skipping Drugs for DMD and Confirmatory Study

As discussed, we are in late preclinical development with two follow-on exon-skipping drugs targeting gene deletions amenable to exons 45 and 53 and we understand the Agency would like us to move these drugs into patients as soon as possible. To this end, we will work with the FDA on the design of this study that would include our drugs, SRPT-4045 and SRPT-4053 and will prepare a design that will include one or both of these drugs against a placebo control. Please note that we are currently at the pre-IND stage for these next 2 compounds and will need to prepare manufacturing scale-up for clinical trials with INDs submissions expected in the 2nd half of this year.

5. Communication to the Public

Upon receipt of formal guidance from the Agency that reflects all of the meetings since November, 2013, as well as FDA's position on the course of activities proposed in this letter, we are prepared to share our planned draft press release with the appropriate contact at the FDA to ensure an alignment of communications between Sarepta and the Agency for this program.

We thank you for your active and broad engagement on this program across the Agency and the extensive time each of you have provided to assist us in evaluating eteplirsen for DMD boys. We look forward to your response to our proposal and we hope you that you find the approach we have outlined above acceptable, so that we, together with the DMD community, can move forward swiftly.

Sincerely,

Chris Garabedian
President & CEO
617.274.3993 direct
(b) (6) mobile
cgarabedian@sarepta.com

SAREPTA THERAPEUTICS

215 First Street, Cambridge MA 02142

Philips, Howard

From: Bastings, Eric
Sent: Thursday, March 27, 2014 3:57 PM
To: Dunn, Billy; Unger, Ellis; Temple, Robert
Subject: A lot of media activity on eteplirsen this week...

http://cbs42.com/2014/03/26/drug-help-kids-duchenne-muscular-dystrophy/

http://www.thedenverchannel.com/news/local-news/ryan-dunnes-white-house-petitionfor-duchene-md-drug-approval-reaches-100000

http://hometownstation.com/santa-clarita-news/petition-names-sought-santa-clarita-boy-duchenne-muscular-dystrophy-41004

Philips, Howard

From: Rao, Ashutosh
Sent: Tuesday, April 08, 2014 1:04 PM
To: Dunn, Billy; Bastings, Eric; Farkas, Ronald; Choy, Fannie (Yuet)
Cc: Unger, Ellis; Moscicki, Richard; Temple, Robert; Jenkins, John K; Woodcock, Janet; Rosenberg, Amy; Kozlowski, Steven
Subject: Update on dystrophin chat

Hi all,

This is a brief update on my discussion today morning with Drs. Moscicki and Unger.

1. We should invite Sarepta's investigator(s) to explain how they obtained the dystrophin IHC quantitation from the images provided.
    a. **Fannie**, please help set up a t-con to discuss having a roundtable discussion with Sarepta and the relevant investigator(s) who obtained and analyzed their IHC images. The goal of this t-con would be to agree on the logistics of the best way for FDA to better understand how their current IHC data was obtained and analyzed. During the round-table, we would want a step-by-step walk-through of the procedure from image acquisition to data quantitation.
    b. We would prefer if the Sponsor/Investigator(s) could come to FDA so more of us can participate but we might be open to a few of us (including OSI) visiting them if it just wouldn't work to present at FDA. We can discuss this during the t-con.
    c. Based on the round-table discussion of their IHC data, the review team will assess the merits of a 4th biopsy and IHC/WB/other dystrophin analysis. We will listen and provide feedback for improving their IHC/WB, based on the strategies document sent earlier and with input from other FDA experts. Emphasis will be made on random image acquisition and analysis, protocol standardization, and quantitation by multiple pathologists. MRI could be considered for guiding the biopsy acquisition. We will also work with Sarepta to standardize their WB SOP based on some validation that is ongoing in OBP laboratories.
2. An FDA workshop/seminar with other investigators (not officially related to Sarepta) is bring organized by the OTS/Critical Path initiative. Input from these investigators will be used to further guide Sarepta confirmatory biomarker approaches such as quantitative MS for dystrophin or MRI.
3. If a 4th biopsy is deemed critical - OBP, in collaboration with Rich, Ellis, and other experts, will assist in confirming some of the IHC/WB data obtained by Sarepta from the 4th biopsy. Sarepta would conduct the primary analysis of their patient samples for regulatory submission, OBP testing would serve as confirmatory only. Ash can lead on the WB confirmatory analyses, Rich/Ellis/other board certified immunohistochemist/pathologist would lead on the IHC confirmation that can be conducted in OBP laboratories. This possibility will be discussed with the Sponsor at the round-table. Sarepta would need to assist in obtaining fresh control samples from DMD and healthy volunteers for comparison with the 4th biopsy samples. Multiple healthy control samples might be required to have an adequate comparison with the 4th biopsy samples.

Rich and Ellis, please let me know if I missed something.
Others, please chime in if you disagree or want to add anything before the t-con.

Best,
Ash

Philips, Howard

From: Rao, Ashutosh
Sent: Friday, April 11, 2014 1:41 PM
To: Unger, Ellis; Temple, Robert
Cc: Jillapalli, Devanand; Choy, Fannie (Yuet); Bastings, Eric; Farkas, Ronald; Dunn, Billy
Subject: RE: Sarepta Proposal for Eteplirsen Program
Attachments: RF off FC April summary minutes IND077429_DRAFT-Advice-Information Request_unger3 _AR.doc.html

Hi all,

Please see minor edits on the revised version. Thanks.

Ash

From: Unger, Ellis
Sent: Thursday, April 10, 2014 7:36 PM
To: Temple, Robert
Cc: Rao, Ashutosh; Jillapalli, Devanand; Choy, Fannie (Yuet); Bastings, Eric; Farkas, Ronald; Dunn, Billy
Subject: RE: Sarepta Proposal for Eteplirsen Program

More edits by RT and me. Thoughts?

From: Unger, Ellis
Sent: Wednesday, April 09, 2014 6:30 PM
To: Temple, Robert
Cc: Rao, Ashutosh; Jillapalli, Devanand; Choy, Fannie (Yuet); Bastings, Eric; Farkas, Ronald; Dunn, Billy
Subject: RE: Sarepta Proposal for Eteplirsen Program

All,

I did some editing and rearranging. I don't think I made fundamental changes. My main contribution was to explain that biomarker studies for AA need to be A&WC, and there are reasons why we think patients in an open-label study will do well - - i.e., I explained bias - - the elephant in the room.

See what you think.

Now to Dr. Temple….

Ellis

From: Dunn, Billy
Sent: Wednesday, April 09, 2014 2:23 PM
To: Unger, Ellis
Cc: Temple, Robert; Rao, Ashutosh; Jillapalli, Devanand; Choy, Fannie (Yuet); Bastings, Eric; Farkas, Ronald
Subject: RE: Sarepta Proposal for Eteplirsen Program

Ellis,

Please find attached the Sarepta letter for your review and continued circulation.

Thanks,
Billy

From: Farkas, Ronald
Sent: Thursday, April 03, 2014 9:29 PM
To: Dunn, Billy; Bastings, Eric
Cc: Unger, Ellis; Temple, Robert; Rao, Ashutosh; Jillapalli, Devanand; Choy, Fannie (Yuet)
Subject: RE: Sarepta Proposal for Eteplirsen Program

Billy and Eric,
Draft eteplirsen meeting minutes attached.
Thanks
Ron

From: Jenkins, John K
Sent: Thursday, April 03, 2014 9:23 AM
To: Dunn, Billy; Unger, Ellis; Temple, Robert; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard; Bastings, Eric; Farkas, Ronald; Choy, Fannie (Yuet)
Cc: Jenkins, John K
Subject: RE: Sarepta Proposal for Eteplirsen Program

I'm sorry that I could not participate in yesterday's meeting in person. Being on the phone and in the car (hands free) was suboptimal for participating in the discussions.

I would add the following thoughts to our plans to draft the letter:

1. In expressing our willingness to consider an NDA application for filing we should be clear that the application should be complete and that a decision to file the application for possible accelerated approval does not suggest the outcome of our review. Also, I would include a statement that based on the available data we expect the application would be discussed at a public AC meeting. The latter will help the sponsor to plan for the review and also signal that this is not a "slam dunk" review.
2. We should clearly state out continued concerns about the strength of the data they have provided related to the dystrophin biomarker. This can be in the context of stating our interest in working with them to better understand the methods/analyses used for the existing data while we also work with them to develop/agree upon methods for use in future studies, including potential biopsies in the boys currently on drug.
3. We should be clear that we are considering them for accelerated approval (based on either the dystrophin biomarker if the data prove to be adequate on our review or under the second pathway using the "stability" of the currently treated boys on 6 minute walk) and that they need to plan for their confirmatory studies. As we discussed there are two pathways, a long-term open-label historically controlled study and/or a confirmation of clinical benefit in another exon of a similar drug in a placebo controlled trial with a clinical endpoint such as 6 MWT. Per the discussion yesterday, I think the long-term historically controlled trial should be the primary confirmatory trial, but the other exon could supplant that if positive. So, they need to start the additional study ASAP and plan to follow the patients long-term on study post-approval (assuming there is an approval).

John

From: Dunn, Billy
Sent: Friday, March 21, 2014 6:34 PM
To: Jenkins, John K; Unger, Ellis; Temple, Robert; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard; Bastings, Eric; Farkas,

Ronald; Choy, Fannie (Yuet)
Subject: RE: Sarepta Proposal for Eteplirsen Program

John,

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Billy

From: Jenkins, John K
Sent: Friday, March 21, 2014 2:44 PM
To: Unger, Ellis; Temple, Robert; Dunn, Billy; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard
Cc: Jenkins, John K
Subject: FW: Sarepta Proposal for Eteplirsen Program

Billy

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Sent: Friday, March 21, 2014 12:44 PM
To: Woodcock, Janet; Temple, Robert; Jenkins, John K; Unger, Ellis; Dunn, Billy
Cc: Shamim Ruff; Ty Howton; Frank J. Sasinowski
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From the views we heard expressed by the Agency, it appears the FDA may be open to an NDA filing based on the clinical outcomes data from the existing Phase 2 study. Sarepta is prepared to submit the NDA this summer, with an understanding that the chances of a positive review would be bolstered by supplemental data, such as 144-week clinical data (e.g., 6MWT, Pulmonary Function tests), early safety data from additional eteplirsen exposed patients in the upcoming studies, and/or a possible fourth muscle biopsy from the ongoing Phase 2 study.

4. Follow-On Exon Skipping Drugs for DMD and Confirmatory Study

As discussed, we are in late preclinical development with two follow-on exon-skipping drugs targeting gene deletions amenable to exons 45 and 53 and we understand the Agency would like us to move these drugs into patients as soon as possible. To this end, we will work with the FDA on the design of this study that would include our drugs, SRPT-4045 and SRPT-4053 and will prepare a design that will include one or both of these drugs against a placebo control. Please note that we are currently at the pre-IND stage for these next 2 compounds and will need to prepare manufacturing scale-up for clinical trials with INDs submissions expected in the 2nd half of this year.

5. Communication to the Public

Upon receipt of formal guidance from the Agency that reflects all of the meetings since November, 2013, as well as FDA's position on the course of activities proposed in this letter, we are prepared to share our planned draft press release with the appropriate contact at the FDA to ensure an alignment of communications between Sarepta and the Agency for this program.

We thank you for your active and broad engagement on this program across the Agency and the extensive time each of you have provided to assist us in evaluating eteplirsen for DMD boys. We look forward to your response to our proposal and we hope you that you find the approach we have outlined above acceptable, so that we, together with the DMD community, can move forward swiftly.

Sincerely,

Chris Garabedian
President & CEO
617.274.3993 direct
(b) (6) mobile
cgarabedian@sarepta.com

215 First Street, Cambridge MA 02142

Philips, Howard

From: Jenkins, John K
Sent: Friday, April 11, 2014 1:21 PM
To: Farkas, Ronald
Cc: Temple, Robert; Dunn, Billy; Unger, Ellis; Jenkins, John K
Subject: RE: Sarepta Proposal for Eteplirsen Program
Attachments: RE Intermediate Clinical Endpoint Discussion in the Accelerated Approval Section of Expedited Programs Final Guidance.html

Well, the problem is that at the current time we are not impressed by their biomarker data (that may change) and I cannot see a way that the clinical data could support regular approval. So, I was considering evidence of a decline in the rate of progression from the small natural history study, which would be considered a clinical benefit in most cases, as a "surrogate" for an actual improvement in long-term disease progression/death. I understand this is tricky, but unless their biomarker data get a lot better soon (as in a 4th biopsy using well-developed methods that show marked dystrophin production) they have very little to stand on for any approval. If we were to conclude the small natural history data provide evidence of clinical benefit for full approval, we will have no authority to require them to do a confirmatory trial to show actual long-term benefit. The intermediate clinical endpoint standard is one we have not used much to date, but we just had a senior level meeting yesterday to reaffirm our views on when to use this pathway and to revise the relevant text of the expedited pathways guidance, which will be published in final soon (see attached).

From: Farkas, Ronald
Sent: Friday, April 11, 2014 12:55 PM
To: Jenkins, John K
Cc: Temple, Robert; Dunn, Billy; Unger, Ellis
Subject: RE: Sarepta Proposal for Eteplirsen Program
Importance: High

John,
I think I understand and agree with what you propose, but if perhaps it's semantics, I very much think that it's problematic to use the term "intermediate clinical endpoint" to describe the clinical eteplirsen data. If we believe the eteplirsen data is "evidence" (weak evidence) but not "substantial evidence", we can approve under subpart H based on the dystrophin surrogate that we think is reasonably likely to predict benefit based, in part, on the therapeutic evidence, or other evidence, as stated directly in the subpart H regulation.

The term "intermediate clinical endpoint", as recently clearly defined in the Expedited Programs Guidance, should perhaps better be left to describe clinical endpoints that can be measured earlier than an effect on irreversible morbidity or mortality.

Subpart H: "FDA may grant marketing approval for a new drug product on the basis of adequate and well-controlled clinical trials establishing that the drug product has an effect on a surrogate endpoint that is reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit or on the basis of an effect on a clinical endpoint other than survival or irreversible morbidity.

Thanks

Ron

From: Unger, Ellis
Sent: Friday, April 11, 2014 12:25 PM
To: Jenkins, John K
Cc: Temple, Robert; Farkas, Ronald; Dunn, Billy
Subject: Re: Sarepta Proposal for Eteplirsen Program

Thanks. Very helpful. We're getting very close (on letter)

Ellis Unger

Sent from my Blackberry

From: Jenkins, John K
Sent: Friday, April 11, 2014 12:19 PM Eastern Standard Time
To: Unger, Ellis
Cc: Temple, Robert; Jenkins, John K
Subject: RE: Sarepta Proposal for Eteplirsen Program

My thought was that if they had very convincing 6MW data, say from a randomized controlled trial that won, that would probably be enough for full approval. Instead, what they have is a failed randomized trial with a post-hoc analysis that looks promising combined with a small natural history study that may, or may not, suggest a change from the expected disease course in the rate of decline in 6MW and other functional parameters. These data, combined with a positive biomarker analysis, which is also in doubt, may be enough to warrant a conclusion to support AA; i.e., these findings are reasonably likely to predict clinical benefit of slowing disease progression. I cannot imagine that we would conclude that their available data are strong enough to show clinical benefit to warrant full approval, but we would have to be open to that possibility on review of the data. All of our conversations about being willing to file an application have been built around AA with a requirement for a confirmatory trial. So, I'm thinking of a hybrid of surrogate approval (e.g., dystrophin) and intermediate clinical endpoint approval (e.g., possible slowed progression in a very small natural history study) where the two sets of data together may be enough for AA, but in and of themselves are not substantial evidence of clinical benefit.

From: Unger, Ellis
Sent: Friday, April 11, 2014 9:16 AM
To: Jenkins, John K
Cc: Temple, Robert
Subject: RE: Sarepta Proposal for Eteplirsen Program

John,

I have to say we've been wrestling with your #3, below. Would we really consider AA based on 6MW data? It's a clinical endpoint. We don't see how we can say that 3 years of data on 6MW is an intermediate endpoint that supports AA. If the positive finding were based on a comparison to historical controls, then we would have major concerns about the validity of the comparison. So to grant AA and ask for confirmation with other 6MW data would be like saying that a weak, historically-controlled comparison between eteplirsen and placebo on 6MW through 3 years is good enough for AA but not good enough for full approval.

Ellis

From: Jenkins, John K
Sent: Thursday, April 03, 2014 9:23 AM
To: Dunn, Billy; Unger, Ellis; Temple, Robert; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard; Bastings, Eric; Farkas, Ronald; Choy, Fannie (Yuet)
Cc: Jenkins, John K
Subject: RE: Sarepta Proposal for Eteplirsen Program

I'm sorry that I could not participate in yesterday's meeting in person. Being on the phone and in the car (hands free) was suboptimal for participating in the discussions.

I would add the following thoughts to our plans to draft the letter:

1. In expressing our willingness to consider an NDA application for filing we should be clear that the application should be complete and that a decision to file the application for possible accelerated approval does not suggest the outcome of our review. Also, I would include a statement that based on the available data we expect the application would be discussed at a public AC meeting. The latter will help the sponsor to plan for the review and also signal that this is not a "slam dunk" review.
2. We should clearly state out continued concerns about the strength of the data they have provided related to the dystrophin biomarker. This can be in the context of stating our interest in working with them to better understand the methods/analyses used for the existing data while we also work with them to develop/agree upon methods for use in future studies, including potential biopsies in the boys currently on drug.
3. We should be clear that we are considering them for accelerated approval (based on either the dystrophin biomarker if the data prove to be adequate on our review or under the second pathway using the "stability" of the currently treated boys on 6 minute walk) and that they need to plan for their confirmatory studies. As we discussed there are two pathways, a long-term open-label historically controlled study and/or a confirmation of clinical benefit in another exon of a similar drug in a placebo controlled trial with a clinical endpoint such as 6 MWT. Per the discussion yesterday, I think the long-term historically controlled trial should be the primary confirmatory trial, but the other exon could supplant that if positive. So, they need to start the additional study ASAP and plan to follow the patients long-term on study post-approval (assuming there is an approval).

John

From: Dunn, Billy
Sent: Friday, March 21, 2014 6:34 PM
To: Jenkins, John K; Unger, Ellis; Temple, Robert; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard; Bastings, Eric; Farkas, Ronald; Choy, Fannie (Yuet)
Subject: RE: Sarepta Proposal for Eteplirsen Program

John,

We began working with Janet's office yesterday on scheduling the internal meeting to discuss the sponsor's proposal and our post-brainstorming meeting comments to the sponsor. That will be on the calendar shortly following Fannie's confirmation of availability. The seminar/workshop idea should work well. We have an independent upcoming CPIM with one of the scientific groups interested in quantitative dystrophin measurements, and we should be able to join the seminar/workshop with the CPIM effort. We will discuss this at the internal meeting.

Billy

From: Jenkins, John K
Sent: Friday, March 21, 2014 2:44 PM
To: Unger, Ellis; Temple, Robert; Dunn, Billy; Woodcock, Janet; Rao, Ashutosh; Moscicki, Richard
Cc: Jenkins, John K
Subject: FW: Sarepta Proposal for Eteplirsen Program

Billy

We will need to schedule an internal meeting to review the sponsor's response to the brainstorming meeting. I discussed this with Janet on Wednesday and I think it is critical that we devote time and resources to understanding the differing conclusions regarding the immunohistochemistry data, since that is a pivotal part of any planned NDA, as well as working together to develop better methods for dystrophin quantification going forward. Janet suggested that we may want to ask Ash and Rich to

help lead an effort for a scientific seminar/workshop (non-public) where we invite in sponsors and experts to better understand available methods and ways to enhance them. This could be an agenda topic at the next internal meeting. I am on leave starting today until Wednesday April 2. It is OK to hold the internal meeting without me to keep this project moving forward.

John

From: Chris Garabedian [mailto:cgarabedian@sarepta.com]
Sent: Friday, March 21, 2014 12:44 PM
To: Woodcock, Janet; Temple, Robert; Jenkins, John K; Unger, Ellis; Dunn, Billy
Cc: Shamim Ruff; Ty Howton; Frank J. Sasinowski
Subject: Sarepta Proposal for Eteplirsen Program

Dear Drs. Woodcock, Temple, Jenkins, Unger, and Dunn:

Thank you for the informal brainstorming meeting and the constructive dialogue that took place on Wednesday, March 19th. We have considered the various kinds of input that you provided at this brainstorming session and we propose the following course of actvities for you to consider. Once we receive formal guidance from the Agency, we will begin to move forward on finalizing these study protocols and begin the process of IRB approvals so we can begin screening and dosing new patients as soon as possible.

1. **Clinical Studies**

   Sarepta proposes the following clinical trials for further discussion with you:

   Proposed Eteplirsen Studies

Study Type	Population	N	Endpoints
Open label with control	Ambulatory exon-51-amenable DMD patients ≥7 years; Concurrent control arm of DMD patients with same inclusion/exclusion criteria that are non-exon-51 amenable	Approx. 60:60	6MWT, dystrophin, safety, exploratory functional endpoints
Open label	Exon-51-amenable DMD patients 4-6 years	Approx. 20	Safety, dystrophin, exploratory functional endpoints
Open label	Nonambulatory exon-51-amenable DMD patients ≥7 years	Approx. 20	Safety, dystrophin, exploratory functional endpoints
Non-interventional natural history	Non-exon-51 amenable DMD patients; all comers	TBD	Safety

2. **Dystrophin Methodology**

   Thank you for your generous offer for us to engage with FDA experts, and also for your additional offer to allow us access to consult experts from the FDA laboratories as well as access to/use of FDA laboratories to work on standardization and refinement of our dystrophin assay methodology.

   In order to clarify the quantification methodology of the dystrophin data from the existing Phase 2 clinical trial, we will ask the pathologistat Nationwide Children's Hospital who generated the dystrophin-positive fiber data to make herself available to you. This may allow you to better understand how these data were generated and interpreted. We look forward to scheduling these meetings as soon as possible.

Sarepta will work closely with the Agency to reach agreement on our dystrophin quantification methods for use in our upcoming studies. We have a protocol to conduct immunofluorescence, western blot, and RT-PCR assessments at a minimum. If the timing of muscle biopsies are expected to occur before agreement on these methodologies are reached with the Agency, Sarepta will explore freezing the blocks for future analysis. Sarepta will also investigate the potential assessment of nNOS as a confirmatory marker of functional dystrophin and use of new dystrophin detection and quantification methods, such as mass spectrometry, which may supplement but not initially replace our existing immunofluorescence-based assay.

3. NDA Submission

From the views we heard expressed by the Agency, it appears the FDA may be open to an NDA filing based on the clinical outcomes data from the existing Phase 2 study. Sarepta is prepared to submit the NDA this summer, with an understanding that the chances of a positive review would be bolstered by supplemental data, such as 144-week clinical data (e.g., 6MWT, Pulmonary Function tests), early safety data from additional eteplirsen exposed patients in the upcoming studies, and/or a possible fourth muscle biopsy from the ongoing Phase 2 study.

4. Follow-On Exon Skipping Drugs for DMD and Confirmatory Study

As discussed, we are in late preclinical development with two follow-on exon-skipping drugs targeting gene deletions amenable to exons 45 and 53 and we understand the Agency would like us to move these drugs into patients as soon as possible. To this end, we will work with the FDA on the design of this study that would include our drugs, SRPT-4045 and SRPT-4053 and will prepare a design that will include one or both of these drugs against a placebo control. Please note that we are currently at the pre-IND stage for these next 2 compounds and will need to prepare manufacturing scale-up for clinical trials with INDs submissions expected in the 2nd half of this year.

5. Communication to the Public

Upon receipt of formal guidance from the Agency that reflects all of the meetings since November, 2013, as well as FDA's position on the course of activities proposed in this letter, we are prepared to share our planned draft press release with the appropriate contact at the FDA to ensure an alignment of communications between Sarepta and the Agency for this program.

We thank you for your active and broad engagement on this program across the Agency and the extensive time each of you have provided to assist us in evaluating eteplirsen for DMD boys. We look forward to your response to our proposal and we hope you that you find the approach we have outlined above acceptable, so that we, together with the DMD community, can move forward swiftly.

Sincerely,

Chris Garabedian
President & CEO
617.274.3993 direct
(b) (6) mobile
cgarabedian@sarepta.com

SAREPTA THERAPEUTICS

215 First Street, Cambridge MA 02142

Philips, Howard

From: Temple, Robert
Sent: Wednesday, July 02, 2014 1:35 PM
To: Locicero, Colleen L; Blount, Aprile; Choy, Fannie (Yuet)
Cc: Shekitka, Barbara; Unger, Ellis
Subject: RE: Program pre-NDA meeting

I can leave for the serepta meeting.

From: Locicero, Colleen L
Sent: Wednesday, July 02, 2014 6:19 AM
To: Blount, Aprile; Choy, Fannie (Yuet)
Cc: Shekitka, Barbara; Temple, Robert; Unger, Ellis
Subject: RE: Program pre-NDA meeting

Aprile,

The DSB is all day. Could Bob miss 1.5 hours of it for this important (Sarepta p-NDA) meeting that needs to be held sooner, rather than later?

RT –could you miss an hour & a half of the DSB? Really hate to have to push this meeting to October or hold it without you….

Thanks,
Colleen

From: Blount, Aprile
Sent: Tuesday, July 01, 2014 5:17 PM
To: Choy, Fannie (Yuet); Locicero, Colleen L
Cc: Shekitka, Barbara
Subject: RE: Program pre-NDA meeting

RT has a drug safety board mtg that he doesn't miss and a potential outside mtg. Ellis has drug safety as well.

From: Choy, Fannie (Yuet)
Sent: Tuesday, July 01, 2014 5:09 PM
To: Locicero, Colleen L
Cc: Blount, Aprile; Shekitka, Barbara
Subject: RE: Program pre-NDA meeting

Thank you so much! What are their availabilities for Thu 9/18 1-2:30 pm? Both calendars are tentatively blocked on that day.

Thanks again!
Fannie

From: Locicero, Colleen L
Sent: Tuesday, July 01, 2014 4:49 PM
To: Choy, Fannie (Yuet)
Cc: Blount, Aprile; Shekitka, Barbara
Subject: RE: Program pre-NDA meeting

Hi Fannie,

Because of the importance/high visibility of this, I consulted with Ellis. He believes it important that both he and Bob attend because of the importance/high visibility and both of their considerable involvement with this program to date. He noted that if you find a date that works for most everybody else and he or Bob has a conflict, he or Bob might be able to miss (or have the other meeting rescheduled) the other meeting (depends on what it is), because of the importance of this meeting. If you need my or Aprile's or Barbara's help in rescheduling, please let us know.

I have some meetings scheduled that include both of them that I would be willing to move/reschedule for the Sarepta meeting. They are:

7/29 at 9 am – Review expectations #2 meeting
7/8 (9 am), 8/9 (1 pm), or 9/15 (1 pm) BP Assessment WG meetings

I can't think of any others, at the moment, but will let you know if I do. If any of the above dates/times work for you, I can reschedule my meeting. Let me know.

Colleen

From: Choy, Fannie (Yuet)
Sent: Tuesday, July 01, 2014 2:51 PM
To: Locicero, Colleen L
Cc: Choy, Fannie (Yuet)
Subject: Program pre-NDA meeting

Hi, Colleen

I'm trying to find an earlier meeting date for the Sarepta pre-NDA meeting. Do we routinely schedule either Dr Unger or Dr Temple as long as one of them is available?

Thanks
Fannie

Philips, Howard

From: Temple, Robert
Sent: Thursday, September 04, 2014 6:01 PM
To: Farkas, Ronald; Dunn, Billy; Bastings, Eric; Unger, Ellis
Cc: Rao, Ashutosh
Subject: RE: FDA Communication: re: IND 77429

Sounds like we need to talk together about how critical each of these is for our action. Mandy 106 will not be very delayed. Am I correct in recalling that that is the most critical?

From: Farkas, Ronald
Sent: Thursday, September 04, 2014 4:35 PM
To: Dunn, Billy; Bastings, Eric; Unger, Ellis; Temple, Robert
Cc: Rao, Ashutosh
Subject: FW: FDA Communication: re: IND 77429

All,

On July 29 we requested information from Sarepta, with some analyses that we need to be submitted in the NDA, and other information that we need to determine how the raw data will be submitted in the NDA. Attached is their response – they don't intend to submit the data until after filing the NDA, and for some requests, estimate not sending the information until close to the likely PDUFA date. Thoughts?

Thanks

Ron

From: Choy, Fannie (Yuet)
Sent: Thursday, September 04, 2014 4:00 PM
To: Farkas, Ronald; Rao, Ashutosh
Cc: Tandon, Veneeta; Choy, Fannie (Yuet)
Subject: FW: FDA Communication: re: IND 77429

IND 77429 / eteplirsen

Please see Sarepta's preliminary response to the FDA request of re-analyses.

Thanks
Fannie

From: Matthew Rael [mailto:MRael@Sarepta.com]
Sent: Thursday, September 04, 2014 3:43 PM
To: Choy, Fannie (Yuet)
Cc: Shamim Ruff
Subject: RE: FDA Communication: re: IND 77429

Dear Fannie,

Please find attached our initial response to the Division's 29-Jul-2014 clinical pharmacology information request, including projected completion dates for the requested deliverables.

We will copy this document to the IND as well.

Best regards,

Matt

Matthew Rael, MS
Manager, Regulatory Affairs
p 617.274.4029 c (b) (6) f 617.812.0509
e mrael@sarepta.com

SAREPTA THERAPEUTICS

215 First Street, Cambridge, MA 02142 USA

From: Choy, Fannie (Yuet) [mailto:Fannie.Choy@fda.hhs.gov]
Sent: Wednesday, September 03, 2014 2:13 PM
To: Matthew Rael
Cc: Shamim Ruff; Choy, Fannie (Yuet)
Subject: FW: FDA Communication: re: IND 77429
Importance: High

Dear Matt,

Reference is made to your IND 77429 for eteplirsen. We also refer to the FDA 7/29/14 communication (attached) and the 8/25/14 phone discussion between Shamim and myself. Shamim informed me that your team is working on the response to the Agency's request regarding re-analysis of dystrophin images, but did not have an update as of 8/25/14.

At the request of the request team, I'd like to convey the following: Please submit a timetable for the estimated completion dates of each request as noted in the 7/29/14 FDA communication. We ask that you provide the timetable by COB 9/4/14.

Regards,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products

From: Choy, Fannie (Yuet)
Sent: Tuesday, July 29, 2014 10:33 AM
To: Matthew Rael
Cc: Choy, Fannie (Yuet); Shamim Ruff
Subject: FDA Communication: re: IND 77429
Importance: High

Dear Matt,

Attached please find the letter following our NCH site visit. Please share with your team and let us know if you have any questions.

Regards,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products
Center for Drug Evaluation and Research
Food and Drug Administration

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Philips, Howard

From: Temple, Robert
Sent: Monday, September 08, 2014 7:04 PM
To: Jenkins, John K; Woodcock, Janet; Unger, Ellis; Whyte, John; Dunn, Billy
Cc: Kweder, Sandra L
Subject: RE: Meeting with Dr. Woodcock

I agree, especially with an NDA imminent and meetings to discussion the submission impending.

From: Jenkins, John K
Sent: Monday, September 08, 2014 12:14 PM
To: Woodcock, Janet; Unger, Ellis; Whyte, John; Dunn, Billy
Cc: Temple, Robert; Kweder, Sandra L; Jenkins, John K
Subject: RE: Meeting with Dr. Woodcock

I don't think it is appropriate to exclude the review division staff from this meeting, assuming Sarepta will be interested in participating, or if it is just a meeting with the patient advocates. Such an exclusion is not consistent with the way we do business in a collaborative environment. It also makes no sense for the staff that will be on the front lines of implementing any policy in this area to not be included/involved in a meeting to help formulate that policy.

John

From: Woodcock, Janet
Sent: Friday, September 05, 2014 6:22 PM
To: Unger, Ellis; Whyte, John; Dunn, Billy
Cc: Jenkins, John K; Temple, Robert; Kweder, Sandra L
Subject: RE: Meeting with Dr. Woodcock

Thanks. I know you folks are overstressed. I have made progress with the salary and hiring issues, we hope to have some substantive changes very soon. jw

---

From: Unger, Ellis
Sent: Friday, September 05, 2014 5:43 PM
To: Woodcock, Janet; Whyte, John; Dunn, Billy
Cc: Jenkins, John K; Temple, Robert; Kweder, Sandra L
Subject: RE: Meeting with Dr. Woodcock

Janet/John,

I'm not sure how all of this transpired – they're talking about two meeting requests in July and a phone call in August, but I only see an email string from this week forward. (I'm not asking to see the complete email string!).

In any case, I think the Division is already doing all that it can – the Division is working on dystrophin quantification, they have already produced much of a first draft of a DMD Guidance, and there's a pre-NDA meeting with Serepta on Monday. And beyond DMD, the Division is loaded with important review work and is short-staffed. Moreover, we made a somewhat unprecedented inspection of the study site in Columbus, OH last spring.

If Christine feels "strongly" about excluding members of the review division, you might ask her to explain her rationale for that if she hasn't done so already. (You might also ask her to state whether she wants anyone from ODE-I or OND to attend.)

But I think the question of granting the meeting is up to you. I don't think the Division would object to being 'left out' (and Billy will opine, no doubt).

Ellis

From: Woodcock, Janet
Sent: Friday, September 05, 2014 5:11 PM
To: Whyte, John; Dunn, Billy; Unger, Ellis
Subject: RE: Meeting with Dr. Woodcock

I'm not sure Serepta will buy into this, but we need to decide if we will agree to meet under these circumstances—just me, John, and the advocates and the firm.

In fact, I'm well aware of their strategy, they have been talking about it for a year. jw

---

From: Whyte, John
Sent: Friday, September 05, 2014 2:42 PM
To: Dunn, Billy; Unger, Ellis
Cc: Woodcock, Janet
Subject: FW: Meeting with Dr. Woodcock

Hi Billy and Ellis.
I wanted to make you aware of this. I was not planning to schedule it without you, and wanted to get your thoughts on this.
John

From: Christine McSherry [mailto:christine@jettfoundation.org]
Sent: Friday, September 05, 2014 2:32 PM
To: Whyte, John
Cc: Ligon, Sharnell (CDER); (b) (6) ; Woodcock, Janet
Subject: Re: Meeting with Dr. Woodcock

Hi John,

I do feel very strongly - I believe that it is important for the CEO of Sarepta to have the opportunity to fully discuss the strategy that is supported by the advocates.

Hopefully, this helps the scheduling easier for you!

Best,
Christine

On Fri, Sep 5, 2014 at 1:48 PM, Whyte, John <John.Whyte@fda.hhs.gov> wrote:

Hi Christine.

I did want to clarify a comment from your previous note.

It was not Dr. Woodcock's understanding that this meeting would occur without members of the division.

Do you feel strongly that members of the review division not be present?

John

John Whyte, MD, MPH

Director, Professional Affairs and Stakeholder Engagement

Acting Director, Office of Communications

Food and Drug Administration

Center for Drug Evaluation and Research

10903 New Hampshire Ave

Silver Spring, MD 20993

✉ **john.whyte@fda.hhs.gov**

☎ **Office: 240-402-4121**

From: Christine [mailto:christine@jettfoundation.org]
Sent: Friday, September 05, 2014 1:40 PM
To: Christine McSherry
Cc: Whyte, John; Ligon, Sharnell ([redacted (b) (6)] Woodcock, Janet

Subject: Re: Meeting with Dr. Woodcock

Hi John,

Any date suggestions?

Christine

On Sep 4, 2014, at 10:16 AM, Christine McSherry <christine@jettfoundation.org> wrote:

> Hi John,
>
> Thank you for your timely reply, as I was just discussing the number of meetings that have been requested and the days that have gone by since then.
>
> - Requests were made July 1 and July 8, 2014.
> - We were granted a phone call on August 15th.
> - It is now September 4th.
> - We have lost at least six (6) young men under the age of 21 in the **63 - sixty-three days** that have passed since our original request.
> - (b) (6) . His life has been changed, possibly forever.
> - (b) (6) was walking last week, now he is not = it happens that fast.
>
> September 15th and 23rd work for this group.
>
> Please let me know, thank you in advance.
>
> Best,
>
> Christine
>
> On Thu, Sep 4, 2014 at 8:49 AM, Whyte, John <John.Whyte@fda.hhs.gov> wrote:
>
> Hi Christine.
> Thank you for your note.
> If you could give me some dates that work for you, I can get to work on coordinating a meeting.
> Best,
> John

From: Christine McSherry [mailto:christine@jettfoundation.org]

Sent: Thursday, September 04, 2014 08:35 AM
To: Ligon, Sharnell (CDER)
Cc: (b) (6) Woodcock, Janet; Whyte, John
Subject: Re: Meeting with Dr. Woodcock

Dear Janet and Sharnell,

Thank you again for your unprecedented level of engagement with the Duchenne community.

We are appreciate your willingness to take into consideration the risk/benefit analysis that we have worked hard to communicate to you specifically, and to the Division of Neurology over the past 18 months. We believe that this work, along with a deeper understanding of our position and the technology - played a significant role in providing an extraordinary amount of flexibility in the guidance issued in April of this year.

To that end, we believe that we are embarking on yet another history changing enterprise that will result in additional innovative guidance and communication that will ultimately change the fate of all children who suffer from Duchenne.

The in-person meeting that has been requested is to provide a platform for the sponsor, the advocates and you - to hold a robust conversation about the plan going forward. It is our position, that the additional color added by the sponsor - and further analysis of risk/benefit (permissible risk), pharmacovigilance discussion with parents/advocates will give you a clear vision of the pathway needed for access to even the rarest populations in Duchenne.

Now that we have a clear path to for access to the first exon skipping drug, we simply can not allow days to slip by wondering what bar will be set by the agency for the other exons - and in reality, children - as we discussed on the phone.

In order to get to these children before it is too late - we need to continue the partnership. It is our position, that the proficient communication that can come from the sponsor, will provide additional clarity, and our advocacy efforts will bolster our support of the path forward. We

need to ensure that you have a complete understanding of the sponsors intent to get drug to these children.

To that end, we urge you to consider calendaring a meeting as quickly as possible.

We truly consider this a remarkable opportunity for the agency to demonstrate even further their amazing willingness to bring innovative drugs to market as quickly as possible.

As you have stated, "We have been very active on this subject, meeting with companies and discussing ways to expedite the drug development process for drugs that show striking early results," and for this we are grateful - however, we believe the patient voice also plays a vital role and needs to be heard.

I understand Dr. Whyte will be reaching out to schedule the meeting - thank you for your prompt attention to this matter.

We look forward to hearing back from you.

Best,

Christine

On Wed, Sep 3, 2014 at 4:07 PM, Ligon, Sharnell (CDER) <Sharnell.Ligon@fda.hhs.gov> wrote:

Hi Christine,

Dr. Woodcock has asked for Dr. Whyte to take the lead in coordinating this meeting. He should be reaching out to you sometime this week.

Kind Regards,

Sharnell

Sharnell M. Ligon

Executive Assistant to Dr. Janet Woodcock

Food and Drug Administration

Center for Drug Evaluation and Research
✉ **sharnell.ligon@fda.hhs.gov**

☎ **Office: 301-796-5253**

🖷 **Fax: 301-595-7910**

From: Christine McSherry [mailto:christine@jettfoundation.org]
Sent: Tuesday, September 02, 2014 9:23 AM
To: Ligon, Sharnell (CDER)
Cc: Woodcock, Janet; (b) (6)
Subject: Meeting with Dr. Woodcock

Hi Sharnell,

Just following up on our request for an in-person meeting with Janet to discuss further the future of personalized medicine, trial design and the approval pathways.

Please give us two dates to choose from - when you can.

Best,

Christine

--

Making…TODAY COUNT - TO CHANGE TOMORROW

Christine McSherry, RN

The Jett Foundation

68 Evergreen Street, Suite One
Kingston, MA 02364
(781) 585-5566

FAX: (781) 585-5233
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Philips, Howard

From: Temple, Robert
Sent: Tuesday, September 09, 2014 11:40 AM
To: Leptak, Christopher; Moscicki, Richard; Unger, Ellis; Dunn, Billy; Bastings, Eric; Kaiser, James; Pacanowski, Michael A
Cc: Gonzalez, Alina; Farkas, Ronald; Rao, Ashutosh; Gross, Mary
Subject: RE: Dystrophin Workshop

Nothing to add to Ellis's comments.

From: Leptak, Christopher
Sent: Monday, September 08, 2014 11:46 AM
To: Moscicki, Richard; Unger, Ellis; Dunn, Billy; Bastings, Eric; Kaiser, James; Pacanowski, Michael A; Temple, Robert
Cc: Gonzalez, Alina; Farkas, Ronald; Rao, Ashutosh; Gross, Mary
Subject: Dystrophin Workshop

Hello everyone,

In preparation for next week's Dystrophin Workshop Steering Committee meeting, Ron, Ash, and I have created a Draft Agenda for your consideration and input. Please review and comment. The document can be found at:

http://sharepoint.fda.gov/orgs/CDER-Center-Wide/dystrophin/SitePages/Home.aspx

The file is under the tab "Workshop Agenda" and the document is titled Draft Agenda Dystrophin Workshop (modified Sept 8, 11:29 AM).

Best,
Chris, Ash, and Ron

Philips, Howard

From: Temple, Robert
Sent: Thursday, September 25, 2014 11:44 AM
To: Choy, Fannie (Yuet)
Subject: RE: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta

I'm not following. When was it to be and when is it now.

From: Choy, Fannie (Yuet)
Sent: Thursday, September 25, 2014 10:21 AM
To: Blount, Aprile; Shekitka, Barbara
Cc: Choy, Fannie (Yuet); Temple, Robert; Unger, Ellis
Subject: RE: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta

Hi,

I have to reschedule this meeting due to conflicts. Dr Temple has a tentative meeting scheduled at the same time, please confirm if he's available.

Thank you,
Fannie

-----Original Appointment-----
From: Choy, Fannie (Yuet)
Sent: Thursday, September 18, 2014 4:20 PM
To: Choy, Fannie (Yuet); Moscicki, Richard; Temple, Robert; Unger, Ellis; Dunn, Billy; Bastings, Eric; Farkas, Ronald; Rao, Ashutosh
Cc: CDER 120 Calendar; Daugherty, Susan B (CSO)
Subject: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta
When: Thursday, October 09, 2014 4:00 PM-5:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: CDER WO 4201 conf rm Bldg22

9/25/14: Meeting rescheduled due to conflicts.
Please confirm your availability. Thank you!

Follow-Up Discussion: Internal discussion on meeting comments to the sponsor. Sponsor Meeting held on 9/18/14.
IND: 077429
Product/Sponsor: eteplirsen / Sarepta Therapeutics
Indication: Duchenne muscular dystrophy

FDA Preliminary Cmts Sponsor's Discussion Slides 4/15/14 FDA Advice Letter 7/29/14 IR (dystrophin)

~scheduled by Fannie Choy on 9/18/14~ 301-796-2899

Philips, Howard

From: Temple, Robert
Sent: Thursday, October 23, 2014 6:46 PM
To: Lu, Wei; Unger, Ellis; Dunn, Billy; Farkas, Ronald
Cc: Shekitka, Barbara; Blount, Aprile
Subject: RE: Please review and provide comments on the Multi-sig letter on DMD and eteplirsen

I don't think the draft is responsive. The congresspeople are urging attention to DMD, of course, but their letter is almost entirely about getting hold of the detailed Mazzone natural history data . This has nothing to do with a pending application. Our answer says that we cannot discuss the substance of matters before the agency, which is irrelevant. It also, by the way, seems to say that our refusal to discuss such matters reflects a policy intended to maintain review process integrity; I always thought it was because we are not legally allowed to discuss trade secret information or confidential commercial information. Anyway, that paragraph is not relevant here. The third paragraph says we really care, etc., again not the question raised by the letter. It could be part of an initial paragraph but doesn't represent an answer.

I think we need to say that good natural history data would be useful in many ways, in designing studies and selecting patients and in evaluating results of controlled trials, and perhaps in creating credible historically controlled trials. We should then say what we can about the Mazzone data and whether we are seeking detailed data, etc. In other words, this needs a "start over"

From: Lu, Wei
Sent: Thursday, October 23, 2014 4:31 PM
To: Unger, Ellis; Temple, Robert
Cc: Shekitka, Barbara; Blount, Aprile
Subject: Please review and provide comments on the Multi-sig letter on DMD and eteplirsen

Dear Dr. Unger and Dr. Temple,

We recently received a letter from Multi-sig Congressional members on DMD and etepliren (incoming letter is attached). Would you please review the draft response letter (attached) and provide comments for ODE I? DNP has cleared it. Please send your comments to me by COB on Friday, 10/24 if all possible.

Many thanks,
Wei

_____

Wei Lu, RN, MS
CDER/OEP/DEO
Bldg 51, Room 6174
Office: 301-796-3448
Fax: 301-847-8753
Email: Wei.Lu@fda.hhs.gov

Philips, Howard

From: Temple, Robert
Sent: Wednesday, November 12, 2014 2:52 PM
To: Lu, Wei; Unger, Ellis
Cc: Shekitka, Barbara; Blount, Aprile; Jenkins, John K; Dunn, Billy
Subject: RE: Please review and provide comments: Inquiry from Remy Brim, staffer for Sen. Warren regarding eteplirsen

I've included others on this. Answers look good

From: Lu, Wei
Sent: Tuesday, November 11, 2014 9:08 AM
To: Unger, Ellis; Temple, Robert
Cc: Shekitka, Barbara; Blount, Aprile
Subject: Please review and provide comments: Inquiry from Remy Brim, staffer for Sen. Warren regarding eteplirsen
Importance: High

Good morning Dr. Unger, Dr. Temple,

We received the following questions from Remy Brim, staffer for Sen. Warren following the recent FDA eteplirsen statement. Please review and provide comments on the draft answers (DNP revised/cleared it) below.

Dr. Woodcock would like us to respond in writing first. Thus, I was told yesterday that Sen. Warren office would like to have a briefing scheduled soon because they heard 'from the company and very upset parents on this issue' as well.

1. How does the clock work on a rolling submission vs a standard submission?

A: The review clock is the same for a rolling submission and a non-rolling submission, and begins when the Agency has received a complete NDA. This is described in more detail in Guidance for Industry*, Expedited Programs for Serious Conditions – Drugs and Biologics,*
http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm358301.pdf

2. If the FDA reviews as data is submitted in a rolling review, but the clock doesn't start until everything is in, does a rolling review generally speed up time to approval? If so by how much on average? Is this different than the agency accepting data after an NDA is filed?

A: A major advantage of rolling review is that it can allow problems to be identified early (i.e., in the sections received prior to the start of the review clock) so that there is more time to successfully resolve them prior to the end of the review period. The Agency similarly tries to resolve problems that are identified in a non-rolling review, but because less time is available to do so, there is a greater likelihood of problems remaining unresolved at the end of the review period and leading to a Complete Response. FDA hasn't tracked the average time saved by rolling review. A rolling review is different than accepting an amendment to a pending NDA after it is filed. For the latter, FDA may or may not need to extend the review clock, which has already started. If FDA determines that the amendment makes it necessary to extend the review clock, the clock is extended by 3 months. The clock can be extended only once during a review cycle.

3. When did the FDA request the samples to be reevaluated, was that in April and was that with the recommendations about how FDA wanted it done? Did the FDA ask to approve a protocol for the reevaluation, and if so, when did that protocol get approved?

A: Under applicable laws and regulations, FDA can't discuss the specifics of the application.

4. How has FDA helped to obtain natural history data from investigators?

A: FDA has contacted multiple data holders to obtain any available natural history data for Duchenne muscular dystrophy; for example we have requested individual patient-level data from Dr. Eugenio Mercuri and his colleagues for the Mazzone et al paper, and from Dr. Craig McDonald and his colleagues for the ongoing Cooperative International Neuromuscular Research Group study.

Thanks a lot. Please provide your comments back to me by noon tomorrow, 11/12 if all possible.

Wei

From: Lu, Wei
Sent: Thursday, November 06, 2014 12:03 PM
To: Farkas, Ronald; Locicero, Colleen L; Ware, Jacqueline H
Cc: Choy, Fannie (Yuet); Dunn, Billy; Bastings, Eric
Subject: Heads up: Inquiry regarding eteplirsen
Importance: High

Hi Ron,

Please find the questions below from Remy Brim, staffer for Sen. Warren regarding eteplirsen. Bob is checking with Dr. Woodcock whether or not she wants to brief them or to provide answers in writing. Whatever the decision is, we'd prepare the answers to the questions (1, 2, and 4). I think we could use standard answer to Q3: Under applicable laws and regulations, FDA can't discuss the specific application.

Colleen and Jackie - do we have general guideline for the different submissions: a rolling submission vs a standard submission? It's more like a regulatory procedure questions for Q1 and Q2.

Thank you.
Wei

_____
Wei Lu, RN, MS
CDER/OEP/DEO
Bldg 51, Room 6174
Office: 301-796-3448
Fax: 301-847-8753
Email: Wei.Lu@fda.hhs.gov

From: Walinsky, Sarah
Sent: Thursday, November 06, 2014 9:48 AM
To: Lu, Wei
Cc: CDER EXSEC; Guidos, Robert; Menon, Ramesh
Subject: Inquiry regarding eteplirsen

Hi Wei,

We received the following questions in follow up to the recent FDA eteplirsen statement. Please let me know if you have language that we can use to respond to these questions, and which questions we cannot respond to. A briefing may be more efficient, depending on how difficult it will be to procure answers to these questions, please let me know if that is the case.

1. How does the clock work on a rolling submission vs a standard submission?
2. If the FDA reviews as data is submitted in a rolling review, but the clock doesn't start until everything is in, does a rolling review generally speed up time to approval? If so by how much on average? Is this different than the agency accepting data after an NDA is filed?
3. When did the FDA request the samples to be reevaluated, was that in April and was that with the recommendations about how FDA wanted it done? Did the FDA ask to approve a protocol for the reevaluation, and if so, when did that protocol get approved?
4. How has FDA helped to obtain natural history data from investigators?

Please let me know if you have any questions.

Thanks,

Sarah

Sarah Walinsky
Congressional Affairs Specialist
Office of Legislation
U.S. Food and Drug Administration
Tel: (240) 402-4075
Fax: (301) 847-8602

Philips, Howard

From: Temple, Robert
Sent: Thursday, February 19, 2015 9:58 AM
To: Unger, Ellis; Dunn, Billy; Moscicki, Richard; Rao, Ashutosh
Subject: FW: Commentary on Dystrophin

You probably all got this, but just in case.

From: Christine McSherry [mailto:christine@jettfoundation.org]
Sent: Thursday, February 19, 2015 8:06 AM
To: Temple, Robert
Subject: Commentary on Dystrophin

Bob,

Please read the below article. I would hope that the authors of this commentary - who are dystrophin experts, will be presenting at the upcoming dystrophin workshop.

Commentary

Citation: *Molecular Therapy Nucleic Acids* (2014) **3**, e152; doi:10.1038/mtna.2014.6
Published online 11 March 2014

What Can We Learn From Clinical Trials of Exon Skipping for DMD?

OPEN

Qi-long Lu[1], Sebahattin Cirak[2] and Terence Partridge[2]

1. [1]Department of Neurology, McColl Lockwood Laboratory for Muscular Dystrophy Research, Carolinas Medical Center, Charlotte, North Carolina, USA

2. [2]Center for Genetic Medicine, Children's National Medical Center, NW, Washington, DC, USA

Correspondence: Qi-long Lu, McColl Lockwood Laboratory for Muscular Dystrophy Research, Department of Neurology, Carolinas Medical Center, 1000 Blythe Blvd., Charlotte, North Carolina 28203, USA. E-mail: Qi.lu@Carolinashealthcare.org; Terence Partridge, Children's National Medical Center, 111 Michigan Ave, NW, Washington, DC 20010, USA. E-mail: TPartridge@childrensnational.org

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Christine McSherry, RN

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FAX: (781) 585-5233
CELL: (781) 254-2669
jettfoundation.org

Philips, Howard

From: Temple, Robert
Sent: Wednesday, April 29, 2015 4:46 PM
To: Blount, Aprile
Subject: FW: Meeting invitation: Dystrophin Meeting Follow Up

Tell her yes if I can. Tx

From: Mary Gross [mailto:messenger@webex.com]
Sent: Wednesday, April 29, 2015 11:08 AM
To: Temple, Robert
Subject: Meeting invitation: Dystrophin Meeting Follow Up

Hello Robert Temple,

Mary Gross invites you to attend this online meeting.

Topic: Dystrophin Meeting Follow Up
Date: Monday, May 4, 2015
Time: 4:00 pm, Eastern Daylight Time (New York, GMT-04:00)
Meeting Number: 742 003 159
Meeting Password: Insider#12

To join the online meeting
---
1. Go to https://fda.webex.com/fda/j.php?MTID=mf1fa2ea68e181276fe5b591a4fd98634
2. If requested, enter your name and email address.
3. If a password is required, enter the meeting password: Insider#12
4. Click "Join".

To view in other time zones or languages, please click the link:
https://fda.webex.com/fda/j.php?MTID=m7db4ab8b945b4433d5d7ed2484a665f3

To join the teleconference only
---
1. Please call one of the following numbers:
Local: 1-301-796-7777
toll free: 1-855-828-1770
2. Follow the instructions that you hear on the phone.
Your Cisco Unified MeetingPlace meeting ID: 742 003 159

For assistance
---
1. Go to https://fda.webex.com/fda/mc
2. On the left navigation bar, click "Support".

You can contact me at:
mary.gross@fda.hhs.gov
1-301-796-3519

To add this meeting to your calendar program (for example Microsoft Outlook), click this link:
https://fda.webex.com/fda/j.php?MTID=m37eb0cbe9ca2e8ccc7871a4547c5f0bb

The playback of UCF (Universal Communications Format) rich media files requires appropriate players. To view this type of rich media files in the meeting, please check whether you have the players installed on your computer by going to https://fda.webex.com/fda/systemdiagnosis.php.

FDARichMedia@fda.hhs.gov

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Philips, Howard

From: Temple, Robert
Sent: Thursday, April 30, 2015 6:01 PM
To: Unger, Ellis
Subject: FW: sharing natural hx data

What is this about???

From: Jenkins, John K
Sent: Thursday, April 30, 2015 12:11 PM
To: Unger, Ellis
Cc: Temple, Robert; Dunn, Billy
Subject: RE: sharing natural hx data

I think we should press to get unrestricted access to the data, and not "matched" patients only. We may need to get JW involved to make the requests and perhaps there can be some agreement that will not use the data outside of our reviews.

From: Unger, Ellis
Sent: Thursday, April 30, 2015 9:01 AM
To: Jenkins, John K
Cc: Temple, Robert; Dunn, Billy
Subject: sharing natural hx data

John,

I guess the future is here, but perhaps we could have done more to plan for it.

We're aware of some consortia with natural history data on DMD patients, but they have some problems with wholesale release of the data. Apparently at least one group would be willing to give us 'matching data' for specific patients. But how could we be assured that they didn't cherry pick the data they provide to us? And by asking them to match patients with specific baseline characteristics, we could tip them off that we're reviewing a specific NDA.

So we're not quite sure how to navigate these waters, but we'd like to do it quickly. And BTW, the drisapersen NDA came in Monday.

Ellis

Philips, Howard

From: Temple, Robert
Sent: Wednesday, May 06, 2015 12:57 PM
To: Moscicki, Richard; Raggio, Miranda; Jenkins, John K
Cc: Unger, Ellis; Dunn, Billy; Choy, Fannie (Yuet)
Subject: RE: Update on GSKs Drisapersen

(b) (4)

)..

From: Moscicki, Richard
Sent: Wednesday, May 06, 2015 12:50 PM
To: Raggio, Miranda; Jenkins, John K
Cc: Temple, Robert; Unger, Ellis; Dunn, Billy; Choy, Fannie (Yuet)
Subject: RE: Update on GSKs Drisapersen

(b) (4) . Rich.

From: Raggio, Miranda
Sent: Wednesday, May 06, 2015 12:35 PM
To: Jenkins, John K
Cc: Moscicki, Richard; Temple, Robert; Unger, Ellis; Dunn, Billy; Choy, Fannie (Yuet); Raggio, Miranda
Subject: RE: Update on GSKs Drisapersen

HI John,

(b) (4)

. Thanks very much,m

Miranda Raggio, BA, BSN, MA
CDER Breakthrough Therapy Program Manager
Regulatory Affairs Team/Office of New Drugs
Center for Drug Evaluation and Research
White Oak Campus/Building 22-Room 6465
301-796-2109
Miranda.Raggio@fda hhs.gov

From: Jenkins, John K
Sent: Wednesday, May 06, 2015 12:30 PM
To: Raggio, Miranda
Cc: Moscicki, Richard; Temple, Robert; Unger, Ellis; Dunn, Billy
Subject: RE: Update on GSKs Drisapersen

Miranda

(b) (4)

John

From: Raggio, Miranda
Sent: Wednesday, May 06, 2015 12:28 PM
To: Jenkins, John K
Cc: Moscicki, Richard; Temple, Robert; Raggio, Miranda
Subject: RE: Update on GSKs Drisapersen

HI..I pursued investigating this, and talked to Ron Farkas and Billy Dunn… (b) (4) .

Just FYI. Thanks,m

Miranda Raggio, BA, BSN, MA
CDER Breakthrough Therapy Program Manager
Regulatory Affairs Team/Office of New Drugs
Center for Drug Evaluation and Research
White Oak Campus/Building 22-Room 6465
301-796-2109
Miranda.Raggio@fda hhs.gov

From: Jenkins, John K
Sent: Wednesday, May 06, 2015 7:47 AM
To: Raggio, Miranda
Cc: Moscicki, Richard; Temple, Robert; Frey, Patrick; Bertha, Amy
Subject: RE: Update on GSKs Drisapersen

Thanks. (b) (4)

From: Raggio, Miranda
Sent: Tuesday, May 05, 2015 6:15 PM
To: Jenkins, John K
Cc: Moscicki, Richard; Temple, Robert; Raggio, Miranda; Frey, Patrick; Bertha, Amy
Subject: Update on GSKs Drisapersen

Hi John,

I checked my records and IND 105284, Drisapersen, (b) (4)).

So, I followed up with the RPM in DNP, who checked with Drs. Farkas and Bastings, (b) (4)

Let me know if you need additional information. Thanks,m

Miranda Raggio, BA, BSN, MA
CDER Breakthrough Therapy Program Manager
Regulatory Affairs Team/Office of New Drugs
Center for Drug Evaluation and Research
White Oak Campus/Building 22-Room 6465
301-796-2109
Miranda.Raggio@fda hhs.gov

Philips, Howard

From: Choy, Fannie (Yuet)
Sent: Friday, July 24, 2015 7:00 PM
To: Temple, Robert; Unger, Ellis
Cc: Dunn, Billy; Bastings, Eric; Locicero, Colleen L
Subject: RE: Internal pre-meeting for Late Cycle: NDA 206031/drisapersen

This is to follow up on our conversation. Your preference is to hold the prep meeting before you're away and leave the late-cycle as is (11/5/15). I'll go ahead and hold the 9/30 block for now.

Thanks

From: Choy, Fannie (Yuet)
Sent: Friday, July 24, 2015 4:43 PM
To: Temple, Robert; Unger, Ellis
Cc: Dunn, Billy; Bastings, Eric; Locicero, Colleen L
Subject: RE: Internal pre-meeting for Late Cycle: NDA 206031/drisapersen

It seems like the prep meeting can be 9/30 (you're away 10/1-10/2), and then hold late-cycle with sponsor 1st week of Nov. That would be a month apart.

Another option is to delay the prep (1st week of Nov) and the late-cycle to 2nd week of Nov, as long as we hold the late cycle 12 days before the AC.

Thanks
Fannie

From: Temple, Robert
Sent: Friday, July 24, 2015 3:47 PM
To: Choy, Fannie (Yuet); Unger, Ellis
Cc: Dunn, Billy; Bastings, Eric; Locicero, Colleen L
Subject: RE: Internal pre-meeting for Late Cycle: NDA 206031/drisapersen

Not sure I'm following. If I'm away on 10/19, could we do the prep before I'm away?

From: Choy, Fannie (Yuet)
Sent: Thursday, July 23, 2015 6:53 PM
To: Temple, Robert; Unger, Ellis
Cc: Choy, Fannie (Yuet); Dunn, Billy; Bastings, Eric; Locicero, Colleen L
Subject: RE: Internal pre-meeting for Late Cycle: NDA 206031/drisapersen

Hi,

I'm checking if the date for the prep-meeting for the late-cycle on 10/19 is acceptable. Dr Temple is out 10/5-10/27 on his calendar and he is the signatory for the NDA. Since Late cycle background is due not less than 20 days before AC, we'd need the prep meeting during Oct. Does that sound okay?

Thanks
Fannie

-----Original Appointment-----

Subject: Internal pre-meeting for Late Cycle: NDA 206031/drisapersen
When: Monday, October 19, 2015 1:00 PM-2:30 PM (UTC-05:00) Eastern Time (US & Canada).

Late Cycle Meeting (internal pre-meeting) for Original NDA

- To brief ODE and Division Director on review issues proposed for discussion at the late-cycle meeting and to plan the meeting.
- Discussion: review outcomes and determination of what issues can be fixed or are amenable to correction in the current review cycle and what issues could affect the outcome of the review.
- Planning: goals of the late-cycle meeting, who should attend and who will chair the meeting, the agenda for the meeting, and plans for the advisory committee meeting.

Application Info: NDA 206031 (IND 105284)
Drug: drisapersen
Sponsor: BioMarin (acquired Prosensa as of 1/16/15)
Proposed Indication: treatment of Duchenne muscular dystrophy (DMD)
Other Info: (b) (4)

EDR link to submissions (also accessible through DARRTS and GlobalSubmit):
\\CDSESUB1\evsprod\NDA206031\206031.enx

Milestone dates:

(b) (4)

Philips, Howard

From: Jenkins, John K
Sent: Thursday, August 06, 2015 3:45 PM
To: Moscicki, Richard; Dunn, Billy; Unger, Ellis; Temple, Robert
Cc: Woodcock, Janet
Subject: RE: DMD

Well, it seems a lot more plausible that, for example, 20% of normal expression might suggest value of dystrophin as a surrogate to support approval, while 1% of normal raises many more questions about clinical significance of the change.

From: Moscicki, Richard
Sent: Thursday, August 06, 2015 1:18 PM
To: Jenkins, John K; Dunn, Billy; Unger, Ellis; Temple, Robert
Cc: Woodcock, Janet
Subject: RE: DMD

I heard this yesterday. The measurement is by Western blot which isn't a great way to quantitate but even so I am not sure that this isn't more than untreated patients, rather I suspect it may be. There has long been consensus that it was unlikely that these therapies would induce dystrophin levels in the range of Becker's patients let alone normal. 1% might even be consistent with the increase observed by IF at 24 wks. It would be good to know what IF shows on these 4 yr bx's and if its consistent with the 24 wk expression. We don't know how much expression would be clinically significant. I still would be interested to know if serum of plasma stored on eteplirsen patients might show CK differences. Rich.

From: Jenkins, John K
Sent: Thursday, August 06, 2015 1:00 PM
To: Dunn, Billy; Unger, Ellis; Temple, Robert
Cc: Moscicki, Richard; Woodcock, Janet
Subject: RE: DMD

How did the sponsor explain the low level of dystrophin?

I added Rich and Janet so they have a heads up of this major new development.

From: Dunn, Billy
Sent: Thursday, August 06, 2015 12:23 PM
To: Jenkins, John K; Unger, Ellis; Temple, Robert
Subject: RE: DMD

John,

The 4th biopsy appears to show very little dystrophin (I believe it was 0.92% of normal levels, if memory serves), though apparently it was minimally elevated, presumably due to drug. How this factors into the overall picture, including the previous dystrophin data and the plausibility of being associated with or predictive of a beneficial effect, will be a matter for review. There was consensus that this was not a filing issue. Bob was also at the meeting, so I've included him.

Billy

From: Jenkins, John K
Sent: Thursday, August 06, 2015 12:17 PM
To: Dunn, Billy; Unger, Ellis
Subject: DMD

Billy

Do you have an update following your filing meeting for eteplirsen? You told me earlier in the week that the 4th biopsy data showed very little dystrophin????

John

Philips, Howard

From: Temple, Robert
Sent: Thursday, September 25, 2014 1:16 PM
To: Blount, Aprile
Subject: FW: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta

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From: Choy, Fannie (Yuet)
Sent: Thursday, September 25, 2014 11:47 AM
To: Temple, Robert
Subject: RE: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta

Was scheduled on 9/30 4 pm. The new date/time is Thu 10/9 4 pm. There is an EOP2 meeting as tentative at the same time. Thanks

---

From: Temple, Robert
Sent: Thursday, September 25, 2014 11:44 AM
To: Choy, Fannie (Yuet)
Subject: RE: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta

I'm not following. When was it to be and when is it now.

From: Choy, Fannie (Yuet)
Sent: Thursday, September 25, 2014 10:21 AM
To: Blount, Aprile; Shekitka, Barbara
Cc: Choy, Fannie (Yuet); Temple, Robert; Unger, Ellis
Subject: RE: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta

Hi,

I have to reschedule this meeting due to conflicts. Dr Temple has a tentative meeting scheduled at the same time, please confirm if he's available.

Thank you,
Fannie

-----Original Appointment-----
From: Choy, Fannie (Yuet)
Sent: Thursday, September 18, 2014 4:20 PM
To: Choy, Fannie (Yuet); Moscicki, Richard; Temple, Robert; Unger, Ellis; Dunn, Billy; Bastings, Eric; Farkas, Ronald; Rao, Ashutosh
Cc: CDER 120 Calendar; Daugherty, Susan B (CSO)
Subject: *Reschedule* Follow-up INTERNAL discussion: IND 77429 / eteplirsen / Sarepta

When: Thursday, October 09, 2014 4:00 PM-5:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: CDER WO 4201 conf rm Bldg22

9/25/14: Meeting rescheduled due to conflicts.
Please confirm your availability. Thank you!

Follow-Up Discussion: Internal discussion on meeting comments to the sponsor. Sponsor Meeting held on 9/18/14.
IND: 077429
Product/Sponsor: eteplirsen / Sarepta Therapeutics
Indication: Duchenne muscular dystrophy

FDA Preliminary Cmts Sponsor's Discussion Slides 4/15/14 FDA Advice Letter 7/29/14 IR (dystrophin)

~scheduled by Fannie Choy on 9/18/14~ 301-796-2899

Philips, Howard

From: Bernstein, Jessica
Sent: Friday, September 25, 2015 10:53 AM
To: Salerno, Mary Jo
Cc: JW Direct Reports & Deputies; CDER-EOS; Behr, Virginia L; Peterson, Jayne E; MedWatch Safety Alerts; Drug Safety Web; Meister, Karen G; Walsh, Sandy; Sherwood, Edward M; Thomas, Kimberly K (ORP); Helmanis, Lisa M; Sullivan, Diane; Stowe, Ginneh D.; Baumgartner, Kristofer; OPQ Policy; CDER-OPQ-Inquiries; Bautista, Philip; Ngo, Diem-Kieu (CDR,USPHS); Farkas, Ronald; Bastings, Eric; Choy, Fannie (Yuet); Dunn, Billy; Ware, Jacqueline H; Unger, Ellis; Locicero, Colleen L; Vail, Victor H; Peddicord, Sarah
Subject: Information Advisory: FDA to Announce a Meeting of Peripheral and Central Nervous Systems Drugs Advisory Committee to Discuss Drisapersen to Treat Duchenne Muscular Dystrophy
Attachments: IA_Public_Mtg_PCNS_AC_Cleared_DNP_ODE1_DEO_OEP_092515.doc

Good morning Mary Jo,

In September or October 2015, FDA will publish a Federal Register notice announcing a meeting of the Peripheral and Central Nervous System Drugs Advisory Committee. The Committee will meet on November 24, 2015 to discuss a new drug application for drisapersen solution for injection, for treatment of patients with Duchenne muscular dystrophy (DMD). FDA expects this announcement to be of interest to patients with DMD and their families, advocacy groups, industry, health care providers including neurologists and therapists working with people with DMD, researchers, and those working in regulatory science. FDA will not issue a press release.

*****PLEASE NOTE THAT THIS INFORMATION IS NOT RELEASABLE TO THE PUBLIC UNTIL ACTION TAKES PLACE*****

Regards,

Jessica

Jessica Bernstein, MPH
Writer-Editor
Division of Executive Operations
Office of Executive Programs
Center for Drug Evaluation and Research
U.S. Food and Drug Administration
(240) 402-0524
jessica.bernstein@fda.hhs.gov

DATE: September 25, 2015

INFORMATION ADVISORY
(CONFIDENTIAL)

SUBJECT/LEAD COMPONENT: FDA to Announce a Meeting of Peripheral and Central Nervous System Drugs Advisory Committee to Discuss Drisapersen to Treat Duchenne Muscular Dystrophy

WHY THIS INFORMATION IS IMPORTANT FOR THE SECRETARY: On or about September XX, FDA will announce a meeting of the Peripheral and Central Nervous System Drugs Advisory Committee. The Committee will meet on November 24, 2015 to discuss a new drug application for drisapersen solution for injection, for treatment of patients with Duchenne muscular dystrophy (DMD). DMD is a rare genetic disease that is found almost exclusively in males. It typically manifests in early childhood and progresses to loss of ambulation and death in young adulthood. Current treatment includes corticosteroids, which may prolong function and survival by several years, and supportive care (e.g., physiotherapy, mechanical supports, orthopedic surgery, and assisted ventilation). Drisapersen is sponsored by BioMarin Pharmaceutical Inc. Drisapersen was granted Breakthrough Therapy Designation by FDA in June 2013, enabling expedited development and review. Another company, Sarepta Therapeutics, Inc., recently submitted a New Drug Application for a competing drug, eteplirsen.

FDA expects this announcement to be of interest to patients with DMD and their families, advocacy groups, industry, health care providers including neurologists and therapists working with people with DMD, researchers, and those working in regulatory science. There is an active community of advocates for research on muscular dystrophy that includes parents of children with DMD. Advocates are well-informed about promising therapies and may be interested in the outcome of this meeting. One advocacy group, Parent Project Muscular Dystrophy, recently submitted a draft guidance to FDA that contributed to the Agency's draft guidance for industry for developing drugs for this rare disease. FDA will not issue a press release. The meeting is expected to garner adequate press coverage such that a press release is not necessary to disseminate the results of the meeting to the community.

SUMMARY OF ISSUE, BACKGROUND, AND DEPARTMENT RESPONSE/ACTIONS:

- Duchenne muscular dystrophy (DMD) is a genetic disorder of muscle, caused by mutations in a gene on the X chromosome that encodes for the dystrophin protein. Dystrophin imparts structural stability to membranes of muscle fiber cells, enabling them to withstand the contraction/relaxation cycles and force generation required of muscle tissue. People with DMD produce little or no functional dystrophin, leading to damage to muscle cells, muscle weakness, and eventually paralysis and early death.

- The prevalence of DMD at birth is about 1 in 3,500 to 1 in 6,000 males. It is among the most common of neuromuscular disorders.

- A number of mutations in the dystrophin gene cause DMD. Drisapersen is targeted to a specific mutation that affects approximately 13 percent of patients with DMD.

- FDA granted drisapersen Breakthrough Therapy Designation in June 2013. This enables expedited development and review of drugs that are intended to treat serious or life-threatening diseases, and for which preliminary clinical evidence indicates that the drug may demonstrate substantial improvement over existing therapies. Drisapersen is also designated as an Orphan Drug because it demonstrates promise for safe and effective treatment of a rare disease. As such, it is eligible for incentives to further advance scientific development.

- In June 2015, FDA released draft guidance for industry on Duchenne Muscular Dystrophy and Related Dystrophinopathies: Developing Drugs for Treatment.

- Three double-blind, placebo-controlled studies have been conducted to establish the efficacy of drisapersen for the treatment of DMD, including 300 patients at more than 50 trial sites. Subsequent to granting drisapersen Breakthrough Therapy Designation, the third and largest trial showed negative results, raising serious questions about the efficacy of drisapersen. Drisapersen is associated with potentially serious adverse effects, such that the Advisory Committee will need to discuss the benefit-risk profile of the product.

- The Committee will discuss the new drug application for drisapersen solution for injection, review safety and efficacy data, including differing clinical trial results, and comment on whether adequate data have been submitted to support approval.

CONTACTS: Alicia Swenson-O'Brien, OS/ES, (202) 205-9953; Mary Jo Salerno, FDA/OES, (240) 402-0420; Jessica Bernstein, CDER/DEO, (240) 402-0524.

Clearance history
Draft: JBernstein, DEO, 9/9/15
Cleared with Edits: EBastings, DNP, 9/11/15
Cleared with Edits: EUnger, ODE-I, 9/11/15
Reviewed/Edited: CBechtel, DEO, 9/14/15
Cleared with Edits: EBastings, DNP, 9/18/15
Reviewed/Minor Edits: CBechtel, DEO, 9/22/15
Reviewed/Minor edits: EUnger, ODE-I, 9/24/15
Cleared: CBechtel, DEO, 9/24/15
Cleared with Edits: HBrown, OEP/CDER, 09/25/15

Philips, Howard

From: Temple, Robert
Sent: Thursday, October 01, 2015 10:03 AM
To: Unger, Ellis
Cc: Dunn, Billy; Bastings, Eric
Subject: RE: Drisapersen update

(b) (4)

From: Unger, Ellis
Sent: Thursday, October 01, 2015 9:29 AM
To: Temple, Robert
Cc: Dunn, Billy; Bastings, Eric
Subject: RE: Drisapersen update

(b) (4)

From: Dunn, Billy
Sent: Wednesday, September 30, 2015 7:56 PM
To: Temple, Robert
Cc: Choy, Fannie (Yuet); Unger, Ellis; Bastings, Eric; Farkas, Ronald
Subject: RE: Drisapersen update

(b) (4)

Billy

From: Hank Fuchs [mailto:HFuchs@bmrn.com]
Sent: Wednesday, September 30, 2015 1:36 PM
To: Dunn, Billy; Temple, Robert; Unger, Ellis
Cc: Choy, Fannie (Yuet)
Subject: Re: Drisapersen update

(b) (4)

(b) (4)

Hank

On Sep 1, 2015, at 7:31 AM, Choy, Fannie (Yuet) <Fannie.Choy@fda.hhs.gov> wrote:

(b) (4)

Regards,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products

From: Hank Fuchs [mailto:HFuchs@bmrn.com]
Sent: Monday, August 31, 2015 10:21 PM
To: Dunn, Billy
Cc: Camilla Simpson; Nicole Persson
Subject: Drisapersen update

(b) (4)

[redacted]

(b) (4)

Philips, Howard

From: Choy, Fannie (Yuet)
Sent: Monday, October 05, 2015 1:08 PM
To: Temple, Robert
Cc: Choy, Fannie (Yuet); Farkas, Ronald
Subject: FW: late cycle communication
Attachments: late cycle communication.doc

NDA 206031 / drisapersen

Dr Temple,

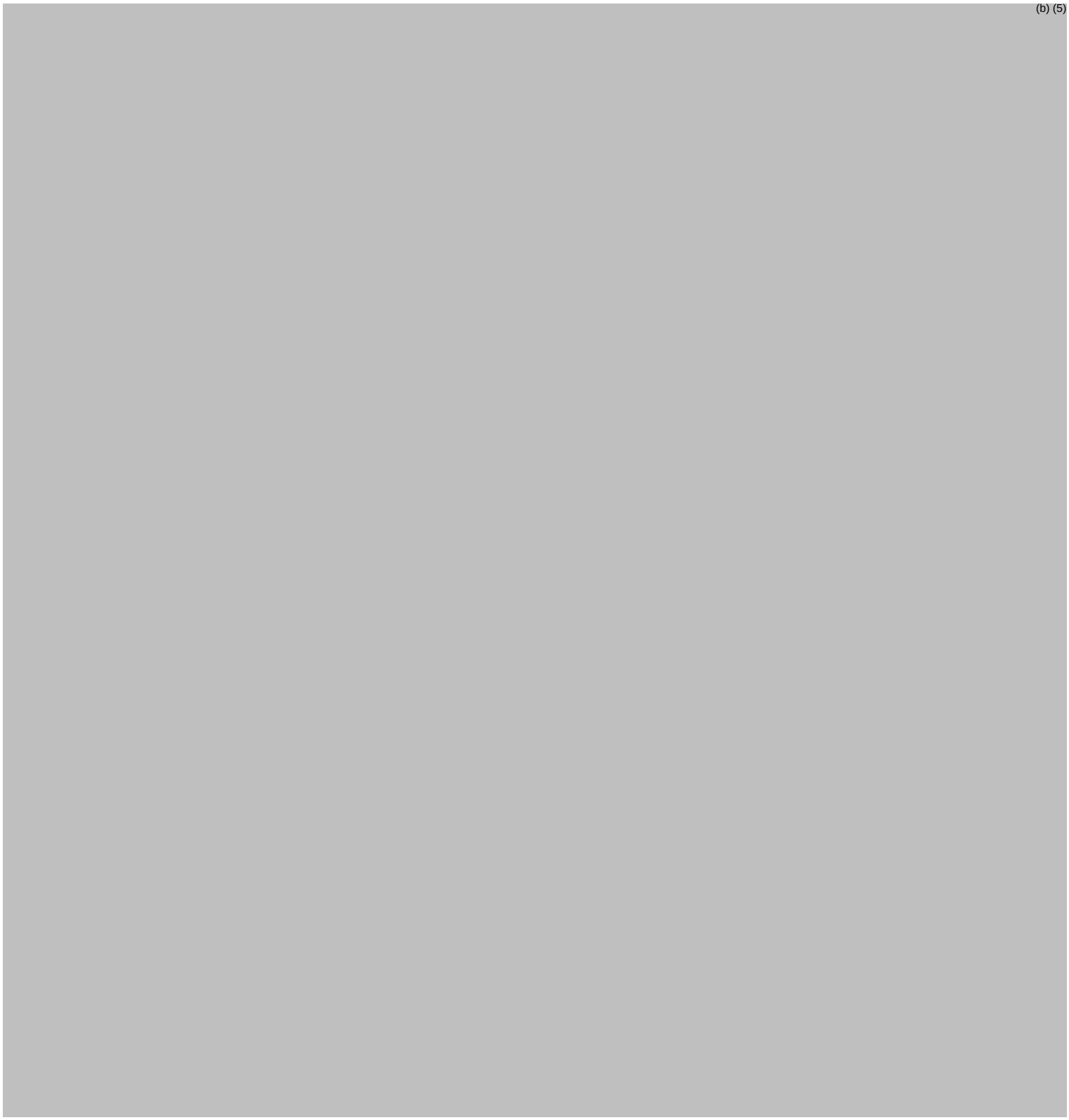
Please see attached for the draft late cycle communication (LCC) as discussed in the internal last week. I have also printed a copy and placed in folder with draft reviews (clinical efficacy, clinical safety, stat, (b) (4) and MRI consult)

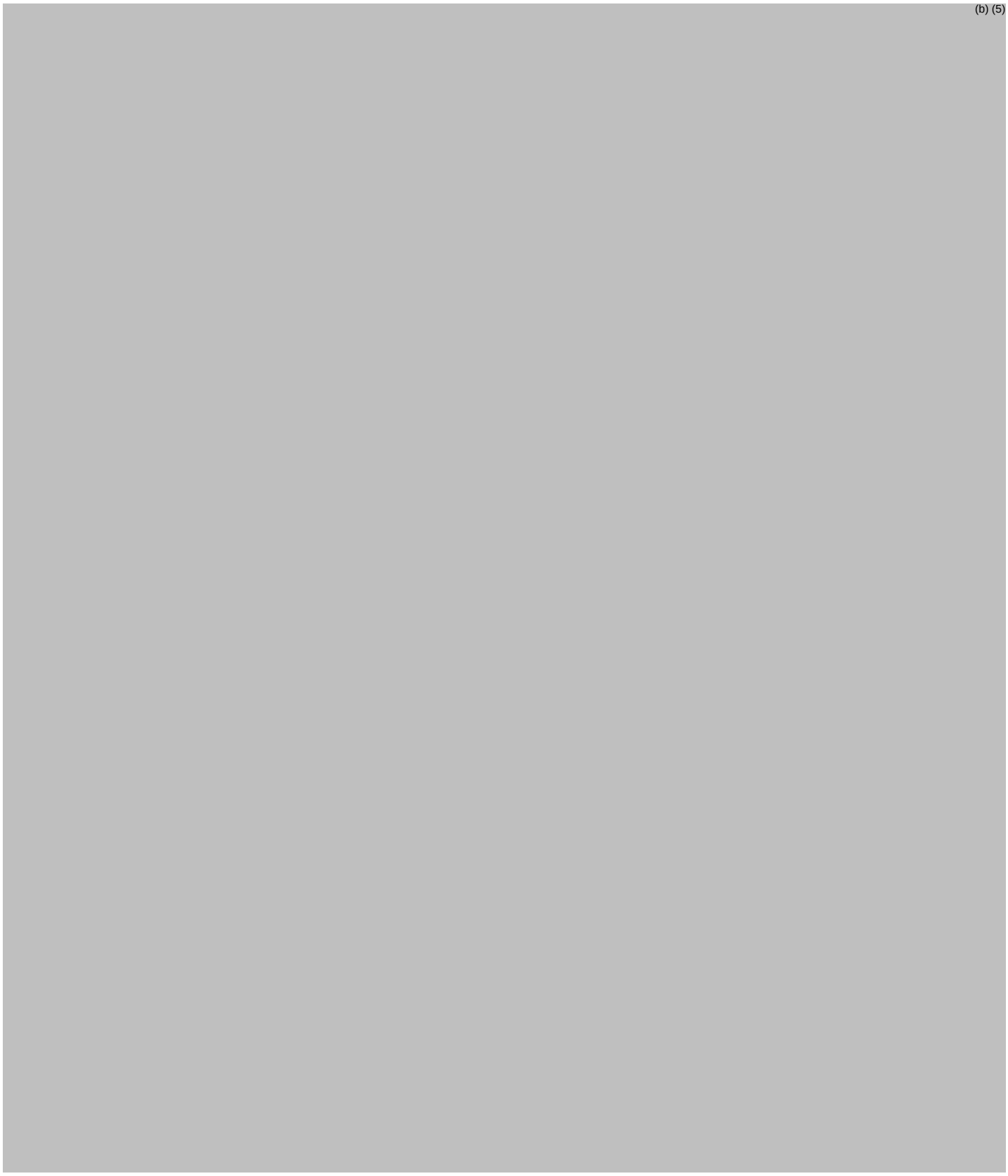
LCC is due to BioMarin on 10/28/15.

Thank you,
Fannie

The concerns of the primary review team are described briefly below.

(b) (5)

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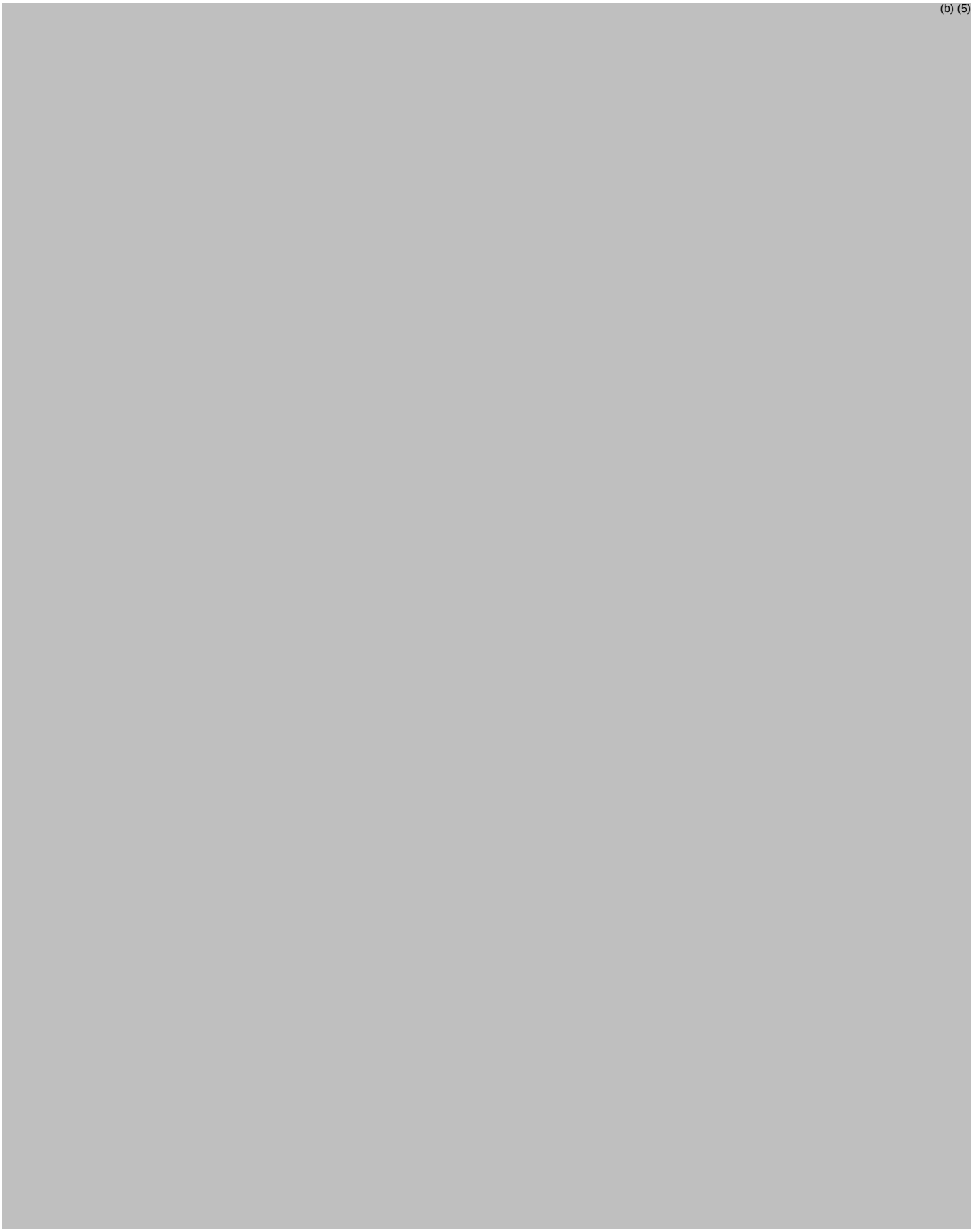
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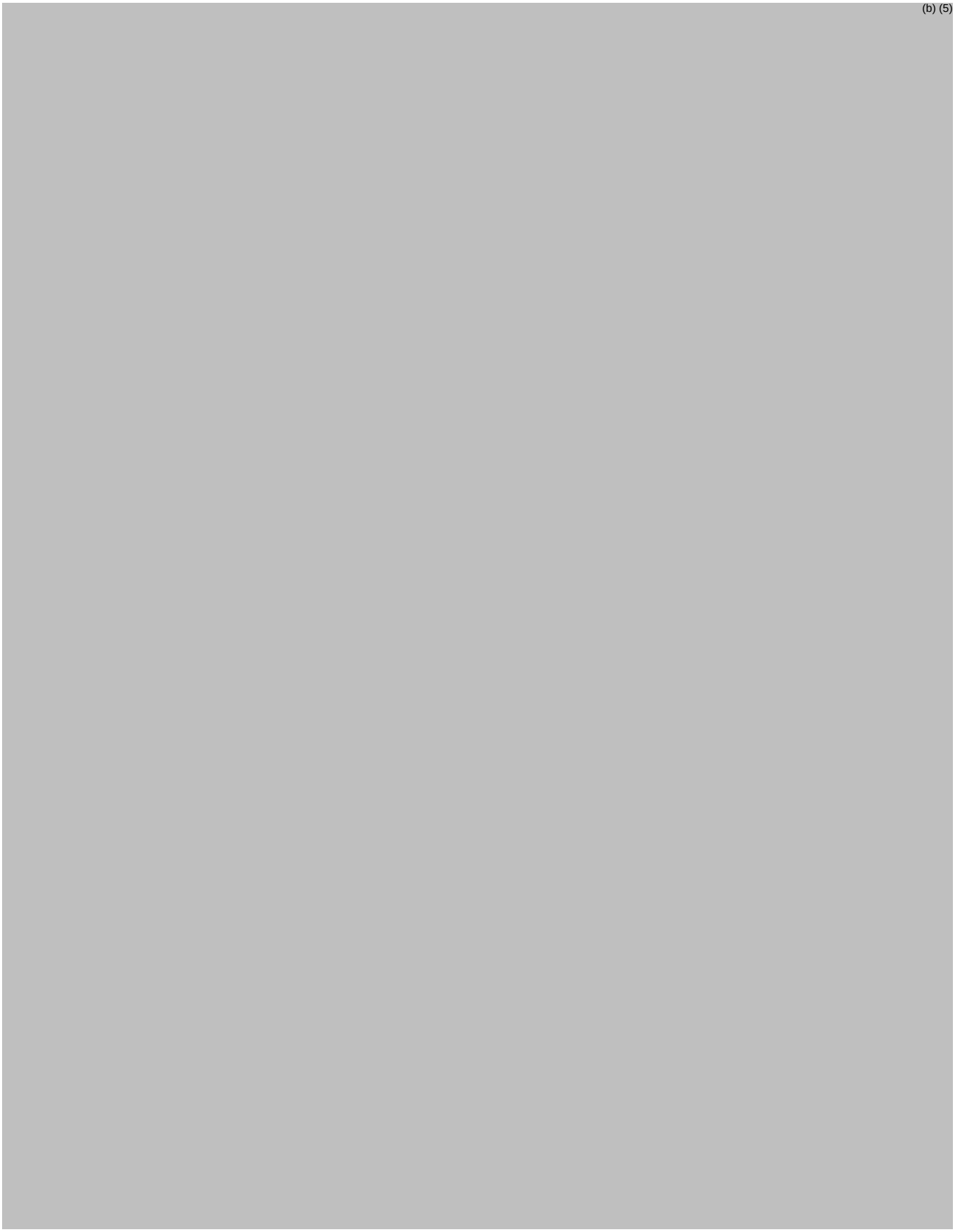
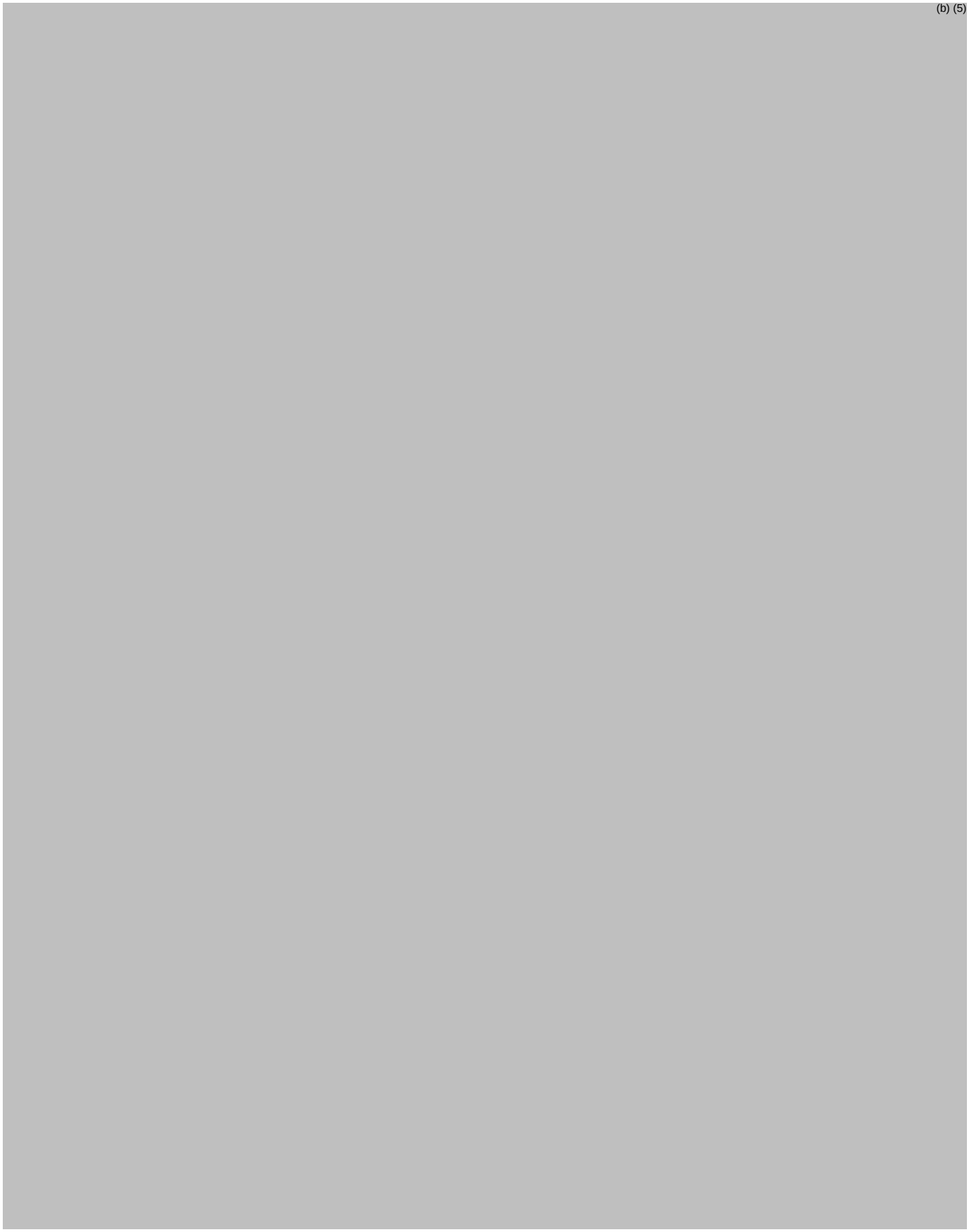

(b) (5)


(b) (5)


(b) (5)

Philips, Howard

From: Temple, Robert
Sent: Thursday, October 15, 2015 4:08 AM
To: Unger, Ellis
Subject: Fw: Sarepta AdComm

Do we have a plan for these? I assume many received this.

Sent from my BlackBerry 10 smartphone.
Original Message
From: (b) (6)
Sent: Thursday, October 15, 2015 7:32 AM
To: Temple, Robert
Subject: Sarepta AdComm

Dear Dr Temple

I am writing this letter in absolute disbelief and disgust at today's announcement that you will wait till late January to hold the Sarepta AdComm.

I am writing this email as I am a parent of a child aged (b) (6) suffering with Duchenne. My son (b) (6) is not amendable to 51 skipping but would benefit from another exon skipping drug being developed for his rare mutation.

Myself and my wife flew all the way from England (b) (6) .
I learned first hand that FDA officials have a poor understanding of Duchenne and although acknowledging our urgency have done nothing but hinder any opportunities to accelerate Sarepta's drug to the boys that desperately need it. You clearly do not understand the clear and desperate unmet medical need of this condition.

I don't want my letter to be "just another desperate parent" who doesn't understand the system. The drug approval system, no matter how you cut it does not help these children in any way. Ever since the 48 week data was announced by the company and eteplirsen has been backed by loving families receiving the drug and fighting for all the other boys, delays have occurred. Let me make this clear. Today is another setback. Sarepta's data beats natural history hands down. The safety data is clean. The parents and the terminally ill patients are screaming for the drug now.

I have seen so many parents try to work with your system over the last 4 years and all came out second best. They changed very little because they were fighting an invisible, non responsive organisation. Please do not continue to appear concerned whilst ignoring all of our pleas. Pfudasia exists in your country and fits this situation. Our regulators are constantly asking "tell us how it feels to have a child with Duchenne" For what? I ask if you don't react to our knowledge. The duchenne community is both knowledgeable and educated. I urge you move the adcom forward for our children and expediate timelines for all other boys that can benefit from this technology. You know you can do so much more. You circulate how you listen more and understand the patients perspective. Truthfully, you don't! This system using bits of the law whilst simply ignoring the ones that can save our children is totally unacceptable.
I will not be a parent that loses his son and says"oh well, I tried"

You are failing us! This is unacceptable. I can't stand by and watch my son wage away knowing this is something we can fix.
I would appreciate that you acknowledge you have received this email

Regards
(b) (6)

Sent from my iPhone

Philips, Howard

From: Temple, Robert
Sent: Monday, October 26, 2015 10:20 PM
To: Bastings, Eric; Unger, Ellis
Subject: Re: Drisapersen AC Background: DUE Tue 1027

Eric, Aprile has some markings I made on the MOR draft. You might look at them. I thoght some comments were excessively negative.

Sent from my BlackBerry 10 smartphone.

From: Bastings, Eric
Sent: Monday, October 26, 2015 10:01 AM
To: Unger, Ellis; Temple, Robert
Subject: FW: Drisapersen AC Background: DUE Tue 1027

Here is the current draft of the documents. There may be some revisions in the documents that go tomorrow (we are still working on it).

Eric

From: Choy, Fannie (Yuet)
Sent: Tuesday, October 20, 2015 7:24 PM
To: Dunn, Billy; Bastings, Eric
Cc: Farkas, Ronald; Choy, Fannie (Yuet)
Subject: Drisapersen AC Background: DUE Tue 1027

Clinical Review	Clinical Safety	Stat Review	OCP	(b) (4)
DRAFT	DRAFT	DRAFT	DARRTS	

Attached are the reviews (draft or DARRTS version) for your reference and prep the DD memo for the AC background. As discussed, it will be decided which reviews will be included in the package. DUE: Tue 10/27

Thanks
Fannie

Philips, Howard

From: Temple, Robert
Sent: Monday, October 26, 2015 10:43 PM
To: Unger, Ellis
Subject: Re: Drisapersen AC Background: DUE Tue 1027

As I suggested to Eric, you might look at my comments. There are not that many. Aprile has them, I am pretty sure. We can certainly live with it as is.

Sent from my BlackBerry 10 smartphone.

From: Unger, Ellis
Sent: Monday, October 26, 2015 4:52 PM
To: Bastings, Eric
Cc: Temple, Robert
Subject: RE: Drisapersen AC Background: DUE Tue 1027

Eric,

In my not comprehensive but more-than-a-quick-look of the clinical (efficacy) and stats reviews, the review work looks pretty solid.

Thank you for sharing.

Ellis

From: Bastings, Eric
Sent: Monday, October 26, 2015 10:00 AM
To: Unger, Ellis; Temple, Robert
Subject: FW: Drisapersen AC Background: DUE Tue 1027

Here is the current draft of the documents. There may be some revisions in the documents that go tomorrow (we are still working on it).

Eric

From: Choy, Fannie (Yuet)
Sent: Tuesday, October 20, 2015 7:24 PM
To: Dunn, Billy; Bastings, Eric
Cc: Farkas, Ronald; Choy, Fannie (Yuet)
Subject: Drisapersen AC Background: DUE Tue 1027

Clinical Review DRAFT	Clinical Safety DRAFT	Stat Review DRAFT	OCP DARRTS	(b) (4)
<< File:	<< File:	<< File:	<< File:	

Drisapersen N206031 Oct1_Clinical (draft).pdf >>	Drisapersen safety review sy 092915 EM final.docm >>	DrisapersenReview_Stat(draft).docx >>	Drisapersen OCP (DARRTS).pdf >>	(b) (4)

Attached are the reviews (draft or DARRTS version) for your reference and prep the DD memo for the AC background. As discussed, it will be decided which reviews will be included in the package. DUE: Tue 10/27

Thanks
Fannie

Philips, Howard

From: Choy, Fannie (Yuet)
Sent: Friday, October 30, 2015 2:27 PM
To: Temple, Robert; Unger, Ellis; Tandon, Veneeta; Freed, Lois M; Wilcox, Barbara; Yasuda, Sally; Mentari, Evelyn; Men, Angela; Jin, Kun; Yan, Sharon; Rao, Ashutosh; Bhattaram, Atul; Rogers, Hobart; Yu, Bei; Sapru, Mohan; Grimstein, Christian; Krudys, Kevin; Peters, Tracy
Cc: Dunn, Billy; Bastings, Eric; Farkas, Ronald; Woody, Dahlia; Ware, Jacqueline H; Zerislassie, Ermias; El Hage, Antoine N; Pratt, Robert; Wilson, Wendy; Heimann, Martha R; Bauer, Larry J; Kozauer, Nicholas; Goldsmith, Jonathan; Harris, Danielle; Wilkins Parker, Jamie; Taylor, Lockwood; White, Lolita; Braver, Elisa; Kulick, Corrinne
Subject: FDA Briefing Document for Nov. 24 PCNS Meeting
Attachments: 102915_AC Background_drisapersen(NDA206031).pdf

Dear drisapersen team,

Please see attached for FDA AC briefing document for drisapersen. This is an unredacted version.

Thank you,
Fannie

Fannie Choy, RPh.
Regulatory Project Manager
Division of Neurology Products

FDA Briefing Document

Peripheral and Central Nervous System Drugs Advisory Committee Meeting

November 24, 2015

NDA 206031
Drisapersen

Disclaimer Statement

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought these issues to this Advisory Committee in order to gain the Committee's insights and opinions, and the background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.

Table of Contents

I. Memorandum to the Committee

MEMORANDUM

DATE: October 28, 2015

FROM: Ronald Farkas, M.D., Ph.D.
Clinical Team Leader
Division of Neurology Products, CDER, FDA

THROUGH: Billy Dunn, M.D.
Director
Division of Neurology Products, CDER, FDA

TO: Members and Invited Guests of the Peripheral and Central Nervous Systems Drugs Advisory Committee (PCNS AC)

SUBJECT: Briefing Memo for New Drug Application (NDA) 206031, for the use of Kyndrisa (drisapersen) for the treatment of Duchenne muscular dystrophy in patients with mutations amenable to exon 51 skipping

The PCNS AC and invited guests will be meeting on November 24, 2015, to discuss the NDA for drisapersen, submitted by BioMarin Pharmaceutical Inc., for the treatment of the subset of Duchenne muscular dystrophy (DMD) patients (≈13%) in whom skipping of exon 51 can restore the reading frame of dystrophin and potentially increase the production of dystrophin, an effect that is theorized to lead to clinical benefit for treated patients.

Disease 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.

Drisapersen Drug Development

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. Drisapersen 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 drisapersen 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.

To support the efficacy of drisapersen, the sponsor undertook two types of studies: biomarker studies to assess whether dystrophin expression was, in fact, increased, and clinical studies, to assess whether the increase in dystrophin had, in fact, resulted in clinical benefit. The design and results of these studies are discussed and reviewed in considerable detail in the draft NDA reviews that we have included in this package. These reviews were conducted by Dr. Veneeta Tandon (efficacy review) and Dr. Evelyn Mentari (safety review), clinical reviewers in the Neurology Division, Dr. Sharon Yan, statistical reviewer in the Office of Biostatistics, Dr. Daniel Krainak, from the Center for Devices and Radiological Health, who acted as a consultant for the Division for the assessment of muscle MRI data, and Drs. Atul Bhattaram (pharmacometrics), Bart Rogers (genomics) and Bei Yu (clinical pharmacology) from the Office of Clinical

Pharmacology. We hope the information in this package will frame the issues we would like you to consider, as well as the briefing materials provided by the sponsor.

A carefully planned and thorough drug development program was undertaken for drisapersen, and we believe that the sponsor and the patients and caregivers who participated in the trials should be recognized for their contributions to the understanding of the drug's safety and efficacy. You will see in the FDA reviews enclosed, however, that it is not clear to the primary review team that substantial evidence of effectiveness has been presented for drisapersen or, consequently, that drisapersen has an acceptable risk-benefit profile. No final decision has been made, however, and the entire review team greatly looks forward to the insights that you can provide at the Advisory Committee meeting.

The concerns of the primary review team are described briefly below.

Biomarkers

It is greatly concerning that a number of biomarker studies suggest that, contrary to initial published reports,[1] drisapersen has little effect on increasing dystrophin levels, the putative mechanism of action. By Western blot, post-treatment dystrophin levels remained very similar to pre-treatment levels, about 1/3rd of 1% of normal. Dystrophin levels with drisapersen treatment thus appear to remain well within the range of the trace levels seen in untreated DMD patients.

We noted that drisapersen *did* decrease creatine kinase (CK) and lactate dehydrogenase (LDH), serum markers of muscle injury in DMD. However, those biomarkers can be affected by many factors that would not predict benefit in DMD, such as decreased physical activity, or that might even indicate harm, such as disease progression.

Some muscle MRI data were included in the NDA, but our internal FDA experts concluded that the MRI studies were not conducted or analyzed with sufficient rigor to be reliable.

Clinical Endpoints

Clinical endpoints were examined in three controlled studies: two Phase 2 studies, and one Phase 3 study. Clinical endpoints were also examined in open-label extension studies that followed controlled studies, including a multi-year extension in 12 patients who participated in one of the early dose-finding studies.

[1] Goemans et al., N Engl J Med 2011;364:1513-1522

The first Phase 2 study, DMD114117 (hereafter Study 117), was a 48-week 3-arm placebo-controlled trial in 53 patients with DMD. Patients were randomized equally to two slightly different dosing regimens, "continuous" or "intermittent", that provided similar overall drug dose and exposure, or to placebo. Blinding to treatment allocation was, by design, only partial in order to decrease the number of placebo injections. Baseline imbalances were present that appeared to favor the continuous treatment arm. The primary endpoint was the 6 minute walk test (6MWT) at 25 weeks, not the later time point of 48 weeks which is arguably of greater interest for understanding efficacy of chronic therapy. The two doses were each tested at $p < 0.025$ according to the prespecified analysis plan, and the continuous arm of the study was positive, with $p = 0.01$ and a treatment difference of 35 meters vs. placebo. However, the intermittent arm was negative, $p = 0.80$, with a treatment difference of 3.5 meters vs. placebo, and secondary endpoints were uniformly negative. Subsequent analyses at 48 weeks were exploratory due to the earlier negative findings, but if each arm was tested according to the same scheme as used at week 25 (testing at $p < 0.025$) the results were nominally negative, with $p = 0.05$ for the continuous arm and $p = 0.15$ for the intermittent arm. Combining the two drug-treated arms did not appreciably strengthen results, yielding a p-value of 0.12 at week 25 and 0.05 at week 48. Thus, given the inconsistencies in its findings and unimpressive statistical strength, the overall persuasiveness of this study appears to be low.

A second Phase 2 study, DMD114876 (hereafter Study 876), was negative by the usual criteria. The study was a 24 week 3-arm placebo-controlled trial in 51 patients comparing 6 mg/kg or 3 mg/kg of drisapersen to placebo. The p-value for the primary endpoint, 6MWT at 24 weeks for the 6 mg/kg arm, was 0.07, with a treatment difference of 27 meters. The 3 mg/kg arm was numerically *inferior* to placebo. Secondary endpoints were uniformly negative, with some leaning towards inferiority of the drug-treated arms. The p-value of the prespecified per protocol sensitivity analysis was 0.23, due to the removal of one placebo patient who was unblinded after a hospital visit. The independent persuasiveness of this study is thus low.

The considerably larger Phase 3 study (Study 044) was negative, and one of the most plausible post hoc analyses yielded similar negative results. The enrollment criteria for the study allowed entry of patients with more advanced disease compared to the Phase 2 studies, which had limited enrollment to patients that could rise from the floor in ≤7 seconds. Therefore, a post hoc analysis was conducted on the subset of patients in Study 044 who would have met the enrollment criteria for the Phase 2 studies. The result of this analysis, when compared with the primary analysis, showed a *smaller* difference between drug and placebo arms, 5 meters, suggesting that differences in enrollment criteria were not likely to have caused the negative results in Study 044. A number of post hoc subgroup analyses proposed by the sponsor were

found to be highly sensitive to small differences in cutoffs for age and 6MWT and to other specific statistical manipulations, and thus lacked independent credibility.

Patients in a multi-year 12-patient single-arm study of drisapersen had unusually well-preserved function at baseline, which is thought to predict less rapid disease progression, and their disease course was generally similar to historical patients. This study does not appear to provide any support for efficacy.

The primary review team is concerned that treatment allocation may have been substantially unmasked in the clinical trials because of a high incidence of outwardly obvious injection site reactions from drisapersen. The distance walked in 6 minutes is clearly related to effort, and might have been affected by patient and investigator expectation bias if treatment assignments could be deduced.

The current thinking of the primary review team is that evidence supporting the effectiveness of drisapersen is inconsistent.

Safety

Even in the context of an invariably disabling and fatal disease such as DMD, the safety profile of drisapersen is concerning, as described briefly below.

Severe toxicity across many organ systems was encountered in the nonclinical studies, and appeared to predict a number of the adverse events that were subsequently observed in the clinical studies.

Major adverse effects identified in the clinical trials include the following:

- Renal injury
- Thrombocytopenia
- Vascular injury
- Dermal toxicity

Possible Approval Pathways

The decision about approvability is necessarily step-wise, requiring first that the drug be found by FDA to be effective prior to any consideration of benefit-risk.

Efficacy is typically established by positive findings on clinically meaningful endpoints in two adequate and well-controlled trials. Factors that either strengthen or weaken the

persuasiveness of any positive findings should be considered, as should the number and persuasiveness of any negative trials.

A single highly persuasive positive trial combined with independent findings that substantiate efficacy might also support approval, but it is critical that the possibility of an incorrect outcome be considered and that all the available data be examined for their potential to either support or undercut reliance on a single trial.

Under the Accelerated Approval provisions, an effect on a surrogate marker that is determined by FDA to be reasonably likely to predict benefit can support approval. For DMD, there is obvious interest in dystrophin expression as a surrogate marker. Whether an effect on a biomarker such as dystrophin might reasonably predict clinical benefit in DMD is inseparable from such factors as the magnitude and character of the effect on the biomarker, and might also depend on patient factors such as age, disease stage, or secondary inflammation or autoimmunity.

Importantly, the evidentiary standards for effectiveness are not lower for biomarker endpoints used to support Accelerated Approval, nor should Accelerated Approval be used to compensate for weak or inconsistent clinical findings. Negative clinical findings in studies of adequate design and conduct to assess such findings would ordinarily preclude Accelerated Approval on the basis of associated biomarker effects.

Finally, if efficacy is established, the next question is whether a drug's benefits justify its risks. This consideration is made in the broader context of the seriousness of the disease, other treatment options, unmet medical need, risk tolerance of the patient population, etc. Risk-benefit assessment should consider that tolerance for risk may vary among individuals, and may be affected by factors such as disease stage and severity.

II. Drafts Points To Consider

FOOD AND DRUG ADMINISTRATION (FDA)

Center for Drug Evaluation and Research (CDER)

Peripheral and Central Nervous System Drugs Advisory Committee Meeting

DRAFT POINTS TO CONSIDER

November 24, 2015

1. Discuss the findings on biomarkers in the clinical studies and consider their relevance to clinical efficacy, particularly in the context of the existing clinical data.
2. Discuss the findings on clinical efficacy endpoints in the clinical studies, particularly with regard to consistency within and between studies.
3. Discuss the major adverse events identified in the clinical trials, particularly with regard to the acceptability of the risk-benefit profile in the context of this disease.

III. Clinical Efficacy Review

CLINICAL REVIEW (EFFICACY)

Application Type	NDA
Application Number(s)	206,031
Priority or Standard	Priority
Submit Date(s)	4/27/15
Received Date(s)	4/27/15
PDUFA Goal Date	12/27/15
Division/Office	DNP/ODE 1
Reviewer Name(s)	Veneeta Tandon Ashutosh Rao (Dystrophin Bioassays)
Review Completion Date	October 1, 2015
Established Name	Drisapersen
(Proposed) Trade Name	KYNDRISA
Applicant	Biomarin
Formulation(s)	Sterile solution in a single use vial for subcutaneous injection
Dosing Regimen	Loading Dose: 6 mg/kg twice weekly subcutaneous injection for first 3 weeks Maintenance Dose: 6 mg/kg once weekly subcutaneous injection
Proposed Indication(s)	Treatment of Duchenne muscular dystrophy with mutations in the dystrophin gene that amenable to treatment with exon 51 skipping
Intended Population(s)	Exon-51 skip amenable DMD boys
Recommendation on Regulatory Action	

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Glossary

DMD	Duchenne muscular Dystrophy
DAPC	Dystrophin-Associated Glycoprotein Complex
CGH	Comparative Genomic Hybridization
CK	Creatine Kinase
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
GCP	Good Clinical Practices
GSK	Glaxo Smith Kline
H-RMCA	High-Resolution Melting Curve Analysis
ICH	International Conference of Harmonization
ITT	Intent-To-Treat
MLPA	Multiplex Ligation-dependent Probe Amplification
MMRM	Mixed Effect Model Repeated Measure
PP	Per Protocol
SC	Subcutaneous

1 Executive Summary

1.1. Product Introduction

Drug and Indication: KYNDRISA (Drisapersen sodium, also known as GSK2402968 or PRO051) is a new molecular entity that is proposed for the treatment of Duchenne muscular dystrophy with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping.

Drisapersen is a chemically-modified antisense oligonucleotide (20-mer) with a sequence specific to bind to exon 51 of the human dystrophin pre-mRNA intended to cause the splicing machinery to skip over exon 51 during splicing of pre-mRNA. This restores the reading frame of the resulting mRNA. Restoration of the open reading frame allows the generation of an internally truncated dystrophin that is partially functional. Skipping exon 51 restores the reading frame in patients that carry a deletion of exons 45–50, 47–50, 48–50, 49–50, 50, 52, or 52–63, which, combined, is 13% of all DMD patients.

Pharmacological Class: The proposed Established Pharmacologic Class (EPC) for drisapersen is: *"exon skipping oligonucleotide inducer of dystrophin synthesis"*

Dosage Form: Drisapersen sodium will be available as a 200mg/mL sterile solution in a single use vial for subcutaneous (SC) injection.

Proposed dosage regimen:

Loading Dose: 6 mg/kg twice weekly subcutaneous injection for first 3 weeks
Maintenance Dose: 6 mg/kg once weekly subcutaneous injection

1.2. Conclusions on the Substantial Evidence of Effectiveness

This review concludes that, while there may be some evidence suggestive of efficacy of drisapersen, the evidence is inconsistent and in some cases contradictory, and does not reach the level of substantial evidence.

1.3. Benefit-Risk Assessment

Note: Risk assessments were conducted by Dr. Evelyn Mentari, MD.

Benefit-Risk Summary and Assessment

KINDRISA or Drisapersen is a chemically-modified antisense oligonucleotide (20-mer) with a sequence specific to bind to exon 51 of the human dystrophin pre-mRNA. It is designed to cause the skipping of exon 51 which results in the generation of an internally truncated dystrophin.

Duchenne muscular dystrophy (DMD) is a severe male pediatric neuromuscular disorder that occurs due to the absence of dystrophin protein. DMD is present at birth, but the disorder becomes apparent between ages 3-5 years. The loss of muscle strength in DMD is progressive, leading to loss of ambulation in the teens. Progressive loss of muscle strength leads to decline in respiratory function, cardiac complications and ultimately death typically in the third decade. Exon 51 skip-amenable DMD constitutes 13% of the DMD population, resulting in a prevalence of 2340 boys in the United States. There are no FDA approved treatments of DMD in the Unites States, but glucocorticoids have been shown to prolong function and survival by a few years. Similarly, improvements in supportive care, including physical therapy and assisted ventilation, have led to a steady but slow increase in survival over the past few decades. Chronic glucocorticoid use is associated with Cushingoid syndrome and obesity. There is significant need to treatment options that prolong ambulation and are better tolerated than steroids.

The conclusion of this review is that substantial evidence of clinical efficacy was not established for drisapersen in the treatment of exon 51-skip amenable DMD. There is no independent substantiation of the positive findings from a small Phase 2 study based 6MWD as a clinical endpoint. A larger study intended to provide the most reliable evidence of effectiveness was negative.

Similarly, this review concludes that there is no substantial evidence of an effect on a biomarker that is reasonably likely to predict clinical benefit. Drisapersen had little, if any effect on increasing dystrophin expression, the proposed mechanism of action. An unexpected finding was reduction in serum creatine kinase (CK), potentially a marker of muscle cell integrity, but CK levels are well known to change because of many other non-beneficial effects, such as loss of muscle or decrease in use of muscle, such that the clinical meaningfulness remains inconclusive at this time.

Drisapersen is associated with severe and potentially life-threatening adverse effects. Drisapersen causes immune thrombocytopenia, renal toxicity, and skin injury at injection sites.

- *Thrombocytopenia:* Six drisapersen subjects (2%) had thrombocytopenia <20 x 10^9/L, levels at which patients are at risk potentially fatal complications, including spontaneous intracranial or intrapulmonary hemorrhage. Most of these patients had confirmed anti-platelet antibodies. These cases occurred 14-26 months after the first dose of drisapersen, suggesting that risk increases with duration of exposure. Platelet monitoring every 2 weeks, patient education regarding the signs and symptoms of thrombocytopenia, and facilitating prompt medical assessment and treatment can mitigate this risk. However, the decrease in platelets occurred precipitously and unpredictably so that even with intensive monitoring, the risk remains. Concomitant use of with antiplatelet, thrombolytic, or anticoagulant drugs is not recommended.
- *Renal Injury:* Renal toxicity was reported in 61% of drisapersen 6 mg/kg/week subjects, compared to 34% of placebo subjects. Proteinuria was the most common renal abnormality and occurred in 44% of drisapersen 6 mg/kg/week subjects, compared to 23% of placebo subjects. One patient developed multiple life-threatening thromboemboli in the setting of glomerulonephritis with nephrotic syndrome. Renal laboratory monitoring every 2 weeks and cessation of drisapersen according to recommended laboratory criteria can mitigate this risk but will not eliminate the risk of severe and potentially fatal renal toxicity.
- *Injection Site Reactions:* Injection site reactions occurred in 79% of drisapersen patients and included ulceration, irreversible scarring, and atrophy. The risk for first injection site reaction occurred throughout the first 72 weeks of exposure. 21% of reactions were not resolved by the end of the studies. Reactions known to resolve lasted for a mean of 58 days and up to 1217 days. Injection site reactions occurred despite administration by a medical professional and rotation of injection sites. No

other strategies to mitigate the risk of injection site reactions are known.

The benefit of drisapersen in exon 51 skip amenable patients is inconclusive at this time. Therefore, the benefit-risk assessments were not made.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Duchenne muscular dystrophy (DMD) is a severe pediatric neuromuscular disorder that occurs exclusively in males. DMD is caused by the absence of functional dystrophin protein that protects 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 restorable by the skipping (removing) of exon-51. • Lack of dystrophin results, through mechanisms not precisely understood, in degeneration of muscle fibers, attracting inflammatory cells and ultimately replacement by fibrotic tissue and adipose tissue. This leads to subsequent loss of muscle strength and function. • Loss of muscle strength is progressive, leaving the patients wheel chair bound by age 10-14. Progressive scoliosis develops that impairs pulmonary and cardiac function. Patients with DMD usually survive until late adolescence but not more than 20 to 25 percent live beyond the twenty-fifth year. • Mutations that are treatable by skipping exon 51 are thought to make up around 13% of the DMD population, resulting in a prevalence of about 2340 boys in the US.	The loss of muscle strength in DMD is progressive leading to loss of ambulation in the teens. Progressive loss of muscle strength leads to decline in respiratory function, cardiac complications and ultimately death typically in the third decade.
Current Treatment Options	• There are no FDA approved treatments of DMD in the Unites States. • The current standard of care is glucocorticoids (prednisone, prednisolone and deflazacort) administered either daily or intermittently with a modest effect. In addition, supportive care such as assisted ventilation and physiotherapy are used to improve quality of life and survival in DMD. • 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.	There is a specific unmet medical need for treatment of exon 51 skip- amenable DMD that will maintain ambulation as long as possible and slow subsequent scoliosis, respiratory and cardiac failure.
Benefit	• KYNDRISA or Drisapersen is a chemically-modified antisense oligonucleotide (20-mer) with a sequence specific to bind to exon 51 of the human dystrophin pre-mRNA. It is designed to cause the skipping of exon 51 which results in the generation of an internally truncated dystrophin. • The development program for drisapersen was exemplary for a severe rare disease, such that beneficial effects on biomarkers and clinical endpoints could have been detected if present. The efficacy of drisapersen was evaluated in one Phase 3 and two Phase 2 randomized placebo controlled trials in ambulant DMD boys ages >5 years in 290 subjects (195 on drisapersen and 95 on placebo) for 24- 48 weeks duration. In addition, two open label extension trials for a total	Studies with drisapersen were adequate and well controlled with an acceptable clinical endpoint, change from baseline 6MWD. No substantial evidence of efficacy was established for drisapersen in the treatment of exon 51-skip amenable ambulant DMD patients There is no independent substantiation of the positive findings from a

<table>
<tr><th>Dimension</th><th>Evidence and Uncertainties</th><th>Conclusions and Reasons</th></tr>
<tr><td></td><td>duration up to 3.5 years intended to provide supportive evidence. Two doses: 3mg/kg/week (n=18) and 6mg/kg/week (n=177) were evaluated in these studies.
• The primary endpoint was change from baseline 6-minute walking distance (6MWD), measured 24 or 48 weeks after treatment. 6MWD is an acceptable, potentially clinically meaningful endpoint. Biomarkers at the mechanistic or molecular level such as dystrophin protein expression, MRI and serum creatine kinase (CK) were measured in some subjects.
• There is no substantial evidence of efficacy for drisapersen. No precise estimate of treatment benefit at 6 mg/kg/week can be established. Note that positive primary endpoint for study DMD114117 was at 24 weeks. At 48 weeks this study showed a decline in change from baseline 6MWD. Study 876 was only placebo-controlled up to 24 weeks. Results below:
<table>
<tr><td rowspan="2">6 mg/kg/week
versus
placebo</td><td colspan="3">6MWD</td></tr>
<tr><td>Study DMD114044
(n=186)</td><td>Study DMD114117
(n=53)</td><td>Study DMD114876
(n=51)</td></tr>
<tr><td>Treatment Difference
(p-value)</td><td>10 m at 48 weeks
(0.42)</td><td>35 m at 24 weeks
(0.01)</td><td>27 m at 24 weeks
(0.07)</td></tr>
</table>
• The weakness of the data include: no independent replication of the results obtained in DMD114117, a decline in change from baseline at Week 49 in the same trial, a different regimen of 6mg/kg dose with identical drug exposure not statistically significant with a p-value of 0.80, a p-value of 0.07 from DMD114876 increased to a p-value of 0.21 after removing a single placebo subject that was unblinded during the study.
• There were multiple secondary endpoints expected to be correlated with the primary endpoint but these generally were not statistically significant in any of the studies, and increase doubt about the robustness of the result on 6MWT.
• Drisapersen was designed to increase dystrophin expression, but any increase in dystrophin protein expression was equivocal. Any effect on dystrophin appears so small as to be unlikely to have resulted in clinical benefit.
• There was a 30-40% reduction in CK in the placebo controlled studies based on percent change from baseline CK, but the clinical meaningfulness of this is uncertain due to the presence of cofounders that reduce CK.
• A small amount of muscle MRI data was collected, but the data was not reliably collected and the results were equivocal.
• The open label studies did not provide evidence of slower progression based on known natural history of DMD patients.</td><td>small Phase 2 study. A larger study intended to provide substantiation of effectiveness was negative. One can argue the reduction in serum creatine kinase to be plausibly treatment related, but it remains inconclusive at this time.</td></tr>
</table>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk	• The safety database for drisapersen includes all patients from the 3 Phase 2 and Phase 3 placebo controlled trials and from the 3 open label studies. Drug exposure is adequate and reflects the intended population for use. • The most common AEs were: Injection site erythema (52%), Proteinuria (44%); injection site discoloration (36%). • Six drisapersen subjects (2%) had thrombocytopenia <20 x 10^9/L, levels at which patients are at risk potentially fatal complications, including spontaneous intracranial or intrapulmonary hemorrhage. These cases occurred 14-26 months after the first dose of drisapersen. Despite routine monitoring of platelets every 2 weeks, thrombocytopenia occurred precipitously in some cases. • Renal abnormalities occurred in 61% of patients taking drisapersen, and the risk for a renal adverse event existed throughout 72 weeks of exposure. Proteinuria occurred in 44% of drisapersen 6 mg/kg/week patients, compared to 23% of placebo patients. Proteinuria was generally reversible on discontinuation, although one patient had life-threatening glomerulonephritis with nephrotic syndrome and multiple thromboemboli. • Injection site reactions, including discoloration, induration, pain, pruritus, bruising, atrophy, hematoma, and swelling, occurred in 79% of drisapersen patients. In 5% of all drisapersen patients, injection site reactions led to ulceration, fibrosis/sclerosis, or calcification. The risk for first injection site reaction occurred throughout the first 72 weeks of exposure. 21% of reactions were not resolved by the end of the studies. Reactions known to resolve lasted for a mean of 58 days and up to 1217 days. • Concomitant use with antiplatelet, thrombolytic, or anticoagulant drugs is not recommended. Patients taking these drugs were excluded from clinical studies. • Safety in the postmarketing setting: Laboratory values as markers of renal and thrombocytopenia adverse events were closely monitored during the clinical studies, and close monitoring will be necessary in the postmarket setting if the drug is approved. • Other uncertainties: The clinical effects of anti-drisapersen antibodies. The clinical effect of pro-inflammatory activity.	Major safety issues of thrombocytopenia, renal adverse events, and injection site reactions occur at the proposed dose of drisapersen. The safety issues can have life-threatening outcomes; the adverse reactions can be mitigated but not completely prevented with monitoring. The magnitude of the potential for serious harm after approval is unknown. Adherence to monitoring of platelets and renal laboratory parameters every two weeks is necessary, and failure to adequately monitor, recognize signs and symptoms, and provide prompt medical treatment in the postmarketing setting would increase the risk of adverse and potentially life-threatening outcomes. Injection site reactions occurred despite administration by a medical professional and rotation of injection sites. No other strategies to mitigate the risk of injection site reactions are known. Based on nonclinical findings and because of limitations due to the small number of patients exposed and duration of exposure in the clinical trials it is likely that adverse reactions not identified to date will occur in the postmarketing setting.
Risk Management	If drisapersen is approved, the following risk management approaches are recommended: • A patient registry as a post-marketing requirement will help to evaluate the main safety risks of drisapersen in the postmarketing setting. • Strong product labeling including a boxed warning and a Medication Guide with recommendations for monitoring of laboratory parameters and for rotation of injection	A patient registry as a post-marketing requirement will help to evaluate the main safety risks of drisapersen in the post-marketing setting. A boxed warning should be included in

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	sites is necessary to mitigate the risks of renal and thrombocytopenia adverse events and of injection site reactions. However, even with adequate monitoring, some patients will likely experience serious adverse events.	labeling to describe the risks of renal adverse events and thrombocytopenia and to provide recommendations for monitoring and to warning of the risk for injection site reactions and provide recommendations for rotation of injection sites. A medication guide should be required to describe these risks and symptoms of concern, and to highlight the need for prompt medical attention.

2 Therapeutic Context

Analysis of Condition

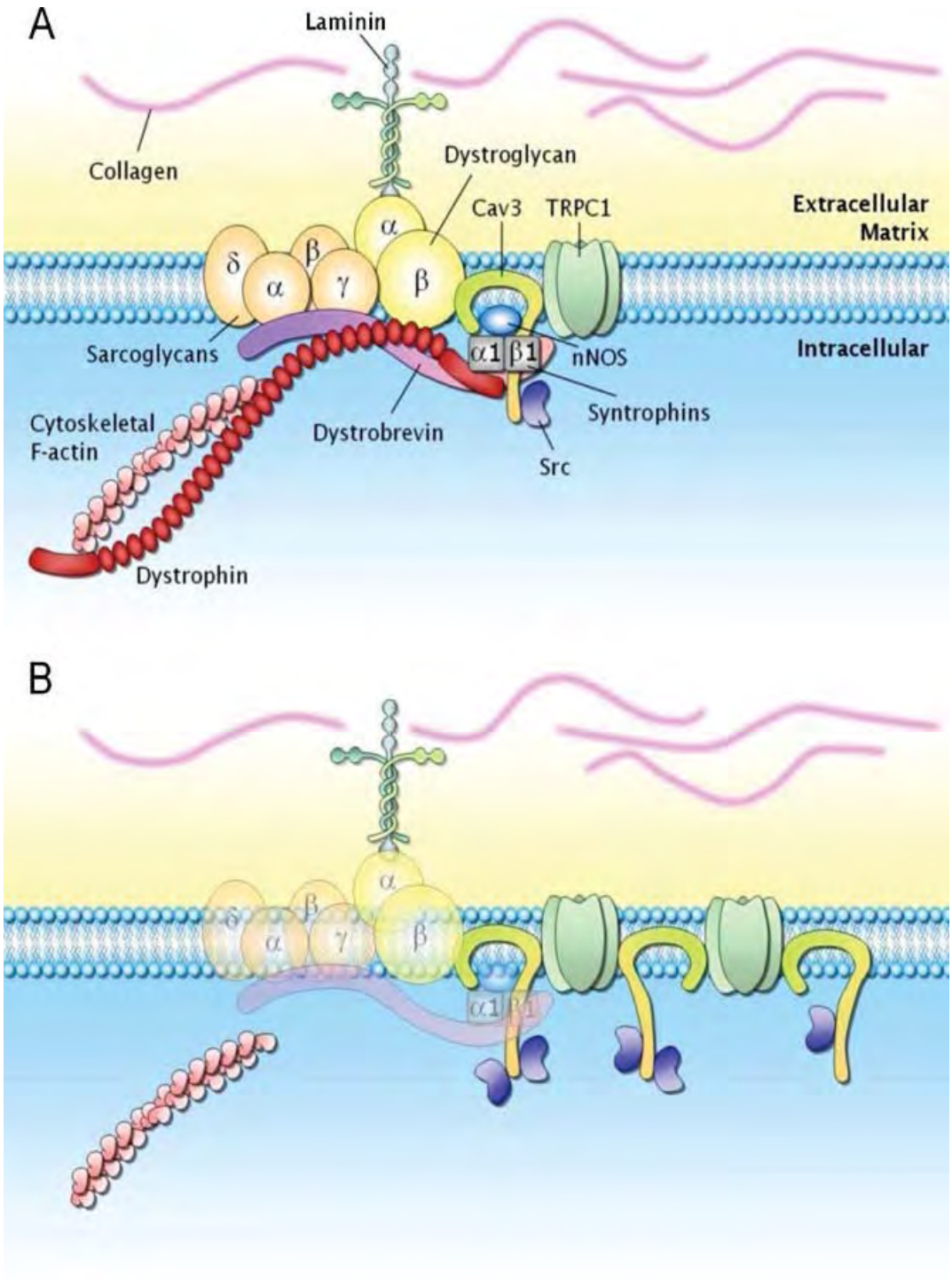
Duchenne muscular dystrophy (DMD) is the most frequent of the early onset muscular dystrophies that occur almost exclusively in males (X-linked recessive disorder). A small percentage of female carriers may exhibit a range of muscle symptoms from the full Duchenne phenotype to milder skeletal muscle weakness. Exon 51 skip-amenable DMD, a subgroup of DMD is defined by the presence of dystrophin exon 51 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 restorable by the skipping (removing) of exon-51.

Etiology: DMD is caused by the absence of functional dystrophin protein due to mutations in the DMD gene. Mutations that disrupt the translational reading frame of the dystrophin transcript, lead to a prematurely aborted dystrophin synthesis. The resulting dystrophin deficiency at the muscle fiber membranes leads to progressive fiber degeneration. The most common mutation is exon deletions (~60%) (Aartsma-Rus 2002). Dystrophin provides structural stability to the dystrogylcan complex on the muscle cell membranes, protecting muscle fibers against contraction induced damage. In addition, the association of dystrophin with catalyzing enzymes (nitric oxide synthases) completes the link between the extracellular matrix and intracellular signal transduction enzymes (Brenman 1995, Allen 2011) (Figure 1). Dystrophin is expressed in the skeletal, cardiac, and smooth muscle, as well as in the brain.

Lack of dystrophin results, through mechanisms not precisely understood, in degeneration of muscle fibers, attracting inflammatory cells and ultimately replacement by fibrotic tissue and adipose tissue. Dystrophin deficiency results in loss of neuronal nitric oxide synthase, which normally is localized to the sarcolemma as part of the dystrophin–glycoprotein complex. The absence of functional dystrophin in DMD results in deterioration of the skeletal musculature with subsequent loss of strength and function (Bushby 2010).

Clinical Features: DMD is present at birth, but the disorder usually becomes apparent between ages 3 and 5 years. There is a proximal-to-distal progression of muscle weakness. The boys fall frequently. Running, jumping, and hopping are invariably abnormal. By age 5 years, muscle weakness is obvious by muscle testing. On getting up from the floor, the patient uses his hands to climb up himself. Contractures of the heel cords and iliotibial bands become apparent by age 6 years, when toe walking is associated with a lordotic posture. Loss of muscle strength is progressive, with predilection for proximal limb muscles and the neck flexors; leg involvement is more severe than arm involvement. Between ages 8 and 10 years, walking may require the use of braces. By age 10-14, patients become wheel chair bound. Contractures become fixed, and a progressive scoliosis often develops. The chest deformity with scoliosis impairs pulmonary function, which is already diminished by muscle weakness. By age 16–18 years, patients are predisposed to serious, sometimes fatal pulmonary infections. In the last years of life the patient becomes bedfast. In general, there is a wide range of functional ability at a given age.

Figure 1 (A) Dystrophin-Associated Glycoprotein Complex (DAPC); (B) Protein changes in dystrophic muscle



Source: Allen 2011

The use of glucocorticoids and the management of spine deformity, pulmonary and cardiac dysfunctions have altered the timing of some of the clinical milestones of the disease.

Life Span: Patients with DMD usually survive until late adolescence but not more than 20 to 25 percent live beyond the twenty-fifth year. Respiratory, orthopedic and cardiac complications emerge, and without intervention the mean age at death is around 19 years (Bushby 2010). Following the introduction in the 1990s of assisted ventilation in the later stages of the disease, the mean age of survival (for those ventilated patients who do not develop early and severe cardiomyopathy) shifted to 24 years, with some surviving to the early thirties (Rall 2012, Eagle 2002).

Incidence: The incidence of DMD is 13 to 35 per 100,000 yearly or about 1 in 3,500 live male births globally. The estimated prevalence in the US is 18,000, with an additional 15,000 cases in EU (McNeil 2009). Mutations that are correctable by skipping exon 51 are thought to make up around 13% of the DMD population, resulting in a prevalence of 2340 boys in the US and 1950 boys in the EU.

Diagnostic Criteria: All boys with a clinical suspicion of a DMD diagnosis are subjected to molecular analysis of their dystrophin gene. Molecular methods that assess DNA copy number are used as the initial step in the diagnosis of DMD. If no deletions are identified, then DNA sequencing is performed to identify point mutations or small insertions or deletions. Three commonly used tests to determine a patient's mutation in the dystrophin gene include Multiplex Ligation-dependent Probe Amplification (MLPA), High-density Array Comparative Genomic Hybridization, and Single-Condition Amplification Internal Primer Sequencing.
Serum CK levels are invariably elevated to between 20 and 100 times normal. The levels are abnormal at birth but decline late in the disease because of inactivity and loss of muscle mass. EMG demonstrates features typical of myopathy.

2.2. Analysis of Current Treatment Options

There are no FDA approved treatments of DMD in the US that will prevent or slow muscle weakness in DMD. The current goals of treatment are to maintain function for as long as possible and to manage associated complications, such as joint contractures, scoliosis, cardiomyopathy, respiratory insufficiency, and weight gain.

The current standard of care is glucocorticoids (prednisone, prednisolone and deflazacort) administered either daily or intermittently. There is no consensus of the dosing regimen of these glucocorticoids globally. Most frequent regimens include 0.75 mg/kg/day prednisone, 10 days on/10 days off, 0.9 mg/kg/day deflazacort, and 5 mg/kg/day on weekends (Griggs 2013). The recent natural history studies have shown that the use of glucocorticoids have changed the natural progression of the disease. Randomized controlled trials published in literature have shown that glucocorticoids improved muscle strength and function for six months to two years. Data from non-randomized studies suggests functional benefit over a five year period in many treated patients, but the overall long-term benefit remains unclear (Cochrane Review). 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.

In addition, supportive care such as assisted ventilation and physiotherapy are used to improve quality of life in DMD.

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Drisapersen is a new molecular entity and is not currently marketed in the US.

3.2. Summary of Presubmission/Submission Regulatory Activity

Drisapersen was initially licensed by Prosensa, who conducted the Phase 1 studies and an open label extension of the Phase 1 study. It was licensed by GSK in 2010. GSK suspended dosing in all studies following the results of the Phase 3 study DMD114044. Subsequently rights were regained by Prosensa and more recently in January 2015 Biomarin acquired Prosensa.

A brief chronology of the regulatory activity with GSK, Prosensa and Biomarin and FDA on the development of drisapersen primarily as it relates to the assessment of efficacy mainly and additional important milestones is tabulated below. The regulatory interactions regarding different review disciplines will be addressed in the respective reviews (i.e. chemistry, nonclinical and safety).

Date	Summary of Regulatory Activity
8 July 2009	Pre-IND meeting with Prosensa; concerns raised by FDA at the meeting were: • 6 mg dose selection based on the 5 week CLIN-02 study may not be adequate, not clear if 6 mg was the MTD. • 6MWD as a primary endpoint acceptable provided 'large enough benefit', supportive data from secondary endpoints will be important due to concerns of unblinding due to injection-site reactions. • Need steps to minimize risk and potential effects of unblinding. • Difficulty in supporting the safety of a 12-month pivotal based on limited human data. • Implementation of adequate safety monitoring for platelet, liver and renal effects. • Need to take confounding factors such as non-invasive ventilation, use of glucocorticoids, scoliosis and surgery into account for randomization scheme or analytical plan.
25 August 2009	FDA grants Orphan Drug Designation to Prosensa
8 April 2010	GSK submitted IND105284 to the FDA Division of Neurology Products, including protocol DMD114118 (single dose PK, safety/tolerability study)

7 June 2010	FDA puts IND105284 on Partial Clinical Hold (only single dose in study DMD114118 is allowed to proceed, not the open label extension) for following reasons: • AEs in previous human studies not described in sufficient detail based on ICH3 Guidelines • Inadequate monitoring of hematological, hepatic and renal effects • Inadequate information on open label extension part DMD 114348
1 November 2010	GSK provides response to the Partial Clinical Hold, including submission of dose ranging protocol DMD114876
6 December 2010	FDA grants Fast-Track Designation to drisapersen
18 January 2011	FDA continues Partial Clinical Hold as 39 week study in monkey induced vascular injury at all doses. There was thrombus formation at two high doses.
4 May 2011	FDA removes Partial Clinical Hold, multiple dosing in protocol DMD114876 is allowed to proceed; Division proposes exclusion, monitoring and discontinuation rules.
4 January 2012	FDA requests additional safety information regarding recent adverse events of proteinuria
2 March 2012	GSK submits new protocol with new safety criteria to monitor and manage future events of proteinuria that is agreed with FDA
7 March 2013	Teleconference to discuss safety monitoring and managing regarding an adverse event of venous sinus thrombosis
23 May 2013	EOP2 Meeting with GSK. Issues discussed at this meeting were: • Pathway of approval: FDA did not consider "accelerated approval" based on Phase II studies as the regulatory course for drisapersen because 6MWD is a clinically meaningful endpoint. Top lines results of the Phase III study were to be available 4 months after the EOP2 meeting. FDA recommended that NDA based on Phase II (DMD114117, DMD114876) and Phase III (DMD 114044) along with results from DMD114763 appear most appropriate. FDA was open to considering dystrophin expression as supportive along with 6MWD to support filing of NDA, but was unclear with the current data were adequate. • FDA recommended immunogenicity be adequately addressed for both drug product and dystrophin. GSK indicated that there was no risk of immunogenicity with AON product. The FDA recommended that GSK provide supportive data explaining why AONs do not need immunogenicity assessments. • FDA agreed to the possibility of a rolling review of the NDA.
26 June 2013	FDA grants Breakthrough Therapy designation for drisapersen based on the results of Study DMD114117 and the 141 week open label study DMD 114673.
02 June 2014	Prosensa receives FDA communication on regulatory path forward. • FDA expresses reservations about the persuasiveness of the available data, but open to filing an NDA for drisapersen for consideration under an accelerated approval pathway. • FDA advised that the 6MWD could be interpreted as an intermediate clinical endpoint, supplemented by relevant evidence supporting reasonably likelihood

	of predicting longer term benefits. • FDA advised on types of confirmatory studies.
22 October 2014	Prosensa participates in Type C meeting with FDA to discuss confirmatory trials. FDA recommends a 2-3 year long confirmatory study. Prosensa agreed with conducting a longer 2-3 years study with an interim analysis. No agreement has been reached on the design of the confirmatory study in subsequent interactions with Biomarin.
12 January 2015	Pre-NDA meeting with FDA, Prosensa and Biomarin. The following agreements were reached: • Antibody data from supportive studies to be submitted at the 120-safety update • DMD natural history data collected by CINRG to be included at the time of NDA submission. • FDA disagreed with Biomarin's proposal to submit only Dr Goeman's natural history data for comparisons based on age and 6MWD and CINRG data for interpretation of pulmonary function in Study DMD114673. FDA explained that totality of available natural history data, including Dr Goeman's data and the CINRG data and any other natural history data would need to be provided at the time of NDA submission to enable appropriate review. FDA did not agree that matching would be adequate if based on 6MWD and age alone. Additional data, such as ability to jump and hop and detailed history corticosteroid use would be necessary. The sponsor agreed to include all natural history data available to the sponsor at the time of NDA submission. **Note: CINRG natural history data was not submitted in the application, and could not be obtained by FDA from CINRG. Matching was only done based on age and 6MWD. Additional data, such as ability to hop, jump etc. from Goeman's natural history data were not provided in the application.**
9 April 2015	FDA did not agree that a 24 week randomized double blind placebo controlled confirmatory study with drisapersen followed by a 72 week open label extension study would provide convincing evidence of benefit given the bias associated with potentially unblinding adverse effects on an effort dependent endpoint.

3.3. Foreign Regulatory Actions and Marketing History

Drisapersen has not been submitted for approval in any other country.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

The review was pending at the time completion of this review.

4.2. Product Quality

The applicant proposes the to-be marketed Drisapersen sodium injection for subcutaneous use be available as 160mg/0.8 mg ad 100 mg/0.5 mg single use vials. The CMC reviewer recommends eliminating the counterion name from the product and expressing the product strength as drisapersen and not as drisapersen sodium This will change the dosage strength to 150.4 mg (equivalent to 160 mg drisapersen sodium)/0.8 mL and 94 mg (equivalent to 100 mg drisapersen sodium)/0.5 mL in single-use vials.

4.3. Clinical Microbiology

Not applicable

4.4. Nonclinical Pharmacology/Toxicology

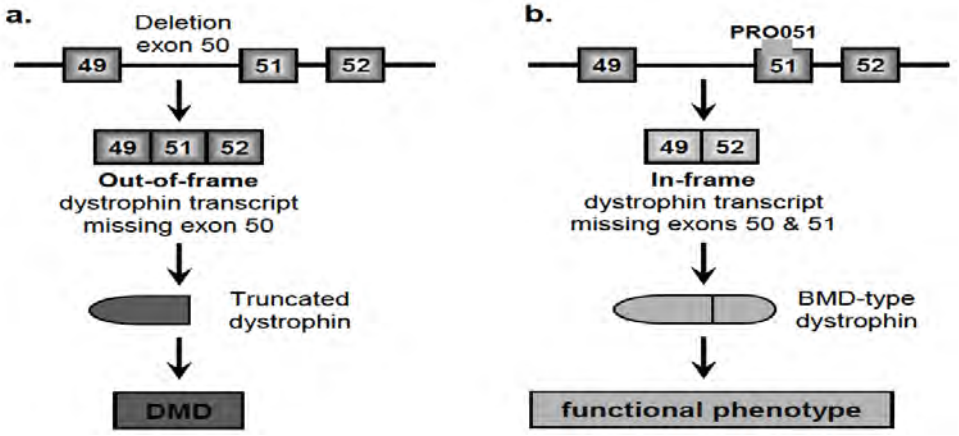
The review was pending at the time of completion of this review.

4.5. Clinical Pharmacology

4.5.1. Mechanism of Action

Drisapersen is a chemically modified oligonucleotide (fully 2'*O*-methyl substituted RNA backbone with phosphorothioate linkages) to promote RNA binding and prevent mRNA breakdown after binding. According the Applicant, drisapersen has high sequence specificity to exon 51. The Applicant's proposed mechanism of action involves disruption of secondary structure and/or interference with the binding of splicing regulatory proteins, resulting in skipping of exon 51 during post-transcriptional splicing and a mature mRNA transcript that is internally shorter but capable of dystrophin production in DMD. The truncated dystrophin lacks amino acids in the central rod domain, but retains the N- and C-terminal domains necessary for its structural and signaling roles. Drisapersen-induced exon skipping has a mutation-dependent corrective approach. Skipping of one specific exon applies to a series of different mutations. Skipping exon 51 with drisapersen would restore the reading frame in patients that carry a deletion of exons 45–50, 47–50, 48–50, 49–50, 50, 52, or 52-63, which comprise a total of 13% of all DMD patients. The exon skipping mechanism by drisapersen is shown schematically in Figure 2 in a patient that has exon 50 deletion.

Figure 2: Schematic representation of exon skipping mechanism



4.5.2. **Pharmacodynamics**

The results of dystrophin analysis are discussed section 6 of the review for each individual study.

4.5.3. **Pharmacokinetics**

- In Plasma:
  - Maximum plasma levels are generally reached between 2 to 4 hours after SC administration, after which the plasma levels decline during a rapid initial tissue (re)distribution phase, followed by a slower elimination phase.
  - After 24 weeks of dosing, the trough plasma concentrations at Week 36 (12 weeks after stopping treatment) were approximately half of the trough concentration at Week 23, indicating drisapersen has a long terminal half-life.
  - In DMD subjects the major drug-related component in plasma after repeated SC administrations was unchanged drisapersen.
  - No studies have been performed specifically to evaluate excretion in humans, but mice studies suggest it is mainly through the urinary route.
- In Skeletal Muscle:
  - Drisapersen concentrations in the muscles reach maximum levels after 39 weeks of dosing.
  - Drisapersen is also eliminated slowly from the muscle tissues with concentrations declining 40% after 12 weeks of stopping treatment.

4.6. **Devices and Companion Diagnostic Issues**

Development of a companion diagnostic was not required because DMD mutation analysis is incorporated in DMD diagnosis, and thus occurs prior to, and separate from, consideration of a therapeutic.

4.7. **Consumer Study Reviews**

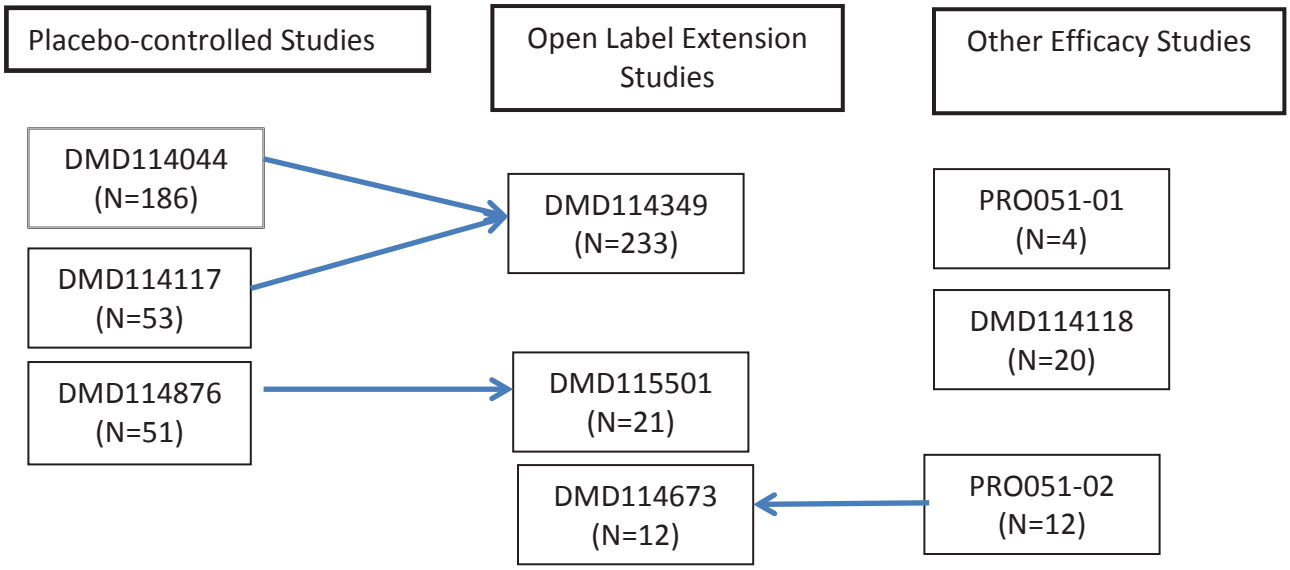
Not applicable.

5 Sources of Clinical Data and Review Strategy

5.1. Table of Clinical Studies

The drisapersen clinical development program consists of nine clinical studies in 326 boys with DMD (Table 1). Of the 326 subjects treated in the clinical development program, 312 received at least one dose of drisapersen. The cut-off date for the NDA submission was 31 August 2014. In September 2013 dosing was halted in all studies after the negative results of the Phase 3 Study DMD114044. No subjects received drisapersen from September 2013 up to the cut-off date. Seven clinical studies were completed by the cut-off date.

A schematic of the development program is shown below:



Clinical Review (Efficacy)
NDA 206, 031 (Drisapersen)

Table 1 Summary of clinical studies of drisapersen for the treatment of DMD

Trial Identity	Trial Design	Regimen/ schedule/ route	Study Endpoints	Treatment Duration/ Follow Up	No. of patients enrolled	Study Population	No. of Centers and Countries
Controlled Studies to Support Efficacy and Safety							
DMD 114117 Completed	Phase II Randomized, double-blind, placebo-controlled, parallel group	Loading dose: 6mg/kg SC drisapersen twice weekly for 3 weeks, then either: Continuous: SC 6 mg/kg/wk or Intermittent: alternating weeks of SC 6 mg/kg twice weekly and 6 mg/kg/wk for 6 weeks followed by 4 weeks off-dose period Dose–matched placebo	1°: 6MWD at week 24	48 weeks	N=53	ambulant subjects, 6MWD≥ 75m, able to Rise from floor ≤7s	13 centers in 9 countries
DMD 114876 Completed	Phase II Randomized, double-blind, placebo-controlled, parallel group	3 mg/kg/wk or 6 mg/kg/wk Volume-matched placebo	1°: 6MWD at Week 24	24 weeks (followed by 24-week post-treatment period with no treatment)	N=51	ambulant subjects, 6MWD≥ 75m, able to Rise from floor ≤15s	13 centers in 1 country
DMD 114044 Completed	Phase II Randomized, double-blind, placebo-controlled, parallel group	6 mg/kg/wk Dose–matched placebo	1°: 6MWD at Week 48	48 weeks	N=186	ambulant subjects, 6MWD≥ 75m	44 centers in 19 countries
Short-term Repeat-dose Open Label Study							
PRO051-02 Completed	Phase I/II Open-label, rising dose	0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg, or 6 mg/kg SC once weekly	1°: Dystrophin	5 weeks	N=12	ambulant and non-ambulant subjects	2 centers in 2 countries
Long-Term Extension Studies to Support Efficacy and Safety							
DMD	Phase I/II	6 mg/kg/wk drisapersen SC	6MWD	Ongoing	N=12	ambulant and non-	2 centers in 2

114673 (Extension to PRO051-02) Ongoing	Open-label uncontrolled extension of PRO051-02	for 72 weeks. After an interval of 8 weeks (Weeks 73-80) off drug, subjects restarted an intermittent treatment regimen of 6 mg/kg/wk drisapersen for 8 weeks, followed by 4 weeks off treatment (12-week cycles) up to 188 weeks. An IV sub study was conducted following Week 188		Planned: Until launch or termination of development. Actual: Dosing in the study was halted in September 2013, but was restarted after the data cut off for this submission (August 31, 2014).	IV sub study N=7	ambulant subjects at start of parent study PRO051-02	countries
DMD 114349 Terminated Completed	Phase III Open-label extension of DMD114117 and DMD114044	6 mg/kg/wk SC drisapersen Subjects with tolerability issues had the option to enter the intermittent arm of 6 mg/kg/wk for 8 weeks followed by 4 weeks off dose. Subjects who did not wish to receive drisapersen or who had to withdraw from both active arms during the study had the option to go into a natural history observation arm.	6MWD	Planned: Until launch or termination of development (minimum 104 weeks). Actual: Up to 101 weeks at time of termination of dosing in September 2013	N=233	ambulant at start of parent study (DMD114044 or DMD114117)	58 centers in 24 countries
DMD 115501 Ongoing (not submitted)	Phase III Open-label extension of DMD114876	6 mg/kg/wk SC drisapersen Subjects with tolerability issues had the option to enter the intermittent arm of 6 mg/kg/wk for 8 weeks followed by 4 weeks off		Planned: Until launch or termination of development Actual: Dosing in the study was halted in	N=21	ambulant at start of parent study (DMD114876)	13 centers in 1 country

		dose.		September 2013, but was restarted after the data cut off for this submission (August 31, 2014).			
Other studies pertinent to the review of efficacy or safety							
PRO051-01 Completed	Phase1 Open label, single dose	0.8 mg IM	Safety, tolerability Dystrophin	Single dose	N=4	Ambulant and non-ambulant	Single center
DMD 114118 Completed	Phase 1 Randomized, placebo-controlled, rising dose	3, 6, 9, 12 mg/kg SC No subjects received 12 mg/kg	Safety, tolerability, PK	Single dose	N=20	Non ambulant	2 centers in 2 countries

5.2. Review Strategy

I reviewed all the clinical efficacy data (placebo controlled studies, open label studies and the exploratory Phase I studies) submitted to the NDA along with the published literature and medical text books to evaluate the efficacy of drisapersen in the treatment of DMD with mutations amenable to exon 51 skipping. I conducted my own analyses of the primary data using graphical explorations and descriptive statistics in JMP, JReview and Excel. The applicant's primary statistical MMRM analyses were confirmed by the statistician Dr. Sharon Yan, Ph.D. I reviewed the dystrophin data from each study, but the methodology for the assessment of dystrophin and its reliability in each study was reviewed by Dr. Ashutosh Rao, Ph.D. His comments were incorporated in this review of the dystrophin data. The safety data were reviewed by Dr. Evelyn Mentari, MD in a separate review. The MRI data were reviewed by Dr. Daniel Krainak, Ph.D from CDRH Imaging Division.

6 Review of Relevant Individual Trials Used to Support Efficacy

Study DMD 114117

6.1.1. Study Design

Overview and Objective

Study DMD114117 was a Phase II placebo controlled clinical study to assess two dosing regimens, continuous and intermittent, of 6mg/kg drisapersen. An intermittent regimen was selected to potentially mitigate liver and kidney toxicities. PK/PD modeling predicted that the selected intermittent regimen would provide similar peak concentrations (Cmax) and total exposure (AUC) over the 48 weeks to the continuous regimen. Both the continuous and the intermittent regimen had comparable total doses administered throughout the study.
Studied period**:** 01 September 2010 to 12 September 2012
Study center(s): 13 centers in 9 countries in Australia, Belgium, France, Germany, Israel, Netherlands, Spain, Turkey, and the United Kingdom.

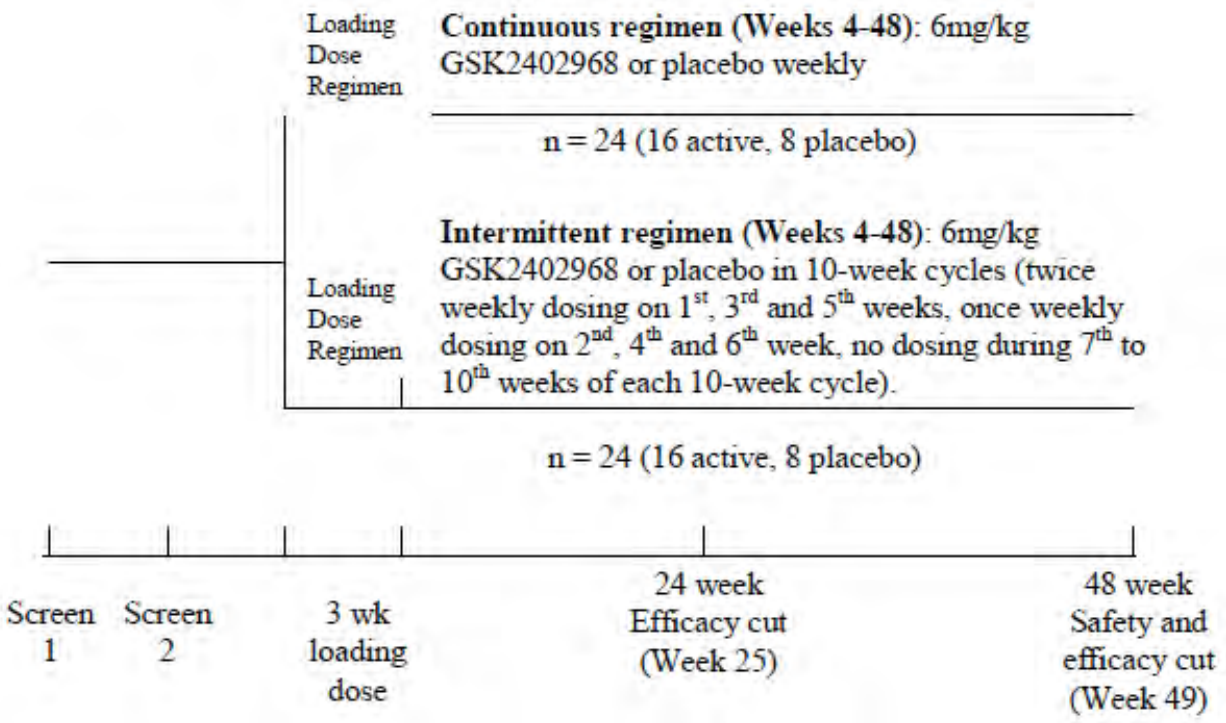
Trial Design

Study DMD114117 was a 48 week double-blind, parallel-group, placebo-controlled clinical study in ambulant DMD boys, with primary efficacy at 24 weeks. The study was fully blinded with respect to active and placebo in each cohort, however the different regimens were not fully blinded, due to the number of dummy doses that would be needed to blind both regimens.
At the end of the treatment period, subjects who completed the study had the option to enter an open-label extension study (Study DMD114349). Additional criteria for entering the open label study are discussed in section 6.4. If subjects did not enter the extension study, they were monitored for a minimum of 20 weeks after the last dose.

Study Schematic is shown in

Figure 3. Screen 1 and 2 were 4 and 2 weeks prior to randomization, respectively.

Figure 3 Study Design Schematic



Population: N=54 ambulant subjects with DMD resulting from a mutation that can be corrected by exon 51 skipping. The sample size was not based on statistical considerations, but would allow the detection of an effect size of 1.1 with 80% power at significance level of 5%

Key Inclusion Criteria:

- Mutation confirmed by a state-of-the-art DNA diagnostic technique covering all DMD gene exons, including but not limited to MLPA (Multiplex Ligation-dependent Probe Amplification), CGH (Comparative Genomic Hybridization) or H-RMCA (High-Resolution Melting Curve Analysis)
- Male of at last 5 years of age
- Able to rise from floor in ≤7 seconds (without aids/orthoses),
- Able to complete the 6MWD test with a distance of at least 75m,
- Results of 6MWD must be reproducible (within 20% for each test) between Screening Visits 1 and 2
- On glucocorticoids for a minimum of 6 months immediately prior to screening, with no significant change in total daily dosage or dosing regimen for a minimum of 3 months immediately prior to screening and a reasonable expectation that total daily dosage and dosing regimen will not change significantly for the duration of the study (unless clinically indicated)
- Life expectancy of at least 1 year

- QTc <450 (based on single or average QTc value of triplicate ECGs obtained over a brief recording period), or <480 msec for subjects with Bundle Branch Block. Note: QTc could be either QT interval corrected for heart rate by Bazett's formula (QTcB) or QT interval corrected for heart rate by Fridericia's formula (QTcF), and machine read or manual overread.

Key Exclusion Criteria:

- Any additional missing exon for DMD
- Current or history of liver or renal disease
- Use of anticoagulants, antithrombotics or antiplatelet agents, previous treatment with investigational drugs, idebenone or other forms of Coenzyme Q10, within 1 month of treatment initiation.
- Positive hepatitis B surface antigen, hepatitis C antibody test, or human immunodeficiency virus (HIV) test at screening,
- Symptomatic cardiomyopathy, to discuss with medical monitor, if subject has a left ventricular ejection fraction <45% at Screening

Key Withdrawal Criteria:

- AE jeopardizing safety of the subject
- Administration of idebenone or Coenzyme Q10 during study
- New evidence of cardiomyopathy.
- QTc>500, to be discussed with Medical Monitor

Dosing regimen:

After a 2-4 week screening period, all subjects received a loading dosing regimen of twice weekly subcutaneous (SC) dosing with 6 mg/kg drisapersen for the first 3 weeks, which was followed by the following regimen in parallel cohorts for 48 weeks. Each cohort included subjects on drisapersen and matched placebo in a 2:1 ratio. Subject randomization was done using Interactive Voice Response System according to the randomization schedule.

- Continuous regimen; 6 mg/kg SC drisapersen once weekly for 48 weeks
- Intermittent regimen; 6 mg/kg SC drisapersen twice weekly on 1st, 3rd and 5th weeks, once weekly on 2nd, 4th and 6th weeks, and no active drug on 7th to 10th week of each 10 week cycle for 48 weeks

Injection sites were rotated to minimize injection site reactions. Some subjects received multiple injections depending on the weight of the subject. There were no food restrictions with regards to dosing. All study treatment was supplied in identical vials. The appearance of the active and placebo solutions were not identical and hence a blinding label was applied to the syringe to minimize the risk of unblinding the subjects. The volumes were different according to the weight of the subject, and were matched for active and placebo. To maintain the blind, the dose was prepared and administered by appropriately trained and qualified unblinded personnel who were not involved in the study's efficacy assessments.

Reviewer's Comment:

Applicant's study design elements mostly appeared reasonable based on the preclinical and clinical information available at the time of study conduct as discussed below:

- **Design:**
  - **Approaches towards blinding of active and placebo treatment were "less than ideal" by design. The placebo solution had a different appearance from the drisapersen solution. The study, by design, was not blinded for the regimens, with a different schedule of doses for the continuous and intermittent arms. Treatment was administered by personnel who knew if drug or placebo was being administered; while these personnel were not involved in efficacy assessments, direct patient contact (and presumably contact with other study personnel) may have jeopardized blinding.**
- **Inclusion/Exclusion Criteria:**
  - **Steroids are the standard of care in the treatment of DMD; therefore enrolling subjects on their stable steroid dose was prudent, but there are many factors associated with steroid use that would render heterogeneity to the selected DMD population. The enrollment criteria required subjects on steroid for a minimum of 6 months, hence some subjects were on steroid for much longer and these subjects may have different disease progression trajectories (Kim, 2014). At this time there is no consensus on the optimal dose, regimen (continuous or intermittent) or the type of steroid. The choice of steroids also varies from country to country. Published data suggest that earlier initiation of steroids provide more sustained improvement in clinical function (Moxley RT III, 2005; Manzur, 2008). Recent 36 month data have shown that patients on daily steroids do better than intermittent steroids (Pane 2014). The impact of age of initiation of steroid use on disease progression is not well established, but in general DMD patients receiving steroid treatment increase ambulation from 2-5 years compared to those not on steroid treatment. All these factors were not controlled in the selection of patients for this study. Understandably, the standard of care being different from country to country enrolling patients with regards to consistency with steroid use would considerably limit the patients meeting the enrollment criteria. These factors are likely to impact the disease trajectory of an individual patient.**
  - **The inclusion criteria required subjects to have no more than 20% difference in 6MWD at the two screening visits 1 and 2 that were separated by 2 weeks. This allows for a lot of variation in the 6MWD that is in fact larger than the difference possibly seen in 1 year in many cases.**
  - **The inclusion criteria of 75 m 6MWD would include subjects that are likely to lose ambulation in 1 year (Mazzone 2011). This criterion was based on available natural history data at the time of study conduct. Recent published data suggest that patients with 6MWD<325 are more likely to lose ambulation in 1 year (McDonald 2013b).**
  - **The inclusion criteria of rise of floor of ≤7 seconds was included for a more homogenous population, although disease trajectories may vary in subjects with rise from floor of <4 or >4 as well. In addition Rise from Floor in conjunction with 6MWD also appears important in disease progression (Mazzone 2010).**
  - **The impact of use of idebenone or other forms of Coenzyme Q10 more than 1 month of treatment initiation in not well established, but may add to variation in disease progression. Other enrollment criteria appear reasonable.**
- **Dose:**

- **A loading dose appears appropriate. PK modeling had suggested that steady state could be achieved 6 weeks earlier with a loading dosing regimen of twice weekly dosing for 3 weeks, given the long half-life of drisapersen (29 days).**
- **There is limited human data on a 9mg/kg /week dose in non-ambulant subjects, but decision was based on pre-clinical pro-inflammatory findings. Applicant has not evaluated longer dose interval with higher doses due to limitations in injectable volume by SC administration.**

- **Assessments:**
  - **Based on what was known at the initiation of this study, efficacy assessments at 24 and 48 week seemed reasonable. PK/PD characteristics of drisapersen had estimated dystrophin protein turnover half-life of 5 weeks. Based on this it was hypothesized that dystrophin expression will reach steady state in 24 weeks, hence a 24 week primary endpoint. Efficacy was also assessed at 48 weeks. (The dystrophin half-life was verified by the sponsor during the review cycle. The Applicant clarified in a response dated June 18, 2015 that this reflected the thought at the time protocol was written. Recent published studies suggest a dystrophin half-life in the range of 2 to 4 months in the skeletal muscle of mdx mice (Verhaart 2014; Wu, 2012). Although, the dystrophin half-life from mice may not predict human dystropin half-life, but likely is longer in humans than in rodents. Given this, studies longer than 48 weeks may be desirable to achieve optimal results to allow adequate time for the attainment of steady state levels of dystrophin.**

Study Endpoints

The following efficacy assessments were done once or twice at screening, baseline, and Weeks 13, 25, 37 and 49, unless specified otherwise.

Primary efficacy endpoint:

- **Muscle function using 6 minute walking distance (6MWD) test**: change from baseline at 25 weeks. Subjects were asked to walk, as quickly but as safely as they could, up and down a fixed distance until they were told to stop after 6 minutes. The subjects were warned of the time and were told that they could stop earlier if they felt unable to continue walking. The total distance walked within 6 minutes (or until the subject stopped in case of early termination of the test) was recorded in meters.

Secondary efficacy endpoints:

- **Timed function tests (times and grading**): These were assessed on a 6-point scale to differentiate those subjects with similarly fast times who may have achieved a ceiling time (Attached to the Appendix A).
  - **Rise from floor**, no aids or orthoses allowed, subjects stood from a standardized supine position as quickly as possible when told to "go". Time was recorded from initiation of movement to upright position.
  - **10m walk/run**, no aids or orthoses allowed: Subject was asked to walk a 10 m measured walkway as quickly as possible. Time was recorded to one tenth of a second. If the subject could not complete the 10-meter walk, the total distance was recorded.
  - **4-stair climb (ascend and descend)**, handrail allowed: subject ascended and descended 4 steps
- **North Star Ambulatory Assessment**: It consists of 17 activities graded 0 (unable to perform), 1 (performs with modifications), 2 (normal movement). NSAA Total Score ranges from 0 to 34, with a

score of 34 implying normal function. The scale assesses activities required to remain functionally ambulant (e.g. rise from the floor), activities that can be difficult even early in the disease (e.g. standing on heels) and activities that are known to progressively deteriorate over time (stand from a chair, walk). (Scale attached in the Appendix A).

- **Muscle strength (total score):** knee flexors, knee extensors, elbow flexors, elbow extensors, shoulder abductors and hip flexors (as determined by handheld myometry using a microFET2 myometer)
- **Frequency of accidental falls during 6MWD**
- **Time to loss of ambulation**
- **Pulmonary function using non-invasive spirometry** (FEV1, FVC, MIP, MEP, PCF, PF)
- **Creatine kinase serum concentrations**: at Screening, baseline and Weeks 3, 5, 9, 13, 17, 21, 25, 33, 41 and 49.
- **Dystrophin expression** (muscle biopsies from tibialis anterior) including percent change from baseline: at baseline, Week 25 and 49 and mRNA production in muscle tissue

Exploratory endpoints:

Pediatric Quality of Life Neuromuscular module, gait characterization by accelometry during 6MWD, percent predicted 6MWD (the percentage of the predicted 6MWD for a healthy boy of the same age and height that the boy with DMD was able to walk), lean body mass by DEXA scan, were conducted as exploratory endpoints. Pharmacokinetics, drisapersen muscle concentration and DNA samples were also taken.

Reviewer's Comment:

6MWD is an effort dependent endpoint. The results may be affected by bias from unblinding due to adverse events such as injection site reaction, which occurred in most patients administered drisapersen. 6MWD was considered a reasonable clinical endpoint in DMD, as little was known on the natural history data of 6MWD in DMD and most data were published on the validity of 6MWD in DMD.

Like the 6MWD, the Timed Function Tests and NSAA are also effort and motivation dependent and measure similar functional capabilities as the 6MWD. In addition, NSAA scoring may be subjective and susceptible to observer bias.

Statistical Analysis Plan

The intent-to-treat (ITT) population was defined as all subjects who are randomized to the study and received at least one dose of study medication and have at least one efficacy assessment. This is the primary population for evaluation of efficacy parameters. The per protocol (PP) population is defined as all ITT subjects and have no major protocol deviations.

There was no interim analysis, although two main analyses were done (1) at week 25 (primary) and (2) at week 49.

Primary Endpoint Analyses: Primary assessment of efficacy data was conducted using a Mixed Effect Model Repeated Measure (MMRM) at week 25 on the Observed case (OC) data. The model included

treatment, visit, treatment by visit interaction, country/country grouping, baseline 6MWD and baseline 6MWD by visit as fixed effects. Comparison of each dosing regimen with placebo (combined group pre-specified) was adjusted using Bonferroni-Holm adjustment for multiplicity. The p-values from the two primary analyses (6 mg/kg drisapersen continuous vs. placebo, and 6 mg/kg drisapersen intermittent vs. placebo), were ordered smallest to largest. The smallest p-value was compared to a significance level of $\alpha/2$ (0.025). If the result was demonstrated to be statistically significant at this level, i.e. $p<0.025$, then the second p-value was compared to a significance level of α (0.05). If the initial comparison shows the result not to reach statistical significance, then the second comparison was also considered not to have reached statistical significance.
If the assumptions of normality were not met, a log-transformation of the data prior to an MMRM analysis was used.
Sensitivity Analyses for primary endpoint: The following sensitivity analyses were performed:

- ANCOVA on OC data for the ITT population
- ANCOVA on LOCF data (missing data for at least 3 months) for the ITT population
- ANCOVA on data imputed via multiple imputations for the ITT population
- MMRM on OC data with PP population

ANCOVA model included fixed terms for treatment, baseline 6MWD and country. The covariates assessed were county, baseline 6MWD, steroid regimen, age, baseline rise time, lean body mass index.
MMRM model included terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit.

Secondary endpoint analyses:

- For continuous endpoints MMRM analyses on OC data, with similar fixed terms
- Kaplan-Meir and Log rank test on time to event endpoints such as loss of ambulation (if ≥4 subjects in either treatment group experienced a loss of ambulation)
- ANCOVA on %predicted 6MWD on OC data

Protocol Amendments

The protocol amendments related to efficacy assessments are summarized below:

Amendment 1 (20 Jul 2010)	Country specific: To remove DEXA scan due to logistics
Amendment 2 (24 Sep 2010)	Country specific: to add conduct gait characterization collected by accelerometric data ta 5 sites (N=20 of 54 subjects) and include 50 healthy control height matched subjects for this.
Amendment 3* (13 Oct 2010)	• Approval of recruitment of healthy control was not granted, hence removed. Intent to enroll in a different protocol. • Removed the inclusion criteria that required reproducibility of Rise From Floor (within 20% at pre-drug visit 1 and 2). • Modified the exclusion criteria regarding concomitant medications: idebenone and Coenzyme Q allowed within 1 month of study administration instead of 6 months • Removed muscle biopsy at 49 weeks • 'Pediatric Quality of Life Neuromuscular module' moved from secondary endpoint to exploratory analyses.
Amendment 4*	• addition of the frequency of accidental falls during the 6MWD and time to

(19 Jul 2010)	loss of ambulation as 2° endpoint

*Also amended safety monitoring and stopping criteria

Reviewer's Comment: These amendments are unlikely to bias the study results as were done within the first month of study initiation when few subjects were enrolled.

Data Quality and Integrity: Sponsor's Assurance

The study was conducted in accordance with ICH GCP (ICH E3 and E6) and applicable country-specific requirements. Written commitments were obtained from investigators to comply with GCP. Study was conducted with written informed consent from subjects and their parents.

6.1.2. Study Results

Patient Disposition

A total of 53 subjects were randomized (Table 2). No subject withdrew from the study.

Protocol Violations/Deviations

The number of subjects with protocol deviations is summarized in Table 2.

Table 2: Number of subjects (%) with major protocol deviations up to week 25

ITT and Safety Population	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)	Total (N=53)
PP Population	17 (94)	17 (94)	15 (88)	49(92)
Subject with major protocol deviation	1(6)	1(6)	2(12)	4(8)
Failed Inclusion criteria (Able to complete the 6MWD test with a distance of at least 75 m and results of 6MWD within 20% of each other at each pre-drug visit.)[a]	1(6)	1(6)	1(6)	3 (6)
Failed Inclusion criteria (Receiving glucocorticoids for ≥ 6 months prior to screening, with no significant change in total daily dosage or dosing regimen for 3 months prior to screening.)[b]	0	0	1(6)	1(2)

Source: NDA DMD114117 study report, page 58

[a]Subject 3055 in the placebo group, Subject 2129 in the 6 mg/kg drisapersen continuous group and Subject 2126 in the 6 mg/kg drisapersen intermittent group. [b]Subject 2103 in the 6 mg/kg drisapersen intermittent

Subject 3055 in the placebo group in addition to not being able to complete the 6MWD test with a distance of at least 75 m at screening also had a single deletion of exon 45 that was not amenable to exon 51 skipping. Given the fact that this subject was on placebo, the applicant included him in the primary analysis with the ITT population. In addition, two placebo subjects on a single visit (week 37 and week 9, respectively) were given active drug based on PK analysis). One subject had lower

drisapersen concentrations at week 9 and according to the applicant probably missed a dose that was not reported.

Reviewer's Comment:

- **The sensitivity analysis (MMRM with the PP population) was also positive with a similar p-value (p=0.013); hence the impact of protocol violation of subject 3055 was not a concern.**
- **Dr. Sharon Yan (Statistician) also conducted an analysis averaging the 2 screening and baseline 6WMD values. A treatment difference over placebo of 30m (p=0.03) was obtained from the drisapersen continuous regimen. Hence, the impact of protocol violation in a few subjects with 6MWD for the 2 screening visits not within 20% was not a concern.**
- **A single missed dose or wrong treatment on a single occasion is unlikely to affect efficacy results, but analysis was done excluding these subjects as well.**

Table of Demographic Characteristics

According to the applicant, the demographic characteristics were similar across treatment groups with the intermittent group being older and having slightly higher mean height and weight values. The demographic characteristics for primary analysis are shown in Table 3.

Table 3 Demographic characteristics of the primary efficacy analysis

	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)	Total (N=53)
Age (yrs)				
Mean (SD)	6.9 (1.2)	7.2 (1.7)	7.7 (1.5)	7.3 (1.5)
Median	7.0	6.5	8.0	7.0
Min., Max.	5, 9	5, 11	5, 10	5, 11
Ethnicity, n (%)[a]				
n	16	17	15	48
Not Hispanic/Latino	16 (100)	17 (100)	14 (93)	47 (98)
Hispanic/Latino	0	0	1 (7)	1 (2)
Race, n (%)[a]				
n	16	17	15	48
White - White/Caucasian/ European Heritage	13 (81)	15 (88)	14 (93)	42 (88)
White - Arabic/North African Heritage	0	2 (12)	0	2 (4)
American Indian or Alaska Native	0	0	1 (7)	1 (2)
Asian - South East Asian Heritage	1 (6)	0	0	1 (2)
Native Hawaiian or other Pacific Islander	1 (6)	0	0	1 (2)
Mixed Race	1 (6)	0	0	1 (2)

Height (cm)				
Mean (SD)	119 (8)	118 (11)	121 (10)	NA
Median	120	120	120	NA
Min., Max.	99, 132	102, 139	106, 139	NA
Weight (kg)				
Mean (SD)	25 (5)	25 (7)	28 (10)	NA
Median	24	23	26	NA
Min., Max.	16, 35	16, 42	18, 51	NA

Source: DMD114117 study report, page 60

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

According to the Applicant, baseline DMD characteristics were relatively balanced across treatment groups (Table 4). The time since first symptoms, diagnosis and first corticosteroid use in the intermittent group were slightly longer than the other two treatment groups which are consistent with the older mean age of this group. Mean baseline values for the 6MWD test were higher in the continuous group (427.61 m) than in the placebo (403.18 m) and intermittent (394.57 m) treatment groups.

Table 4 Baseline disease characteristics

	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)	Total (N=53)
Time Since First Symptoms (months)				
Mean (SD)	63 (24)	61 (25)	64 (24)	63 (24)
Median	73	57	63	62
Min, Max	15, 95	27, 112	27, 105	15, 112
Time Since Diagnosis (months)				
Mean (SD)	44 (22)	45 (28)	48 (26)	45 (25)
Median	35	41	47	43
Min, Max	12, 82	3, 96	3, 105	3, 105
Time Since First Corticosteroid Taken (months)				
Mean (SD)	24 (14)	26 (21)	33 (17)	27 (18)
Median	22	14	34	26
Min, Max	7, 60	7, 69	7, 63	7, 69
Corticosteroid Regimen				
Continuous	11 (61)	12 (67)	9 (53)	32 (60)
Intermittent	7 (39)	6 (33)	8 (47)	21 (40)
6MWD (m)				
Mean	403	428	395	NA
(SD)	(45)	(70)	(67)	
Exon Mutation, n (%)				

DMD 45-50 deletion	7 (39)	6 (33)	5 (29)	18 (34)
DMD 48-50 deletion	3 (17)	6 (33)	6 (35)	15 (28)
DMD 49-50 deletion	1 (6)	4 (22)	3 (18)	8 (15)
DMD 50 deletion	4 (22)	1 (6)	3 (18)	8 (15)
DMD 52 deletion	3 (17)	1 (6)	0	4 (8)

Source: NDA DMD114117 study report, page 61

Reviewer's Analysis and Comments on Baseline Characteristics:

The heterogeneity in the DMD population is well known (Humbertclaude 2012, McDonald 2013). Some advantage for 6mg/kg/week group was observed. Higher baseline function is almost always associated with slower long term decline (McDonald: http://www.treatnmd.eu/downloads/file/meetings/2013/workshop/Session1/McDonald_NH.pdf

Subjects in the continuous treatment regimen appeared to have greater number of subjects that were < 7 years, on continuous steroids, higher mean baseline 6 MWD, greater number of subjects with baseline 6MWD of >400m, shortest time since first symptoms that is consistent with a younger more functional population that are all likely to have a slower decline, even though the average age was slightly higher for the continuous treatment. In addition, I looked for other disease characteristics that would enable the assessment of functional capabilities at baseline. Greater number of subjects on continuous treatment could jump with both feet up at the same time, hop with clearing foot and heel from floor at the same time, rise from floor <4 seconds and had the ability to rise from floor without gower's maneuver. These are considered milestones that can predict loss of ambulation (McDonald). The percentage of subjects across the treatment groups are shown in Table 5, suggesting subjects in the 6mg/kg/week group have higher functional capabilities and likely to have a slower decline in the 6MWD. The randomization was conducted as per the master randomization schedule generated via an interactive voice response system. Hence, these differences in treatment groups occur by random chance.

Table 5 Percentage of subjects with Baseline Characteristics

Baseline Factors	Continuous 6mg/kg/week	Intermittent 6 mg/kg/week	Placebo
Age <7 years	50%	30%	39%
6MWD>400m	61%	41%	50%
Rise From Floor <4 secs	44%	18%	22%
On continuous regimen	67%	53%	61%
Other factors: Ability to jump with both feet up at the same time	100%	88%	77%
Ability to hop with clearing foot and heel from floor	50%	29%	27%
Ability to rise from floor without gower's maneuver	27%	6%	11%

Interaction between treatment and baseline 6MWD, age and steroids in a statistical model did not lead to any statistically significant interaction, but the study size is small and not powered to show any differences due to covariates.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

No imbalances in concomitant medications were noted. All subjects were 80% to 120% compliant with dosing. The median dose (in mg) was the lowest in the continuous drisapersen treatment arm. These exposure differences likely arise due the higher % of younger kids in the continuous treatment arm as dosing is based on mg/kg. The duration of exposure was similar across treatment arms.

Efficacy Results – Primary Endpoint

Applicant's Primary Analysis and conclusions:
Based on the primary efficacy MMRM analysis for change from baseline in 6MWD at Week 25, there was a statistically significant treatment benefit for the continuous regimen [35 m; p=0.01 (p<0.025)] over placebo, but not for the intermittent regimen (Table 6). A similar analysis was also done at week 49. A treatment differences between drisapersen and placebo of 36 m and 27 m were observed for the continuous and intermittent groups respectively at Week 49. The continuous group showed some decline towards baseline after the initial increase in 6MWD up to Week 25, whereas the intermittent group was relatively stable over the 48 week time-period (Table 6).
Note: After Week 25, the biostatistics and programming team was unblinded, but the subjects, investigators and monitors were blinded. There was also a blinded, independent Medical Monitor assigned to the study from the point of unblinding for the Week 25 analysis, to maintain the integrity of medical decisions.

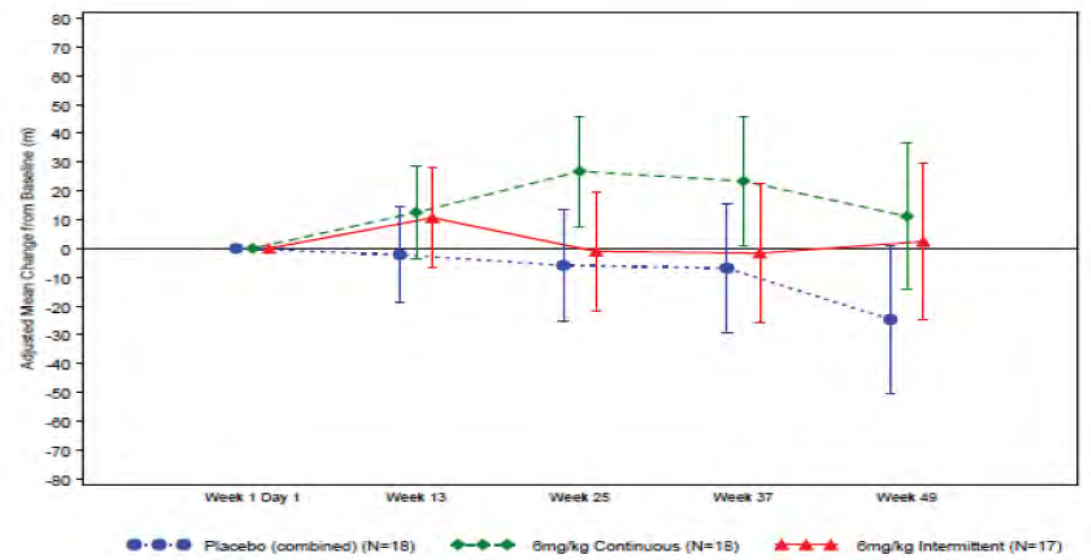
Table 6 Summary of MMRM Analysis of Change from Baseline in 6MWD (m)

	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)
Baseline			
n	18	18	17
Mean (SD)	403 (45)	428 (70)	396 (70)
Week 25			
n	16	16	15
Adjusted mean change (SE)	-4 (10)	31 (10)	-0.1 (10)
Adjusted mean difference vs. placebo		35	4
95% CI		(8, 62)	(-24, 31)
p-value		0.01	0.80

Week 49			
n	17	18	15
Adjusted mean change (SE)	-25 (13)	11 (13)	2 (14)
Adjusted mean difference vs. placebo		36	27
95% CI		(-0.11, 72)	(-10, 64)
p-value		0.05	0.15

Source: DMD114117 study report page 64

Figure 4 MMRM Analysis of Change from Baseline in 6MWD (m) at Week 49



Source: Study DMD114117 Study Report page 68

Interactions between treatment and age, baseline rise from floor, baseline lean body mass, baseline 6MWD, corticosteroid regimen and country grouping at week 25 showed only a statistically significant interaction ($p \leq 0.10$) for baseline rise from floor. The confidence interval was larger, so the applicant cautions the interpretation of this. In addition, the study size is small.

Reviewer's Comment: Note that not all subjects were included in the primary analysis at Week 25, since five subjects had their 6MWD assessment done at Week 24 and one subject at Week 27. A sensitivity analysis including these subjects gives a p value of 0.02 with a treatment difference of 31m for the continuous regimen compared to placebo (Table 7).
The analysis at Week 49 was not a planned analysis and cannot be evaluated while controlling type 1 error.

Applicant's Sensitivity Analyses for the primary endpoint: The planned sensitivity analyses supported the primary analysis as shown in Table 7. Sensitivity analyses at week 49 support the MMRM analysis at week 49 (not shown in Table).

Table 7 Sensitivity analyses on change from baseline 6MWD at week 25 for drisapersen continuous regimen

Analysis	Population	Dataset	Treatment Difference	95% CI	P-value
MMRM	PP	OC	38	(8, 68)	0.01
ANCOVA	ITT	OC	35	(7, 63)	0.01
ANCOVA	ITT	LOCF	31	(4, 57)	0.03
MMRM	ITT	OC (unslotted)[a]	31	(5, 58)	0.02
MMRM	ITT	OC (exc. outlier)[b]	31	(4, 58)	0.03
MMRM, Uncombined Placebo	ITT	OC	47	(12, 82)	0.01

Source: DMD114117 study report, page 66

a. For reporting purposes, efficacy data were slotted to pre-defined visit windows, based on the time of the assessment since first dose. Where a visit was attending particularly early or late, the assessment would have fallen outside of the visit window and was thus excluded from analyses. This analysis of unslotted data, analyzed the data according to the investigator recorded visit, regardless of the time at which this occurred.

b. Subject 3002, randomized to placebo intermittent was determined to be an outlier by the applicant (6MWD of 322 m, 432.2 m and 265 m at Baseline, Week 13 and Week 25 respectively): gained 110 m at Week 13.

Additional Analyses on 6MWD:

1. MMRM analyses with placebo groups analyzed separately (continuous and intermittent placebo): The treatment difference for the continuous regimen compared to continuous placebo was 47m. The placebo continuous and placebo intermittent performed differently (Table 8). The applicant is unclear of the difference in placebo response in the two regimens, but explains that this could be due to chance due to small group size combined with inherent inter-subject variability (including age).

Table 8: MMRM analyses with placebo groups analyzed separately

	Placebo Continuous (N=9)	Placebo Intermittent (N=9)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)
Baseline				
n	9	9	18	17
Mean (SD)	406 (49)	400 (43)	428 (70)	395 (70)
Week 25				
n	7	9	16	15
Adjusted mean change (SE)	-15 (14)	6 (13)	32 (10)	0.3 (10)
Adjusted mean difference vs. placebo			47	-6
p-value			0.01	0.72
Week 49				
n	9	8	18	15
Adjusted mean change (SE)	-36 (18)	-13 (18)	11 (13)	3 (13)
Adjusted mean difference vs. placebo			47	16
p-value			0.04	0.48

Source: NDA DMD114117 study report, page 69

2. MMRM analysis in combined drisapersen regimens (continuous and intermittent combine):
No statistically significant difference (p=0.12) was observed at Week 25 when the two drisapersen treatment regimen groups were combined, but a nominal p-value of 0.05 was observed at Week 49 (Table 9).

Table 9 MMRM analyses of the combined drisapersen regimens

	Placebo (combined) (N=18)	6 mg/kg Drisapersen (combined) (N=35)
Week 25		
n	16	31
Adjusted mean change (SE)	-3 (10)	16 (7.4)
Adjusted mean difference vs. placebo		20
95% CI		(-5, 44)
p-value		0.12
Week 49		
n	17	33
Adjusted mean change (SE)	-25 (13)	7 (9)
Adjusted mean difference vs. placebo		31
95% CI		(0.5, 62)
p-value		0.05

Source: NDA DMD114117 study report, page 71

3. Change from baseline in 6MWD by response category:
Response was measured as the percentage of subjects that achieved a fixed change in 6MWD (≥-30 m, ≥0 m, ≥30 m, ≥60 m or ≥-10%, ≥0%, ≥10%, ≥20% and≥30% change). Subjects could be included in more than 1 category. There were 19% subjects in the placebo and 38% drisapersen continuous arm and 20% in the intermittent arm that showed ≥ 30 m change in 6MWD at Week 25. A total of 31% subjects (n=5) in the drisapersen continuous arm and 13% (n=2) in the placebo group showed a ≥10% change in 6MWD.
4. Change from baseline in percent-predicted 6MWD
As 6MWD distance is influenced by age and development, analysis of the percent-predicted 6MWD that takes these factors into account were conducted (Henricson 2012). The percent-predicted 6MWD in DMD provides an estimate of performance relative to a healthy control population. An increase in the percent-predicted 6MWD is consistent with functional improvement. A treatment benefit (~6% difference) in favor of the continuous group over placebo was observed at both Week 25 (p=0.01) and Week 49 (p=0.05). The treatment difference over placebo with the intermittent regimen was 0.7% at Week 25 (p=0.78) and 5% at Week 49 (p=0.14).

Applicant's post-hoc analyses:

- MMRM analysis in subjects ≤7 years and > 7 years DMD:

The 6MWD generally improves in boys until the age of 7 years (Henricson 2012). The sponsor's analysis showed a treatment benefit of 77 m in >7 years at Week 25 compared to 22 m for boys ≤7 years for the continuous regimen.

Reviewer's Comment: The sample size is too small for any interpretable subgroup analysis of age in this study.
A post-hoc analysis, when combining the patients on continuous and intermittent shows a treatment difference in favor of drisapersen at each time point and statistically significant p of 0.05 at Week 49. Combining the treatments appears reasonable as they have the same drug exposure at Week 49. This may suggest treatment benefit at Week 48, given a more heterogeneous population.

Data Quality and Integrity – Reviewers' Assessment

There are no issues with data integrity.

Durability of Response

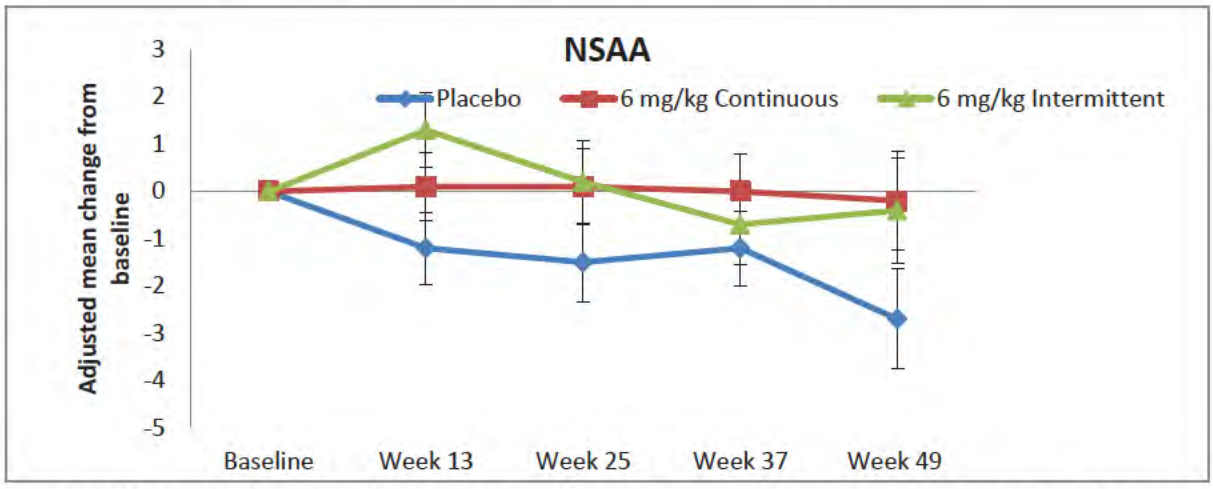
The treatment difference of 35 m for the continuous drisapersen regimen is maintained for the 49 weeks. This is probably because the placebo declines more at Week 49, such that the effect size remains the same at Week 48. For the continuous arm, the change from baseline in 6MWD declines after Week 25. It is unclear if this is due to the variability in the measure or a true treatment effect (Figure 9).

Efficacy Results – Secondary and other relevant endpoints

Applicant's key conclusions:

- None of the secondary endpoints showed a statistically significant treatment difference from placebo at Week 25 or 49 (Table 11, Week 49 summary). The frequency of worsening was variable and greater at Week 49 for most endpoints.
  - **Timed Function Tests (rise from floor, 10 m walk/run, and 4-stair climb/descent**):
    - The primary efficacy was supported by directionally favorable trends (non-significant) for the continuous group compared with placebo at Week 25 and 49 (Table 11) for Timed Function Tests
    - The intermittent regimen showed inconsistent and variable results for different timed functions tests at Week 25, but a trend favoring treatment at Week 49.
  - **NSAA**: Both treatment groups showed a favorable treatment difference relative to placebo in NSAA total scores, which was more pronounced at Week 49. The applicant states that when assessed against natural history, the differences between drisapersen and placebo at 49 weeks (continuous, 2.50; intermittent, 2.29) appear to be clinically meaningful.

Figure 5 MMRM Analysis of change from baseline for NSAA at each visit



- **Muscle Strength and Pulmonary Function Tests:** Small decrease from baseline in mean total muscle strength was observed in the continuous and intermittent treatment groups, compared to an increase in the placebo group at Week 25. At Week 49, small improvements in mean total muscle strength was observed in the continuous group, but the intermittent group muscle strength remained similar to that at baseline and overall still worse than placebo.
  The variable small changes in pulmonary function tests were similar across treatment groups.
- **PedsQL neuromuscular module questionnaire:** PedsQL showed a trend for improvement (higher scores indicating better quality of life) for the continuous group compared with placebo but not for the intermittent regimen (Table 10).

Table 10 Summary of Change from Baseline in PedsQL Total Score for both regimens

Assessor	Change from Baseline in PedsQL Total Score					
	Placebo (combined) (N=18)		6 mg/kg Drisapersen Continuous (N=18)		6 mg/kg Drisaperse n Intermittent	
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Child						
Baseline	18	80 (10)	18	78 (9)	16	82 (10)
Change from Baseline at Week 25	17	-4 (11)	17	4 (6)	15	-4 (10)
Change from Baseline at Week 49	17	0.4 (12)	18	7 (9)	13	-0.2 (8)
Parent						
Baseline	18	76 (11)	18	78 (11)	17	77 (14)
Change from Baseline at Week 25	18	-2 (5)	18	-0.9 (7)	16	-3 (6)
Change from Baseline at Week 49	18	-4 (12)	17	0.3 (7)	15	-2 (7)

Source: Adapted from Study DMD114117 Study Report
Note: The PedsQL total score ranges from 0 to 100, where higher scores indicate better health-related QoL

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Table 11: Secondary Endpoints at week 49

Treatment	n	Baseline Mean (SD)	Adj. Mean Δ from Baseline (SE) at Week 49	Treatment Difference	95% CI	p-value
NSAA Total Score						
Placebo Drisapersen 6 mg/kg/wk	17 18	26 (4.4) 27 (4.5)	-2.7 (1.1) -0.2 (1.0)	2.5	(-0.4, 5)	0.09
4 Stair Climb Ascent Velocity (stairs/s)						
Placebo Drisapersen 6 mg/kg/wk	17 18	3.5 (1.6) 3.1 (1.2)	1.0 (0.5) 0.8 (0.5)	-0.2	(-1.7, 1.2)	0.72
10 m Walk/Run Velocity (m/s)						
Placebo Drisapersen 6 mg/kg/wk	17 18	5 (0.8) 5 (1.2)	0.8 (0.3) 0.1 (0.3)	-0.7	(-1.3, 0.05)	0.07
Rise from Floor (s)c						
Placebo Drisapersen 6 mg/kg/wk	17 18	5 (1.0) 5 (1.7)	3.8 (1.2) 0.9 (1.2)	-3	(-6.2, 0.4)	0.08
4 Stair Climb Descent Velocity (stairs/s)						
Placebo Drisapersen 6 mg/kg/wk	17 18	3 (0.9) 3 (0.7)	0.3 (0.4) 0.0 (0.4)	-0.3	(-1.5, 0.8)	0.57
Muscle Strength Total Score (lbs)						
Placebo Drisapersen 6 mg/kg/wk	17 18	122 (28) 124 (23)	7.5 (5) 5.9 (5)	-1.6	(-15, 12)	0.82

Source: Adapted from Study DMD114117 Study Report

- **Serum Creatine Kinase:**

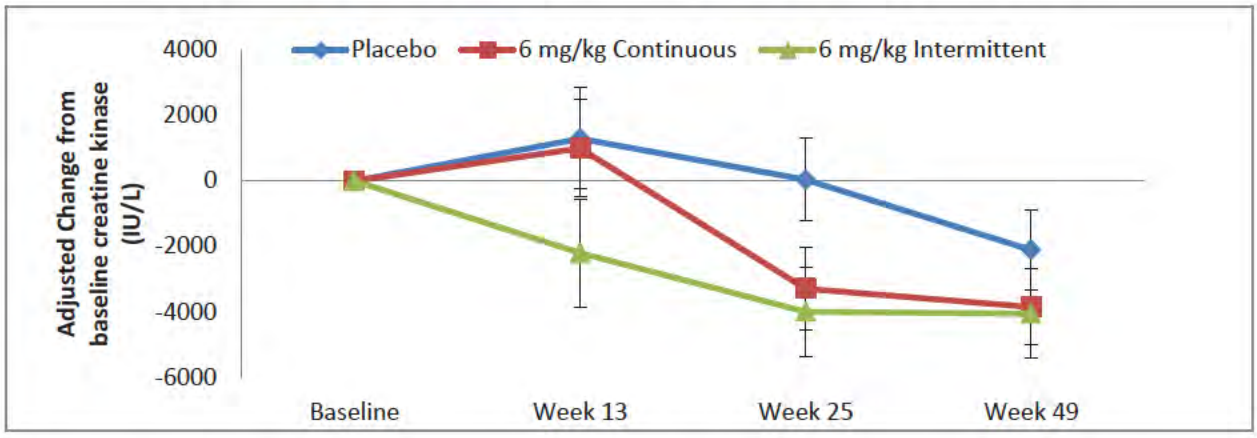
In early stages of DMD, increased muscle cell permeability (due to absence of dystrophin and increased membrane fragility) causes muscle specific enzymes such as creatine kinase to leak out of the cell. It is hypothesized that an improved membrane integrity induced by production of dystrophin can result in reduction of serum CK. At Week 25, there was a decline in CK for both drisapersen treatment arms compared with placebo, with a slightly greater treatment difference for the intermittent treatment group than the continuous treatment group. At Week 49, there was a trend of CK decline in both drisapersen arms compared with placebo, though the effect was smaller at Week 49 compared to Week 25 (Figure 6).

Table 12 Summary of MMRM Analysis of Change from Baseline in Creatine Kinase Serum Concentration (IU/L)

	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)
Baseline			
n	18	18	17
Mean (SD)	9525 (5415)	12267(6297)	14023 (7561)
Week 25			
n	18	17	16
Adjusted mean change (SE)	-62 (1290)	-3268 (1273)	-4093 (1390)
Adjusted mean difference vs. placebo		-3206	-4031
95% CI		(-6779, 366)	(-7844, -218)
p-value		0.08	0.04
Week 49			
n	18	18	14
Adjusted mean change (SE)	-2115 (1210)	-3851 (1169)	-4056 (1361)
Adjusted mean difference vs. placebo		-1736	-1941
95% CI		(-5090, 1618)	(-5601.86, 1720.15)
p-value		0.30	0.29

Source: page 88; check log transformed data

Figure 6 MMRM Analysis of change from baseline of serum creatine kinsase (IU/L)

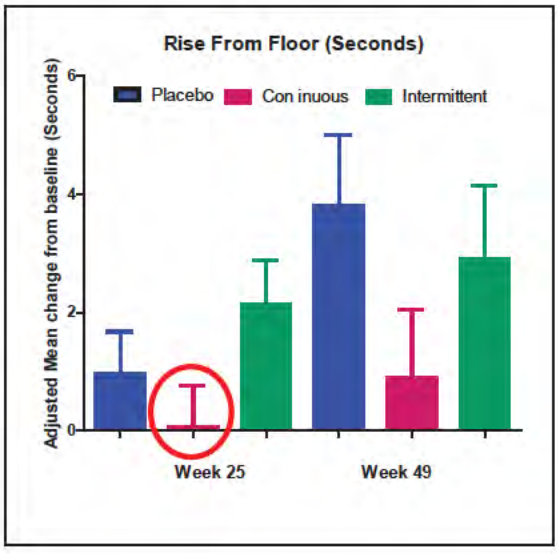


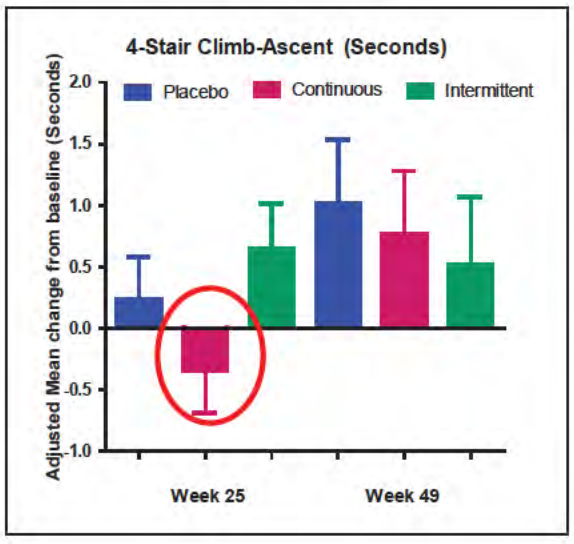
Reviewer's Assessment of Secondary Endpoints:

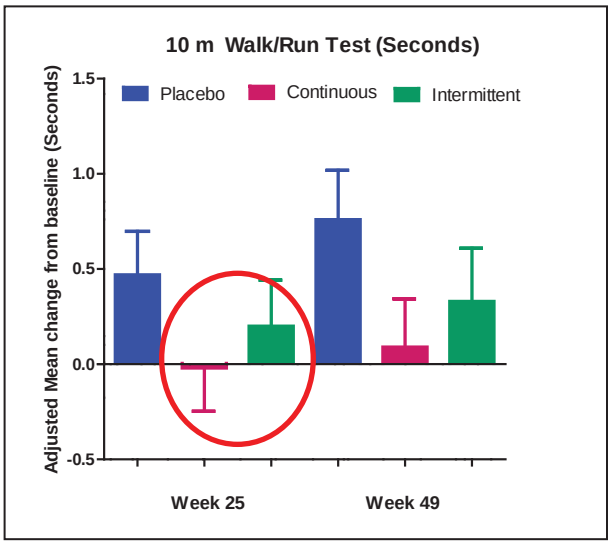
The secondary endpoints were not analyzed in a hierarchical manner and no endpoint was assigned as key endpoint, therefore the interpretation of these multiple endpoints is difficult.

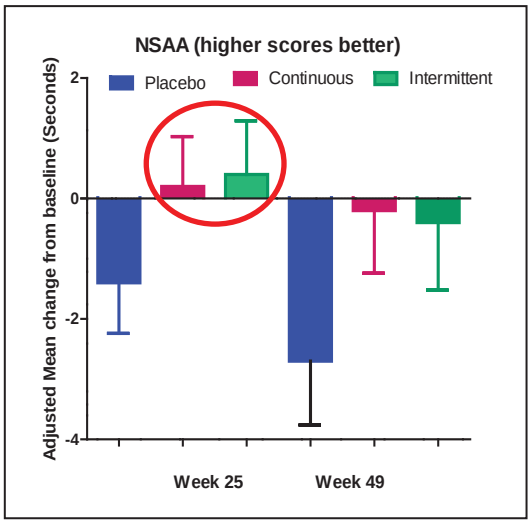
Timed Function Tests: The adjusted mean changes from baseline for the Timed Function tests and NSAA are graphically presented in Figure 7. NSAA scores stay stable for the 48 weeks for the continuous arm, but NSAA is shown to be stable in patients below the age of 7 years in the first year [+0.15 (SD 4.8)] (Mazzone 2013) and the continuous arm had higher percentage of subjects <7 years. All endpoints show a trend of improvement with the continuous regimen at Week 25 as shown by the circled data, which worsens at week 49, but remains better than placebo.

Figure 7 MMRM analysis of secondary endpoints at Week 25 and 49









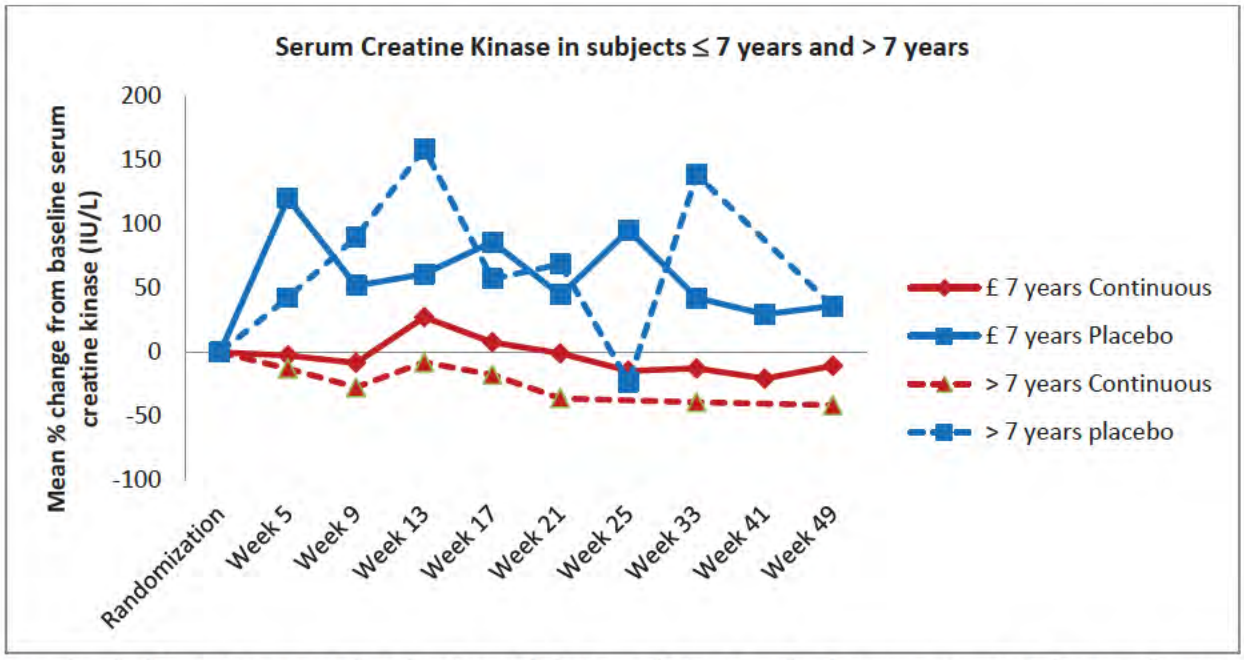
All Timed Function scores had 6 gradings (with 1 being unable to do the task and 6 easily do the task with least maneuvers). Very few subjects showed improvement in the grading scores (n ≤3) for Timed Function Tests, with the exception of 4-stair climb descent where 6 subjects showed improvement in the continuous group (e.g. holding one handrail vs. two handrails) compared to 2-3 subjects in the placebo and intermittent groups.

<u>Peds QL:</u> Parents perception of the problem was greater than that of the child's (e.g. More parent felt that their Child's leg hurts, child feels tired, child's back is stiff, wakes up tired etc.) at baseline. There was no systematic trend in the change from baseline at different visits. Many items on the PedQL neuromuscular module related to upper extremity weakness that are not likely to be impacted in an early ambulatory population (such as, My hands are weak, Hard to use my hands, long time to eat, hard to breath) and hence insensitive to overall meaningful change.

<u>Creatine Kinase:</u> Both drisapersen treatment arms showed a decline in CK compared to placebo, suggesting improvement in muscle cell integrity. However, this finding is associated with several confounders: CK levels are remarkably variable, with large within patient and between patient variability and are known to fluctuate day to day. It is affected by diurnal variation (increase during the day and decrease at night), exercise (reduced with inactivity), steroid use, and age (reduced with advancing age of the DMD boys as the muscle fibers are replaced with fat and fibrous tissue). There is no evidence of inactivity of the subjects from the Patient or Parent Reported Outcome measures, but the day to day activity level is unknown. The pain and discomfort from the injection site reactions could plausibly make the subjects less active.

Since CK peaks at ages 3-5 years and declines with age with levels reduced to 50% by age 7 years, I looked at the mean percent reduction of CK based on age. The following Figure shows the percent reduction in CK with treatment compared to placebo in subjects, ≤ 7 years and > 7 years. It is interesting to note that in this study Subjects >7 years had larger mean reduction in CK (-41%)

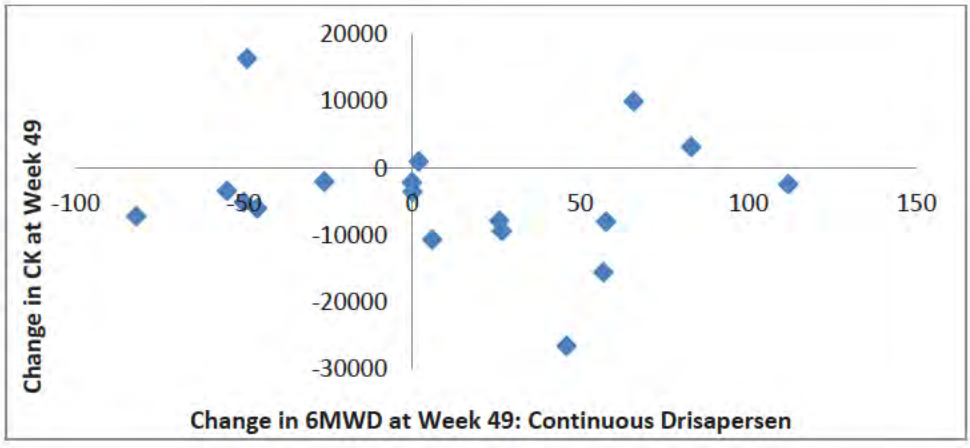
compared to subjects ≤ 7 years (-11%). This is consistent with a sub-group post-hoc analysis conducted by the applicant showing larger treatment benefit in older subjects. Given the small number of subjects and the large variability in the CK levels, this could be a chance finding and unreliable being post-hoc in nature. As a result of these confounding factors, a true treatment effect of a reduction in CK is difficult to discern.

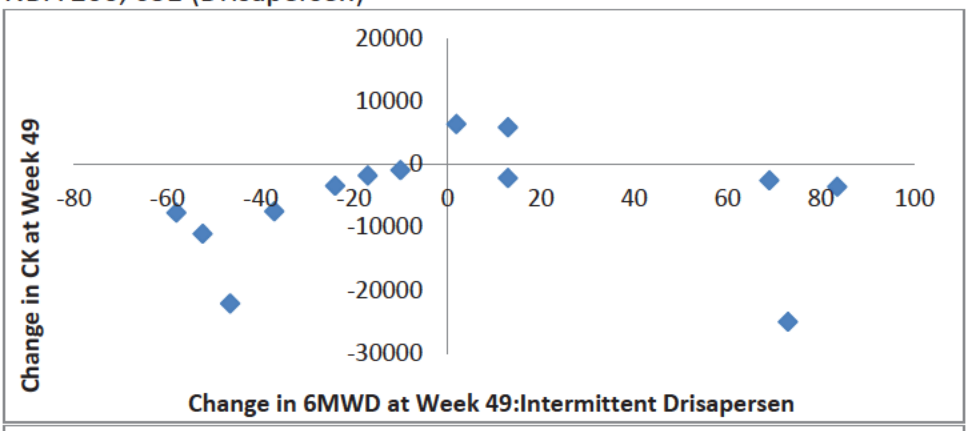


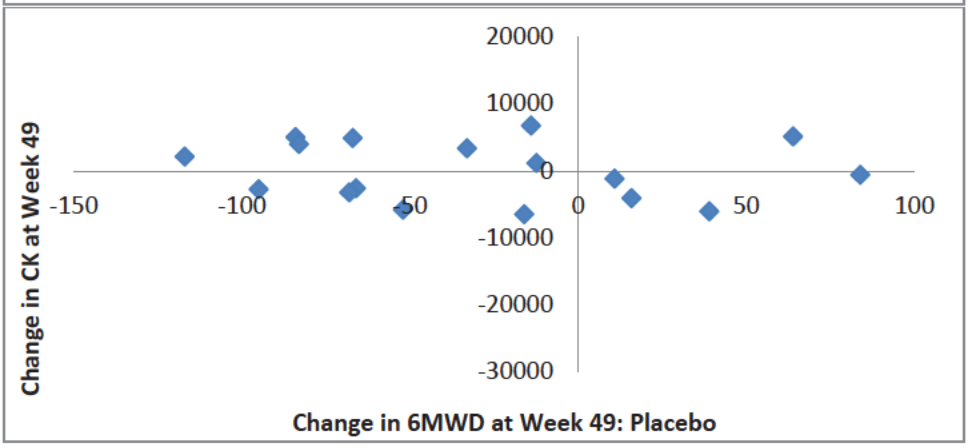
Note: Standard Deviations are not plotted as the variability is very high and overlaps between groups, including error bars constricts the graphs considerably

The following figures show the relationship of change in 6MWD and change in CK at Week 49.

- **No clear relationship between change in 6MWD and CK was observed at Week 49. The sample size of this study and the variability in the endpoints may preclude the ability to see any relationship in this study.**
- **There were a higher number of subjects showing a decline in CK in the drisapersen arm compared to the placebo arm.**







Dystrophin Measurements

Muscle biopsies from the tibialis anterior (TA) were obtained from most subjects and from the quadriceps of 5 subjects at baseline and at Week 25 and were analyzed using the following methods:

- Detection of exon 51 skipped mRNA by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)
- Detection of dystrophin protein expression by:
  - Qualitative Western blot analysis (WB).
  - Qualitative immunofluorescence assay (IFA), including percent change from baseline.

Dystrophin exon 51 skipped-mRNA measured by RT-PCR

Exon 51 skipped mRNA was detected in 2/18 (12%) of subjects in the continuous group and 5/17 (29%) of subjects in the intermittent group compared to 0/18 subjects in the placebo group at week 25, as shown by increased intensity. Increase in exon skip was detected more in quadriceps biopsy (3/4, 75%) compared to TA muscle biopsy (4/30, 13%). An increase in Exon 51 skip intensity was defined as: skip copies/ non skipped copies (Post treatment –pre-treatment/pre-treatment)*100>1%

OBP Reviewer Dr. Rao's Comment on methodology:

The gel-based PCR method used by the applicant was not quantitative because it involved a visual assessment of whether or not a band for the skipped mRNA product was observed. In addition to treated samples, baseline samples also showed exon 51 skipped band in 100% of

patients. See Appendix B for additional comments on the impact of revertants on the PCR data and the limitations of measuring dystrophin mRNA.

With nested RT-PCR there was no apparent increase in median PCR fragment intensity at week 25 when comparing to pre-treatment biopsies for the drisapersen continuous group (Table 13). The applicant attributes this variable finding to the low number of subjects. There is also large variability in the samples in each group that obscures any conclusion of increase in dystrophin.

Table 13 Intensity of exon 51 skipped dystrophin mRNA product by nested RT-PCR

	Exon Skip (a.u) Mean (SD)	
Study Treatment	Week 0	Week 25
Placebo	1.30 (1.01)	0.73 (0.41)
Weekly 6mg/kg	1.29 (2.42)	1.30 (1.50)
Intermittent 6mg/kg	1.72 (2.38)	2.60 (1.29)

OBP Reviewer Dr. Rao's Comment on methodology:

The nested PCR approach used should provide the applicant greater specificity for detecting their exon skipped product because a second set of primers and PCR run were used to amplify a narrower region of the same target mRNA. However, this method does not provide absolute quantitation because no reference standard was used to provide a calibration curve. The quantitation provided with arbitrary units is derived from the relative band density of other samples and does not necessarily reflect copy numbers of the skipped product. See Appendix B for additional comments and supporting references.

Dystrophin Protein by IFA:

With IFA, an increase in the mean dystrophin of the entire fiber population was observed in 9/15 (60%) subjects in the continuous group and 4/15 (54%) subjects in the intermittent group compared with only 1/17 (6%) subject in the placebo group. An increase in dystrophin was defined as an increase in mean membrane intensity of more than 4% at week 25 compared to pre-treatment biopsy.
The dystrophin intensity as measured by IFA is shown in Table 14. Mean percent change shows a 3% increase in dystrophin in the drisapersen continuous group compared to a 3% decrease in placebo. The Mean % change in the intermittent group was minimal.

Table 14 Dystrophin Intensity measurement by IFA (change from baseline at week 25)

	Muscle	Placebo (combined) (N=18)		6 mg/kg Drisapersen Continuous (N=18)		6 mg/kg Drisapersen Intermittent (N=17)	
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Non-Qualified Mean	Quadriceps	0	0	2	4.0 (6)	1	30.0 (0)*

Percentage Change (%)	Tibialis Anterior	17	-3 (5.3)	14	3.5 (6.9)	15	0.9 (8.6)
	All	17	-3.1 (5.3)	16	3.5 (6.7)	16	2.7 (11.0)
Non-Qualified Q90 Percentage Change (%)	Quadriceps	0	0	2	0.5 (6.4)	1	46.0 (0)
	Tibialis Anterior Muscle	17	-2.6 (7.1)	14	5.7 (10.0)	15	2.6 (10)
	All	17	-2.6 (7.1)	16	5.0 (9.6)	16	5.3 (14.3)

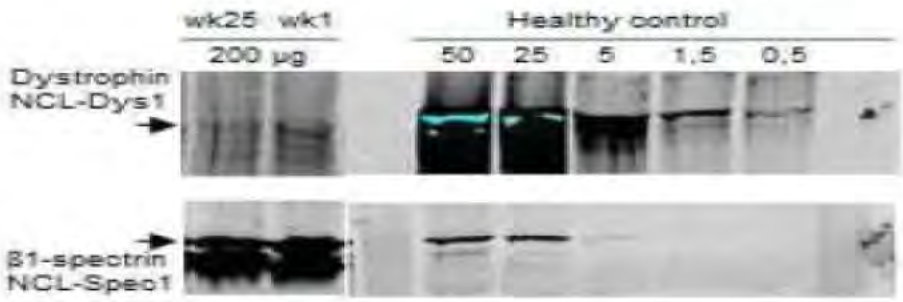
Q90 mean is the mean fiber membrane of the 10% brightest pixels in the membrane
*Subject 2101 had the highest drisapersen concentration in the quadriceps (130 µg/g) and also the highest dystrophin

OBP Reviewer Dr. Rao's Comment on methodology:

No correlative human endpoint data (e.g. muscle function or dystrophin-associated protein complex (DAPC) protein co-localization) has been presented to support the biological/clinical significance of this scoring. See Appendix B for additional comments.

Dystrophin Protein by Western Blot:

With WB, an increase in the mean dystrophin intensity was observed in 5/17 (29%) subjects in the continuous group and 5/16 (31%) subjects in the intermittent group compared with 0/17 (0%) subject in the placebo group. An increase was defined as: (post treatment –pre-treatment/pre-treatment)*100>30%.



For western blotting, the applicant included serial dilution of a healthy control sample on each gel, although comparison between pre- and post-treatment samples from the same patient was used for scoring. No comparison was made to the values between the healthy controls dystrophin and the test samples.

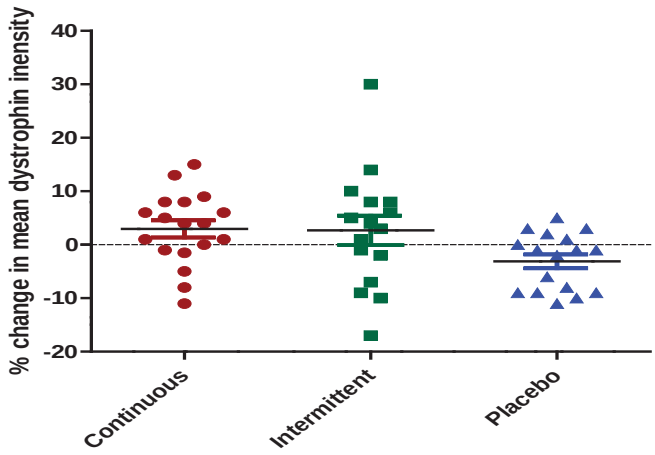
Reviewer's Assessment:

With WB, because baseline expression was at or below the lower limit of detection, the ratio of pre- to post-treatment expression in some cases was similar to "dividing by zero" leading to very high percent increases (up to 2500%) when the post-treatment expression level was only trace (about 1/3rd of 1% of normal levels). Some subjects similarly showed a large percent decrease in expression post-treatment likely due to small variations in measurements that were near zero.

In response to an FDA request about relative dystrophin in healthy tissue, the applicant provided data from five donor samples that show a range of 58-115% of the mean dystrophin value in quadriceps and 87-118% in tibialis anterior. Immunofluorescence results also showed similar variability and have been published by Beekman et al (2014). The applicant also states that the mean dystrophin intensity in the pre-treatment DMD biopsies can be estimated to be 0.3% of a particular control. In all but one post-treatment sample, the applicant estimates that the percentage of dystrophin detected was less than 1%, which they claim is the lower limit of detection for their assay.

The individual subject change in mean dystrophin intensity by IFA in the three groups is shown in Figure 8. There is a trend towards greater mean dystrophin intensity (by IFA) in the drisapersen treatment groups compared to placebo. There is an increase in dystrophin expression in higher number of subjects in the continuous drisapersen regimen. However, the small mean *increase* of 3-5% *from baseline* is unlikely to be biologically relevant.

Figure 8 % change in mean dystrophin intensity in individual subjects (IFA)



There was no clear relationship between the changes in dystrophin expression (as measured by IFA at Week 25) in the biopsy of the TA and the primary endpoint, the 6MWD at Week 25 or Week 49 (not shown).

A pharmacodynamic response was not detected in 30-40% treated patients. The applicant speculates that this could be related in part to: differences in characteristics of individual subjects (including but not limited to: stage and rate of disease progression; age; total weight; and muscle exposure on an mg/kg basis); treatment regimen, continuous vs. intermittent treatment (continuous showed a more consistent pharmacodynamic effect); and muscle group analyzed. TA muscle is relatively well preserved in the disease and therefore possibly less permeable to drug delivery and histological improvement difficult to detect in TA muscle. It is noteworthy that only 2 subjects in the continuous group and 2 subjects in the intermittent group showed an increase by all three methods. Dr. Rao's assessment of this overall conclusion is given below.

OBP Reviewer Dr. Rao's Comment:

A clear, consistent, and positive correlation between all three assays - IFA, WB, and Exon skip has not been established by the Applicant or published literature in the field using appropriate positive/negative controls (e.g. with BMD, DMD, healthy samples in the linear range). Taylor et al have reported a correlation between immunofluorescence-based intensity ratios of dystrophin/spectrin and dystrophin protein levels measured by Western blotting and normalized to actin. As presented by the applicant, the WB data is likely to be most reliable because a serial dilution with a healthy positive control was used for comparison and pre-treatment and post-treatment samples were run on the same gel in most instances. The IFA can suggest protein localization but is likely to be less meaningful for protein level quantitation. See Appendix B for additional comments on the caveats about each method.

Reviewer's overall assessment/discussion:

The strengths of Study DMD114117 are:

- **Primary endpoint (change from baseline 6MWD at Week 25) was positive for the continuous drisapersen treatment regimen with a treatment difference from placebo of 35 m (p=0.014) based on Applicant's pre-specified analysis.**
- **At Week 49 a similar *treatment difference* from placebo of 36 m was observed for the continuous drisapersen treatment regimen.**
- **Secondary endpoints measuring functions similar to walking [timed function tests (rise from floor, 10 m walk/run, and 4-stair climb/descent), and NSAA] also had directionally favorable trends for continuous treatment group at both Week 25 and 49, although not statistically significant. The secondary endpoints were not analyzed in a hierarchical manner, therefore the interpretation of these endpoints are difficult. Muscle strength did not favor drisapersen. Small changes in pulmonary function were similar across treatment groups.**
- **The percent reduction in serum creatinine kinase concentrations was 30-40% in the drisapersen treatment groups (p=0.08 and 0.04 at Week 25 for the continuous and intermittent regimen, respectively) compared to placebo.**

Some of the weaknesses of the study results are:

- **The intermittent drisapersen treatment group did not show a statistically significant treatment difference.**
    - **Both the continuous and the intermittent regimen have comparable total doses administered for the duration of the study.**
    - **Both regimens have identical plasma-concentration time profiles and should perform similarly (15% higher plasma AUC0-24 at Week 29 for the intermittent regimen).**
    - **The applicant states in the ISE that the difference between the intermittent and continuous treatment may have reflected the differences in age and advanced disease (time since diagnosis and duration of steroid treatment) in the intermittent group.**
        - **A post-hoc MMRM analysis with the two regimens combined showed a non-statistical difference from placebo of 20 m (p=0.12) at Week 25 and a treatment difference from placebo of 31 m (p=0.05) at Week 49. Given the**

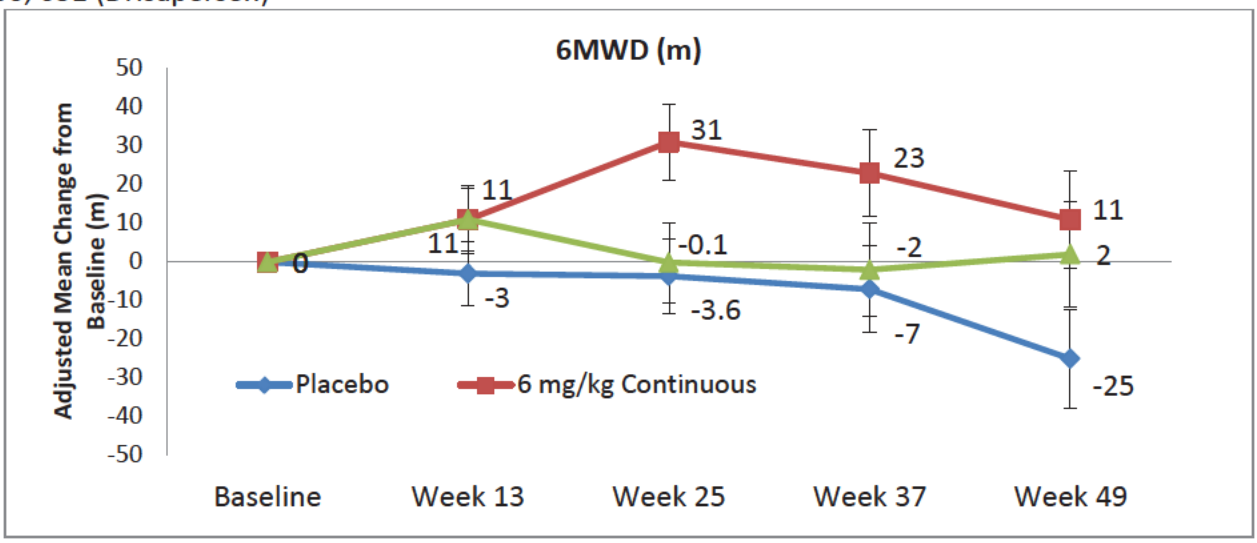
heterogeneity in the DMD population and in the study arms (as discussed below), a combining the two regimens suggest a treatment benefit with drisapersen at Week 49 and may be viewed as a strength of this study, even though post-hoc in nature.

- **Potential partial unblinding to the assigned treatment due to injection site reactions: Unblinding could affect the performance of 6MWD, which is considered an effort dependent endpoint. The incidence and the duration of injection site reactions was the largest for the 6 mg/kg continuous group and the least for placebo.**
- **Dystrophin expression increased *very slightly from baseline* in drisapersen treated subjects compared to placebo in some subjects**

Discussion on primary endpoint 6MWD:

- **The drisapersen continuous group appeared to consist of boys with less functional impairment compared to other treatment arms as discussed on page 37.**
- **The continuous group shows an initial increase in *change from baseline* 6MWD at Week 13 and 25 and declined in subsequent weeks.**
-
- Figure 9 **shows the magnitude of change from baseline at each time point. Improvement at Week 13 with both regimens could also be consistent with unblinding bias or the natural variation in the population. The change from baseline in the intermittent group on the other hand showed stability in the 48 weeks. The natural history studies also show that some subjects can remain stable in their ability to walk as assessed by 6MWD for 1-2 years before they begin to decline. It is uncertain if the improvement at Week 25 followed by decline is due to variability or a treatment effect.**
  The applicant includes in their discussion of the study: "It is not known whether the apparent increase and decline in 6MWD in the continuous regimen versus the relative stability in the intermittent regimen is due to the drug effect or simply a result of natural variation in the population. Longer term treatment data and data from a larger number of subjects are needed to determine such an effect (page 134 of study report)."

Figure 9 MMRM Analysis of change from baseline in 6MWD



- **The natural history data as published in the literature also show variation on the reported decline in 6MWD (-22 to - 58m per year based on literature). Given these concerns the benefit seen with the continuous drisapersen treatment group at week 25 may be a result of population variation (as shown in differences in disease characteristics at baseline), especially given that the intermittent regimen had a different treatment effect, as also speculated by the applicant. In DMD, fatigue and falls have been shown to be critical factors of six-minute walk distance variability. Motivation, concentration are other factors of variability for an effort dependent endpoint. The injection site reactions experienced by the subjects could add to the bias as well. Recent published data (Bello 2105) suggest that there are genetic modifiers that can either be associated with milder (LTBP4) of more progressive phenotypes (osteopontin gene-SPP1).**

<u>On secondary endpoints:</u>

- **The baseline imbalances in the continuous group would affect the secondary endpoints in a similar way.**
- **There was a greater decline in CK concentrations in the drisapersen intermittent treatment group compared to the continuous group, which is inconsistent with what was seen with 6MWD, NSAA, Rise From Floor, 10 m walk/run, nevertheless there was greater reduction in CK in drisapersen treated groups compared to placebo. There is a lot of variability in the assessment of CK that this may not be as concerning.**

6.2. Study DMD 114876

6.2.1. Study Design

Overview and Objective

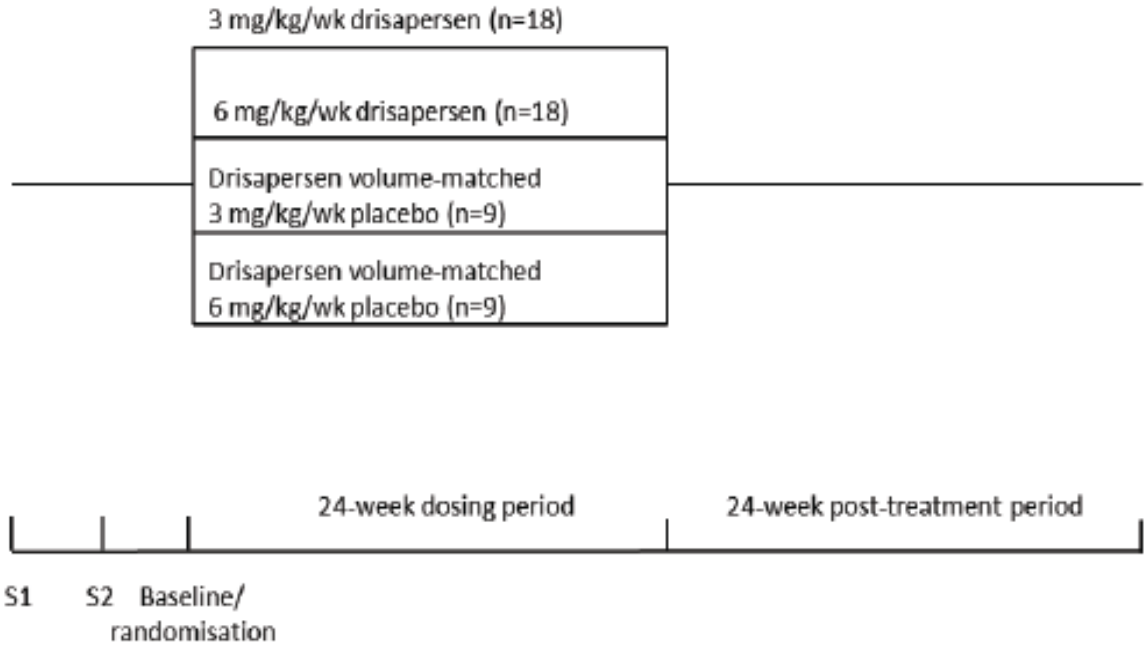
The primary objective of this study was to assess efficacy of 2 different doses of subcutaneous drisapersen versus placebo administered over 24 weeks in ambulant subjects with DMD. The

Clinical Review (Efficacy)
NDA 206, 031 (Drisapersen)
secondary objectives were to assess safety, tolerability, PK, dystrophin half-life and persistence of efficacy at 48 weeks.
Studied period**:** 26 Oct 2011-4 Nov 2103
Study center(s): 13 centers in the United States

Trial Design

The study design was similar to study DMD114117, with the exception of a treatment period of only 24 weeks: and included 2 doses. The study design schematic is given in Figure 10. Screen 1 and 2 were 4 and 2 weeks prior to randomization, respectively. After the last dose of drisapersen /placebo, subjects continued into a 24 week post-treatment period off drug, after which subjects had the option to enter an open label extension study with drug treatment or have a 20 week follow up if the patient did not enter the open label extension. There were no life style restrictions during the study.

Figure 10 Study design schematic for Study DMD114876



Population: N=54 ambulant DMD boys with a mutation corrected by exon 51 skipping. The sample size is not based on statistical considerations.
Key Inclusion Criteria: Same as Study DMD114117.
Amendment 3 of the protocol changed the rise time from floor to ≤15 seconds from ≤7 seconds (without aids/orthoses)
Key Exclusion Criteria: Same as Study DMD114117 with the addition of:

- Baseline platelet count below the Lower Limit of Normal
- aPPT above the Upper Limit of Normal
- History of significant medical disorder which may confound the interpretation of either efficacy or safety data e.g. inflammatory disease

Dosing Regimen:

Subjects were randomized to the following in 2:2:1:1 ratio using Interactive Voice Response System:

- 3 mg/kg/week SC drisapersen for 24 weeks (N=18)
- 6 mg/kg/week SC drisapersen for 24 weeks (N=18)
- 3 mg/kg/week SC placebo for 24 weeks (N=9)
- 6 mg/kg/week SC placebo for 24 weeks (N=9)

Injections were rotated on different sites in the abdomen, back, arm and thighs. Volume of placebo was matched to the dose to maintain the blind and limit bias. The different doses were not fully blinded, but administered by personnel not involved in efficacy assessments (Subjects on 3 mg received a lower volume of injection). Blinding of the study was maintained until completion of Week 24 assessment. After the 24 week analysis, the results were not communicated to the investigators or monitors.

The purpose of the post-treatment phase was to model the half-life of dystrophin, assess maintenance of response, and provided information about resolution of adverse event and laboratory abnormalities following cessation of treatment.

Study Endpoints

The following efficacy assessments were assessed at screening (1 and 2), randomization, week 24 or early withdrawal and week 48 or follow-up unless specified otherwise.

The primary efficacy endpoint were the same as Study DMD114117: 6MWD at week 24

6MWD

The secondary efficacy endpoints were also the same with the exception of the following additional secondary endpoints:

- The pulmonary function tests included (FEV_1, FVC, PCF, PF, sniff pressure test)
  (Study DMD114117 had MIP and MEP instead of sniff pressure test)
- Clinical global Impression of Improvement
  The CGI-I was measured on a 7-point Likert scale (1 = 'very much improved', 2 = 'much improved', 3 = 'minimally improved', 4 = 'no change', 5 = 'minimally worse', 6 = 'much worse', 7 = 'very much worse').
- Functional Outcome Assessments:
  - Physician Assessment of Daily Living [the ability of the subject to perform usual day-to-day activities (e.g., general health, mobility, general daily activities)]
  - Functional Outcomes Survey by Family/caregiver

Most secondary endpoints were not assessed at week 48, with the exception of CGI and Functional Outcomes Survey. Pediatric Quality of Life was not a secondary endpoint in this study.

Other:

- Dystrophin expression (muscle biopsies from tibialis anterior) was done at baseline, Week 24 in all subjects, and an randomly assigned third biopsy at Week 12 or Week 36 visit
- mRNA production in muscle tissue.
- Drisapersen in muscle tissue

Exploratory endpoints: at baseline, week 24 and 48

- A set of T1-weighted (T1w) images to assess the level of fat infiltration and quantified according to the Mercuri scale and apparent fat fraction and set of T2-weighted (T2w) images to assess the combined effects of fat infiltration and edema and quantified according to the normalized T2-weighted signal intensity and T2 relaxation rate in skeletal muscle in the thigh as determined by structural MRI measures over time (optional for subjects). MRI scans of the mid-thigh were performed.
- 6MWD at week 48

Statistical Analysis Plan

Efficacy parameters were analyzed using the ITT population (same definition as Study DMD114117).

Primary Endpoint Analyses: Primary assessment of efficacy data was conducted using MMRM at week 24 on the Observed case (OC) data. The analysis was similar to StudyDMD114117. Due to the two different doses, Type 1 error rate was preserved by utilizing a hierarchical approach, with 6 mg being assessed first. If no statistically significant difference was observed between the 6 mg/kg dose and placebo, then further analyses of the 3mg/kg dose were considered exploratory.
Sensitivity Analyses for primary endpoint: Same as Study DMD114117
Secondary endpoint analyses:

- For continuous endpoints ANCOVA analyses on OC data, with fixed terms of treatment center and baseline score (For Study DMD114117 MMRM analysis on OC data was used)
- Kaplan-Meir and Log rank test on time to event endpoints such as loss of ambulation

Protocol Amendments

Protocol amendments related to efficacy assessments are summarized below.

Amendment 1 ((09 Sep 2011)	No significant change that would affect efficacy assessments
Amendment 2 (27 April 2012)	Muscle Biopsies to be conducted after functional efficacy assessments, no more than seven days after the scheduled visit.
Amendment 3 (09 Aug 2012)	Able to rise from floor in ≤15 seconds (without aids/orthoses) at Screening Visit 1 and Screening Visit 2 instead of in ≤7 seconds (without aids/orthoses) in the original protocol. Sponsor's rationale: A review of blinded data from other ongoing Drisapersen studies suggests an increase to the RFF inclusion criteria to include subjects with RFF up to 15 seconds at screening should not increase the likelihood of subjects losing ambulation during the study or increase variability in the 6MWD significantly. It will enable more potential subjects in this rare disease to be eligible.

Reviewer's Comment: The impact of including subjects with rise from floor in ≤15 seconds will be discussed in the results section.

Management of clinical data was performed in accordance with GSK standards. The study was conducted in accordance with ICH GCP (ICH E3 and E6) and applicable country-specific requirements. Written commitments were obtained from investigators to comply with GCP. Study was conducted with written informed consent from subjects and their parents.

6.2.2. Study Results

Patient Disposition

All 51 subjects completed the study. The distribution of subjects in each group is given in Table 15.

Protocol Violations/Deviations

A total of 4 subjects had major protocol violations. (Table 15)

Table 15 Protocol Violations

	Number (%) of Subjects			
	Placebo (combined) (N=16)	Drisapersen 3 mg/kg/week (N=17)	Drisapersen 6 mg/kg/week (N=18)	Total (N=51)
Intent-to-Treat Population	16 (100)	17 (100)	18 (100)	51 (100)
Per Protocol Population	15 (94)	14 (82)	18 (100)	47 (92)
Total Number of Subjects with Major	1 (6)	3 (18)	0	4 (8)
Subject 000226 had history of aortic root dilatation	0	2 (12)	0	2 (4)
Subject 000176 had idebenone until Day 3	0	1 (6)	0	1 (2)
Subject 000176 missed 5 doses (<80% compliant)**	0	1 (6)	0	1 (2)
Subject 000201 with history of cardiomyopathy being managed with lisnopril	0	1 (6)	0	1 (2)
Subject 000158 Treatment blind broken*	1 (6)	0	0	1 (2)

*due to ER visit for flu-like symptoms (a member of the investigators staff was unblinded). The family stated the ER staff did not unblind them to the treatment assignment; however this could not be positively confirmed.
**protocol required follow-up for lab values falling outside the study reference ranges (proteinuria)

Reviewer's Comment: The Protocol deviations were all in the 3 mg/kg group, except one in the placebo group. The impact of this subject is shown in the Sensitivity analysis.

Table of Demographic Characteristics

Baseline demographic characteristics were similar across treatment groups with the drisapersen 6 mg/kg group having slightly lower median age (Table 16).

Table 16 Summary of demographic characteristics

	Number (%) of Subjects			
	Placebo (combined) (N=16)	Drisapersen 3 mg/kg/week (N=17)	Drisapersen 6 mg/kg/week (N=18)	Total (N=51)
Age (yrs)				
Mean	8.0	7.8	7.6	7.8
SD	1.79	1.91	2.70	2.15
Median	8.0	8.0	6.5	8.0
Min., Max.	5, 11	5, 11	5, 13	5,13
Ethnicity				
Hispanic/Latino	4 (25)	1 (6)	1 (6)	6 (12)
Not Hispanic/Latino	12 (75)	16 (94)	17 (94)	45 (88)
Race				
African American/African Heritage	1 (6)	1(6)	1(6)	3 (6)
Asian – East Asian Heritage	0	0	1 (6)	1 (2)
Asian – South East Asian Heritage	0	0	1 (6)	1 (2)
White – White/Caucasian/European Heritage	14 (88)	16 (94)	15 (83)	45 (88)
Mixed Race	1 (6)	0	0	1 (2)
Height (cm)				
Mean (SD)	123 (6)	120(8)	120 (14)	NA
Median	122	120	117	NA
Min., Max.	109, 133	102, 130	101, 146	NA
Weight (kg)				
Mean (SD)	30 (9)	29 (6)	30 (13)	NA
Median	28	31	25	NA
Min., Max.	19, 51	18, 42	17, 64	NA

Source: Study report DMD114876, page 69

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Other baseline characteristics are summarized in Table 17. There were some differences in corticosteroid use and duration across treatment groups. The subjects in the 6 mg/kg group had the shortest time since first symptoms.

Table 17 Summary of disease characteristics

	Number (%) of Subjects			
	Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)	Total (N=51)
Time Since First Symptoms (months)[a]				
Mean (SD)	57 (30)	67 (27)	59 (30)	61 (29)
Median	60	60	60	60
Min., Max	13, 112	25, 124	8, 112	8, 124
Time Since Diagnosis (months)[a]				
Mean (SD)	46 (30)	47 (26)	46 (27)	46.4 (27)
Median	45	38	46	43
Min., Max.	9, 111	13, 108	7, 96	7, 111
Time Since First Corticosteroid Taken (months)[a]				
Mean (SD)	37 (24)	33 (16)	27 (22.51)	32 (21)
Median	39	28	19	27
Min., Max.	7, 85	8, 58	6, 81	6, 85
Corticosteroid Regimen[b]				
Continuous	15 (94)	15 (88)	18 (100)	48 (94)
Intermittent	1 (6)	2 (12)	0	3 (6)
6MWD (m)				
Mean	416	415	396	NA
(SD)	(57)	(58)	(61)	NA
EXON Mutation, n (%)				
DMD 43-50 deletion	0	0	1 (6)	1 (2)
DMD 45-50 deletion	9 (56)	4 (24)	4 (22)	17 (33)
DMD 47-50 deletion	1 (6)	0	0	1 (2)
DMD 48-50 deletion	4 (25)	5 (29)	3 (17)	12 (24)
DMD 49-50 deletion	1 (6)	5 (29)	6 (33)	12 (24)
DMD 50 deletion	1 (6)	2 (12)	1 (6)	4 (8)
DMD 52 deletion	0	1 (6)	3 (17)	4 (8)

Source: Study report DMD11487, page 70

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Concomitant medication usage was similar across groups. Fifty subjects (98%) were >80% compliant. Duration of exposure was similar across groups.

Reviewer's Assessment of Baseline characteristics:

The baseline assessment of functional capabilities in each treatment group is summarized below:

Baseline Factors	Continuous 6mg/kg/week	Continuous 3 mg/kg/week	Placebo
Age <7 years	50%	24%	25%
6MWD>400m	44%	52%	75%
Rise From Floor <4 secs	28%	35%	50%
On continuous regimen	100%	88%	94%
Other factors: Ability to jump with both feet up at the same time	61%	82%	81%
Ability to hop with clearing foot and heel from floor	38%	58%	56%
Ability to rise from floor without gower's maneuver	0%	0%	13%

A higher percentage of subjects were of age <7 years in the 6 mg/kg/week group that tend to improve in function. The 3 mg/kg/week group has some factors that could suggest lower functional capability compared to the placebo group, such as percent of subjects with Rise from Floor<4s and 6MWD>400m.

Efficacy Results - Primary Endpoint

Applicant's analysis:

In the primary efficacy MMRM analysis of change from baseline in 6MWD (m) at Week 24, a non-statistically significant (p=0.07) difference of 27 m was observed for the 6mg/kg group compared to placebo. The difference observed at 24 weeks was maintained at 48 weeks (28 m) during the drug free period and so was the change from baseline 6MWD. A decrease of baseline of 11-13 m was observed at week 24 and 48 in the placebo group. The applicant considers this modest decrease consistent with the range expected in an early ambulant population (Table 18).

Table 18 Summary of Repeated Measures Analysis of Change from Baseline in 6MWD (m) by Visit

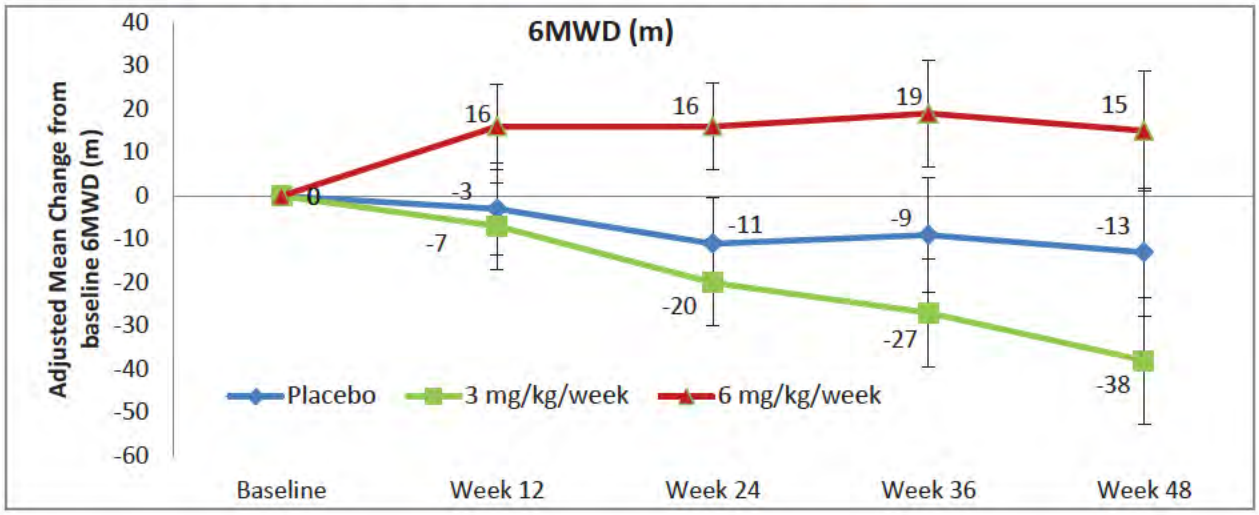
	Placebo (combined) (N=16)	Drisapersen 3 mg/kg/week (N=17)	Drisapersen 6 mg/kg/week (N=18)
Baseline n Mean (SD)	 16 416 (57)	 17 415 (58)	 18 396 (61)
Week 24 primary analysis n Adjusted mean change (SE) Adjusted mean difference vs. placebo 95% CI p-value	 16 -11(11)	 17 -20 (10) -9 (-39, 21) 0.55	 18 16 (10) 27 (-2, 56) 0.07

Week 48 (end of post treatment period)			
n	15	17	18
Adjusted mean change (SE)	-13 (15)	-38 (14)	15 (14)
Adjusted mean difference vs. placebo		-25	28
95% CI		(-66, 17)	(-13, 69)
p-value		0.24	0.18

Source: DMD114876 study report, Page 74

None of covariates (visit, treatment, center grouping, baseline 6MWD, age, and age group) tested were significant at the 5% or 10% level.

Figure 11 MMRM analysis of change from baseline at week 24 and 48



Applicant's Sensitivity Analysis:

Sensitivity analyses with the ITT population show similar magnitude of treatment difference. A lower magnitude of treatment difference was observed with the PP population after removing the protocol violators.

Drisapersen 6 mg/kg/week Sensitivity Analyses: Change from Baseline in 6MWD (m) at Week 24

Analysis	Population	Dataset	Treatment Difference	95% CI	P-value
Drisapersen 6 mg/kg/wk					
MMRM	ITT	OC	27	(-2, 56)	0.07
MMRM	PP	OC	19	(-11, 50)	0.21
ANCOVA	ITT	OC	27	(-3, 57)	0.07
ANCOVA	ITT	LOCF	27	(-3, 57)	0.07

Source: DMD114876 study report, page 76

Reviewer's Comment: One subject in the placebo group made this difference. The subject # 158 in the placebo group had a 6MWD decline of 70 m and had the second largest decline in the placebo group (-127m was the largest decline at Week 24). This subject had his treatment unblinded due to an ER visit.

Clinical Review (Efficacy)
NDA 206, 031 (Drisapersen)

Additional Analyses on 6MWD:

1. Change from baseline in 6MWD by response category
   Response was measured as the percentage of subjects that achieved a fixed change in 6MWD (≥-10%, ≥0%, ≥10%, ≥20% and≥30% change). Subjects could be included in more than 1 category. At week 24, 3 subjects showed ≥10% change in the 6 mg/kg/week group and none in the placebo. At 48 weeks 8 subjects showed ≥10% change in the 6 mg/kg/week group and one in the placebo group. 8 subjects in the 6 mg/kg/week group and 2 subjects in the placebo group had ≥30 m change in 6MWD.
2. Change from baseline in percent-predicted 6MWD
   As 6MWD distance is influenced by age and development, analysis of the percent-predicted 6MWD that takes these factors into account were conducted (Henricson 2012). A treatment benefit (~5% difference) in favor of the 6 mg/kg group over placebo was observed at Week 24 (p=0.05). A treatment difference of -1.5% was observed with the 3mg/kg group at Week 24.
3. Number of Falls: Number of falls were similar across treatment groups
4. Loss of ambulation: No subject in any group lost ambulation during the study.

Post-hoc analyses on 6MWD:

By age group (≤7 and > 7 years): No statistically significant treatment differences were noted for either drisapersen treatment group compared to placebo, but the mean treatment difference from placebo was 31m in subjects ≤ 7years.

Table 19 Summary of Repeated Measures Analysis of Change from Baseline in 6MWD by Visit Split by Age Group

	Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)
≤ 7 years at baseline			
Baseline			
n	6	8	10
Mean (SD)	445 (63)	439 (50)	385 (60)
Week 24			
n	6	8	10
Adjusted mean change (SE)	-14 (21)	-22(18)	17 (17)
Adjusted mean difference vs. placebo		-8	31
95% CI		(-66, 49)	(-29, 90)
p-value		0.77	0.29
>7 years at baseline			
Baseline			
n	10	9	8
Mean (SD)	399 (48)	394 (59)	410 (62)
Week 24			
n	10	9	8
Adjusted mean change (SE)	-16 (12)	-16 (12)	12 (14)
Adjusted mean difference vs. placebo	NA	-0.14	28
95% CI	NA	(-39, 39)	(-9, 65)
p-value	NA	0.99	0.13

Source: DMD114876 study report, page 82

Reviewer's Comment:

- **This post-hoc analysis is un-interpretable due to small number of subjects. It is noteworthy, that Study DMD114117 showed larger treatment difference in subjects >7 years.**
- **In this study the impact of including subjects with Rise from Floor of ≤15 seconds was not significant as only two subjects were enrolled with RT of >7 seconds (one in the 3 mg/kg group with RT of 10 seconds and one in the 6 mg/kg group with RT of 12 seconds.**

Data Quality and Integrity - Reviewers' Assessment

There are no data integrity issues.

Dose/Dose Response

There was no clear dose-response established. It is unclear why the 3 mg/kg performed worse than placebo and may suggest random noise.

Efficacy Results - Secondary and other relevant endpoints

Applicant's Analysis:

Timed Function tests, NSAA, Muscle Strength, and Pulmonary Function:
At Week 24, changes on timed function tests (rise from floor, 10 m walk/run, 4-stair climb) were small (generally less than one second). Rise from floor, 4-stair climb, muscle strength and NSAA favored the 3mg/kg group more (Table 20). The number of subjects improving or worsening was similar for each test or showed greater number of subjects worsening from baseline in the Rise from floor and 10 m walk/run in the 6 mg/kg group. Changes in pulmonary function measures were small and variable across both treatment groups. The secondary endpoints were not measured in the post treatment period at Week 48.

Table 20 Secondary Endpoints, unadjusted mean baseline (SD), adjusted treatment difference (95% CI), p-value at Week 24

	Placebo (n=16)	3mg/kg/week (n=17)		6 mg/kg/week (n=18)	
Rise from Floor (s)					
Baseline (SD) Adj. treatment difference	4.49 (1.61)	4.96 (2.21) 0.39	p=0.69	5.19(2.46) 0.83	p=0.38
10 m walk/run (s)					
Baseline (SD) Adj. treatment difference	5.12 (1.35)	4.97 (1.17) 0.56	p=0.05	5.38 (1.35) 0.04	p=0.89
4 step ascent (s)					
Baseline (SD) Adj. treatment difference	3.53 (1.80)	3.14 (1.29) 0.002	p=0.99	4.60 (3.17) -0.80	p=0.06
4 step descent (s)					

Baseline (SD) Adj. treatment difference	2.94 (1.2)	3.34 (2.2) -0.68	p=0.31	4.05 (2.3) -0.41	p=0.52
NSAA (total score)					
Baseline (SD) Adj. treatment difference	26.5 (5.0)	26.5 (5.2) -0.49	p=0.68	24.6 (5.7) -0.18	p=0.87
Muscle Strength					
Baseline (SD) Adj. treatment difference	128.4	129.2 (22) 2.36	p=0.72	125.2 (43) 0.82	p=0.89

Source: Adapted from applicant's analysis.

Creatine Kinase:

A decline in CK was observed from baseline at Week 24 for both active treatment arms compared to placebo, with a greater treatment difference for the drisapersen 6 mg/kg/week group than for the drisapersen 3 mg/kg/week group. However at the end of the post-treatment period at week 48, a continued decrease in CK compared with placebo was observed for the drisapersen 3 mg/kg/week arm, but not for the drisapersen 6 mg/kg/week arm (Table 21).

Table 21 **Summary of Repeated Measures Analysis of Change from Baseline in Creatine Kinase Serum Concentration (IU/L) by Visit**

	Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)
Baseline			
n	16	15	18
Mean (SD)	12715 (5606)	14553 (7810)	13059 (8288)
Week 24			
n	16	17	17
Adjusted mean change (SE)	-3479 (1230)	-4079 (1173)	-4537 (1189)
Adjusted mean difference vs. placebo	NA	-600	-1058
95% CI	NA	(-4121, 2920)	(-4456, 2340)
p-value	NA	0.73	0.53
Week 48			
n	15	17	18
Adjusted mean change (SE)	-2783 (1249)	-4838 (1171)	-2616 (1149)
Adjusted mean difference vs. placebo	NA	-2055	167
95% CI	NA	(-5587, 1478)	(-3209, 3544)
p-value	NA	0.25	0.92

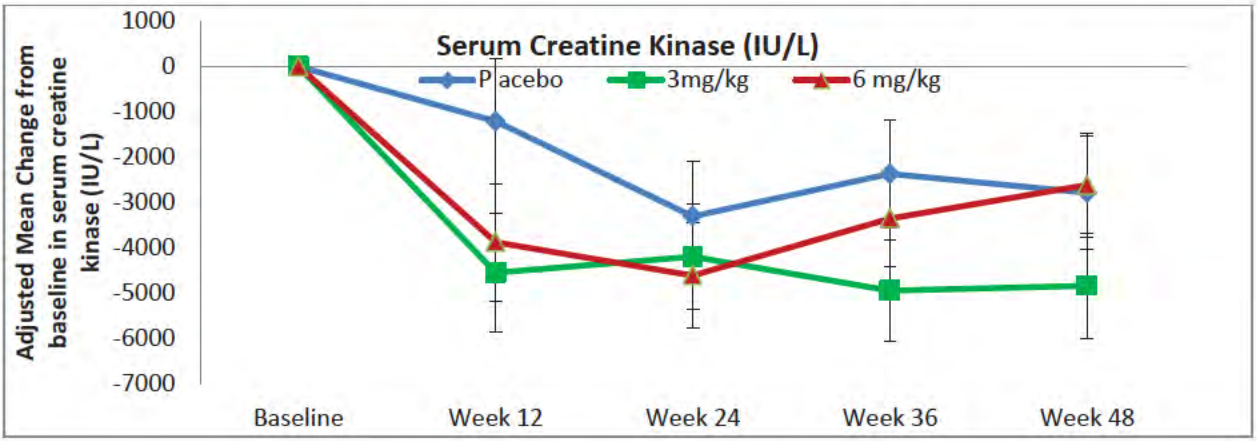
Source: DMD114876 Study Report, page 96

Reviewer's Comment:

The 3 mg/kg dose group continues decline during the drug free period up to Week 48, but the 6 mg/kg dose group shows an increase in CK. The reliability of this finding is unclear. Exercise induced changes in CK are difficult to ascertain, nevertheless CK declined in drisapersen treated groups.

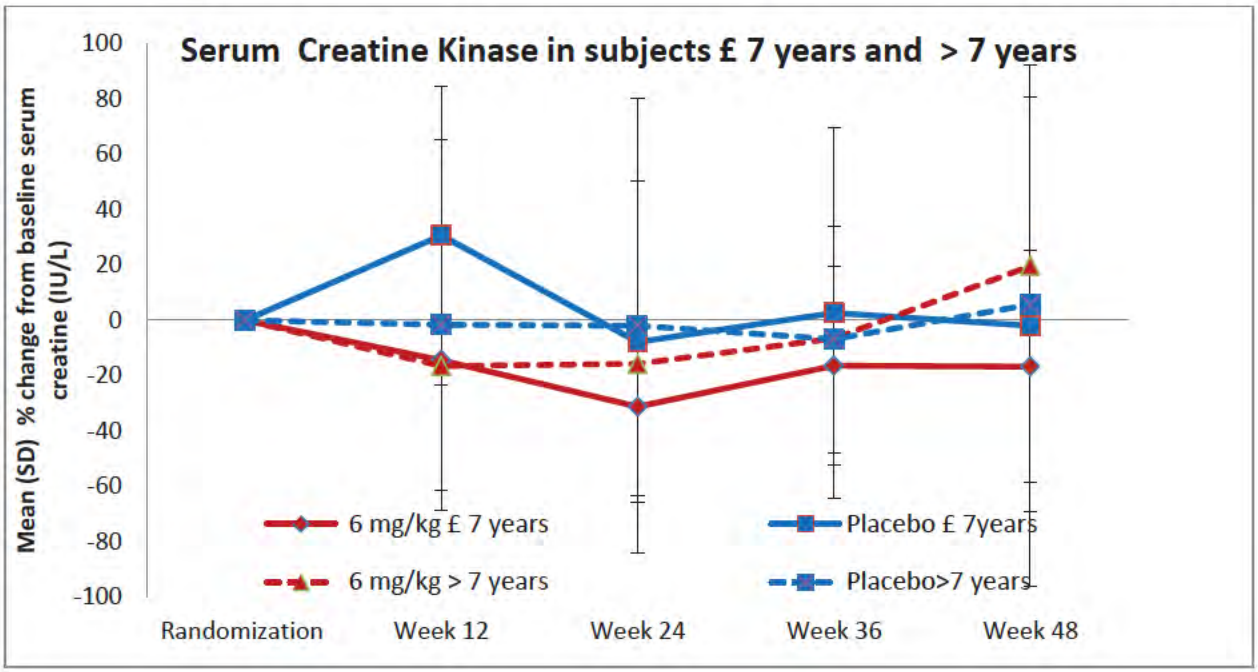
Clinical Review (Efficacy)
NDA 206, 031 (Drisapersen)
Figure 12 shows the time course of adjusted mean change in CK.

Figure 12: MMRM analysis of change from baseline in serum creatine kinase concentrations



In a post-hoc subgroup analysis, subjects ≤7 years showed greater treatment difference on 6MWD compared to subjects >7 years, therefore I looked at percent reduction of CK in these subgroups. Consistent with the finding with 6MWD, the reduction in CK was greater in subjects ≤7 years compared to subjects >7 years (Figure 13). The sample size is small and variability is large, therefore these findings should be interpreted with caution.

Figure 13 Change from baseline serum creatine kinase in subjects ages ≤7 years and > 7 years



CGI, Functional Outcome Survey, Physician assessment of daily living:

No differences among treatment groups were observed on CGI-I and Functional Outcome Survey (29 assessed by parents (including general health, mobility, physical activities, hand dexterity and use of assistive devices domains). For CGI-I, there were 11% (2/18) with "much improved" or "very much improved" response in the 6 mg/kg group and 14% (2/14 responders in the placebo group. For the Functional outcome survey, some trends in favor of the 6 mg/kg group were observed for "general health" and "hand dexterity" compared to placebo and 3 mg/kg group. On some questions in the functional outcome survey, placebo and 3 mg/kg were rated as "improvement".

MRI:

The applicant cites MRI data showing trends for reduction in edema (swelling) and adipose (fatty tissue) replacement signals at Week 24 and Week 48, based on T2 mapping and fat fraction methodology, in the small number of subjects. The applicant concludes:

- T2-weighted signal decreased (-0.07 to -0.23; N = 14) compared to controls (0.07 – 0.14; N = 10)
- Apparent fat fraction increased (2.7 – 5.2%; N = 5) in placebo group compared to 6 mg/kg/week treatment (0.9 – 3.8%; N = 6), suggesting a reduced rate of fat infiltration in subjects in 6 mg/kg.
- Effects persisted up to 24 weeks post-treatment

Consult review of the MRI data conducted by Dr. Daniel Krainak (CDRH Imaging Division) concludes that the data presented in the application are unconvincing for several reasons: the small number of subjects with fat fraction data at baseline, 24 weeks and 48 weeks, variability in the MR systems used, and lack of data concerning the actual quality control measurements from phantoms. The limited data show substantial overlap between treatment groups, large variability by muscle and a wide range of fat fraction observed at baseline. The magnitude of the changes observed in the study population is on the order of the uncertainty in the measurement technique (approximately 3%). Dr. Krainak concludes greater uncertainty about quantitative T2 measures in the context of edema as T2 may be influenced by many physiologic factors (including inflammation/edema, local bleeding/hematocrit, fat, [fat effects T1 more than T2] and more). Most of the commonly seen pathologies (infection/inflammation, tumor [benign or malignant], etc.) lead to an increase in T2 values. Therefore, an altered T2 is sensitive but not specific unless correctly interpreted in the context of the underlying pathophysiology.

Dystrophin Measurements

Muscle biopsies were obtained from the tibialis anterior (TA) muscle from each subject at baseline, Week 24 and either week 12 or 36. The following analyses were done:

- Detection of exon 51 skipped mRNA by RT-PCR
- Detection of dystrophin protein expression at baseline and Weak 24 (N=36) and at Week 12 by IFA (N=4)
- Detection of dystrophin protein expression at baseline and Week 24 (N=9) by WB analysis, when IFA was not possible because of poor sample quality for IFA. For 2 subjects WB analysis was performed to confirm IFA results.

Exon 51 skipped mRNA by RT-PCR

A total of 10/17 (59%) subjects in each of the drisapersen 3 mg/kg/week group and 6 mg/kg/week group compared to 2/15 (13%) subjects in the placebo group had an increase in exon 51 skipping

compared to baseline (Table 22). For about 24-60% of the subjects, the relative intensity was below the reporting level across treatment groups. The applicant notes that the increase was more pronounced at week 36, where 43% of the 3 mg/kg and 100% of the 6 mg/kg subjects and 14% in the placebo group showed an increase in exon skipping (Note: sample size, N=7 at week 36). Note that doses were administered only up to 24 weeks.

Table 22 Summary of DMD Exon 51 Skip Muscle Biopsy Data at Weeks 12, 24, and 36

Parameter/ Visit	Result	Number (%) of Subjects		
		Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)
Relative Intensity				
Week 12	n Increase	7 1 (14)	6 3(50)	8 4 (50)
Week 24	n Increase	15 2 (13)	17 10 (59)	17 10 (59)
Week 36	n Increase	7 1 (14)	7 3 (43)	7 7 (100)

Source: adapted from Page 98

The mean PCR fragment intensity (a.u) for exon 51 skipped product was increased for both 3 and 6 mg/kg at Week 24 (**Table 23**).

Table 23 Intensity of exon 51 skipped dystrophin mRNA product by nested RT-PCR and capillary electrophoreses

Study Treatment	Exon Skip (a.u) Mean (SD)	
	Week 0	Week 25
Placebo	2.0 (1.5)	1.5 (1.2)
Weekly 3mg/kg	2.7 (4.1)	4.4 (3.6)
Weekly 6mg/kg	2.4 (4.4)	4.4 (6.6)

The applicant also provided Digital Doppler PCR (ddPCR) data with supporting information that the assay precision was >25% and the limit of reporting for DMD biopsies carrying exon 50 deletion was >140 for skipped copy numbers in total 1500 ng RNA. The RNA integrity number (RIN) value for many samples was lower than 5, suggesting moderate to low RNA quality (the highest possible value being 10). The applicant has provided ddPCR data for all RIN value samples and those with RIN >5. No appreciable increase in skip numbers relative to placebo group was observed in majority of the samples of the treated groups.

OBP Reviewer Dr. Rao's Comment on Methodology:

Given that the applicant identified a high inter-assay variation of 25%, it is unlikely that this method, as performed, could provide the Applicant with any meaningful quantitative data regarding dystrophin mRNA because the relative increases in mRNA are small and in most instances within the applicant's proposed variability. Additionally, the very small sample

number, low RIN values for many samples, and different skip numbers across various exon deletion subgroups, do not allow a clear interpretation of a trend in dystrophin skip product increase between groups.

Dystrophin expression by IFA:

The results of dystrophin analysis by IFA were not supportive for the treatment group. The relative intensity was increased in maximum number of subjects in the placebo group and only 1 subject in the 6 mg/kg group (Table 24).

Table 24 Summary of Immunofluorescence Assay and Western Blot Qualitative Muscle Biopsy Data

Parameter/ Testing Method	Result	Number (%) of Subjects		
		Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)
Relative Intensity (All muscles)				
Week 24 Immunofluorescence assay (IFA)	n	12	11	13
	Increase	7 (58)	5 (45)	1 (8)
Western blot (WB) Week 24	n	5	5	1
	Increase	0	1 (20)	0

Source: Adapted from page 99
Predefined criteria for increase: (>4% increase compared to baseline)

Based on an initial analysis at Week 24, the mean percent increase from baseline in dystrophin were observed in both the drisapersen 3 mg/kg/week group (2%) and placebo group (6%) compared with a decrease in the drisapersen 6 mg/kg/week group (-3%) as shown in Table 25.

Table 25 Summary of Dystrophin Intensity Measurement by IFA (change from Baseline to Week 24)

Parameter		Placebo (combined) (N=16)		Drisapersen 3 mg/kg (N=17)		Drisapersen 6 mg/kg (N=18)	
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Non-Qualified Mean Percentage Change (%)	Week 12	0	NA	2	-2 (6)	2	-0.5 (5)
	Week 24	12	6 (10)	11	2 (11)	13	-3 (6)
	Week 36	0	NA	0	NA	0	NA
Non-Qualified Q90 Percentage Change (%)	Week 12	0	NA	2	-6 (4)	2	-2 (3)
	Week 24	12	8 (13)	11	-0.2 (11)	13	- 4 (8)
	Week 36	0	NA	0	NA	0	NA

Source: Study DMD114876 report, page 100

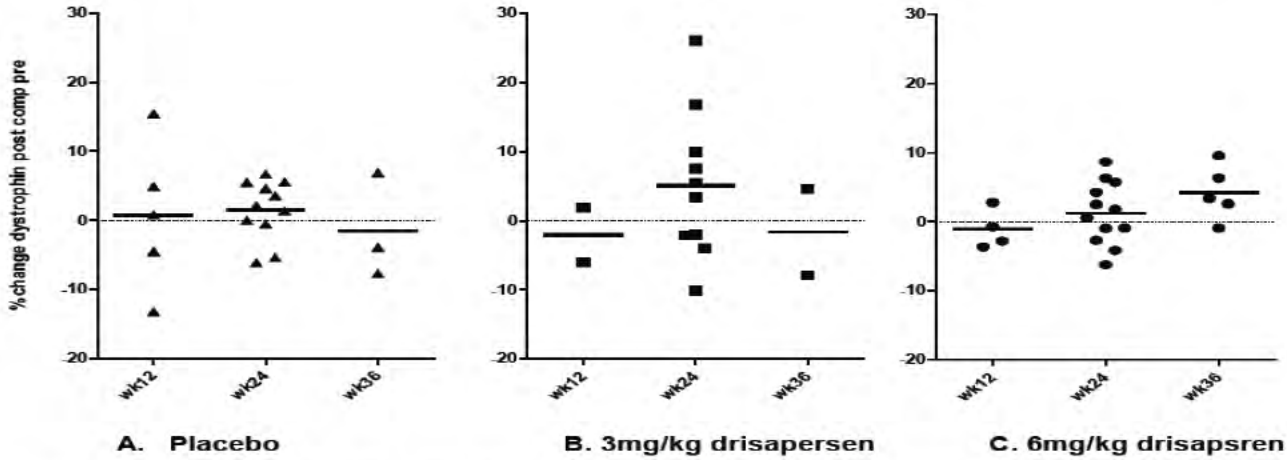
After this initial analysis, due to the large variability, no difference between the treated and placebo group and large changes in spectrin between 2 biopsies, the applicant further investigated the variability and presented the results in a separate report. A repeat analysis of these biopsies was conducted in 2-3 experiments. An average of the 2-3 repeat experiments was reported for each subject. These were used to calculate the non-qualified mean percentage change as shown in Table 26 and Figure 14. The highest increase at Week 24 was observed in the 3 mg/kg group.

Table 26 Summary if dystrophin intensity measurement (by average by 2-3 experiments for each subject)

Parameter	Visit	Placebo (N=16)		Drisapersen 3 mg/kg (N=17)		Drisapersen 6 mg/kg (N=18)	
		n	Mean (SD)	n	Mean (SD)	n	Mean (SD)
Non-Qualified Mean Percentage Change (%)	wk12	5	1% (11%)	2	-2% (6%)	4	-1% (3%)
	wk24	11	2% (4%)	10	5% (11%)	12	1% (4%)
	wk36	3	-2% (8%)	2	-2% (9%)	5	4% (4%)
Non-Qualified Q90 Percentage Change (%)	wk12	5	1% (17%)	2	-6% (5%)	4	-2% (1%)
	wk24	11	1% (6%)	10	5% (11%)	12	0% (6%)
	wk36	3	0% (14%)	2	-6% (3%)	5	-13% (12%)

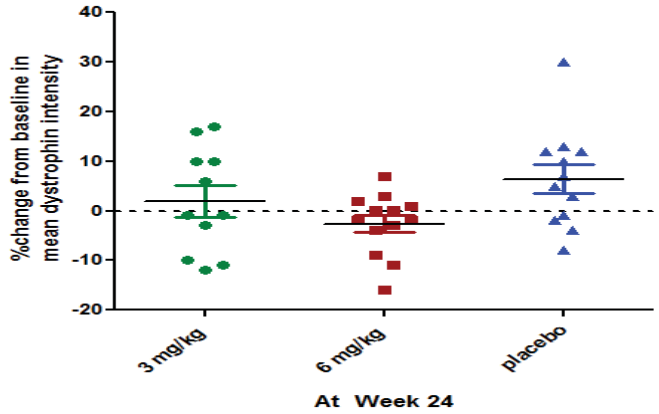
Source: DMD114876-LAB-01 report, page

Figure 14 % change in mean dystrophin intensity in individual subjects from an average of 2-3 experiments (IFA)



Reviewer's Comment: **The applicant's scatter plot in Figure 14 shows larger variability in the % change in mean dystrophin intensity in the 3 mg/kg group (-10% to 26%) compared to the 6 mg/kg group (-4% to 9%), driving a higher mean % change for the 3 mg/kg group at Week 24. There is also no appreciable differences in dystrophin expression between placebo (-6 to 7%) and 6 mg/kg group (-4% to 9%). While there appears to be an upward trend in the mean values, the values are within the known assay variability and not apparently or statistically significant. In addition, no dose was given beyond 24 weeks, hence the reason for the upward trend observed is unclear. The scatter plot based on their original analysis is shown in Figure 15**

Figure 15: % change in mean dystrophin intensity in individual subjects from original experiment (IFA)



OBP Reviewer Dr. Rao's Comment on IFA:

The applicant states that the inter-assay reproducibility of their IFA assay between experiments for placebo and drisapersen-treated subjects combined was 5% and ranged between 0-16% for the study. It is not clear if the mean values reported in Table 26 that are below 5% represent biologically-relevant responses or expected assay variability. There also appear to be several critical deficiencies in the applicant's design of experiments that preclude an interpretable assessment of dystrophin increase: (1) the Applicant states that the IFA analyses were repeated up to 3 times to "*investigate further the inter-biopsy and inter-assay precision from one* subject 027; however repeated analyses were carried out for several other subjects without any accompanying justification regarding why 2 or, in some cases, 3 replicates were obtained, (2) the Applicant states that the operators and managers were blinded to the treatment groups at the time of the 2013 analysis, after which the treatment regime was unblinded. The IFA repeat analyses were carried out in 2014 and after the study was unblinded, (3) in the case of each of the 2nd or 3rd replicate values, it was noted that the placebo samples showed lower dystrophin intensity values and the treatment samples showed higher values in the re-analysis. In the absence of a systematic, consistent, and prospective design of experiment, it may not possible to conclude that the repeated analyses generated unbiased and robust mean dystrophin values. See Appendix B for additional deficiencies.

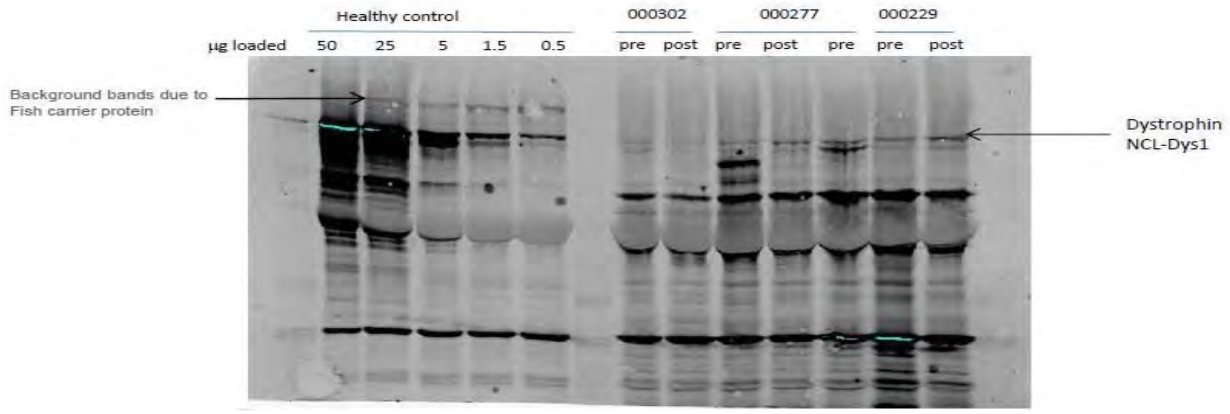
Dystrophin expression by WB:

The applicant has provided limited western blot analysis data for this study. They claim that in most instances the biopsies were of too poor quality to generate data. In the 3 mg/kg/wk group, 5 subjects were anlayzed and one showed an increase in dystrophin protein. The one subject in the 6 mg/kg/wk group that was analyzed did not show an increase.

OBP Reviewer Dr. Rao's Comment on WB:

In addition to the poor quality of biopsies and very low sample number, the Applicant chose to examine the 11 subjects for western blotting whose samples were originally deemed unsuitable for IFA analysis.

The applicant did include a loading control healthy sample in their western blots and used beta3-spectrin as a loading control. However, there is no clear trend showing an increased band in post-treatment samples. The dystrophin band appears to diminish in some post-treatment samples (e.g. subject 277). Image below was provided by the Applicant upon request for raw data from this study. While the applicant has not compared their dystrophin values to the healthy control samples on the same gel, in most cases the dystrophin band appears to be at or lower than the lowest dilution of healthy control sample tested. The applicant was asked to clarify how the dystrophin values compared to the healthy controls on the same gels. They stated that while it is not possible to accurately calculate the relative dystrophin signal due to a lack of linearity at the low levels of signal from the DMD samples, they estimate that the dystrophin expression they observed is generally around 0.3% or below of the highest control dilution. They further state that only 4/18 *samples* tested showed dystrophin levels above their lower limit of detection of 1% of normal, and that those 4 samples with 2.1-4.1% of normal dystrophin levels did not show an appreciable treatment effect when compared to their corresponding pre-treatment sample.



Persistence of Effect

<u>Reviewer's Comment:</u> One of the objectives of this study was to determine the persistence of effect during the drug free observation period from week 25- Week 48. The treatment difference was maintained at Week 48 for the 6mg/kg group.
Also interesting to note that the change from baseline was not maintained for the 6 mg/kg group in Study DMD114117, which in treatment was given for 48 weeks. So it is unclear if the persistence of effect observed in this study is a true treatment effect or the natural variation in the DMD population.

<u>Reviewer's Analysis and Discussion:</u>

The study results in general appear weak, some weak trends in favor of treatment observed were:

- **The primary endpoint (change from baseline in 6MWD at Week 24) had a numerical advantage on 6MWD with a treatment difference of placebo of 27 m for the 6mg/kg dose, but the study was negative (p=0.07).**

- **The reduction in creatinine kinase was in favor of the drisapersen treated groups. There was a dose related response in the reduction of CK at week 24, with a greater decline in the 6 mg/kg group compared to the 3 mg/kg group, but only the 3 mg/kg group continued to decline during the drug free period at Week 28.**

The weaknesses of the observed results from Study DMD114876 are:

- **Based on the pre-specified primary efficacy analysis for the change from baseline 6MWD, the study was negative with a p-value of 0.07**
- **Removing one subject in the placebo group who was unblinded due to a hospital visit, the treatment difference was 19 m, p=0.21**
- **There was no dose response:**
  - **6 mg/kg/week was superior than placebo (27m)**
  - **3 mg/kg/week was worse than placebo (-9 m)**

  This could be suggestive of noise in the data or difference in disease characteristics of the two dose groups as discussed earlier.
- **The secondary endpoints in this study were not supportive of the primary endpoint. There was no dose related trends in the secondary endpoints, with 3 mg/kg trending better compared to 6mg/kg in some endpoints (10m walk/run, 4 stair climb, muscle strength, NSAA).**
- **Potential partial unblinding to the assigned treatment due to injection site reactions: Unblinding could affect the performance of 6MWD, which is an effort dependent endpoint. The incidence and the duration of injection site reactions was the largest for the 6 mg/kg group and the least for placebo.**
- **The dystrophin expression (IFA and WB) did not favor treatment benefit. The dystrophin expression as evaluated by IFA showed the similar response in the placebo group and 6 mg/kg group. The RT-PCR exon skipping data showed higher percent of exon skip subjects in favor of the 6mg/kg group, but this was not supported by IFA analysis. The WB analysis did not show any treatment response.**

6.3. Study DMD114044

6.3.1. Study Design

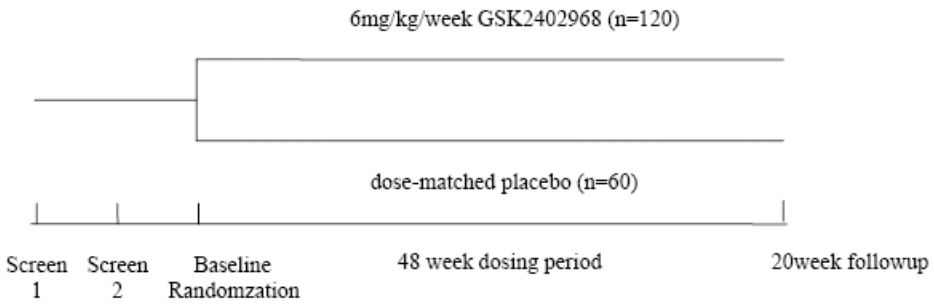
Overview and Objective

This study was designed to assess the efficacy of drisapersen 6 mg/kg once weekly for 48 weeks in ambulant subjects with DMD compared to placebo. The secondary objectives were to assess safety, tolerability, PK and impact on quality of life.
Studied period**:** 02 December 2010 to 28 June 2013
Study center(s): 44 centers in 19 countries: Argentina, Belgium, Brazil, Canada, Chile, Czech Republic, Denmark, France, Germany, Italy, Japan, Korea, Netherlands, Norway, Poland, Russia Federation, Spain, Taiwan, and Turkey.

Trial Design

This phase III, randomized, double-blind, parallel-group clinical study similar in design to Studies DMD114117 and DMD114876, with the exception of primary endpoint assessments at Week 48. Any differences will be outlined in this section. Subject completing this study entered an open label extension (Study DMD114349). The study design schematic is shown in Figure 16.

Figure 16 Study design schematic for Study DMD114044



Population: N= 186 DMD boys with a mutation corrected by exon 51 skipping. The study was designed to have 90% power to detect a difference in 6MWD between drisapersen and placebo of 30 meters, assuming a common standard deviation of 55 meters.
Key Inclusion/Exclusion Criteria: Same as Study DMD114117 and DMD114876, with the exception of:

- There was no restriction on ability to rise from floor (in $\leq$ 7 seconds for Study DMD114117, and $\leq$ 15 seconds for Study DMD114876). Subjects with any rise time could be enrolled in this study.

Dosing Regimen:
Subjects were randomized to the following in 2:1 ratio using Interactive Voice Response System. Similar blinding approaches were adopted as the Phase 2 studies.

- 6 mg/kg/week SC drisapersen for 48 weeks (N=125)
- SC placebo for 48 weeks (N=61)

To minimize injection site reactions, rotation of site on a weekly basis was recommended. Abdomen, arms, thighs, back and buttocks were all used as injection site.
Applicant's Dose Rationale: Prior to randomization in this study, 12 subjects from open label study DMD114673 had received 6 mg/kg/week for 48 weeks. A mean change from baseline in the 6MWD at Week 24 was 37 m (range -58 m to +115 m). The applicant considered this supportive of the choice of the dose. In addition, due to early signs of potential subclinical renal effects (mild proteinuria) in the open label study and the preclinical pro-inflammatory findings (including data from the 39 week monkey study), 6 mg/kg was considered to be the maximum tolerated dose (MTD) and selected for this study.

Reviewer's Comment: This study was initiated 3 months after the initiation of the 48 week Study DMD114117.

Study Endpoints

The primary efficacy endpoint was 6MWD at week 48. 6MWD was assessed at screening (1 and 2), randomization, at weeks 24, 36 48 or follow-up.

The secondary efficacy endpoints were same as Study DMD114117 and DMD114876. Some of the differences between the endpoints were:

- For pulmonary function tests, MIP, MEP (Study DMD114117 only) and sniff nasal pressure (Study DMD114876 only) were not measured in this study.
- The quality of life assessments in this study were:
  - Pediatric Quality of Life Neuromuscular module (also on Study DMD114117)
  - Clinician Global Impression of Improvement (CGI-I) (also in study DMD114876)
  - Health Utilities Index
  - Activities of Daily Living

Key Secondary Endpoints:

1. Change from baseline in the NSAA linearized score
2. Change from baseline in the 4-stair climb (ascent) velocity
3. Change from baseline in the 10-meter walk/run velocity

Other exploratory endpoints:

- MRI and DEXA

Statistical Analysis Plan

Primary Analysis: Change from baseline in the 6MWD at week 48 was analyzed for the OC dataset on ITT population using MMRM with fixed effects of treatment, visit, treatment by visit interaction, country grouping, and continuous fixed covariates of baseline 6MWD and baseline 6MWD by visit. Other supportive information was derived from Week 12, 24, and 36. The covariates assessed were: country group, baseline 6MWD, age, age group (≤7 and >7 years), corticosteroid regimen (Continuous or Intermittent), Baseline rise from floor group (Less than or equal to 7 seconds or greater than 7 seconds), 117 Data dissemination group (Week 48 visit before or after 117 dissemination), country, and race. Statistical significance tests were 2 sided.

Sensitivity analyses were also similar to Study DMD114117 and DMD114876.

Secondary Analysis: If a statistically significant treatment difference at the 5% level was observed for the primary efficacy endpoint, then the key secondary endpoints were tested in a hierarchical manner. For all other endpoints no adjustment for multiplicity was made and all were performed at α of 5% and considered supportive.

Protocol Amendments

Amendment 1 (Sep 2010)	• If subjects become non ambulatory and are unable to perform the 6MWD assessment for this reason, their distance will be imputed to be 0m for the purposes of analysis • Clarified that the primary analysis will be on OC data. • Sensitivity analyses will be on LOCF data • Efficacy analysis using PP population will be a sensitivity analysis • MMRM analysis for PP population will be a sensitivity analysis.

	• ANCOVA with the LOCF and OC dataset will be carried out for the 6MWD at week 48
Amendment 2 (21 Jun 2011)	• Allow subjects who withdrew for safety reasons to enter the extension study • MRI sub-study as requested by EMA. This would be performed only in Argentina and Brazil due to logistical and timing issues

Reviewer's Comment: Amendments related to study analysis were made prior to initiation of enrollment

Data Quality and Integrity: Sponsor's Assurance

Management of clinical data was performed in accordance with GSK standards. The study was conducted in accordance with ICH GCP (ICH E3 and E6) and applicable country-specific requirements. Written commitments were obtained from investigators to comply with GCP. Study was conducted with written informed consent from subjects and their parents.

6.3.2. Study Results

Patient Disposition

A total of 181 subjects completed the study (Table 27).

Table 27 Summary of subject disposition

Subject Status	Number (%) of Subjects		
	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)	Total (N=186)
Completed	60 (98)	121 (97)	181 (97)
Withdrawn	1 (2)	4 (3)	5 (3)
Primary reason for study withdrawal			
Adverse event[a]	0	2 (2)	2 (1)
Withdrew consent	0	2 (2)	2 (1)
Protocol deviation[b]	1 (2)	0	1 (<1)

Source: Study DMD114044 report, page 64

a. An AE of glomerulonephritis leading to discontinuation was reported in 1 subject (Subject 527). Intracranial venous sinus thrombosis and spinal pain AEs leading to discontinuation were reported for 1 subject (Subject 1270).

b. Subject 1266 in the placebo group had mutation not correctable by exon 51 skipping

Protocol Violations/Deviations

A total of 7 (11%) subjects in the placebo group and 22 (18%) subjects in the drisapersen group had a major protocol deviation. Summary of major protocol violations leading to exclusion from the PP population is shown in Table 28.

Table 28 Protocol Deviations

	Number (%) of Subjects		
	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)	Total (N=186)
ITT Population	61 (100)	125 (100)	186 (100)
PP Population	54 (89)	103 (82)	157 (84)
Total Number of Subjects with Major Protocol Deviations	7 (11)	22 (18)	29 (16)
Mutation not correctable by exon 51 skipping.	1 (2)	0	1 (<1)
Not able to complete 6MWD test with minimal distance of at least 75 m at each pre-drug visit. In addition, results must be within 20% of each other at each pre-drug visit.)	1 (2)	6 (5)	7 (4)
Symptomatic cardiomyopathy.	0	2 (2)	2 (1)
Use of prohibited concomitant medication	2 (3)	9 (7)	11 (6)
Significant change in corticosteroid regimen	2 (3)	2 (2)	4 (2)
Subjects not adequately complying with dosing specifications of the study	1 (2)	6 (5)	7 (4)

Source: Study DMD114044 report, page 65

Reviewer's Comment: A sensitivity analysis removing protocol violators did not change the overall conclusions, but treatment difference was reduced (Table 32).

Table of Demographic Characteristics

Most demographic characteristics were similar across treatment group, with numerically higher weight in drisapersen group (Table 29).

Table 29 Summary of demographic characteristics

	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)	Total (N=186)
Age (yrs)			
Mean (SD)	8.0 (2.4)	8.3 (2.4)	8.2 (2.4)
Median	8.0	8.0	8.0
Min., Max.	5, 16	5, 16	5, 16
Ethnicity, n (%)			
Hispanic/Latino	10 (16)	23 (18)	33 (18)
Not Hispanic/Latino	51 (84)	102 (82)	153 (82)

Race, n (%)			
African American/African Heritage	1 (2)	0	1 (<1)
Asian - Central/South Asian Heritage	1 (2)	3 (2)	4 (2)
Asian - East Asian Heritage	3 (5)	6 (5)	9 (5)
Asian - Japanese Heritage	5 (8)	9 (7)	14 (8)
Asian - South East Asian Heritage	0	2 (2)	2 (1)
White - Arabic/North African Heritage	4 (7)	5 (4)	9 (5)
White - White/Caucasian/European Heritage	46 (75)	95 (76)	141 (76)
White - Mixed Race	0	1 (<1)	1 (<1)
Mixed Race	1 (2)	4 (3)	5 (3)
Height (cm)			
Mean (SD)	122 (10)	124 (11)	NA
Median	122	123	NA
Min., Max.	102, 145	101, 148	NA
Weight (kg)			
Mean (SD)	27(7)	30 (10)	NA
Median	24	27	NA
Min., Max.	17, 46	17, 68	NA

Source: Study DMD114044 report, page 67

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Mean baseline values for the 6MWD was lower, time since first corticosteroid taken, time since first symptoms and time since diagnosis were all was numerically longer in the drisapersen group than in the placebo group.

Table 30 Summary of Muscular Dystrophy Disease Baseline Characteristics

	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)	Total (N=186)
Time Since First Symptoms (months)[a]			
n	58	122	180
Mean (SD)	66.7 (31)	71.8 (31.5)	70.2 (31.5)
Median	60.8	70.2	66.8
Min., Max.	11, 168	12, 176	11, 176
Time Since Diagnosis (months)[a]			
n	61	125	186
Mean (SD)	54.2 (32.8)	58.0 (35.2)	56.7 (34.4)
Median	49.8	54.5	53.1
Min., Max.	6, 148	6, 163	6, 163
Time Since First Corticosteroid Taken (months)[a]	61	125	186
n	29.1 (25.8)	35.6 (28.9)	33.5 (28.1)
Mean (SD)	18.9	26.6	25.6
Median	7, 135	6, 146	6, 146
Min., Max.			

Corticosteroid Regimen, n (%)[b]			
n	61	125	186
Continuous	52 (85)	108 (86)	160 (86)
Intermittent	9 (15)	17 (14)	26 (14)
6MWD (m)	61	125	NA
Mean (SD)	348 (92)	337 (95)	NA
Method of Diagnosis, n (%)[c]			
n	61	125	186
Clinical symptoms	56 (92)	110 (88)	166 (89)
Muscle biopsy	21 (34)	47 (38)	68 (37)
Genetic testing	61 (100)	125 (100)	186 (100)
Multiplex Ligation-dependent Probe Amplification	59 (97)	117 (94)	176 (95)
Comparative Genomic Hybridisation	0	7 (6)	7 (4)
Single Condition Amplification/Internal Primer	1 (2)	1 (<1)	2 (1)
Other	1 (2)	0	1 (<1)
EXON Mutation, n (%)			
n	61	125	186
DMD 45-50 deletion	16 (26)	40 (32)	56 (30)
DMD 47-50 deletion	1 (2)	0	1 (<1)
DMD 48-50 deletion	7 (11)	26 (21)	33 (18)
DMD 49-50 deletion	20 (33)	31 (25)	51 (27)
DMD 50 deletion	5 (8)	11 (9)	16 (9)
DMD 52 deletion	10 (16)	16 (13)	26 (14)
Other	2 (3)	1 (<1)	3 (2)

Source: Study DMD114044 report, page 68

Reviewer's Assessment of baseline characteristics:

The baseline characteristics were generally balanced across treatment groups, with a small number of subjects with greater functional impairment in the drisapersen group based on age and 6MWD. The mean time since first diagnosis and time since first symptoms was greater in the drisapersen group. For a large study these differences are not likely to adversely affect the study results.

- **Impact of age distribution: The subjects <7 years are balanced between the two groups. Subjects ≥ 7 years are also balanced between the two groups, but there were 17% subjects >11 years in the drisapersen group compared to 11% in the placebo group.**
- **Impact of 6MWD: Mean baseline 6MWD was lower in the drisapersen group. I looked at the distribution of 6MWD in the treatment groups. There were higher number of subjects with baseline 6MWD <150m and in between 250-300m in the drisapersen group (18%) compared to placebo (12%), and a higher % of subjects with 6MWD greater than 350 m in the placebo group (54%) compared to drisapersen group (49%).**

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

About 60 (98%) subjects in the placebo group and 119 (95%) subjects in the drisapersen group were >80% compliant. The total dose and duration of exposure were similar.

Efficacy Results - Primary Endpoint

Applicant's Analysis: A statistically non-significant (p=0.415) treatment difference of 10.3m for drisapersen over placebo at week 48 was observed for the primary endpoint change from baseline in 6MWD.

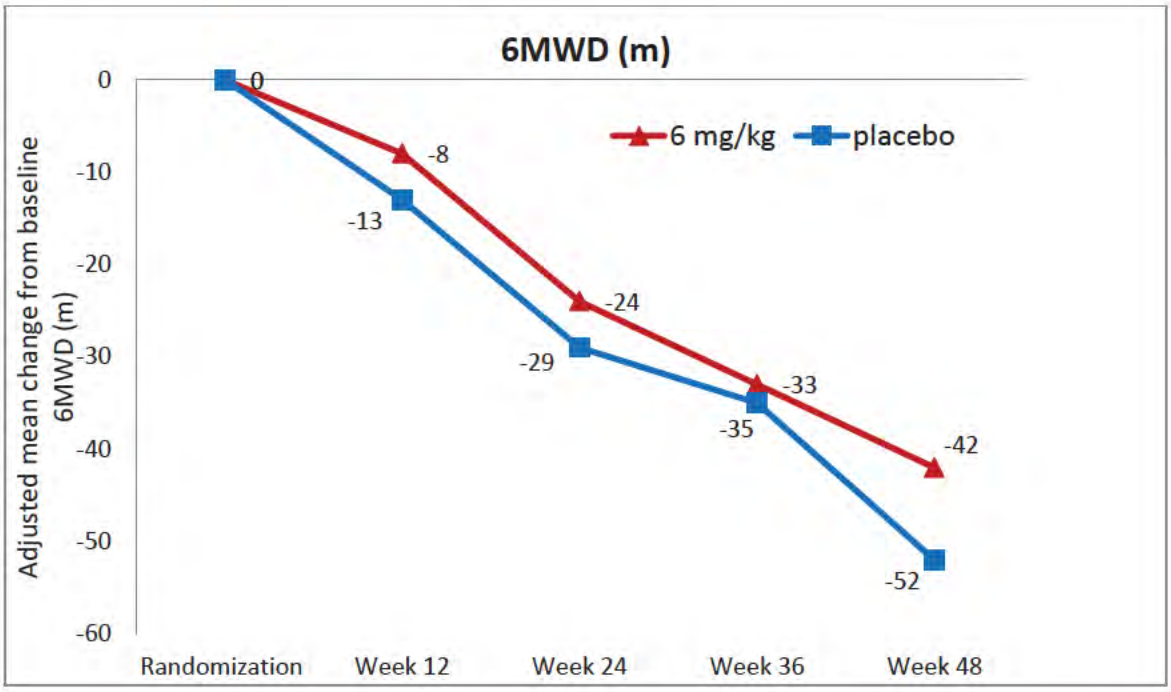
Table 31 MMRM Analysis of change from baseline in 6MWD (m)

	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)
Baseline		
n	61	125
Mean (SD)	348 (92)	337(95)
Week 48		
n	59	117
Adjusted mean change (SE)	-52 (10)	-42 (7)
Adjusted mean difference vs. placebo		10
95% CI		(-15, 35)
p-value		0.42

Source: Study DMD114044 report, page 77

I generated the following Figure that shows the time course of the change from baseline for drisapersen 6 mg/kg/week and placebo at each visit.

Figure 17 MMRM Analysis of change from baseline in 6MWD (m)



None of the covariates evaluated showed a significant treatment interaction. Exon mutation and baseline CK were fitted as covariates retrospectively and did not show any significant interaction as well.

Clinical Review (Efficacy)
NDA 206, 031 (Drisapersen)

Sensitivity Analyses:

Most sensitivity analyses were consistent with the primary analyses and gave similar treatment differences, with the exception of MMRM analysis with the PP population (removing protocol violators), where the treatment difference was reduced to **4.9 m (p=0.703) (Table 32)**

Table 32 Sensitivity Analyses for Change from Baseline in 6MWD (m) at Week 48 (ITT Population)

Analysis/Treatment	n	Adjusted Mean Change from Baseline (SE)	Treatment Difference[a]	95% CI	p-value
MMRM OC (ITT)[b]					
Placebo	59	-52 (10)			
Drisapersen 6 mg/kg/wk	117	-42 (7)	10	(-15, 35)	0.42
MMRM OC (PP)[b]					
Placebo	53	-45 (10)			
Drisapersen 6 mg/kg/wk	99	-40 (7)	5	(-20, 30)	0.70
ANCOVA OC (ITT)[c]					
Placebo	59	-50 (11)			
Drisapersen 6 mg/kg/wk	117	-38 (8)	12	(-14, 37)	0.36
ANCOVA LOCF (ITT)[c]					
Placebo	60	-50 (10)			
Drisapersen 6 mg/kg/wk	125	-38 (7)	11	(-13, 36)	0.36
ANCOVA Multiple Imputed (MAR approach) (ITT)[d]					
Placebo	59	-50 (10)			
Drisapersen 6 mg/kg/wk	117	-40 (8)	10	(-15, 35)	0.43
ANCOVA Multiple Imputed (CIR approach) (ITT)[d]					
Placebo	59	-50 (10)			
Drisapersen 6 mg/kg/wk	117	-40 (8)	10	(-15, 35)	0.44

Additional analyses on 6MWD:

1. Percent-predicted 6MWD: The percent predicted 6MWD in DMD provides an estimate of performance relative to a healthy control population to account for age and development differences. An increase in the percent-predicted 6MWD is consistent with functional improvement. The results of this analysis showed a 2 % difference between the placebo and drisapersen group at Week 48 (95% CI: -2, 6) which was not statistically significant (p=0.32)
2. Change from baseline in 6MWD by response category
   At week 48, the percent of subjects with ≥ 30 m change in 6MWD was 18% in the drisapersen group and 12% in the placebo group. The percent of subjects with ≥ 60 m change in 6MWD were similar in the two groups.
3. Time to persistent 10% decrease in 6MWD: 43% subjects show persistent 10% decrease in placebo compared to 36% subjects in drisapersen group.
4. Sub-group Analyses: Post-hoc analyses on various subgroups were conducted. Only sub-groups that showed a greater treatment difference at week 48 in the change from baseline in 6MWD over placebo are summarized in Table 33. These large numbers of post-hoc analyses in smaller number subjects are uninterpretable. Sub-group analysis (as summary statistics) in subjects ≤ 7 years and > 7 years was pre-specified, other sub-group analyses were post hoc. Subjects

>7 years showed a treatment difference of 7 m, whereas subjects less than 7 years showed a treatment benefit of 21 m (Table 33). Other sub-group analyses showing treatment difference in favor of 6 mg/kg is summarized in the following Table.

Table 33 Sub-group Analyses

Treatment	N	Baseline Mean (SD)	n	Adjusted Mean Change from Baseline (SE) at Week 48	Treatment Difference	95% CI
Age ≤7 years at Baseline (pre-specified)						
Placebo	29	383 (66)	29	-25 (11)	21	(-6, 48)
Drisapersen 6 mg/kg/wk	51	368 (65)	50	-4 (8)		
Rise from Floor >7s at Baseline						
Placebo	26	318 (77)	25	-88 (16)	21	(-20, 61)
Drisapersen 6 mg/kg/wk	47	307 (75)	43	-67 (12)		
Able to Stand from Supine at Baseline (Rise from Floor Grade >1)						
Placebo	55	363 (82)	53	-43 (10)	15	(-10, 40)
Drisapersen 6 mg/kg/wk	106	360 (80)	102	- 28 (7)		
≤330 m at baseline						
Placebo	22	256 (67)	21	-93 (22)	18	(-32, 69)
Drisapersen 6 mg/kg/wk	57	253 (66)	50	-75 (14)		
Centers previously enrolled in other studies						
Placebo	9		9	-109(25)	62	(-2, 126)
Drisapersen 6 mg/kg/wk	17		17	-47(18)		

Change from baseline by country grouping showed largest treatment difference in Northern Europe and Russia-Eastern Europe grouping, but the number of subjects was small.

5. Time to a 25, 50% and 75 % decrease in 6MWD: These similar across treatment groups.
6. 6MWD in subjects with Rise from Floor ≤7 seconds: In this study there were no restrictions on rise from floor time. As a post-hoc analysis in this subgroup, the treatment difference (5m) was smaller than in the DMD114044 population as a whole (10m). In fact the treatment difference was larger in subjects with rise from floor >7 seconds (21m). The applicant indicates that the greater range of rise from floor values at baseline was not the primary reason for the differing results (Table 34).

Table 34: MMRM Analysis of Change from baseline in 6MWD at week 48 by baseline Rise from Floor

Treatment	N	Baseline Mean (SD)	n	Adjusted Mean Change from Baseline (SE) at Week 48	Treatment Difference	95% CI
Rise from Floor ≤7s at Baseline						
Placebo	29	404 (62.520)	28	-4 (10)	5	(-18, 28)
Drisapersen 6 mg/kg/wk	59	402 (55.540)	59	1 (7)		
Rise from Floor >7s at Baseline						
Placebo	26	318 (77)	25	-88 (16)	21	(-20, 61)
Drisapersen 6 mg/kg/wk	47	307 (75)	43	-67 (12)		

Source: Study DMD114044 Study Report, page 83

Data Quality and Integrity - Reviewers' Assessment

There were no issues with data quality or integrity.

Efficacy Results - Secondary and other relevant endpoints

No significant treatment differences or consistent trends in favor of drisapersen were observed for any key or other secondary endpoints (Table 35)

Table 35 Secondary endpoints

Treatment	n	Baseline Mean (SD)	Adjusted Mean Change from Baseline (SE) at Week 48	Treatment Difference	95% CI	p-value
Key Secondary Efficacy Endpoints						
Linearized NSAA Total Score						
Placebo	58	58.4 (17.71)	-6.7 (1.43)			
Drisapersen 6 mg/kg/wk	117	56.6 (18.85)	-7.2 (1.01)	-0.53[a]	(-3.95, 2.88)	0.757
4 Stair Climb Ascent Velocity (stairs/s)						
Placebo	55	0.88 (0.573)	-0.12 (0.049)			
Drisapersen 6 mg/kg/wk	111	0.86 (0.658)	-0.14 (0.035)	-0.021[a]	(-0.137, 0.095)	0.718
10 m Walk/Run Velocity (m/s)						
Placebo	58	1.57 (0.542)	-0.20 (0.050)			
Drisapersen 6 mg/kg/wk	117	1.54 (0.575)	-0.21 (0.035)	-0.009[a]	(-0.129, 0.111)	0.881
Other Secondary Efficacy Endpoints						
Rise from Floor (s)[c]						
Placebo	44	13.41 (15.882)	7.48 (2.080)			
Drisapersen 6 mg/kg/wk	91	12.34 (14.984)	6.36 (1.463)	-1.115[b]	(-6.097, 3.866)	0.658
4 Stair Climb Descent Velocity (stairs/s)						
Placebo	55	0.98 (0.601)	-0.15 (0.052)			
Drisapersen 6 mg/kg/wk	109	1.02 (0.634)	-0.11 (0.037)	0.041[a]	(-0.082, 0.164)	0.513
Muscle Strength Total Score (lbs)						
Placebo	58	98.55 (30.527)	-1.21 (2.729)			
Drisapersen 6 mg/kg/wk	118	102.49 (34.941)	-2.18 (1.926)	-0.965[a]	(-7.446, 5.516)	0.769
Creatine Kinase (IU/L)						
Placebo	60	11901.1 (7133.32)	-1228.5 (500.59)		(-5232.21,	
Drisapersen 6 mg/kg/wk	118	10956.5 (7316.07)	-5273.5 (359.05)	-4044.99[b]	-2857.77)	<0.001

Source: Adapted from Study DMD114044 report

There were no meaningful changes in pulmonary functions tests (not shown in the Table).

Creatine Kinase:

A statistically significant treatment difference in favor of drisapersen in change from baseline in creatinine kinase serum concentration was observed at week 48 with a 48% decrease in absolute mean CK in the drisapersen group compared to 10% in the placebo group.

Table 36 Summary of MMRM analyses of Serum creatine kinase (IU/L)

Treatment	N	Baseline Mean (SD)	n	Adjusted Mean Change from Baseline (SE) at Week 48	Treatment Difference	95% CI P-value
Placebo	61	11901 (7133)	60	-1228 (500)	-4044	(-5232, -2858) <0.001
Drisapersen 6 mg/kg/wk	125	10956 (7316)	118	-5273 (359)		

Source: page 91

Post-hoc analyses on CK: Post-hoc analyses showed a greater decline in CK compared to placebo in subjects ≤7 years vs. > 7 years, consistent with a larger treatment difference with 6MWD observed in ≤7 years (Table 37).

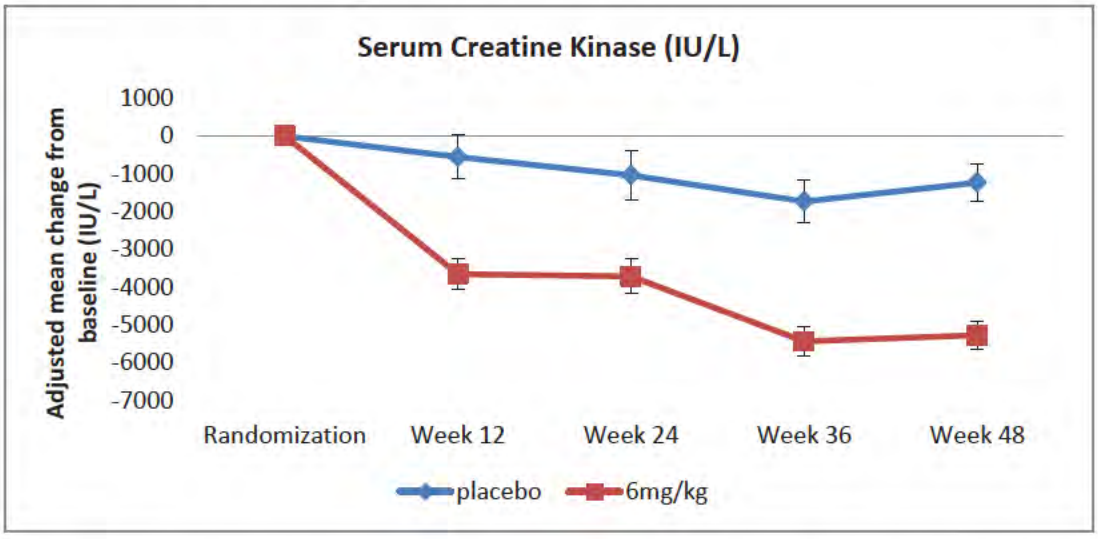
Table 37 MMRM analysis of CK based on age group

Treatment	N	Baseline Mean (SD)	n	Adjusted Mean Change from Baseline (SE) at Week 48	Treatment Difference	95% CI
≤7 years at baseline						
Placebo	29	15471 (6834)	29	-1314 (831)		
Drisapersen 6 mg/kg/wk	51	15626 (8422)	49	-7230 (613)	-5916	(-7890, -3942)
>7 years at baseline						
Placebo	32	8666 (5794)	31	-1439 (570)		
Drisapersen 6 mg/kg/wk	74	7738 (4075)	69	-3589 (407)	-2149.	(-3455, -845)

Source: Study DMD114044 report, page

I generated the following Figure 18 that shows the time course of reduction in CK.

Figure 18 MMRM analysis of change from baseline in CK



CGI-I, PedsQL, Health Utility Index Scores, and Activities of Daily Living: A total of 1 (2%) subject in the placebo group and 12 (10%) subjects in the drisapersen group were considered responders (much improved or very much improved) on the CGI-I at Week 48. Two (4%) subjects in the placebo group and 23 (20%) subjects in the drisapersen group minimally improved on the CGI-I at Week 48. There were no clinically meaningful treatment differences between drisapersen and placebo for the PedsQL Neuromuscular Module, HUI health outcomes assessments and Activities of Daily Living.

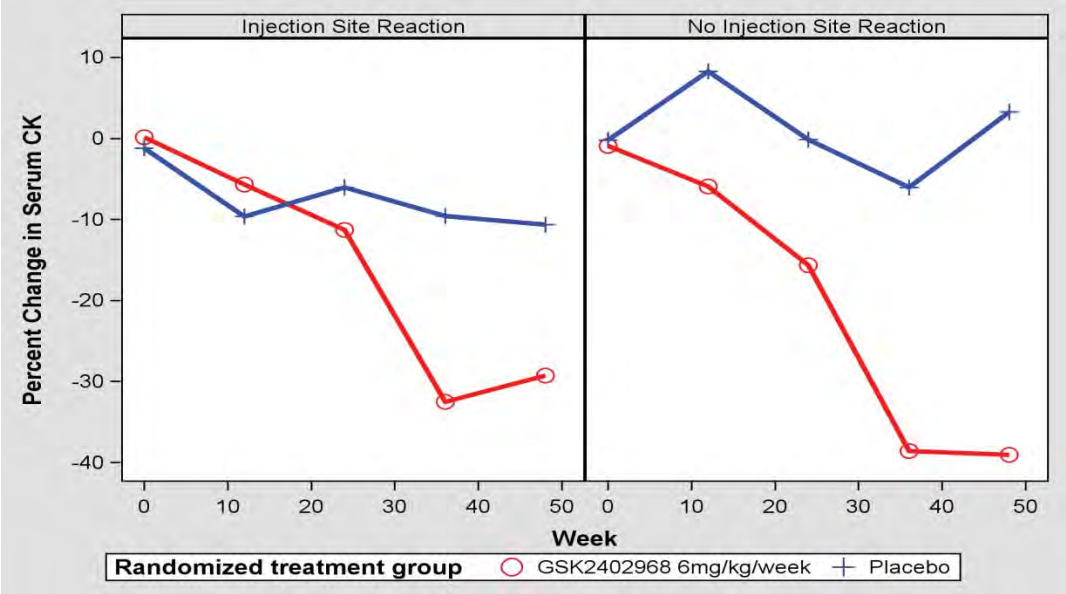
Time to loss of ambulation: A total of 6 (10%) subjects in the placebo group and 15 (12%) subjects in the drisapersen group lost ambulation during the study. A Kaplan-Meier analysis of Time to loss of ambulation did not show any difference (Figure not shown). There was a complete overlap of the 95%

confidence interval between treatment groups. Subjects who lost ambulation had a higher mean age in both treatment groups (placebo: 9 years; drisapersen: 10 years) compared with subjects who remained ambulant during the study (placebo: 8 years; drisapersen: 8 years).

Reviewer's Analysis and Comment:

Since the difference in CK between the treatment and placebo groups were statistically significant (p<0.001), I wanted to explore if this difference was due to inactivity resulting from injection site reactions. The reduction of CK was greater in the treatment group compared to placebo whether subjects had an injection site reaction or not, as depicted in Figure 19. The level of activity/inactivity could not be assessed by patient reported outcomes or other means. Overall CK was reduced by 32% in the drisapersen group.

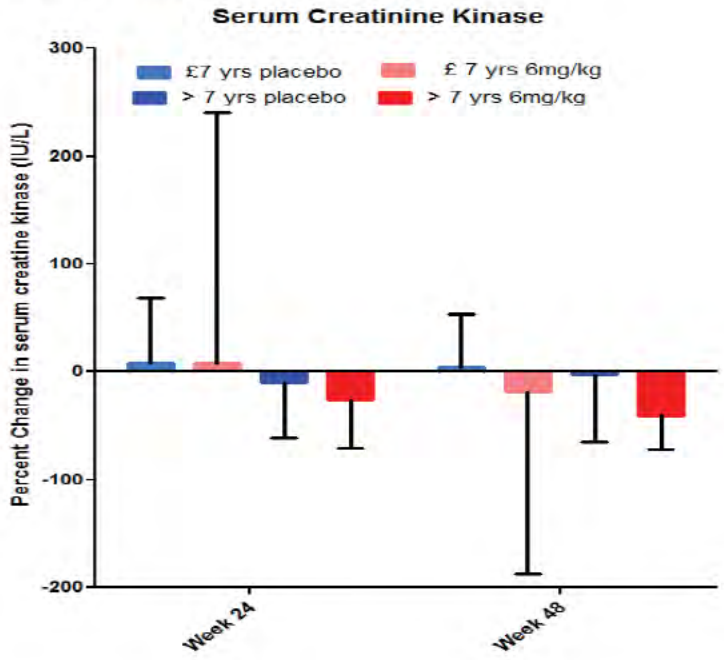
Figure 19 Change from baseline serum CK in subjects with or without injection site reactions



Source: Dr. Bhattaram

The MMRM analysis of serum CK based on age groups (≤7 years and > 7 years), showed greater reduction in CK in subjects ≤7 years, consistent with the age based analysis on 6MWD.
Since, the baseline CK is higher in the younger age group, I looked at percent reduction in these groups. At Week 48, the subjects > 7 years showed greater mean reduction in serum CK (-41%) compared to the subjects ≤7 years (-18%), opposite to what was observed when looking at adjusted mean change from baseline. There was much larger variability in the CK in subjects ≤7 years.

Figure 20 Percent change in serum creatine kinase in subjects ≤7 years and >7years



Dystrophin Measurements

Two muscle biopsies were obtained from each subject, one at Week 48, the other biopsy at either Week 8, 12, 24 or 36 from the tibialis anterior in most subjects with the exception of quadriceps in 3 subjects. No muscle biopsies were performed at baseline. Therefore the applicant analyzed the muscle biopsies for drisapersen concentration, detection on exon 51 skipping by non-quantitative RT-PCR only and the number of revertant fibers (as it could influence the amount of exon skip observed in total RNA).

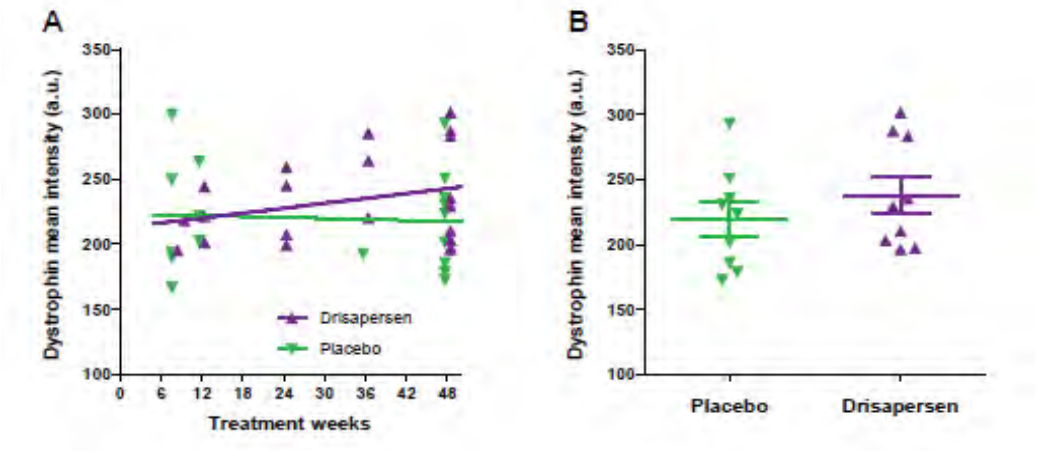
Exon 51 Skipped mRNA:
With a non-quantitative RT-PCR, skipping of exon 51 was detected in 48 (81%) subjects in the placebo group and 106 (89%) subjects in the drisapersen group at Week 48. There was a weak trend to higher intensities in the drisapersen treated group beyond 24 weeks of treatment. (Table 38).

Table 38 Exon 51 skip with non-quantitative RT-PCR

Test/ Visit	Placebo (N=61)		Drisapersen 6 mg/kg/week (N=125)	
	n	Mean (SD)	n	Mean (SD)
DMD Exon 51 skip - Integrated intensity (units)				
Week 8	20	1.75 (1.25)	26	1.25 (1.55)
Week 12	16	2.02 (1.78)	30	1.64 (1.79)
Week 24	9	1.52 (1.03)	34	2.32 (2.90)
Week 36	13	0.91 (0.56)	26	2.86 (5.18)
Week 48	56	1.43 (1.69)	114	2.49 (3.46)

Dystrophin expression by IFA:
According to the Applicant, many biopsies were not acceptable for dystrophin analysis: due to freeze damage, small size and/or relative large areas of adipose and connective tissue (indicative of advanced disease state), an insufficient number of fibers with intact membranes can be identified for a representative and reproducible result. A summary of dystrophin analysis on selected biopsies were performed by the operators that were blinded to study treatment during analysis is presented in Figure 21. The results in placebo patients show the presence of dystrophin both with IFA and WB, which is considered a result of trace dystrophin expression in many fibers and/or dystrophin expression in revertant fibers. Therefore, the applicant concluded that in the absence of pre-treatment biopsies, the IFA results would not provide relevant information in this study.

Figure 21 Dystrophin mean intensities on selected biopsies



Reviewer's Comment: The numbers of subjects were few at each time point. There was a trend of increased dystrophin intensity with a large inter-subject variability in the drisapersen group.

OBP Reviewer Dr. Rao's comments:

> **The applicant reports several problems with the quality of the biopsy samples. They state that repeated freeze-thaws of the tissue samples being shipped from multiple international sites in an aluminum tube container to a central re-distributing laboratory for analysis led to rejection of a large number of samples with freezing-related artifacts that were deemed unsuitable for IFA or WB. The absence of pre-treatment samples from the same patients probably renders any treatment-related conclusions questionable.**

MRI:
According to the Applicant, MRI data were only acquired in a small number of subjects and data were not analyzed due to technology issues at acquisition.

Applicant's discussion on lack of response in this study:

The applicant attributes the following factors to have led to a less favorable outcome in this Phase 3 study:

- **Demographic and baseline population makeup** – Subjects in DMD114044 had, on average, more functional impairment than subjects in the Phase II studies (as measured by baseline 6MWD). Also, unlike the Phase II trials, DMD114044 did not include a baseline requirement for performance on the rise from floor assessment. This change led to further enrolment of older, more severely progressed subjects in DMD114044, most of whom would not have met the criteria for enrolment in the Phase II studies. According to the applicant, these patients would have had fewer intact muscle cells where increased dystrophin levels due to exon skipping would have an impact. Pre-specified subgroup analyses of the DMD114044 data show that subjects ≤7 years performed similarly at Week 48 to subjects in the Phase II studies, with a 21 meter treatment effect in favor of 6 mg/kg/week drisapersen (not statistically significant). Subjects >7 years in DMD114044 showed a small numerically favorable treatment effect (7 meters) and a large decline from baseline 6MWD performance (-76 meters), possibly indicating subjects in the decline phase of their ambulatory capacity.
- **Treatment duration-** 48 weeks not adequate to demonstrate treatment benefit in a heterogeneous population
- **Lack of a loading dose** – unlike Study DMD114117, there was no loading dose in DMD114044. As a result, subjects likely took longer to achieve steady state for drug effect. This could be important in an already more progressed population.
- **Multiple centers** – because of the size of the study, DMD114044 required more study sites in more countries (44 sites in 19 countries) than were employed in the Phase II studies, including some sites that were not specialized in DMD management. This global reach is likely to have led to increased variability in standard of care in areas such as steroid usage and frequency of formal physiotherapy. A post hoc analysis of DMD114044 sites previously participating in study DMD114117 showed a difference of 62 m (p=0.06) in change from baseline 6MWD at Week 48 between drisapersen 6 mg/kg/week (-47m; n=17) and placebo (–109m; n=9) suggesting that experience in management of DMD patients and in conducting assessments in the context of a clinical trial, may help to decrease variability and enable detection of a treatment effect.

Reviewer's analysis/discussion:

The only strength of this study was:

- **A favorable effect of serum creatine kinase at Week 48 (p=<0.001). No apparent relationship was observed between reduction in CK from baseline and 6MWD response. There is a 30-40% reduction in CK across studies.**
- **There were some differences observed in the patient population that could have an unfavorable prognosis in the drisapersen group in this study, although it is unlikely that these differences could account for some modest differences in the outcome in a large study. In a large study these differences in baseline characteristics are difficult to interpret.**
    - **There were 17% subjects >11 years in the drisapersen group compared to 11% in the placebo group.**

  - **There were 18% of subjects with baseline 6MWD <150m and in between 250-300m in the drisapersen group compared to 12% in the placebo group.**
  - **There were 49% subjects with baseline 6MWD >350 m in the drisapersen group compared to 54% in the placebo group.**
- **The study design was identical to the phase 2 studies, with the exception of no restriction on the ability to rise from floor. Hence, subjects >7 seconds were also enrolled in this study. In a post-hoc analysis in subjects with Rise from Floor <7seconds, the treatment difference of 5 m was observed, which was smaller than in the DMD114044 population as a whole (10 m). The applicant indicates that the greater range of rise from floor values at baseline was not the primary reason for the differing results.**
- **In a post-hoc analysis, the applicant states there was a treatment difference of 62m in favor of drisapersen in study sites that participated in prior drisapersen studies, and might have been more experienced at collecting data. However, I assessed the quality of data for the primary and key secondary endpoints across studies and centers and there did not appear to be any meaningful or systematic differences in the values or trajectories between centers that would suggest differences in the way the data was collected (See Appendix C). The sponsor's analysis therefore does not appear reasonable. Furthermore, the remaining sample size of this entirely post-hoc analysis is so small, 9 subjects in the placebo group and 17 in the drisapersen group, that it could have readily arisen by chance alone.**

The weaknesses of this study are:

- **The primary endpoint (change from baseline in 6MWD at Week 48) did not show a statistically significant treatment difference from placebo, although the study was a well-designed and executed study with good statistical power to detect a small effect.**
- **Percent change from baseline in CK based on age group is not consistent with age group effect on treatment response on 6MWD, but so was reduction in CK not consistent with treatment effect in the primary analysis. (i.e. treatment difference with 6MWD negative, treatment difference with CK positive)**
- **No consistent trends in favor of drisapersen were observed for any key or other secondary endpoints.**
- **There could have been partial unblinding due to differences in injection site reactions between the drisapersen (78%) and placebo groups (16%) leading to lean in 6MWD in favor of drisapersen. Although, the impact of potential unblinding is not as apparent in this study as though directionally favorable, the effect size was small. Nevertheless, a small numerical difference between treatments could be due to unblinding.**
- **There was a weak trend of higher intensity of exon 51 skipping.**

6.4. Study DMD114349 (Open label extension study)

6.4.1. Study Design

Overview and Objective

This study was designed to assess the long-term safety, efficacy, and tolerability of drisapersen for at least 104 weeks in subjects with DMD who previously participated in either feeder study (DMD114117 or DMD114044). The study was terminated early because results of Study DMD114044 showed that the primary efficacy endpoint was not achieved.
Study Dates: 19 September 2011 to 17 March 2014 (termination) (no dosing occurred after 20 September 2013)
Study Centers: 58 centers in 24 countries: Argentina, Australia, Belgium,
Brazil, Bulgaria, Canada, Chile, Czech Republic, Denmark, France, Germany, Hungary, Israel, Italy, Japan, Republic of Korea, The Netherlands, Norway, Poland, Russian Federation, Spain, Taiwan, Turkey, and United Kingdom.

Trial Design

This was a Phase III, multicenter, open-label, uncontrolled extension study in subjects with DMD who had completed the double-blind treatment phase in DMD114117 or DMD114044. Eligible subjects entered into a 4-week Run-in period of the study. No assessments were conducted and no drug was administered during this Run-in period.
Study Population: 6- to 17-year-old (median of 9 years males were enrolled. Subjects entering this study had mean times since first symptoms, diagnosis, and first corticosteroid use of 80.4, 66.1, and 43.9 months, respectively. The corticosteroid regimen given during the feeder studies (DMD114117 or DMD114044) was continuous for 80% of the subjects and intermittent for 20%.
Dosing Regimen: The primary dosing arm for all subjects was 6 mg/kg/week continuous dosing for at least 104 weeks, but subjects had the option of intermittent dosing (6 mg/kg/week for 8 weeks and 4 weeks off drug) based on safety and tolerability issues. At any point during the study, subjects could discontinue active treatment and move to the natural history observation arm for the duration of the study or until Early Withdrawal.
Primary endpoint: Change from baseline in muscle function using the 6MWD test assessed at Week 104. (Note: Baseline efficacy assessments were only required for those subjects who had withdrawn early from the feeder studies and who did not have these assessments performed within the previous 3 calendar months, for other last assessment from feeder studies were used as baseline).
Secondary endpoints: same as feeder studies.
Muscle Biopsy for dystrophin expression: Only subjects who showed an unexpected decrease in efficacy required a muscle biopsy, defined as two consecutive 20% decreases in 6MWD after Week 24 in those subjects previously demonstrating improvement or maintenance of 6MWD (unless an alternative explanation (e.g., fall)
MRI: MRI was to be conducted in those subjects where it was obtained in Study DMD114044

Statistical Analysis Plan

No formal interim analysis, but the first data-cut was planned for 05 June 2013.
The primary population for evaluation of efficacy parameters was the Modified ITT population, defined as all subjects enrolled in the study that received at least 1 dose of investigational product, or entered the natural history arm at the start of the study and as such did not take any investigational product, and had at least one post-baseline efficacy assessment. A Modified Ambulant ITT population, defined

as above, but only in the ambulant population was used for analysis using pre-specified MMRM model with a 5% significance level was used for 6MWD, NSAA and timed function tests. An analysis pooling all the subjects from the two studies was uninterpretable; hence the applicant conducted post-hoc analyses based on parent studies.

Data Quality and Integrity: Sponsor's Assurance

This study was conducted in accordance with ICH GCP and all applicable subject privacy requirements, and the ethical principles that are outlined in the Declaration of Helsinki 2008. Management of clinical data was performed in accordance with applicable GSK standards. The study was monitored in accordance with ICH E6.

6.4.2. Study Results

Patient Disposition

A total of 233 subjects were enrolled. 228 subjects were assigned to continuous treatment with drisapersen 6 mg/kg/week, 4 subjects were assigned to intermittent treatment with drisapersen 6 mg/kg (12 week cycles of drisapersen 6 mg/kg/week for 8 weeks followed by 4 weeks of no dosing), and 1 subject was assigned to the natural history arm (no treatment). Of the 228 subjects assigned to continuous treatment with drisapersen 6 mg/kg/week at the start of the long-term extension study, 8 switched to intermittent treatment, 15 subjects switched to the natural history arm, and 1 of the subjects who initially switched to intermittent treatment switched to the natural history arm due to an AE. The final numbers in each arm after switching regimens during study were: Continuous N=205, Continuous to Intermittent N=7, Continuous to Intermittent to natural History N=1, Continuous to Natural History N=15, Intermittent N=4, Natural History N=1. The number of subjects that withdrew during the study and the reason is shown in Table 39. The rest of the subject's attrition was due to termination of the study.

Table 39 Subjects withdrawn

	Number of subjects
Subjects Withdrawn	17
Reason	
AE	3
Lack of efficacy	2
Protocol Deviation	1
Withdrew Consent	11

Note: only 1 subject who withdrew was from Study DMD114117

Subjects were treated with 6 mg/kg drisapersen continuously over a mean total duration of 353 days; 74%, 57%, and 24% of the subjects received investigational product for ≥24 weeks, ≥48 weeks, and ≤72 weeks, respectively. Due to the small numbers of subjects in the drisapersen intermittent treatment group or the natural history arm at the time of early study termination, applicant's discussion focuses

on the drisapersen continuous treatment group through 72 weeks (N=55). The cumulative exposure of each regimen is given in Table 40.

Table 40 Summary of cumulative exposure

	6 mg/kg Drisapersen Continuous	6 mg/kg Drisapersen Intermittent	6 mg/kg Drisapersen (Combined)	Natural History
Cumulative Duration of Exposure (weeks), n (%)				
n	228	12	232	17
0 weeks	0	2 (17)	0	0
>0 weeks	228 (100)	10 (83)	232 (100)	17 (100)
≥6 weeks	226 (>99)	9 (75)	229 (99)	15 (88)
≥12 weeks	201 (88)	8 (67)	205 (88)	13 (76)
≥18 weeks	178 (78)	7 (58)	181 (78)	11 (65)
≥24 weeks	168 (74)	5 (42)	171 (74)	9 (53)
≥48 weeks	131 (57)	3 (25)	136 (59)	3 (18)
≥72 weeks	55 (24)	0	60 (26)	0
≥104 weeks	0	0	0	0

Source: NDA 206, 031 Study Report DMD114379

Protocol Violations/Deviations

Protocol deviations were reported by the site for 230 (99%) subjects overall, mostly related to study procedures/assessments not performed on the exact day, but reviewing the data they were mostly within 1 week of the scheduled visit. A total of 23% of the subjects eitherhad an extra dose, did not get a dose, got a wrong dose or dose was given at an incorrect time.

Table of Demographic Characteristics

The study population consisted of 6- to 17-year-old (median of 9 years), predominantly White (78%), non-Hispanic (86%) males. Baseline age characteristics are shown in Table 41.

Table 41 Baseline Age

Demographic Characteristic	6 mg/kg Drisapersen Continuous (N=205)	6 mg/kg Drisapersen Intermittent (N=11)	Natural History (N=17)	Total (N=233)
Age (years)				
Mean (SD)	8.9 (2.12)	9.9 (1.73)	8.7 (1.69)	9.0 (2.14)
Median	9.0	9.5	9.0	9.0
Min, Max	6, 17	8, 14	6, 12	6, 17

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

81% of the subjects were more than 80% compliant.

Efficacy Results - Primary Endpoint

Applicant's Analysis:
The applicant considered the results pooling subjects from the two parent studies uninterpretable, because the parent studies showed different results and the population was more progressed in Study DMD114044, an analysis based on each parent study was considered appropriate by the applicant. The applicant considers subjects from study DMD114117 more functional population and subjects in Study DMD114044 as a more progressed population. The change from original baseline in 6MWD from parent studies is shown in Table 42 and Table 43.

Table 42 Change from original baseline in 6MWD (m) by parent study (Study DMD 114117)

Treatment in parent study (DMD114117)	Placebo	Drisapersen 6 mg/kg/week	
Treatment in DMD114349	Drisapersen 6 mg/kg weekly or intermittent	Drisapersen 6 mg/kg/week	Drisapersen 6 mg/kg weekly or intermittent
Original baseline, n Mean (SD)	18 403 (45)	18 428(70)	18 428 (70)
Change to Study 349 Week 48, n Mean (SD)	14 –55 (78)	16 1.24 (69)	17 –5 (68)
Change to Study 349 Week 72, n Mean (SD)	11 –89 (126)	12 -33 (71)	13 –39 (71)

Source ISE Table 10.1.3

Table 43 Change from original baseline in 6MWD (m) by parent study (Study DMD 114044)

Treatment in parent study	Placebo	Drisapersen 6 mg/kg/week	
Treatment in DMD114349	Drisapersen 6 mg/kg weekly or intermittent	Drisapersen 6 mg/kg/week	Drisapersen 6 mg/kg weekly or intermittent

Original baseline, n Mean (SD)	60 348 (93)	116 346 (92)	119 343 (94)
Change to Study 349 Week 48, n Mean (SD)	37 –123 (135)	61 –92 (130)	61 –92 (130)
Change to Study 349 Week 72, n Mean (SD)	25 –173 (132)	35 –108 (134)	37 –106 (132)

Source ISE Table 10.1.3

According to the applicant, subjects who received drisapersen in both the parent study were combined, the subjects on drisapersen continuous regimen in both parent and the extension study had a smaller decline in 6MWD (-89 m) than those who received placebo in the parent study followed by drisapersen in the extension study (-142 m) at Week 72 (overall Week 120). The treatment difference in the two groups (continuous and delayed start was, 31m at Week 96 (from study baseline) and 59m at Week 120.

Based on these results, the applicant believes that this reinforces the notion that subjects who are treated younger (and presumably prior to the onset of more serious functional impairment) will get a greater benefit of treatment with drisapersen in the long-term. At the time of study termination, 21 (10 %) subjects in the continuous treatment arm lost ambulation. Four subjects required respiratory support during sleep.

Efficacy Results - Secondary and other relevant endpoints:

Timed Tests, Muscle strength and NSAA: Small changes from baseline were observed on secondary endpoints (e.g., muscle strength, timed muscle function tests [rise from floor, 10-meter walk/run, 4-stair climb (ascent/descent)], NSAA total score) in both the delayed placebo and drisapersen arms in both studies at different visits. The applicant only discusses the results in terms of the continuous regimen. Subject on the intermittent regimen when switched to continuous regimen continued to show larger decline, consistent with the greater impairment in this group at baseline.
Pulmonary Function tests: No clinically meaningful change was seen in any pulmonary function test.
PedsQL, Health Utilities Index, CGI-I, Functional Outcome Assessment: No meaningful differences in the continuous drisapersen arm and the delayed drisapersen arm
Serum CK: CK showed a decline over 72 weeks, but was very variable.
Dystrophin and MRI: No subjects demonstrated an unexpected decrease in efficacy that required a muscle biopsy to quantitate dystrophin expression. No MRI or dystrophin expression data has been provided from this study.

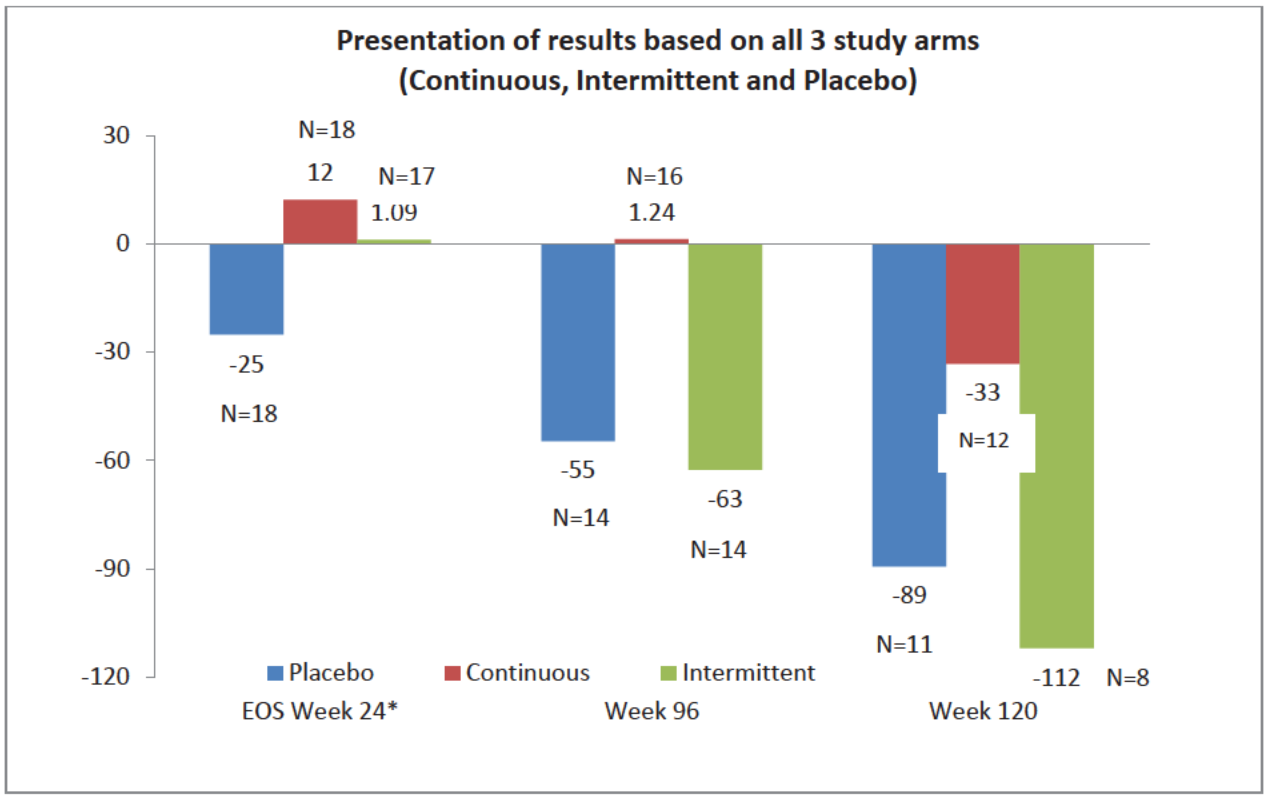
Reviewer's Comment: The durability of response is uninterpretable from this study as not all subjects completed the study.

Reviewer's Comments/Discussion:
The dropout of subjects during the study and the lack of control make this study uninterpretable, even if the analyses are on parent studies. A total of 26%, 43% and 76% of the subjects dropped out from the study at 24 weeks, 48 weeks and 72 weeks due to AE or termination of the study.

The Applicant has discussed the open label extension results based on the parent studies. For Study DMD114117, the results have been discussed only for the continuous 6 mg/kg/week drisapersen arm and the placebo arm switched to the 6mg/kg/week regimen. The parent study DMD114117 was a three arm study with 18 subjects each on intermittent and continuous drisapersen 6mg/kg regimen. The subjects that were in intermittent regimen in the parent study, when switched to continuous 6mg/kg/week regimen under Study DMD114349, continued to decline (Figure 22). These subjects appeared to have more functional impairment compared to the continuous treatment arm at baseline of the parent study as also stated by the applicant in the ISE and their probable reason that the intermittent group did not show treatment difference from placebo. The applicant has not explained the evidence supporting efficacy in context of the deterioration seen in these subjects when switched to continuous regimen from the intermittent regimen in the parent study. While these data are not completely interpretable because not all subjects completed their assigned treatments during the study, but do suggest the subjects appear to follow their course of progression. The disease trajectories appeared to be different in the three treatment arms at the start of the study.

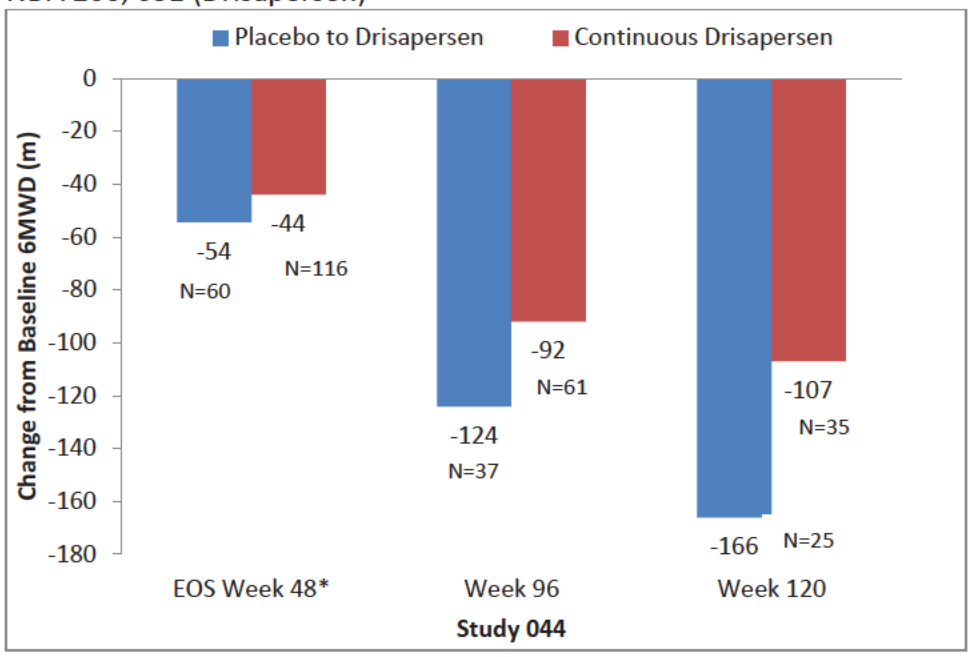
Based on these results of extension of Study DMD114117, the applicant believes that this reinforces the notion that subjects who are treated younger (and presumably prior to the onset of more serious functional impairment) will get a greater benefit in the long-term of treatment with drisapersen. While it may seem logical that it may be pharmacologically easier to restore dystrophin before much muscle damage occurs, and hence benefit may be discernable when treated young, there is no clear evidence supporting this hypothesis. Published natural history data suggest that some subject can have better prognosis than other based on baseline 6MWD, age and genetic disposition (LTBP4 genotype predicts age of ambulatory loss in DMD). In addition, experts in the area have also shown that higher baseline function is almost always associated with slower long-term decline in DMD. (McDonald:
http://www.treatnmd.eu/downloads/file/meetings/2013/workshop/Session1/McDonald_NH.pdf

Figure 22: Adjusted mean change from baseline 6MWD in the open label extension of Study DMD114117 (all three arms presented)



At weeks 96 and 120, treated subjects from parent Study DMD114044 had larger decline in the extension phase (Figure 23). This is consistent with a more impaired population. The applicant also asserts that that treatment benefit is slower to emerge in subjects with advanced age. While this could be true, the drop out of subjects during the study (N's shown in the figure), preclude reliable conclusions from this study. Therefore, there is no convincing evidence suggesting longer treatment would be needed to show treatment benefit in subjects with advanced age and more functionally impaired subjects.

Figure 23 Adjusted mean change from baseline 6MWD in the open label extension of Study DMD114044



6.5. Study DMD114673 (Open Label Extension Study)

6.5.1. Study Design

Overview and Objective

This was an open label long-term extension of Study PRO051-02 (a 5 week dose escalation study with 0.5, 2, 4 and 6 mg/kg doses) in patients with Duchenne muscular dystrophy. The Applicant has used this as a historically controlled Study.

Study Date: 30 July 2009 (start of Continued Treatment Phase) to 26 Mar 2013 (completion of 188 week), 25-Jun 2013 (completion of IV sub-study)
Study Centers: 2 centers (Belgium and Sweden)

Trial Design

All subjects from Study DMDPRO51-02 were moved to a Continued Treatment Phase with 6 mg/kg/week dose. The applicant's intension is to continue treatment for 216 week. Data up to 188 week has been included in this NDA. Baseline assessments were conducted again prior to the first dose in the Continued Treatment Phase at Visit 13. Safety, efficacy and pharmacokinetic assessments were conducted at regular intervals throughout the Continued Treatment phase. Glucocorticoid use was to be kept constant during the study unless medical reasons dictated otherwise.
Inclusion Criteria:

- Boys aged between 5 and 16 years
- Not ventilator dependent
- Life expectancy of at least six months

Exclusion Criteria:

- Any subject who did not complete the initial Study Period of PRO051-CLIN-02
- Aberrant RNA splicing and/or aberrant response to drisapersen, detected by in vitro drisapersen assay during screening.
- Known presence of dystrophin in ≥5% of fibers in a pre-study diagnostic muscle biopsy
- Severe muscle abnormalities defined as increased signal intensity in >50% of the tibialis anterior muscle at MRI
- FEV1 and/or FVC <60% of predicted, history of liver or renal disease, severe cardiac myopathy

Dosing Regimen:

- Between PRO051-02 and DMD114673 subjects received no drisapersen treatment for 6-15 months.
- From Visit 13 to Visit 85 (Study Week 72), all subjects received a weekly s.c. dose of 6 mg/kg.
- Starting Visits 86 to 93 (Study week 73-80), all subjects had a 8 week wash-out period due to the emerging safety data from other studies. It was anticipated that the washout would not compromise efficacy due to the predicted retention of drug in muscle and the known long half-life of dystrophin.
- At Visit 94 (Week 81) subjects restarted drisapersen on a 12 week cycle intermittent regimen (8 weeks treatment of 6 mg/kg followed by 4 weeks off-treatment). Based on PK/PD, the intermittent regimen, the dystrophin levels were predicted to be 70% of the level prior to wash-out and 80% at the end of the 8 week treatment period. This measure was taken to minimize the hepatotoxicity and nephrotoxicity.
  If a perceived continuous decline in efficacy was observed and where safety and tolerability was acceptable, subjects was given the option to return to continuous regimen. Treatment is currently ongoing in all subjects according to the intermittent dosing schedule and no subjects have reverted to continuous weekly dosing up to Visit 201 (Week 188).

A weight cap on dosing was implemented:

- subjects with body weight <50 kg continued to be dosed with 6 mg/kg drisapersen;
- subjects with body weight ≥50 kg received a fixed maximal dose of 300 mg drisapersen.

Injection was preferred in the abdominal subcutis but alternate sites were allowed. Injections had to be separated by at least 4 inches, if two or more injections were required at a given time.

Study Endpoints

- 6MWD
- Timed Function Tests (10m walk/run, Rise from Floor, Stair Climb)
- Muscle Strength by handheld myometry
- Pulmonary Function (FVC, FEV1, MEP, MIP, PC and PCF)
- Muscle Biopsy at Visit 37 (week 24) and Visit 65 (Week 52, optional), Visit 81, optional
    - mRNA production
    - Dystrophin Expression
- Parent Questionnaire to capture:
    - loss of any skills or daily activities,
    - improvements in daily activities,

- development of new skills

Statistical Analysis Plan

Study was analyzed by only descriptive statistics.

Data Quality and Integrity: Sponsor's Assurance

The study was conducted according to ICH GCP guidelines for assuring proper study conduct with regard to protocol adherence and validity of the data recorded on the CRFs. The study was monitored in accordance with ICH E6.

6.5.2. Study Results

Patient Disposition

All 12 subjects who participated in the initial 5 week Study DMD PRO051-02 were subsequently enrolled into the Continued Treatment phase and the study is ongoing.

Protocol Violations/Deviations

There were no major protocol deviations; hence ITT and PP population are the same. All subjects had missed one or more doses up to Visit 202. 8 subjects had ≤ 10 missed doses. The number of missed doses in the 4 other subjects were 11, 15, 25 and 32.

Table of Demographic Characteristics

At the end of Visit 190, subjects are between the ages 9-18 years

	N	Mean	Min	Max
Age at Screening	12	9	5	13
Age at Visit 190	12	13	9	18

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

The time interval between the two baseline visits was approximately 6 to 15 months. At entry, subjects were classified as either “Stable” or “Declining” based on the investigators’ clinical examinations combined with judgments from the subjects’ parents, home physiotherapists or other relevant individuals (e.g. teachers). Subjects in the ‘decline’ group were on average older, taller and heavier than subjects in the ‘stable’ group. The subjects’ baseline classification is presented in Table 44. The applicant notes that the median walking distance (N=11) had reduced by ~20 m in the time between their inclusion in the initial Study Period and Baseline (Visit 13) in the Continued Treatment Phase.

Table 44 Baseline Status of Subjects

Demographics at Visit 13 by baseline status	N	Mean	SD	Median	Min	Max
Stable						
Age (years)	7	9	2	9	6	11
Body height (cm)	7	116	9	118	97	124
Body weight (kg)	7	24	4	25	15	28
Body Mass Index (kg/m^2)	7	18	2	18	15	21
Decline						
Age (years)	5	12	2	12	10	14
Body height (cm)	5	135	6	136	125	141
Body weight (kg)	5	39	13	37	28	61
Body Mass Index (kg/m^2)	5	21	5	18	18	31

*Stable subjects included Subjects 101, 102, 104, 105, 202, 206 and 207.
*Declining subjects included Subjects 103, 106, 107, 201 and 205 (Subject 103 had the maximum number (N=32) of missed dose, subject 201 was non ambulant).

<u>Reviewer's Comment:</u> The "Stable" and "Decline" groups differed in their baseline 6MWD and Rise from Floor Time as shown below. The "Stable" subjects were atypical in the entire development program, with very Rise From Floor Time.

Baseline	Mean (SD) 6MWD (m)	Mean (SD) Rise From Floor (s)
Stable	435 (108)	2.4 (0.36)
Decline	217 (96)	8.5 (1.85)

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

All subjects received corticosteroids during the Continued Treatment phase and no changes in dosing regimen, other than for routine weight adjustment, except Subject 207 who had his steroid treatment changed to an intermittent dosing regimen.

All subjects missed at least one dose. None of the subjects were dosed at Visits 86 to 93 (washout) and Visits 102 to 105, 114 to 117, 126 to 129, 138 to 141, 150 to 153, 162 to 165, 174 to 177, 186 to 189 and 198 to 201 (off-drug periods). These were not counted as missing dose. Overall compliance was considered to be 92%

Efficacy Results - Primary Endpoint

<u>Applicant's results and Conclusions:</u>

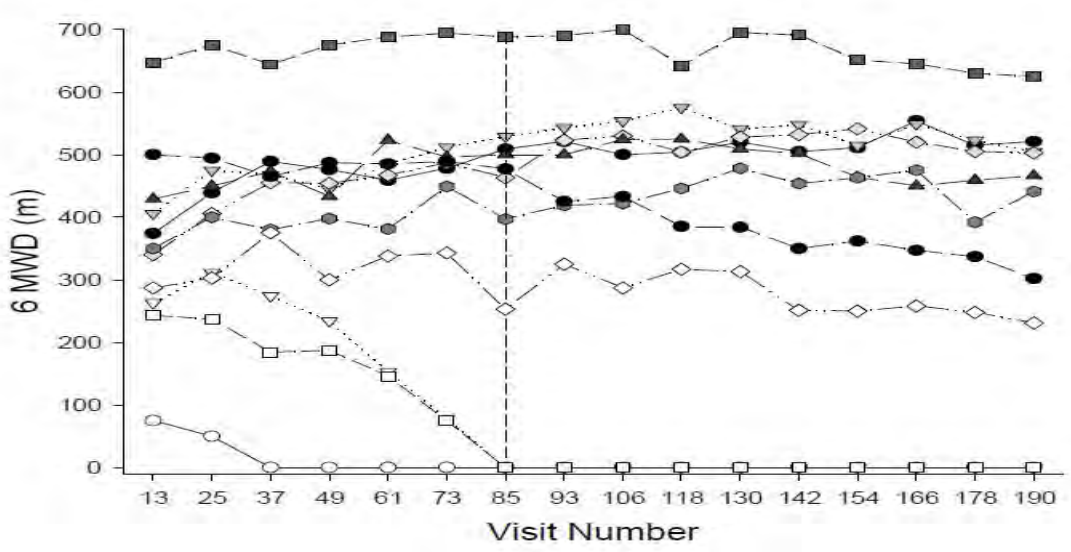
<u>6MWD:</u>

- 10 subjects completed the 6MWD, two subjects (103 and 201) lost ambulation at Visit 37 and 85 respectively.

- 7 subjects were classified as "stable" and 5 as "decliners" at entry to the study, based on baseline 6MWD and medical judgment.
  - Amongst the "stable" subjects, one subject (#207), continued to decline (500 m to 302 m). The mean change from baseline for the 6MWD at Week 177 in the stable group showed an improvement of 45m (range -198 to +163m). In general improvement appears to be maintained until Visit 142 (Week 129) (Table 45)
  - Amongst the 5 "decliners",
    - 2 subject became non ambulant.
    - The remaining 3 subjects had continued to decline to the extent that they could not attempt the 6MWD at later visits.
    - A reduction in those in the decline group (excluding Subject #103 who couldn't complete the assessment at entry) of -187m (range -263 to -56m) (Table 45).
- In the 10 subjects who were able to complete the 6MWD at Visit 13 (study baseline), the median change in 6MWD from Visit 13 to Visit 190 (Week 177) was 8 m (mean change: –25m).
- Five of the 10 subjects who could complete the 6MWD at baseline (Visit 13), could still walk further (range 37 to 163 m) at Week 177 with 2 subjects still being able to walk over 140 meters further at Visit 190 (Week 177) than they could at baseline (Visit 13).
- Introduction of an intermittent dosing regimen following Week 72 (Visit 85) did not appear to adversely affect efficacy parameters.

The absolute change from baseline in 6MWD is shown in Figure 24

Figure 24 Absolute 6MWD over 190 Weeks



Source: Study DMD114673 report, page 65, Note: Stable subjects have filled symbols; 'Decline' subjects have open symbols. Vertical line denotes start of intermittent dosing regimen at Visit 85

Table 45 6MWD – Only Subjects Who Completed Test at Visit 13 (Split by Baseline Status)

Visit	Stable Subjects					Declining Subjects				
	N	Change from Baseline in Continued Treatment Phase (Visit 13) (m)		Change from Baseline in Main Study (m)		N	Change from Baseline in Continued Treatment Phase (m) (Visit 13) (m)		Change from Baseline in Main Study	
		Mean (SD)	Median	Mean (SD)	Median		Mean (SD)	Median	Mean (SD)	Median
13	7	-	-	5.7 (53.97)	-5.0	3	-	-	-73.0 (15.52)	-74.0
25	7	41.9 (28.40)	50.0	47.6 (42.48)	39.0	3	19.7 (27.68)	16.0	-53.3 (24.95)	-63.0
37	7	46.9 (56.27)	44.0	52.6 (40.76)	42.0	3	13.3 (73.53)	11.0	-59.7 (58.07)	-63.0
49	7	46.0 (47.23)	39.0	51.7 (47.14)	28.0	3	-24.0 (34.77)	-29.0	-97.0 (19.70)	-103.0
61	7	63.1 (47.24)	79.0	68.9 (55.58)	59.0	3	-52.0 (89.44)	-97.0	-125.0 (77.67)	-154.0
73	7	79.9 (51.06)	99.0	85.6 (49.12)	86.0	3	-99.0 (134.50)	-168.0	-172.0 (122.43)	-225.0
85	7	73.9 (57.39)	71.0	79.6 (47.13)	87.0	3	-180.0 (126.84)	-243.0	-253.0 (114.95)	-300.0
93	7	82.1 (85.55)	71.0	87.9 (71.27)	87.0	3	-156.0 (168.31)	-243.0	-229.0 (156.12)	-300.0
106	7	88.1 (82.67)	95.0	93.9 (74.10)	111.0	3	-168.7 (146.41)	-243.0	-241.7 (134.36)	-300.0
118	7	76.4 (102.59)	96.0	82.1 (81.09)	97.0	3	-158.7 (163.70)	-243.0	-231.7 (151.53)	-300.0
130	7	87.0 (100.37)	128.0	92.7 (85.82)	115.0	3	-160.0 (161.39)	-243.0	-233.0 (149.24)	-300.0
142	7	76.6 (110.85)	104.0	82.3 (97.91)	93.0	3	-180.7 (125.68)	-243.0	-253.7 (113.81)	-300.0
154	7	65.9 (110.86)	109.0	71.6 (91.22)	89.0	3	-181.0 (125.11)	-243.0	-254.0 (113.24)	-300.0
166	7	70.6 (122.38)	125.0	76.3 (95.97)	106.0	3	-178.3 (129.71)	-243.0	-251.3 (117.80)	-300.0
178	7	45.0 (112.65)	42.0	50.7 (90.33)	67.0	3	-181.7 (123.96)	-243.0	-254.7 (112.10)	-300.0
190	7	45.3 (124.47)	91.0	51.0 (101.24)	79.0	3	-187.3 (114.18)	-243.0	-260.3 (102.43)	-300.0

Source: Study DMD114673 report, page 68

Note: Treatment was halted from Visit 86-93, after which subjects were on intermittent regimen (shown as grey shaded area)

Reviewer's Comment: There is a wide range of change in 6MWD for all subjects (-263 to 163m)

Efficacy Results - Secondary and other relevant endpoints

Timed Tests:

Subjects in the stable group were all able to complete the tests rapidly (<5 secs), compared to the subjects in the declining group in which the ability to complete the tests continued to diminish. The ability to rise from the floor was the first of these functions to deteriorate. The changes in timed tests were small Table 46.

Table 46 Timed Tests – Change from continued treatment phase baseline

Visit	N	10m Walk/run (sec)		Rising from floor (sec)		Stair Climb (sec)	
		Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
21	11[a]	0.08 (1.184)	0.14	0.50 (1.723)	0.43	-0.85 (1.791)	-0.14
25	11[a]	0.27 (0.515)	0.30	-0.20 (0.577)	-0.08	-1.07 (2.110)	-0.34
29	11[a]	0.61 (0.621)	0.53	0.06 (0.655)	0.07	-0.40 (1.603)	-0.14
33	10[a]	0.56 (0.761)	0.33	1.000 (2.632)	0.22	-0.45 (1.064)	-0.34
37	10[a,b]	0.40 (0.388)	0.30	-0.08 (0.923)	-0.06	-0.18 (1.554)	-0.04
41	10[a]	0.79 (1.133)	0.58	0.55 (0.789)	0.31	-0.61 (1.690)	-0.15
49	10[a,b]	0.51 (1.010)	0.31	0.82 (1.620)	0.24	-0.37 (1.248)	-0.21
61	10[b,c]	0.82 (1.181)	0.38	0.30 (0.656)	0.14	-0.50 (0.996)	-0.30
73	10[c]	0.94 (1.696)	0.12	1.04 (2.620)	0.20	-0.15 (0.677)	-0.21
85	9[c]	1.32 (2.446)	0.38	1.75 (4.031)	0.48	0.04 (1.201)	-0.04
93	9[c]	2.21 (5.229)	0.35	1.27 (2.472)	0.58	0.32 (0.859)	0.00
106	8[b]	0.49 (1.080)	0.12	1.75 (4.588)	0.12	0.76 (1.422)	0.24
118	8	0.72 (1.360)	0.24	3.09 (6.439)	0.35	1.34 (3.041)	-0.03
130	8[d]	0.99 (1.695)	0.28	1.06(1.976)	0.32	0.85 (2.063)	-0.12
142	8[e]	1.00 (1.360)	0.40	0.67 (0.904)	0.24	1.62 (3.469)	0.26
154	8[e]	1.29 (1.925)	0.66	0.44 (0.497)	0.44	1.73 (3.446)	0.59
166	8[e,f]	1.52 (1.789)	0.65	0.79 (0.625)	0.73	1.78 (4.061)	0.43
178	8[e]	1.91 (2.059)	0.78	1.12 (1.097)	0.78	3.48 (5.654)	1.01
190	8[e,f]	1.61 (2.039)	0.86	1.05 (0.914)	0.94	4.23 (10.382)	0.74

Source: DMD114673 Study Report

[a] N=9 for rising from floor
[b] N=9 for stair climb
[c] N=8 for rising from floor
[d] N=7 for rising from floor
[e] N=6 for rising from floor
[f] N=7 for stair climb

In order to interpret the results from all subjects, missing values were replaced by an arbitrary number of 30 seconds (timed considered to be maximum likely).

Reviewer's Comment: In the Table presented, the applicant excluded the worst performing subjects from the summary statistics at later visits, potentially biasing the results.

Muscle Strength: The data were highly variable both between and within subjects.
Pulmonary Function tests: The data were highly variable both between and within subjects. FVC (% predicted) and FEV1 (% predicted) tended to be lower in the declining subjects than the stable subjects. There was a trend in increase in absolute values over time, which the applicant presumes is related to growth over Visit 190. The applicant acknowledges that both the absolute and '% predicted' values have their limitations. The absolute values do not take into account subject growth, however the '% predicted' values use algorithms based on healthy subjects with a 'normal' correlation between age and height. There are no data on how well these correlations apply to DMD subjects whose growth may be stunted. In addition, accurate measurement of DMD subjects' height becomes more difficult if they have contractures and/or lose ambulation. When not measured directly, height may be over-estimated by measuring arm span, which in turn may lead to an apparent reduction in '% predicted' values compared to when their standing height could be measured.
Parent Questionnaire: In general, the majority of parents felt that their child's general condition, walking ability, and endurance was either the same or better than at the beginning of the Continued Treatment phase, with the exception of Visits 154-157 where the majority felt their child's general condition was worse than at the beginning of the Continued Treatment phase. The majority of parents considered their child's ability to climb stairs was the same or better than at the beginning of the Continued Treatment phase up to Visit 123, although subsequently the perception of this ability appeared to worsen through to Visit 178.

Table 47 Summary of Responses to Parent Questionnaire

Visits	N	Response	Questions: change in condition over the Continued Treatment phase			
			General condition	Walking	Taking stairs	Endurance
81-89	11	Better	4 (36%)	4 (36%)	3 (27%)	4 (36%)
		Same	5 (45%)	5 (45%)	3 (27%)	7 (64%)
		Worse	2 (18%)	2 (18%)	5 (45%)	0
109-123	12	Better	3 (25%)	3 (25%)	1 (8%)	0
		Same	5 (42%)	5 (42%)	6 (50%)	10 (83%)
		Worse	4 (33%)	4 (33%)	5 (42%)	2 (17%)
154-157	12	Better	1 (9%)	2 (17%)	1 (8%)	2 (17%)
		Same	4 (36%)	6 (50%)	4 (33%)	7 (58%)
		Worse	6 (55%)	4 (33%)	7 (58%)	3 (25%)
178	12	Better	1 (8%)	1 (8%)	1 (8%)	2 (17%)
		Same	8 (67%)	6 (50%)	3 (25%)	8 (67%)
		Worse	3 (25%)	5 (42%)	8 (67%)	2 (17%)

OBP Reviewer Dr. Rao's assessment of dystrophin expression:
Dystrophin expression by IFA:

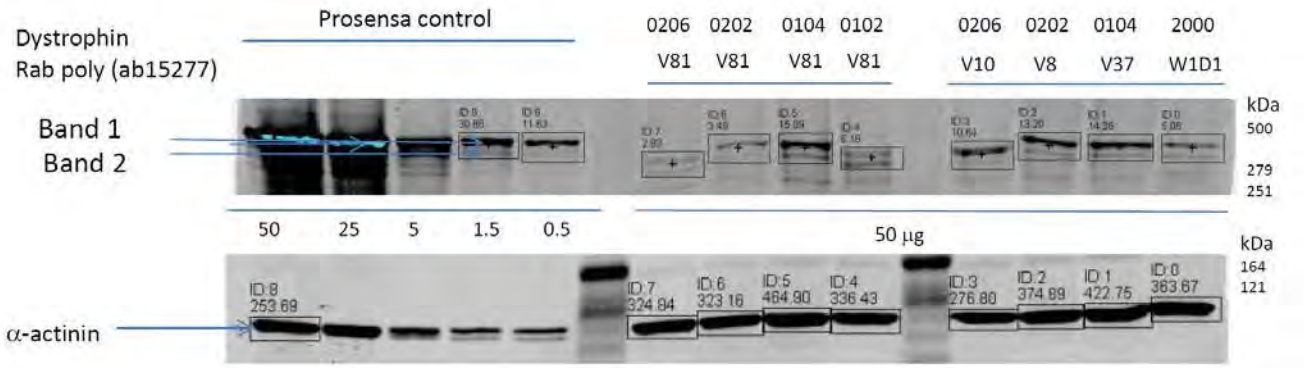
- **No reliable estimate of dystrophin expression was obtained at Week 24 of the open label**

phase due to poor muscle biopsy quality in the 12 subjects.

- **8/12 subjects had additional biopsy at Week 72. For IFA analysis, 6/8 subjects had baseline biopsies. Compared to baseline, 3/6 subjects showed slight increase (2 to 8%) in mean membrane intensity, 3/6 subjects showed a decrease (1 to 9%) at Week 72.**

Dystrophin expression by WB:

- **WB analysis was conducted on 8 patients. An increase of 21-46% at week 24 over previous time point was observed; however, at week 72 the same patients did not consistently show an increase.**
- **Each western blot image included a serial dilution of a healthy positive control. One representative image shown below shows the serial dilution between 0.5-50 μg of healthy control and 50 μg of DMD patient samples. Based on reviewer estimation, the observed dystrophin levels approximately ranged between 0.24 to 0.28 % (for subject 0206/V81 sample) and 1.27 to 1.47% (for subject 0104/V81 sample) of healthy control in the gels (looking at the lightest and the darkest bands on the gel). However, importantly, the α-actinin loading for these two samples do not appear to be comparable to each other, so the dystrophin expression in these two subjects cannot be reliably compared to each other**.



It appeared that most DMD samples showed a reasonable dystrophin band that was within the tested range of the positive control serial dilution shown on each gel; although, densitometric numbers are only provided for the 0.5 and 1.5 μg loaded bands for all gels suggesting saturation beyond these samples. The α-actinin bands appeared to be reasonably resolved, consistent between samples and adequately clear to allow a quantitative estimation of protein loading. Overall, for all the gel images provided, it is not possible to get an exact quantitation of the relative dystrophin due to the saturation in the dystrophin bands at higher concentrations of the healthy controls and no quantitation provided for α-actinin at the lower concentrations. The densitometric quantitations for all samples suggest a slight increase in normalized dystrophin compared to the prior time-point for some samples but a decrease for others. The modest increase appear to be below the applicant's stated threshold of >30% above baseline for a positive response.

Reviewer's Comment: This suggests that there is no evidence of increased dystrophin intensity over time, i.e. 72 week after the start of the open label study. The Applicant suggests that problems with the quality of the sample preclude conclusive interpretation. However, it appears that the quality of

the samples and Western blots was adequate to exclude that there was anything other than a very small increase in dystrophin expression due to treatment.

OBP Reviewer Dr. Rao's comment:

> **Upon request, the applicant clarified that, with the exception of one sample at 1.1% the dystrophin levels observed in this study were all below 1%, which is their lower limit of detection by WB.**

Persistence of Effect

No dose was given between Visits 86-93, after which subjects were switched to intermittent regimen. Change from baseline appeared to be the same in the "Stable" subjects. However, it is unclear if this is persistence of effect (see Reviewer discussion below)

Additional Analyses Conducted on the Individual Trial

Comparison to Natural History (NH):

Applicant's conclusion on natural history comparison:

- Most NH subjects had worse functional trajectory compared to matching drisapersen subjects. Three subjects were excluded from the matching analysis:
  - Subject 104 was considered atypical, had baseline 6MWD of >600m and functional capacity was maintained for 3.4 years
  - Subject 201 was non ambulant at baseline
  - Subject 103 had a single match and only one assessment from the point of matching
- Substantial gains up to 192 m from baseline were reported. Increases of this magnitude were not seen in NH controls.
- The difference in subjects that declined and NH was less easy to discern.

FDA Analysis based on natural history

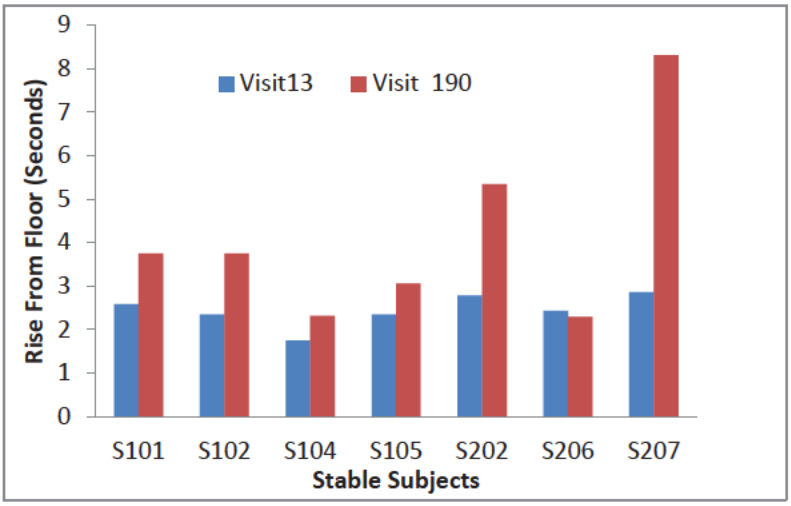
This analysis was conducted by Dr. Bhattaram (Pharmacometrics). FDA analysis suggested that there were insufficient number of patient matches (based on age, 6MWD, rise from floor and exon 51) from the natural history dataset provided to obtain reliable comparisons. Please refer to Dr. Bhattaram's review for details on the analysis.

Reviewer's Analysis and Discussion:

The "Stable" subjects (N=7) were between 5 and 10 years of age. The "Stable" subjects had Rise Time From Floor between 1.7 -2.8 seconds at study baseline (Week 13) and between 2.3-5.3 seconds at end of study at Week 190. Only one subject had a Rise from Floor Time of 8.3 seconds at Week 190. Five of these subjects had baseline 6MWD between 374-647 m and two subjects had baseline 6MWD of 340 and 350 m. (Figure 25). These subjects appear to have a milder prognosis. The mean (SD) change from baseline in the "Stable" subjects was 45 (124)m at Week 190.

The "Decline" subjects (N=3) had baseline Rise from Floor of 7.2-9.4 seconds and a maximum of 28.5 seconds at Week 118. No subject could perform the test beyond Week 118 (See Figure and Table below for the Rise from Floor Time and the baseline 6MWD in the "Stable" Subjects.

Figure 25 Rise from Floor and 6MWD in "Stable" subjects

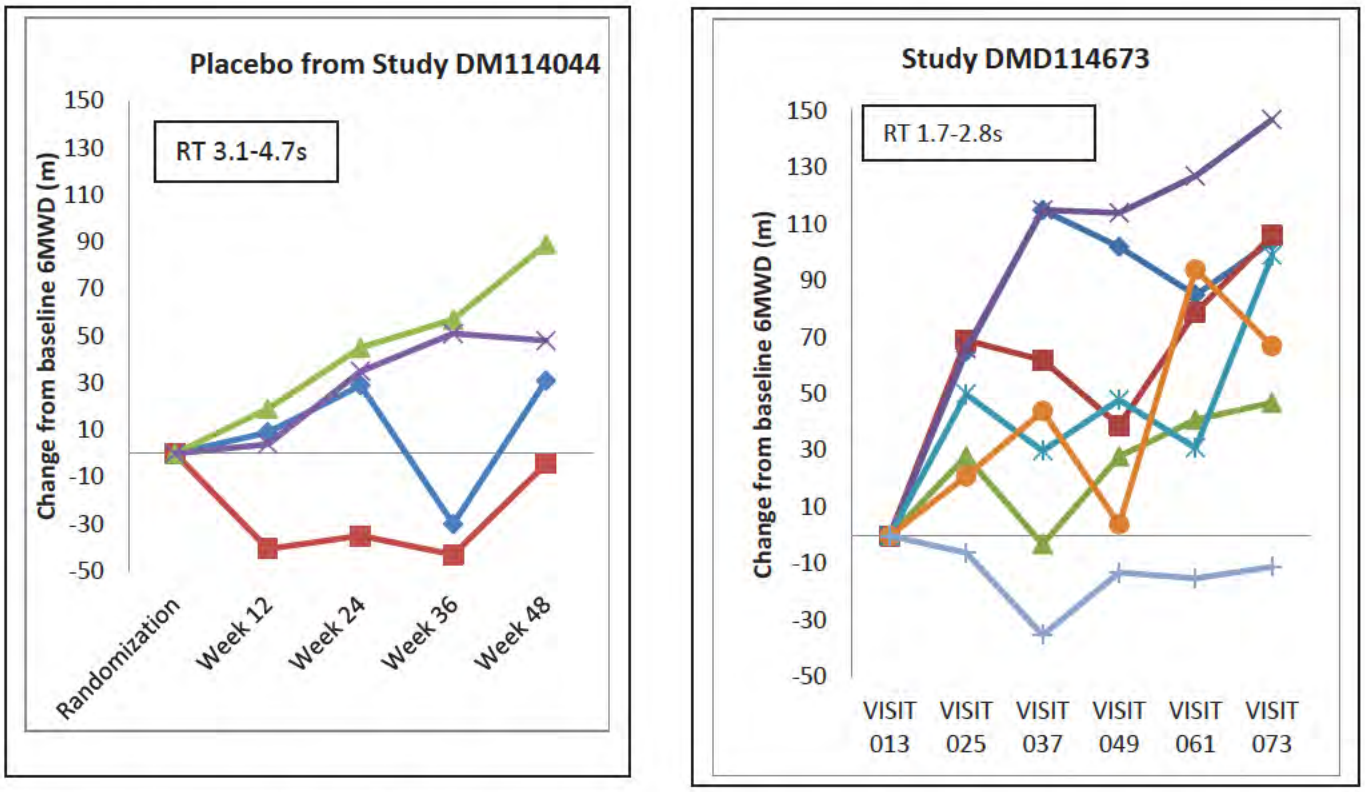


Subject	Baseline Rise Time	Baseline 6MWD
101	2.58	374
102	2.35	406
104	1.75	647
105	2.35	340
202	2.78	429
206	2.44	350
207	2.86	500

I looked at placebo subjects from Study DMD114044 that would be between the ages 8 and 14 years (for the 3+ years of study duration) and would have a rise time of < 5 seconds to match the baseline characteristics of the stable population in this study to explore the disease trajectory in such subjects. There were no matching subjects >10 years with similar characteristics. The "Stable" subjects in the open label study were atypical, that had extraordinarily low rise time from floor time, at an age when many patients with this genotype lose ambulation. As can be seen the Figure 26, placebo subjects in the similar baseline (age, 6MWD and Rise time) characteristics also show a gain in the 6MWD *over a year* of similar magnitude (up to 90 m). Subjects with low Rise from Floor Time appear to have a milder prognosis and can remain more functional for couple years before they begin to decline. For comparisons, I plotted the disease trajectory for the patients in this study up to 1 year.

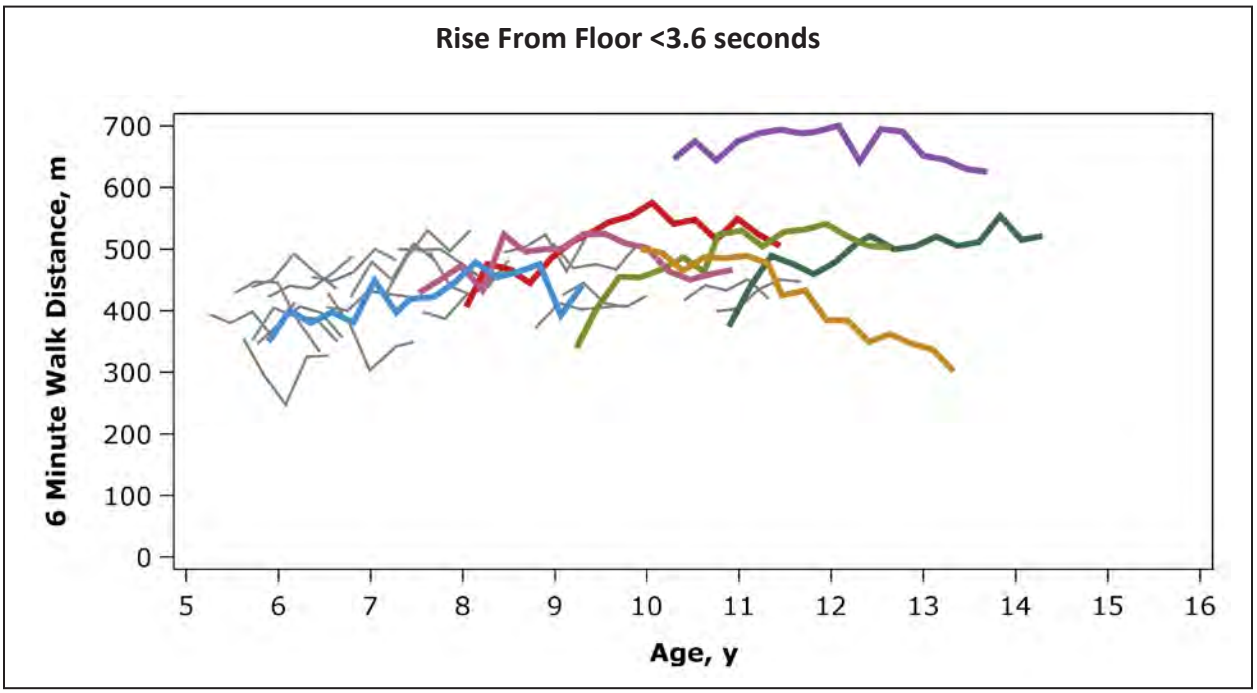
The disease trajectory in this one year duration appear similar, even though the placebo subjects from Study DMD114044 have baseline Rise from Floor *higher* (worse prognosis) than those in Study DMD114673. No other study in the application has a mean rise from floor time of 2.4s (Stable group). The mean rises from floor time in other studies range from 4.8-12.3s.

Figure 26 Comparisons of subjects with similar Time to Rise From Floor

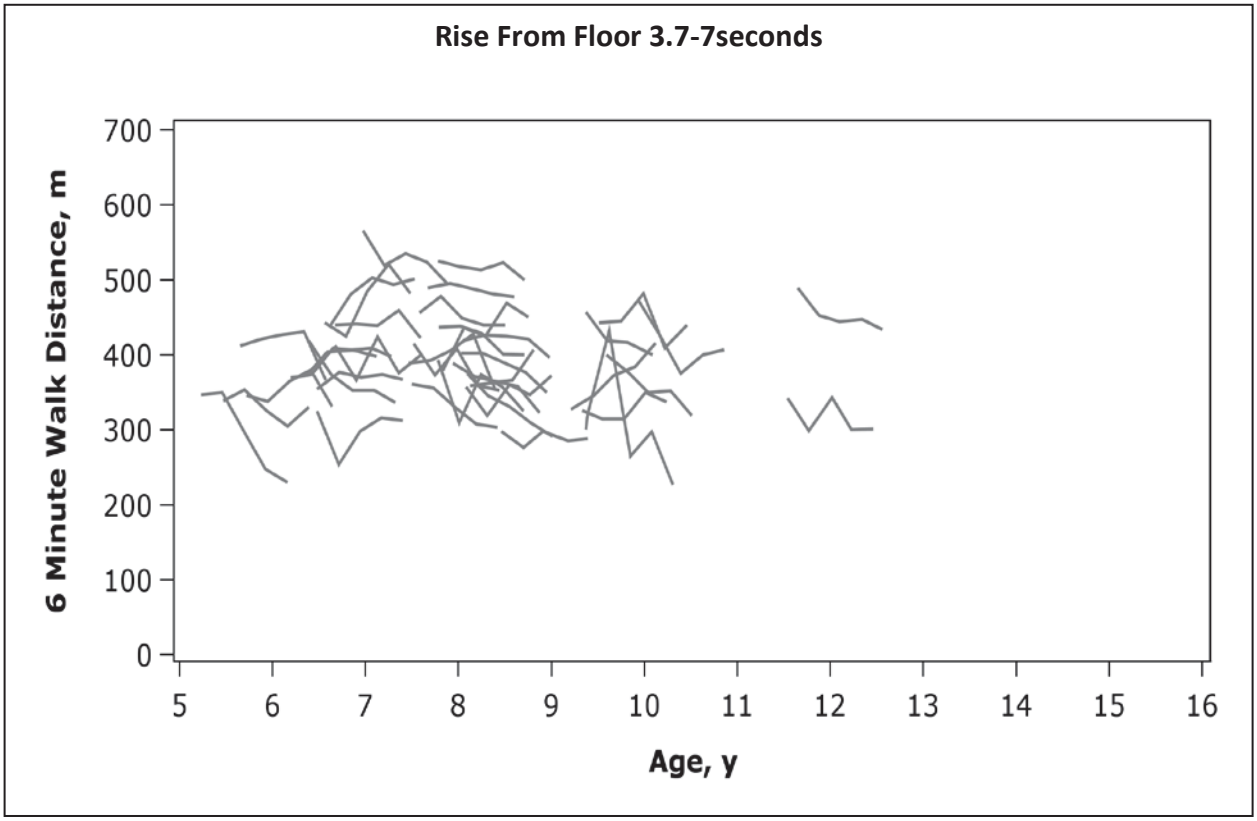


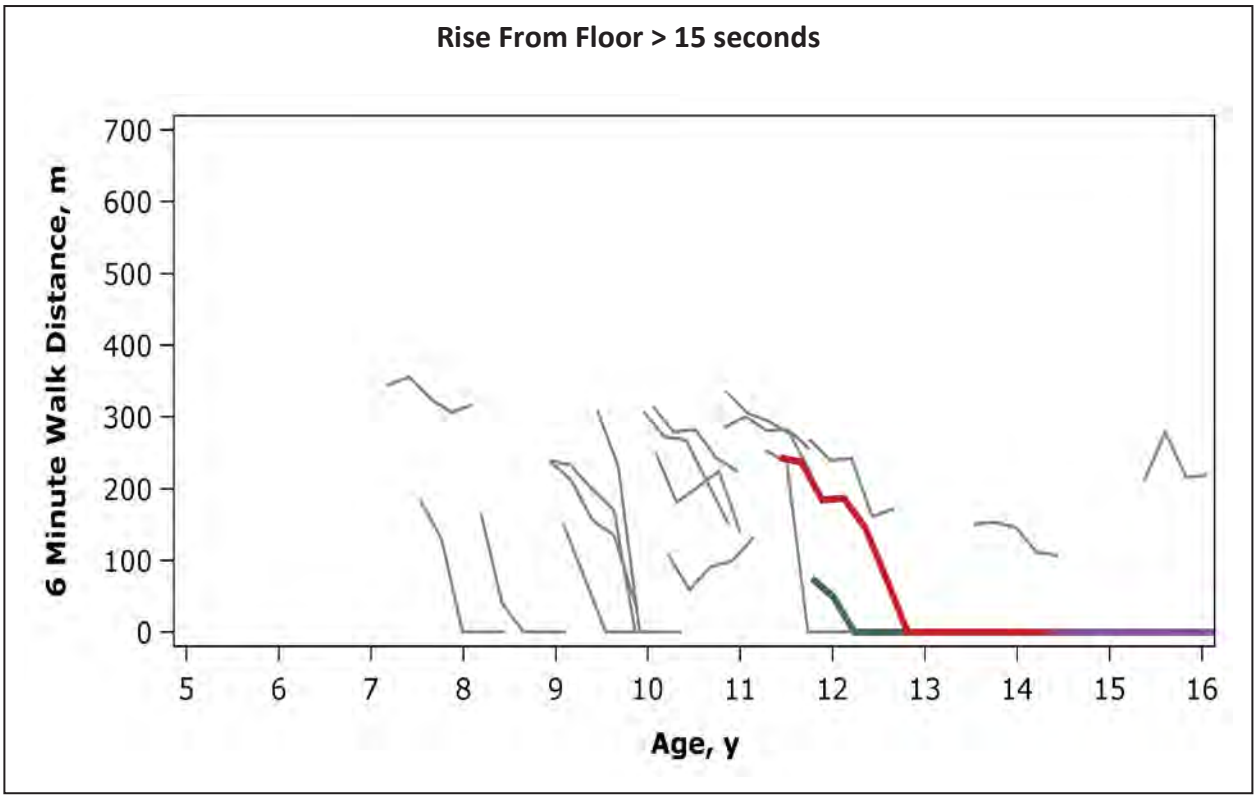
The 6MWD across all the placebo subjects from drisapersen studies, with Rise from Floor that match those from this Study DMD114673 was also looked at to evaluate the disease progression in these subjects. Rise from Floor from Study DMD114673 were lower than those found in the placebo subjects. The four different groups of baseline Rise from Floor were generated and the 6MWD was plotted as a function of age. The four groups were subjects with baseline Rise From Floor <3.6 seconds (to match the stable patients), 3.7-7 seconds, 7.1-15 seconds and >15 seconds. The 6MWD for each patient from this open label study was overlaid on the placebo subjects, to visualize the disease course of the subjects as shown in Figure 27. The figure shows that the disease progression of the subjects in Study DMD114673 is not different from placebo subjects from the drisapersen studies. The subjects that have higher function at baseline tend to show slower progression for few years.

Figure 27: 6MWD matching analysis with placebo subjects from drisapersen studies matched for baseline Rise From Floor Time



Note: Placebo data are shown in grey lines and all subjects from Study DMD114673 are shown in colored lines



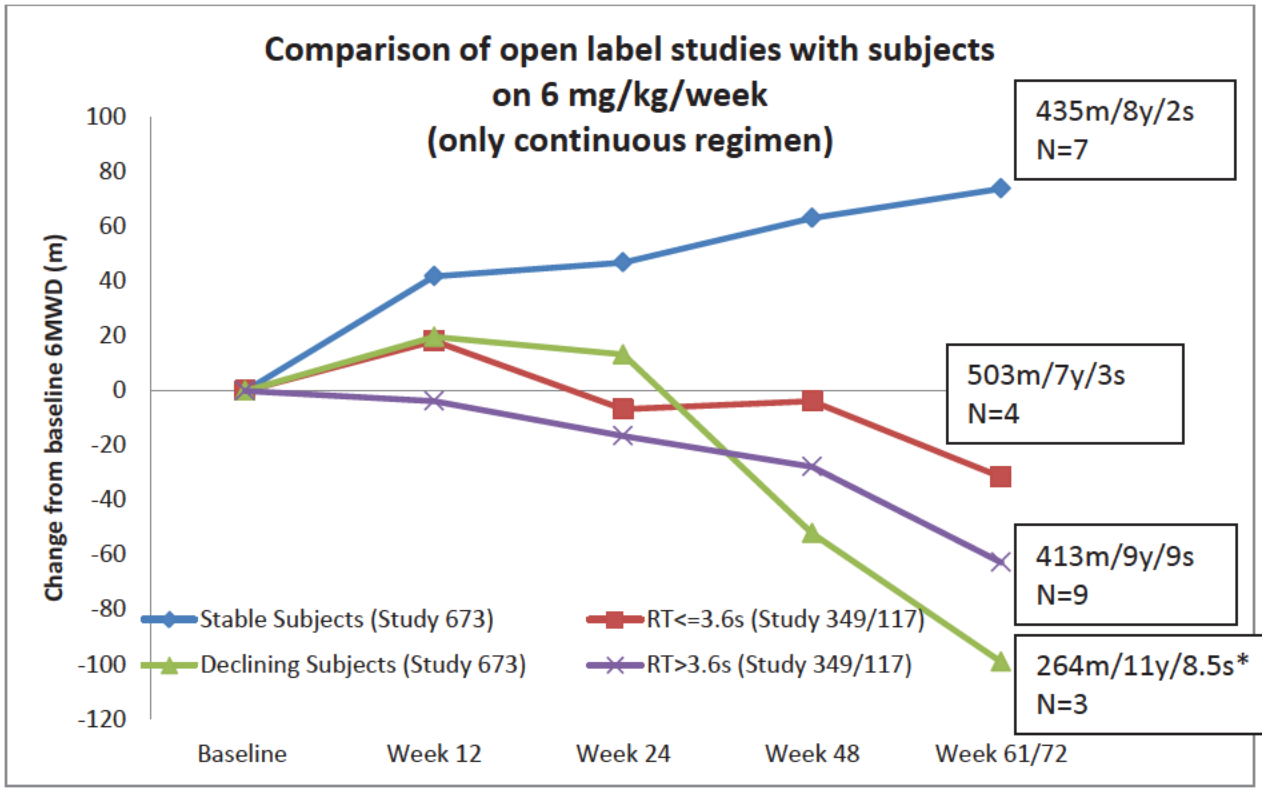


Source: Dr. Bhattaram

In addition, I also explored the differences in disease progression across the two open label studies (Study DMD114349 and Study DMD114673). I graphically compared the means profiles of "Stable" and "Declining" subjects from Study DMD114673 to the subjects from the Phase 2 study DMD114117 that were evaluated for 2+ years in the open label extension study DMD11349. Baseline 6MWD, age and Rise from Floor are provided in boxes for each group of patients. Three observations were made in this comparison based on the mean data for each group:

1. **Two small groups of patients in an open label setting can behave quiet differently such as the interpretation of small open label studies may be problematic (Figure 28, blue and red profiles.**
2. **The disease progression appears to be dependent on the baseline Rise from Floor, 6MWD and age. Subjects with milder disease at baseline tend to have a slower progression, as shown by grouping subjects with Rise from Floor <3.6 and >3.6 seconds. This makes it difficult to conclude that the slow progression is due to drug effect and not due to the natural course of the disease in such patients. With the lack of adequate long term natural history data in such patients with mild disease at baseline, any conclusions of drug effect appears problematic (Figure 28 and Figure 29).**
3. **The magnitude of decline also depends on the composition of the patients in a group. When subjects from the continuous and intermittent regimens were combined the magnitude of decline was greater (Figure 29 and Figure 30).**

Figure 28 Comparisons of open label studies with subjects on 6mg/kg/week



*Note: one subject was unable to Rise from Floor, two that lost ambulation not included, week 61 plotted for declining subjects

Figure 29 Comparisons of open label studies (Study DMD114117 regimens combined)

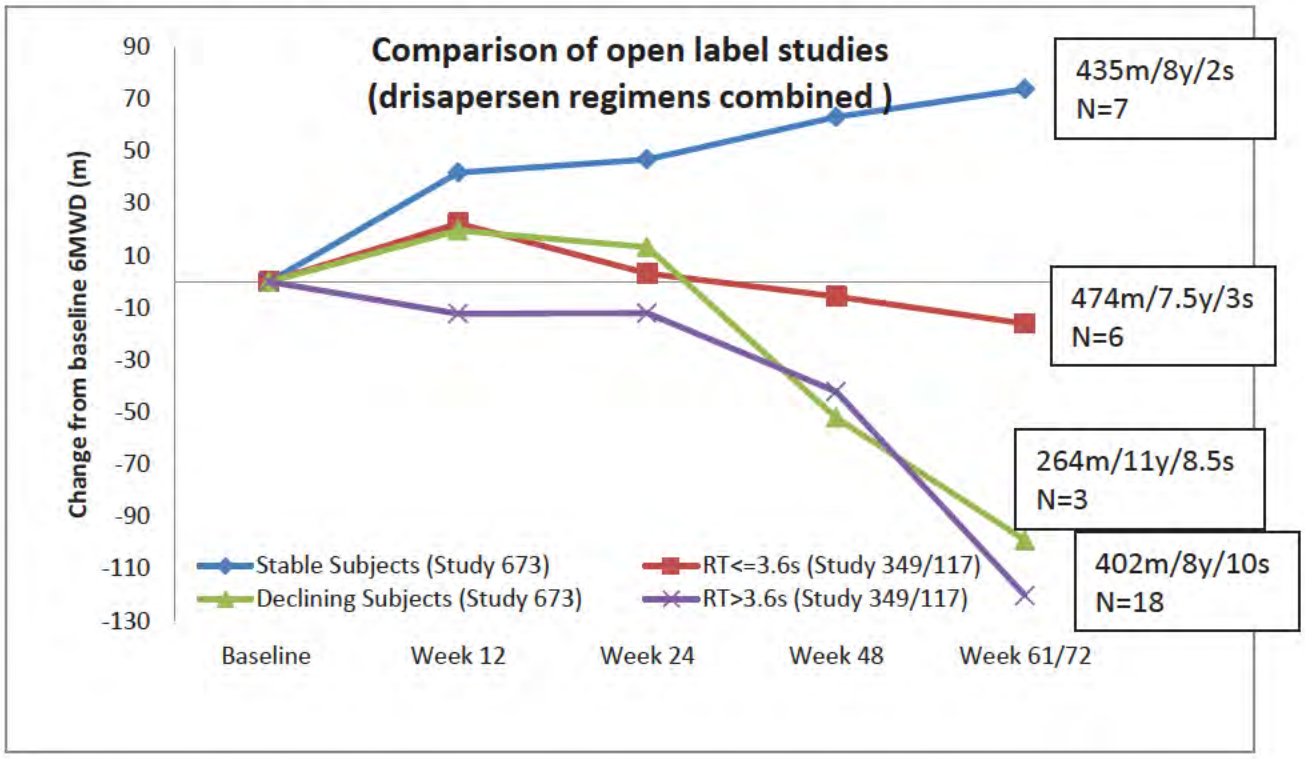
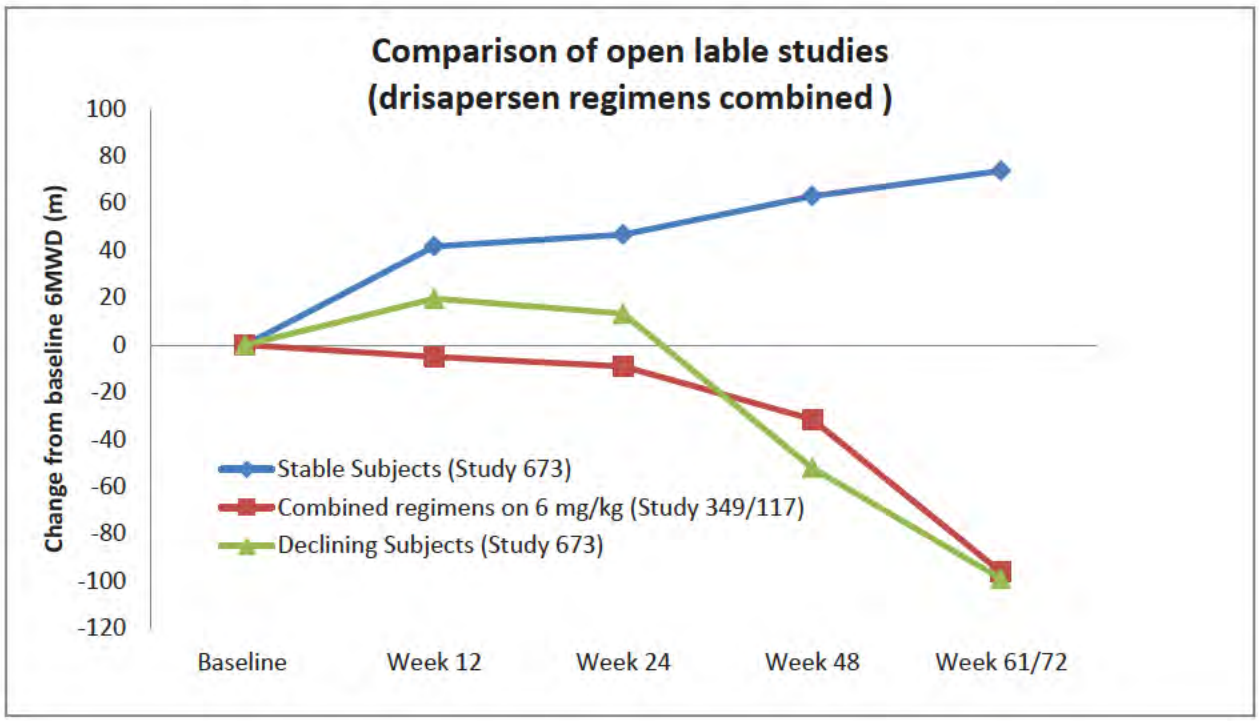


Figure 30 Comparisons of open label studies (Study DMD114117 regimens combined, not separated by Rise from Floor)



Therefore, age, 6MWD and rise from floor time all are important factors to determining the disease trajectory of a given patient, in additional to many other known and unknown factors, genetic modifiers etc. The subjects in this study appear to have a better disease prognosis. In the absence of a control group, this study therefore, does not show any interpretable evidence of a benefit from treatment with drisapersen.

6.6. PRO051-02

6.6.1. Study Design

Overview and Objective

This was a phase I/II, open label, escalating dose pilot study to assess the effect, safety, tolerability and pharmacokinetics of multiple subcutaneous doses of PRO051 in patients with Duchenne muscular dystrophy.
Study Period: 25 March 2008 to 25 May 2009
Study Centers: UZ Leuven (campus Gasthuisberg), Leuven, Belgium
The Queen Silvia Children's Hospital, Goteborg, Sweden

Trial Design

Doses: Dose escalation study with subcutaneous injection of 0.5, 2, 4 and 6 mg/kg once per week (N=3 in each group)
Medication was injected in the abdominal subcutis by a maximum of two subcutaneous injections.
Population: 12 DMD boys with mutation correctable by skipping exon 51

- Ages 5-16 years that had a life expectancy of at least 6 months and were not on ventilator support.
- Glucocorticoid use was to be stable for at 2 two months prior to enrolment, and was to be kept constant during the study.

At screening, an MRI was performed to assess the quality of the muscle in which the biopsy was planned.
Duration/Assessments: Treatment: 5 weeks (Days 1, 8, 15, 22 and 29), Follow up: 13 weeks (Days 36, 43, 57, 78, 99 and 120)
In the 0.5 mg/kg dose group, a muscle biopsy was taken at Visit 1 (Screening) and at Visit 8 (Day 43). In the other dose groups, a muscle biopsy was taken at Visit 8 (Day 43) and at Visit 10 (Day 78).

Study Endpoints

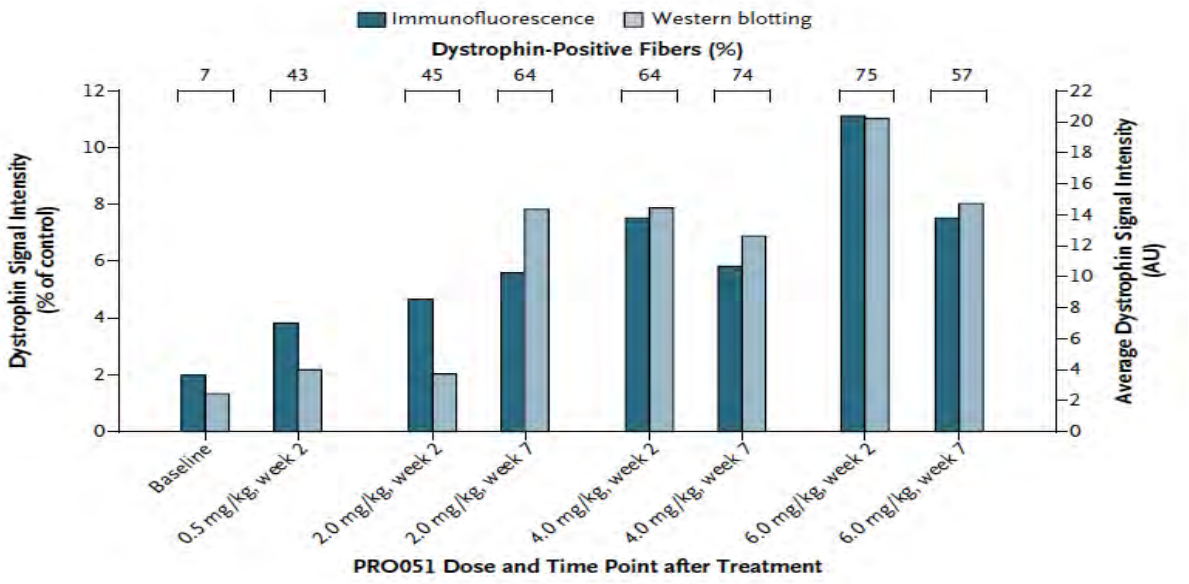
- Production of exon skip 51 mRNA and dystrophin expression by IFA and WB
- Presence of dystrophin expression (immunofluorescence analysis of cross-sections derived from muscle biopsy and Western blot analysis of total protein extracts from muscle biopsy)
- Muscle function (10m walk/run test, timed rising from floor, stair climb and 6MWT)
- Muscle strength

6.6.2. **Study Results**

In the 0.5 mg/kg dose group none of the subjects showed exon 51 skipping by RT-PCR. The effect in the 6 mg/kg dose group was not as prominent. Only the 4 mg/kg group showed exon 51 skip in all subjects. Average number of dystrophin positive fibers and average dystrophin signal intensity increased with increasing dose at 2 and 7 weeks after treatment.

The applicant refers to the published article by Dr. Goemans, which states that new dystrophin expression was observed between 60-100% of muscle fibers in 10 of the 12 patients, which increased in a dose dependent manner to up to 15.6% of the expression of healthy muscle.

<u>Reviewer's Comment:</u> It is unclear how dystrophin was detected 2 and 7 weeks after 5 doses in this study, when in no other study dystrophin was detected after 12 weeks of continuous dose of 6 mg/kg/week. The applicant has not provided individual raw data for this study to support the numbers for the dystrophin positive fibers, but refers only to the published article. There were no pre-treatment assessments at all doses, but only for the low dose (0.5 mg/kg) which is represented as the baseline in the above figure.

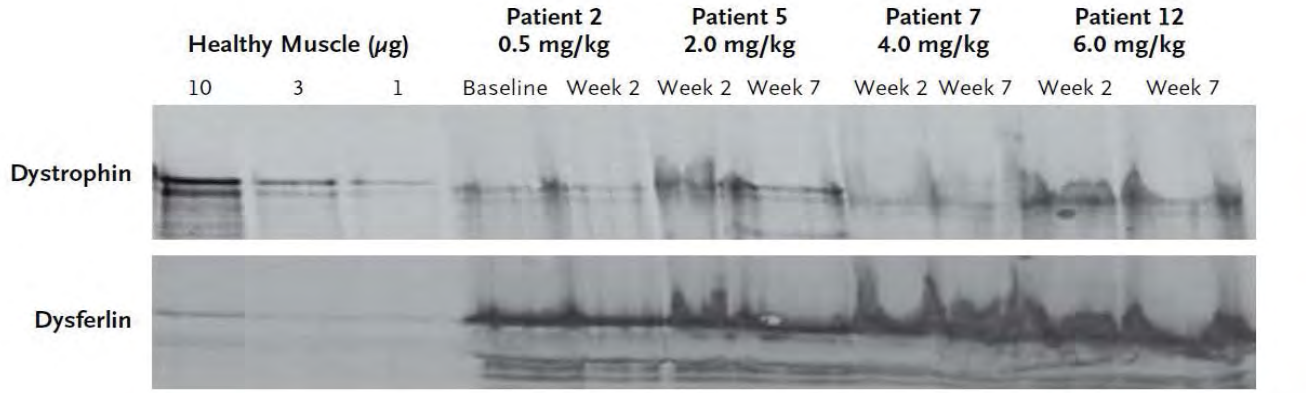


Source: Goemans NEJM 2010

<u>OBP Reviewer Dr. Rao comment on methodology:</u>

The publication states that "a signal intensity of 15.6% of the control intensity in Patient 8." However, no raw data for patient 8 is provided in the publication. Overall, they comment that "The amount of dystrophin ranged from 17 to 35% of control levels." However, a baseline sample was not tested in 9 out of the 12 patients. It is also noted that the western blot image in the NEJM publication is of <u>*extremely poor quality*</u> because the dystrophin bands are not clearly discernable, there is substantial smearing of the bands into other lanes, air bubbles or other artifacts, and the loading control (dysferlin) appears to be <u>*unusable*</u> for densitometric

quantitation and normalization of dystrophin. While the healthy control dilutions appear to suggest that dystrophin levels comparable to or higher than these dilutions were observed in post-treated samples, the dilution factor between the healthy and DMD samples was between 300-500 fold, suggesting that post-treatment levels were extremely low compared to normal. Furthermore, linearity cannot be assumed at this high dilution.



6.7. Other Studies: DMD PRO051-01

Study PRO051-01 was an exploratory Phase 1 single dose, open label study in 4 ambulant and non-ambulant DMD subjects to evaluate the local dystrophin production after localized intramuscular injection. The primary data was not submitted to the NDA, and the following description is based solely on what was reported by the authors of the published report. Subjects were administered 0.8 mg drisapersen intramuscularly. The amount of dystrophin in total protein extracts ranged from 3 to 12% of that found in the control specimen and from 17 to 35% of that of the control specimen in the quantitative ratio of dystrophin to laminin α2.

7 Integrated Review of Effectiveness

7.1. Assessment of Efficacy across Trials

7.1.1. Primary Endpoints

The Applicant conducted three randomized, double blind placebo controlled trials:

1. Study DMD114117 (n=53): evaluated two different regimens (continuous and intermittent) of the same dose (6mg/kg); Primary endpoint was change from baseline 6MWD at Week 25.
2. Study DMD11476 (n=51): evaluated two doses(3 mg/kg/week and 6 mg/kg/week); primary endpoint was at Week 24
3. DMD114044 (n=186):evaluated a single dose (6mg/kg/week), primary endpoint was at Week 48

In addition, the NDA included two open label extension studies Study DMD114349 (the 120 week extension of the placebo controlled studies: DMD114117 and DMD114044), and Study DMD114763

(the 3.5 years extension of a 5 week proof of concept study PRO-051-02). In the extension studies subjects were given 6 mg/kg/week, but subjects had the option of moving to the intermittent regimen or to discontinue for safety related reasons.

6MWD is considered an effort dependent endpoint. The unblinding due to injection site reactions observed in the drisapersen treated subjects could potentially bias the results obtained from an effort dependent endpoint.

The strength and weaknesses of the analysis of the primary endpoint for the three placebo controlled studies are summarized below:

- Study DMD114117: The primary endpoint, change from baseline 6MWD at Week 25 was statistically significantly different from placebo for the continuous 6mg/kg/week dose with a treatment difference of 35 m (p=0.01) over placebo. Some of the weaknesses of the study include:
    - *Lack of treatment benefit with intermittent regimen*: a non- significant p-value of 0.80 at 24 weeks for the intermittent regimen which had comparable total doses and identical plasma concentration time profile and should seemingly produce similar response to treatment.
    - *Decline at Week 49*: The change from baseline in 6MWD of 31 m observed at Week 25 does not appear sustained at Week 49 with a decline to 11 m. Such differences could also be observed due to variability that may make the results look worse than they are (such as at Week 49) and make the results look better than they are (such as at Week 25). The improvement seen at Week 25 followed by a decline at Week 49 in the continuous drisapersen treatment group is concerning.

The subjects in the continuous treatment arm comprised of patients that were more functional at baseline compared to the intermittent and placebo arms as discussed in section 6.1.2. Differences in the baseline functional abilities of subjects in the treatment groups could bias the interpretation of the results. A small study increases the risk that efficacy may reflect baseline imbalances. Subjects with milder disease progression and younger age may remain stable or improve for the duration of 48 weeks or more. (McDonald 2013).

Since the continuous and intermittent regimen, both consisted of the same 6 mg/kg dose, a post-hoc analyses combining the two treatment regimen, showed a treatment difference over placebo of 31 m (nominal p=0.05) at Week 49, but a treatment difference of 20 m (p=0.12) at Week 25. This post-hoc analysis may suggest a lean towards treatment benefit at week 48 with drisapersen for a phenotypic heterogeneous DMD population, although unblinding due to injection site reaction remains a concern.

- Study DMD114876: The primary endpoint, change from baseline 6MWD at Week 24 for the 6mg/kg/week dose showed a treatment difference over placebo of 27 m (p=0.07) that was not statistically significantly different from placebo. Ordinarily this would be considered a negative study, but for a disease with no approved treatment, this could be considered as supportive evidence of benefit. Additional weaknesses of the study include:
    - In applicant's sensitivity analysis, removing a single placebo subject whose treatment was unblinded due to a hospital visit for flu-like symptoms, a treatment difference of 19 m (p= 0.21) was observed, further weakening the confidence in the evidence towards efficacy.

- A lower 3 mg/kg/week dose was worse than placebo. This suggests that the disease trajectory of patients with DMD is dependent on their baseline disease characteristics as discussed in section 6.2.2.
- This was a 24 week study. A 48 week Study DMD114117, showed at decline from baseline at Week 48. In Study DMD114876, the 6 mg/kg/week group showed stability in 6MWD up to Week 48, even when no treatment was given beyond 24 weeks. This could be due to variability or that subjects follow their own disease trajectories based on the baseline characteristics. Natural history studies by experts in the area suggest that earlier functional abilities can predict later functional abilities.

- Study DMD114044 (2:1 randomization): The change from baseline at week 48 for the 6 mg/kg/week dose was 10 m (p=0.42) that was not statistically significantly different from placebo. Applicant's discussion on the lack of response in study DMD11404 is given in section 6.3.3. Overall, this study was a well-designed and executed study with good statistical power to detect a small treatment effect.

The change from baseline and magnitude of treatment difference in these placebo controlled studies is summarized in Table 48 on page 116:

The open label studies with drisapersen are not supportive of treatment benefit.

- A 120 week open-label extension Study DMD114349 appears uninterpretable as many subjects dropped out either due AE or the study being terminated early (4/18 on placebo and 5/35 on treatment from Study DMD114117). The decline in 6MWD in each treatment arm based on the parent study (DMD114117 and DMD114044) appears to be consistent with the phenotypic heterogeneity of each arm (see discussion in section 6.4.4), with the more functional subjects at baseline showing a slower progression and less functional patients at baseline showing a larger decline in a year. The applicant asserts a treatment difference of 50m at Week 96 for subjects on the continuous regimen compared to the placebo group that switched to treatment. The Applicant discusses the extension of DMD114117 only based on the subjects that were on continuous 6mg/kg/week regimen, that appeared more functional at baseline compared to the subjects on the intermittent regimen. The subjects on intermittent regimen when switched to the continuous regimen after 48 weeks show a treatment difference of 8m at Week 96 compared to the placebo group that switched to treatment. The subjects on intermittent regimen showed a mean decline in 6MWD of 63m at Week 96. It is known that the disease course is highly variable between affected individuals, a striking example being the age for the loss of ambulation, which can range from 6 to 15 years (Flanigan 2013, Hembertclaude 2102) and higher baseline function is associated with slower long-term decline in DMD (McDonald).
- A 3.5 years long open label study DMD114673 in 12 subjects showed that only 5 subjects did not decline during this study and 3 lost ambulation. The subjects that declined did not appear to be different from the typical natural history control. The subjects that did not decline in Study DMD114673 were atypical. They were highly functional at baseline with Time to Rise from Floor of <3seconds. ***There were no other patients in the entire drisapersen development program with such low Time to Rise from Floor***. Consequently, it is entirely unconvincing that the stability observed in these 5 subjects is a treatment effect.

Table 48 Primary endpoint analysis from the three placebo controlled studies (primary endpoint in orange colored boxes)

	Phase II studies						Phase III study	
	DMD114117			DMD114876			DMD114044	
	Placebo (combined) (N=18)	Drisapersen 6 mg/kg/wk (N=18)	Drisapersen 6 mg/kg intermittent (N=17)	Placebo (combined) (N=16)	Drisapersen 3 mg/kg/wk (N=17)	Drisapersen 6 mg/kg/wk (N=18)	Placebo (N=61)	Drisapersen 6 mg/kg/wk (N=125)
Baseline, n								
Mean								
Week 24								
n	16	16	15	16	17	18	59	122
Mean change	–4	31	–0.1	–11	–20	16	–29	–24
Treatment difference		35	4	–	–9	27	–	5
p-value		0.01	0.80	–	0.55	0.07	–	0.63
Week 48								
n	17	18	15	15	17	18	59	117
Mean change	–25	11	2	-13.17	-38	15	–52	–42
Treatment difference	–	36	27.	–	-25	28	–	10
p-value	-	0.05	0.15	–	0.24	0.18	–	0.42

No treatment

Source: Adapted from Applicant's analysis with FDA re-evaluation

The conflicting, results from Study DMD114044 diminish the strength of the smaller studies. One of the explanations by Applicant for this negative study has been the demographic and baseline population make-up of the Phase II and Phase III studies. As shown in the Table below, the smaller studies had a slight younger and more functional population in the continuous 6 mg/kg/week arm compared to the larger negative study.

Study	Prognostic Factor	Placebo	Drisapersen 6 mg/kg/week
DMD114117 (N=53)	Median Age (years) Baseline 6MWD (m)	7 years 403 m	6.5 years 428 m
DMD114876 (N-51)	Median Age (years) Baseline 6MWD (m)	8 years 416 m	6.5 years 396 m
DMD114044 (N-186)	Median Age (years) Baseline 6MWD (m)	8 years 348 m	8 years 337 m

Based on mean/median age and mean 6MWD at baseline, it appears true that the DMD subjects in Study DMD 114044 are likely to be more progressed in their disease. No restriction on the rise from floor was the only difference in the enrollment criteria between these studies. I looked at a subset of subjects from this study that had the similar age distribution (5-13 years), same range of 6MWD at baseline (330-561m) and subjects with rise time of ≤7 seconds as in the smaller studies to match the patient population characteristics in the Phase 2 and 3 studies. The MMRM analyses were conducted by Dr Sharon Yang (Statistician). The subjects in this subgroup of Study DMD114044 are balanced with regards to their age, baseline 6MWD and rise from floor time as shown below.

Study DMD114044 Subset with Subjects that meet the following criteria: Ages 5-13 years 6MWD 300-561m RT ≤7 sec	Prognostic Factor at baseline	Placebo N=27	Drisapersen 6 mg/kg/week N=59
	Median Age (years)	7 years	7 years
	Mean Age (years)	6.8 years	7.2 years
	Mean 6MWD (m)	401 m	402 m
	Median 6MWD (m)	399 m	402 m
	Mean Rise from Floor (s)	4.44 s	4.84 s
	Median Rise from Floor (s)	4.1 s	4.9 s

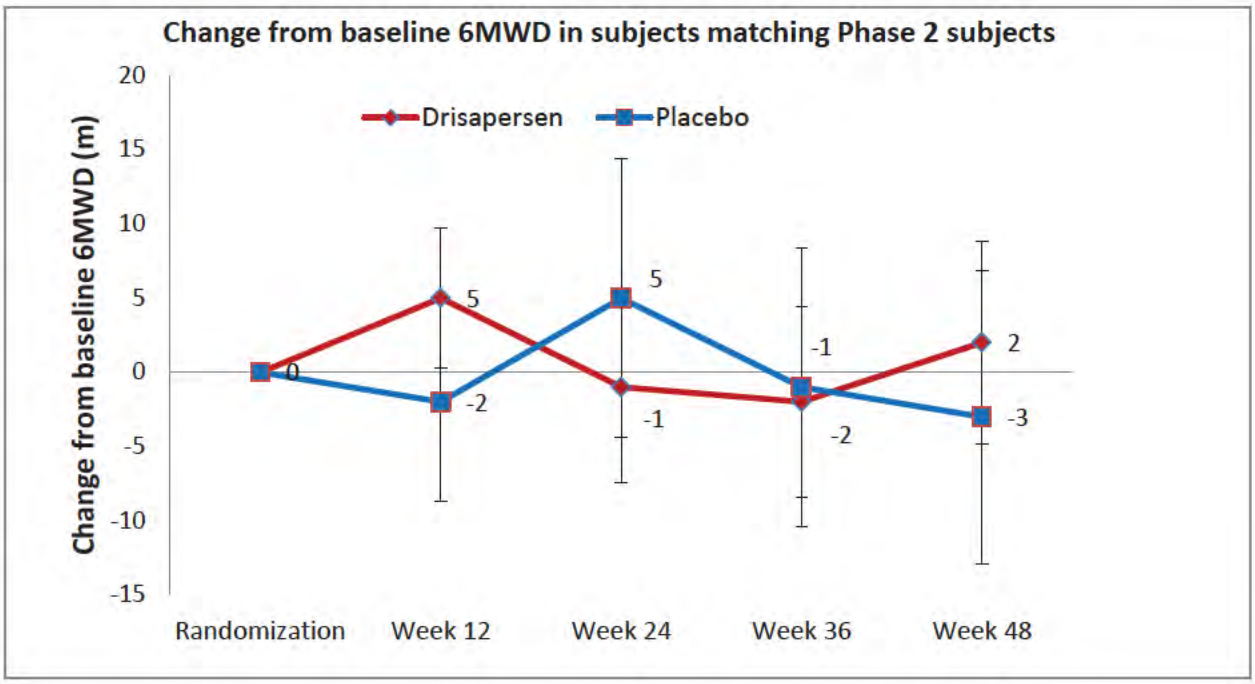
This subset of patients from Study DMD114044, showed a mean treatment difference of 5m, suggesting that the severity of the disease is not the reason for the negative results in study DMD114044 (Table 49). The results from this post-hoc analysis are contradictory to the results obtained from Study DMD114117. This also suggests that chance baseline imbalances, not drug effect, led to the positive findings in study 117.

Table 49 FDA analysis of subjects from Study DMD114044 that match the subjects from Studies DMD114117 and DMD114876

	Placebo (n=27)	Drisapersen 6 mg/kg/week (n=59)
Week 48		
Adjusted mean change	-3	2
Adjusted mean difference vs. placebo		5
p-value		0.71

Figure 31 shows the adjusted mean change from baseline in subset of subjects from Study DMD114044 that match the baseline characteristics of subjects from the Phase 2 studies.

Figure 31: FDA analysis of subjects from Study DMD114044 that match the subjects from Studies DMD114117 and DMD114876



I also conducted Kaplan-Meier analysis showing time to persistent 10% worsening in 6MWD in subjects with rise from floor ≤7 seconds. No meaningful differences between treatment groups were observed. Hence, the applicant's argument of a more functionally impaired population of the Phase III study as the reason for a negative study is not convincing.

The applicant presents the argument in the ISE that certain subgroups based on age (≤ and >7 years) and 6MWD (±330) may respond differently. The applicant's post-hoc analyses showing the treatment

differences in the four subgroups based on age and 6MWD are presented in Table 50.

Table 50 Mean/Median Change of baseline 6MWD by age group and 6MWD (applicant's post-hoc analysis of Study DMD114044)

	Age ≤ 7 years				Age > 7 years			
	6MWD ≤ 330 meters		6MWD > 330 meters		6MWD ≤ 330 meters		6MWD > 330 meters	
	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo
N	17	4	33	25	33	17	34	13
Mean	11	-62	-12	-18	-124	-108	-25	-52
SD	49	99	63	51	94	108	61	70
Median	23	-55	-7	-26	-109	-109	-22	-55
Min	-109	-184	-215	-96	-303	-311	-194	-185
Max	68	42	112	106	48	89	102	48
Difference in Medians	+77 p=0.01		+20 p=0.66		-0.2 p=0.48		+33 p=0.24	
Difference in Means	+75 m		+6 m		-16 m		+27m	

Source: NDA module 2.5 Clinical Overview page 52

A treatment difference of 75 m (p=0.012) was observed for the sub-group age ≤7 years and 6MWD of ≤330 m. The treatment effect observed in the smaller studies was not likely driven by this subset of the patients, as there was only 1 subject each in the 6 mg/kg/week treatment group in both the Phase II studies with a baseline 6MWD of ≤330 m. Secondly, the median treatment difference of 75 m in Age ≤7 years + 6MWD of ≤330 m subgroup is driven by one placebo subject (#1256) with a baseline 6MWD of 184 m who could not perform the 6MWD due to gait loss after Week 12, hence at all subsequent visits the 6MWD was imputed to zero. Removing this subject, the treatment difference in the group of patients Age ≤ 7 years + 6MWD≤ 330 m is 10 m. The other subgroups did not have a nominally positive p-value, including the subgroups that would likely have a milder progression (Age ≤7 years + 6MWD of >330 m, Age >7 years +6MWD of >330 m). Lastly a 6MWD of 330 m is a subjective cutoff. In the published literature some experts have presented natural history data in DMD with a cutoff of 350 m.

In an amendment to the ISE submitted on August 31st 2015, the applicant conducted additional analysis using an enhanced MMRM model including age and 6MWD subgroup and treatment by age and subgroup interaction as terms in the model. This analysis showed a treatment difference of 20 m (p=0.12) (Table 51). Baseline 6MWD was accounted for redundantly as terms in this model. Please refer to the statistical review for limitations on the statistical model.

Table 51: Applicant's post-hoc analyses of Study DMD114044

Model	Population	Placebo (adjusted mean)	6mg/kg (adjusted mean)	Treatment effect vs. Placebo	P-value of Treatment Difference
MMRM	Overall*	-60 m	-40m	+20 m	0.12
MMRM	Overall ** minus older/more severe group based on 6MWD cut off of **330m**	-44 m	-11m	+33 m	0.01

Source: Sponsor ISE amendment, Aug 31st 2015

The applicant asserts a treatment benefit of 33 m (nominal p-value 0.01) after removing subjects that are of Age > 7 years + 6MWD≤ 330 m as it was the only subgroup that did not show a treatment benefit. By removing only one placebo subject that could not perform the test the p-value increases to 0.06. Using a 6MWD cutoff of 350m p-value increases to 0.06 with a treatment difference of 23 m and removing the single placebo subject (#1256) the p-value increases to 0.12 (Table 52), further suggesting that the results are dependent on the way the cutoff points are drawn are appear unstable.

Table 52 FDA Analysis with a 6MWD cutoff of 350m

Model	Population	Placebo (adjusted mean)	6mg/kg (adjusted mean)	Treatment effect vs. Placebo	Nominal P-value of Treatment Difference
MMRM	Overall ** minus older/more severe group based on 6MWD cut off of **350m**	n=39 -33.8 m	n=81 -10.4m	+23.4 m	0.062
MMRM	Above population minus subject #1256	n=38 -26.9	n=81 -10.7	+16.3	0.196

Source: Dr. Sharon Yan

Hence, the sponsor's argument of removing a subgroup to show treatment benefit is subjective, as the effect size appears unstable and the p-value changes.

Overall, the evidence of efficacy from these studies based on the primary endpoint change from baseline 6MWD appears uncertain. The increase from baseline in 6MWD observed at Week 25 does not appear sustained at Week 49. The results from the other two studies further mitigate the positive findings from Study DMD114117, although a numerical advantage at Week 48 can be argued. It is difficult to separate a treatment effect from unblinding biases. Some of the many factors that could introduce bias studies could be clear unblinding due to injection site and physical training. Physical training might delay the functional deterioration caused by disuse in boys with DMD (Jansen 2013). From the open label studies, there is no reliable estimate of what happens to the subjects over several years due to dropouts. Overall, a precise estimate of treatment benefit cannot be established. Even if we assume a short term treatment benefit of 10-20m based on the overall results, there is no clear way to estimate whether this would delay the time to loss of ambulation. In addition, switching subjects from an intermittent regimen from Study DMD114117 to continuous regimen in the second year, subjects appear to be headed to losing ambulation at an age not older than expected from

natural history. The prognosis of subjects is dependent on their baseline function.

Given the heterogeneity in disease progression, stratification of patients was important to ensure the groups were balanced not only for age, but for baseline 6MWD and corticosteroid use (McDonald 2103). It also appears that other factors such as Time to Rise from Floor, Ability to jump or hop clearing both feet up, ability to rise with gower's maneuver may also be important factors in predicting disease progression. The knowledge of these factors became apparent after these studies were initiated. In general, the pathophysiological mechanisms that underlie phenotypic variability are not fully understood. Recent publications also suggest genetic modifiers such as LTBP4 and osteopontin that could predict the disease trajectory in individual subjects. Such information is not available from the drisapersen program.

7.1.2. Secondary and Other Endpoints

None of the secondary endpoints showed statistically significant treatment difference at either Week 24 or 28 in the three placebo controlled studies. The secondary endpoints were not analyzed in a hierarchical manner in the small studies (DMD114117 and DMD114876) and no key secondary endpoint was identified. In the large Study DMD114044, NSAA, 4-stair climb-ascent and 10 m walk/run were assigned as key secondary endpoints. The applicant does not propose labeling claims based on any secondary endpoints. All conclusions from all secondary endpoints are briefly summarized below:

Timed Function tests (rise from floor, 10 m walk/run, and 4-stair climb/ascent-descent):

- In Study DMD114117, the timed function tests were in the same direction as the primary endpoint for the continuous 6 mg/kg/week group compared with placebo was observed at Week 25 and 49. The trend of improvement worsened at Week 49, but remained numerically better than placebo. The clinical meaningfulness of these trends is unclear as the changes in the Timed Function Tests were less than 1 second, with the exception of rise from floor where a treatment difference of 3 seconds was observed. The intermittent regimen showed a favorable trend only for the 10 m walk/run.
- In study DMD114876, the timed function tests were variable with no consistent dose trends and the treatment difference was<1 second.
- In Study DMD114044, no consistent trends in favor of drisapersen were observed across all tests that are correlated with each other.

NSAA

- In Study DMD114117, NSAA showed favorable trends at Week 25 for both continuous and the intermittent regimen, that worsened at Week 49. The change from baseline in favor of drisapersen was greater for the intermittent regimen at Week 25, which does not follow the same direction as that of the 6MWD. The changes are small at week 49 (-0.2 and -0.4, respectively for the continuous and intermittent) in a total NSAA score of 34, with higher scores being better). Mazzone et al 2013 have shown that younger subjects can remain stable in NSAA assessments in the first year and there were a larger number of younger subjects in this study. Therefore, it is unclear if the stability is related to treatment effect or the natural progression of the disease as measured by NSAA.
- In study DMD114876 and DMD114044, no significant or consistent treatment difference in favor of drisapersen was observed.

Pulmonary Function Tests

- The changes in pulmonary function tests were small, not clinically meaningful in any study and not in favor of drisapersen. Changes in pulmonary function are not expected in early ambulant population.

Muscle Strength

- Unfavorable and variable trends in muscle tests were observed in all studies, with greater decline in muscle strength observed in the drisapersen group in Study DMD114044.

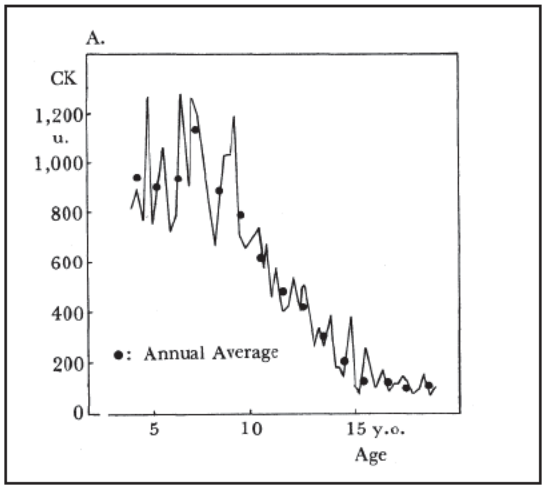
Serum Creatine Kinase:

Serum creatine kinase, a marker of muscle cell injury was the only secondary endpoint that was consistently reduced by 30-40% across the three studies based on percent change from baseline with nominal p-values of 0.08, 0.53 and <0.001 for the Study DMD114117, DMD 114876 and DMD114044 respectively, suggesting a possible improvement in muscle cell integrity. However, this finding is associated with several confounders: CK levels are remarkably variable, with large within patient and between patient variability and are known to fluctuate day to day. It is reduced with inactivity, steroid use, advancing age of the DMD boys due to reduction in muscle mass as the muscle fibers are replaced with fat and fibrous tissue, clinical progression and diurnal variation (increase during the day and decrease at night) and inflammation. I will evaluate each of these factors as possible confounder of a treatment effect.

- *Effect of variability on CK:* Statistically significant reductions in CK were observed despite the variability. CK increases during the day and decreases at night. All CK assessments in the studies were generally conducted between 8 am to 5 pm.
- *Effect of inactivity on CK:* Florence et. al. (1985) have shown that on complete bed rest days the mean decrease in CK level was 13,000 IU/L compared to the active days in DMD boys. The mean decrease in the drisapersen treated subjects in all the three studies was between 4000-5000 IU/L. There was no evidence of inactivity of the subjects from the Patient or Parent reported outcomes but the activity level of subjects are hard to discern from such studies and activity levels were not recorded systematically. The greater number of subjects with injection site reactions in the drisapersen treated patients could plausibly make the subjects less active, but CK reduced in subjects with or without injection site reactions to the same extent of 30-40%.
- *Effect of steroids on CK:* Steroid use reduces CK. Both drisapersen and placebo subjects were on steroids for a minimum of 6 months. It is not known if the dose, regimen and duration of steroid use would impact the reduction in CK. The impact of these on the reduction in CK across treatment groups are difficult to discern from the studies. There were some differences in steroids use across studies as shown below, but the impact of these difference is unknown:

	Study DMD114044		Study DMD114117		Study DMD114876	
	Placebo	Drisapersen	Placebo	Drisapersen	Placebo	Drisapersen
Median Time on steroids (months)	19	27	23	14	39	19
Steroid Regimen: Continuous (%) Intermittent (%)	 85% 15%	 86% 14%	 61% 39%	 67% 33%	 94% 6%	 100% 0%

- *Effect of age on CK:* Published studies suggest that CK peaks at ages 3-5 years and declines with age with levels reduced to 50% by age 7 years. Konagaya et. al. (Figure on the left) suggest that in the second half of the first decade, due to relatively higher rate of muscle degeneration, there is an abrupt drop of serum CK levels. Hence, CK decreases with age and disease progression. In study DMD114044, there were 17% subjects that were greater than 11 years in the drisapersen group compared to 11% in the placebo group. The Phase 2 studies had only couple subjects that were older than 11 years, about an age when there is an abrupt drop in CK levels. To explore if the statistically significant reduction in CK in Study DMD114044 was driven by the slightly higher number of subjects >11 years in the drisapersen group, I also calculated the mean percent change from baseline in CK in subjects >7 to ≤11 years in both drisapersen and placebo groups. Irrespective of the age groups CK was consistently reduced in the drisapersen treated patients compared to placebo patients as shown:



Mean percent change from baseline in CK	Study DMD114044		Study DMD114117 At Week 48		Study DMD114876 At Week 24	
	Placebo	Drisapersen	Placebo	Drisapersen	Placebo	Drisapersen
≤ 7 years	4.5%	-19%	36%	-11%	-2%	-31%
>7 years	-2%	-41%	36%	-41%	5.7%	-16%
>7 to ≤11 years	0.4%	-43%				

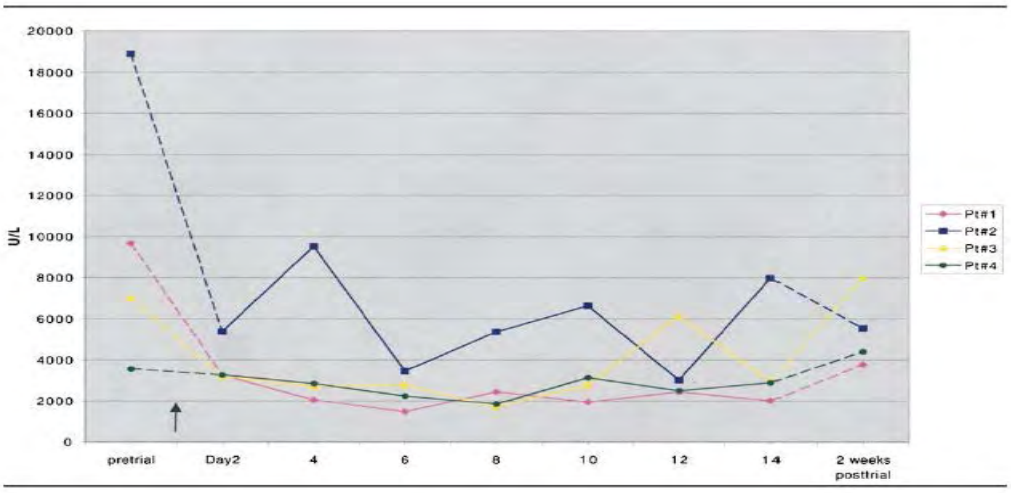
There was no consistent trend in mean percent reduction of CK in subjects <7 or >7 years and ranged between 30-40% reduction in these groups with wide variability. The percent reduction in the >7 years of age was 40% in Studies DMD114117 and DMD114044, and was 11-19% in subjects ≤7 years of age. Opposite trend was observed in Study DMD114876 subjects where subjects ≤7 years of age had greater reduction in CK (30%) compared to 16% in subjects >7 years of age. In addition, impact of reduction in muscle mass due to muscle wasting with increasing age is unknown from these studies, but muscle mass will decline with age and disease progression.

Nevertheless, CK reduction was greater in the drisapersen treated subjects in all three subjects with nominal p-values of <0.001, 0.08 and 0.53 from Studies DMD114044, DMD114117 and DMD114876, respectively.

- *Effect of inflammation on CK:* Reduced CK activity in inflammatory diseases such as rheumatoid arthritis is linked to an inflammatory response, where an inverse correlation has been reported between CK activity and inflammatory markers (Lee 2000). I explored if the decrease in CK in the drisapersen treated subjects was due to an increase in inflammation and not an improvement in muscle cell integrity. Only ≤10% of subjects on drisapersen showed elevation in

one of the inflammatory markers. The markers of inflammation, C-reactive protein and Complement C3 measured in the drisapersen program did not appear to be inversely correlated to CK. It is uncertain if the inflammatory response due to injection site reactions could be associated with a reduction in CK, although CK reduced by 30-40% in subjects with or without injection site reaction.

- In a 14-day study with gentamicin, CK dropped by 50% in DMD boys (Malik 2010), while dystrophin increased after 6 months of dosing. In another 14-day study the highest drop in CK with gentamicin was after 1 day of dosing (Wagner 2001).



Gentamicin has a short half-life of 2-3 hours, but uncertain is this reduction in one day is mechanistically plausible. CK was assessed at earlier time points in some subjects on drisapersen. The CK reduction appeared to be lower at Week 3 compared to Week 49, but there was a large variability between time points. This is somewhat reassuring given the long half-life of drisapersen. Therefore, it is uncertain to what extent these factors could play a role in reducing serum CK levels and if CK change are essentially caused by improvement in muscle integrity or due to other unknown factors such an increase in metabolism of CK caused by the drug. While some factors could be explained by data, the others remain unknown. In addition, there was no correlation of 6MWD and CK, as in the Phase 3 study. Therefore, given all these caveats, a treatment effect due to reduction in CK is difficult to discern, but a plausible treatment effect cannot be ruled out.

MRI: Consult review of the MRI data conducted by Dr. Daniel Krainak (CDRH Imaging Division) concludes that the data presented in the application are unconvincing of treatment benefit.

Dystrophin:

The utility and caveats of dystrophin measurement in DMD:
Genetic mutation and deficiency of dystrophin leads to DMD in humans. Dystrophin has a structural role in the dystrophin-associated glycoprotein complex as a cytoskeletal stabilization protein and protects muscle fibers against contraction induced damage. Dystrophin also has a signaling role that includes mechanotransduction of forces and localization of proteins. Mutations in the DMD gene disrupt the open reading frame and prevent the full translation of dystrophin. Hence, there appears to

an intrinsic biological reason to measure dystrophin in DMD patients. Drisapersen is designed and targeted to restore the open reading frame with the production of a truncated but functional dystrophin. However, in humans, a quantitative, linear, and direct correlation between restoration of functional dystrophin and treatment benefit in terms of muscle function has not been clearly established (see discussions by Wilton2014 and Lu 2014). Qualitatively, the existence of Becker patients with detectable levels of dystrophin and milder phenotype than Duchenne patients suggests that dystrophin is, at least in part, related to the muscle function. Morandi and others have unsuccessfully attempted to establish this quantitative relationship using BMD cohorts (Morandi 1995).

In the mdx animal model for DMD, dystrophin restoration at levels of ~25-80% of normal have been achieved with antisense oligonucleotides (Gao 2014) or exon-skipping morpholino oligomers (Goyenvalle 2010). In these and other studies, dystrophin restoration was observed along with prevention of dystrophic pathology and restoration of muscle strength in the animal muscles examined.

In humans, some caveats that complicate a clear relationship between dystrophin protein and functional outcome are:

1. The presence of variable levels of trace and revertant fiber dystrophin. The low levels found in DMD also suggest that trace levels of dystrophin are unlikely to limit muscle degeneration.
2. The heterogeneity of the dystrophin between muscle sub-groups (e.g. biceps vs quadriceps) and within the same muscle biopsy.
3. The genotype and specific gene mutation of a BMD or DMD appears to impact basal levels of dystrophin but a systematic study hasn't been presented.
4. Inconsistent or heterogeneous measurement of dystrophin across laboratories that claim a quantitative relationship. Lack of a reference standard and proper control samples also make robust quantitative claims questionable.
5. The severity of the disease as a consequence of the chronic inflammatory environment. Lack of dystrophin also stimulates an inflammatory response that is an important mechanistic driver for the muscle degenerative process over time. With increasing age, the interplay between chronic activation of innate immunity and asynchronous bouts of degeneration/regeneration combine to yield a poorly orchestrated repair response that may itself drive disease progression (Rosenberg 2015). It is possible that the chronic inflammatory environment and repeated muscle damage over time presents a point-of-no-return that cannot be overcome simply based on restoration of extremely low levels of dystrophin.
6. Restoration of truncated dystrophin in DMD patients likely triggers an auto-immune response to the new protein in these patients. Even though these patients have revertant fibers, Flanigan and Mendel have previously published that these patients are not "tolerized" to novel dystrophin production and show dystrophin-specific T-cell immunity that increases with age (Fanigan 2013). In animal models, this was mitigated with administration of anti-inflammatory agents (Villalta 2014 and Rosenberg 2015). How the autoimmune response to the new dystrophin impacts its availability and function within muscle tissue is unclear.

Detection of dystrophin was assessed by exon 51 skipping by RT-PCR, qualitative IFA and WB in Study

DMD114117, DMD114876, DMD114044, DMD114673 and PRO051-01. A small to non-existent dystrophin increase in dystrophin from baseline was observed; however, this increase was not consistent across all the placebo controlled studies. A relatively reasonable assessment of dystrophin increase was only obtained from Study DMD114117. The results across placebo controlled studies and methodologies are summarized below.

Dystrophin mRNA by PCR: The applicant provided a qualitative assessment of dystrophin mRNA by either nested, lab-on-chip, or droplet digital PCR. These methods do not indicate that mRNA was translated into functional protein.

- *Nested RT-PCR:* Nested RT-PCR assessments were qualitative with assessments of visual bands (yes/no). A small increase in exon 51 skipped dystrophin mRNA was observed only in some patients across studies with no consistent trend between treatment groups as summarized below.

Study	Number of subjects/total number analyzed		
	Placebo	6 mg/kg/week	6 mg/kg Inter OR 3 mg/kg/week
Study 114117 (Wk 24)	0/18	2/18	5/17
Study 114876 (Wk 24)	2/16	10/18	10/17
Study 114044 (Wk 48)	56/61	114/125	-

- *The Lab-on-chip capillary electrophoresis PCR:* This method showed some increase compared to baseline but the standard deviations were high. Due to several analytical deficiencies, this quantitation has limited reliability.

Study Treatment	Exon skip (a.u) (mean(SD))	
	Week 0	Week 25, 24 or 48
Study 114117		
Placebo	1.30 (1.01)	0.73 (0.41)
Weekly 6 mg/kg/week	1.29 (2.42)	1.30 (1.50)
Intermittent 6 mg/kg	1.72 (2.38)	2.60 (5.34)
Study 114876		
Placebo	2.01 (1.52)	1.53 (1.20)
Weekly 6 mg/kg/week	2.69 (4.09)	4.44 (3.55)
Weekly 3 mg/kg/week	2.41 (4.38)	4.37 (6.78)
Study 114044		
Placebo		1.45 (1.68)
Weekly 6 mg/kg/week		2.83 (5.21)

Dystrophin Protein by Immunofluorescence:. Immunofluorescence assessments suggest small increases in mean membrane-associated dystrophin intensity in some treated patients from study 114117 but not from studies 114876 or 114044 as summarized below. IFA quantitation of dystrophin has high

background and may increase more than linearly with small changes in dystrophin which could lead to an overestimation of amount of dystrophin compared to WB. The IFA method can suggest protein localization but is less meaningful for protein level quantitation.

Study	Number of subjects/total number analyzed		
	Placebo	6 mg/kg/week	6 mg/kg Inter OR 3mg/kg/week
Study 117 (Wk 24)	1/18	9/15	4/15
Study 876 (Wk 24)	7/12	1/18	5/11
Study 044 (Wk 48)	9/61	9/125	

Dystrophin Protein by Western Blot: Some patients in study 114117 showed a small increase compared to baseline but with very low dystrophin signal compared to healthy samples.

Study	Number of subjects/total number analyzed		
	Placebo	6 mg/kg/week	6 mg/kg Inter OR 3 mg/kg/week
Study 114117 (Wk 24)	0/14	5/17	5/16
Study 114876 (Wk 24)	0/5	0/1	1/5
Study 114044 (Wk 48)	-	-	-

The applicant's WB method was more quantitative than IFA because (1) a serial dilution of healthy tissue sample was used alongside test samples and control samples and (2) a reasonably muscle-specific α-actinin loading control was included.

Open-label studies: The dystrophin increase was unreliable and unimpressive from the open label studies as discussed below:

Study DMD114673: Dystrophin was assessed at Week 24 and Week 72 of this extension study.

- For IFA analysis, at week 24, no reliable estimate of dystrophin expression was obtained due to poor muscle biopsy quality in the 12 subjects. However, 8/12 subjects had additional biopsy at Week 72. Some subjects showed a slight increase (2 to 8%) in mean membrane intensity, while other showed a slight decrease (1 to 9%) at Week 72, leading to no consistent evidence of increase in dystrophin expression.
- For WB analysis, an increase of 21-46% at week 24 over previous time point was observed; however, at week 72 the same patients did not consistently show an increase. Upon request, the applicant clarified that, with the exception of one sample at 1.1% the dystrophin levels observed in this study were all below 1%, which is their lower limit of detection by WB.

Study PRO051-02: Dystrophin expression was evaluated 2 and 7 weeks AFTER a 5 week dosing period in 12 subjects.

- This study does not give reliable estimates of dystrophin. There were no baseline samples in 9/12 subjects. This is the only study where dystrophin expressions have been reported by

the investigator after 5 weeks of dosing with drisapersen.

7.1.3. Subpopulations

Age:

No conclusive evidence of efficacy in any age group can be established from the placebo controlled studies. Age was post-hoc subgroup analysis for studies DMD114117 and DMD114876, but was pre-specified as a subgroup for Study DMD114044. The age groups evaluated were ≤ 7 years and > 7 years, as published data suggest that there are differences in disease progression among these subgroups, but these differences are also dependent on the baseline 6MWD.

McDonald et al and many other experts have shown that *"there is a trend for the 6MWD to improve or be stable over the first 7 years of age and patients who have lower initial 6MWD tend to show greater declines over the course of 48 weeks. Patients ≥7 year of age may also show stable function or even improving function, but they are almost those with higher levels of baseline function (6MWD >350m)"* (Excerpt from McDonald 2013). Please note that there are imbalances in the 6MWD between treatments in some of these age comparisons such that it complicates the interpretation of these age differences in treatment effect.

The age subgroup analyses in the placebo controlled studies are shown in Table 53. Age subgroup analysis was not nominally positive in any study. The age related differences seen in the post-hoc analyses of small phase II studies (DMD114117, DMD114876) are unreliable due to small number of subjects in each group and due to an imbalance in the baseline 6MWD in the treatment and placebo arm within some age comparisons.

Table 53: Age subgroup analyses in placebo controlled studies

Treatment	N	Baseline Mean (SD)	Adjusted Mean Change from Baseline (SE) at Week 48*	Treatment Difference	95% CI
Age ≤7 years at Baseline (Study DMD114044)					
Placebo	29	383 (66)	-25 (11)	21	(-6, 48)
Drisapersen 6 mg/kg/wk	51	368 (65)	-4 (8)		
Age ≤7 years at Baseline (Study DMD114117)					
Placebo	13	409 (40)	-13 (16.54)	38	(-12, 89)
Drisapersen 6 mg/kg/wk	11	413 (62)	25 (18.20)		
Age ≤7 years at Baseline (Study DMD114876)* 24 weeks					
Placebo	6	445 (63)	-14 (21)	31	(-29, 90)
Drisapersen 6 mg/kg/wk	10	385 (60)	17 (17)		
Age >7 years at Baseline (Study DMD114044)					

Placebo	32	316 (101)	- 84 (15)	7	(-29, 43)
Drisapersen 6 mg/kg/wk	74	316 (107)	-77 (11)		
Age >7 years at Baseline (Study DMD114117)					
Placebo	4	386 (57)	-61 (24)	56	(-6, 114)
Drisapersen 6 mg/kg/wk	7	450 (81)	-5 (18)		
Age >7 years at Baseline (Study DMD114876)* 24 weeks					
Placebo	10	399 (48)	- 16(12)	28	(-9, 64)
Drisapersen 6 mg/kg/wk	8	410 (62)	12 (14)		

In study DMD 044, the subjects ≤ 7 years had larger treatment benefit (21 m) than subjects > 7 years (6.9m). The applicant believes that younger subjects may have greater treatment benefit with drisapersen. In this comparison, one 7 year old subject (#1256) on placebo with a baseline 6MWD of 184m could not perform the test due to gait loss after Week 12. This inability to perform the test is not likely to do the lack of response to treatment after Week 12, but more likely to the disease condition of this subject. A treatment difference of 10 m was observed in subject's ≤ 7 years of age after removing this single subject. This further weakens the notion that younger subjects may receive greater treatment benefit.

In addition, pooling the two Phase II studies, which the applicant asserts was a more functional population, the treatment difference over placebo was greater for the older subjects (40m), compared to the younger subjects (27m) at Week 24, further weakening the notion that treatment effect is greater in the younger population.

Pooling the 48 week placebo controlled studies, a larger treatment difference of 24m was observed for the younger population compared to a 7m treatment difference in the older subjects. Pooling the 48 week studies, I also conducted an exploratory post-hoc analysis by age in subjects with Rise from Floor ≤ 7 seconds which is summarized in Table 54. Given the few number of subjects that were >7 years, the interpretation of the data across all ages are not reliable. Nevertheless, the 5-6 year old subjects showed greater treatment difference.

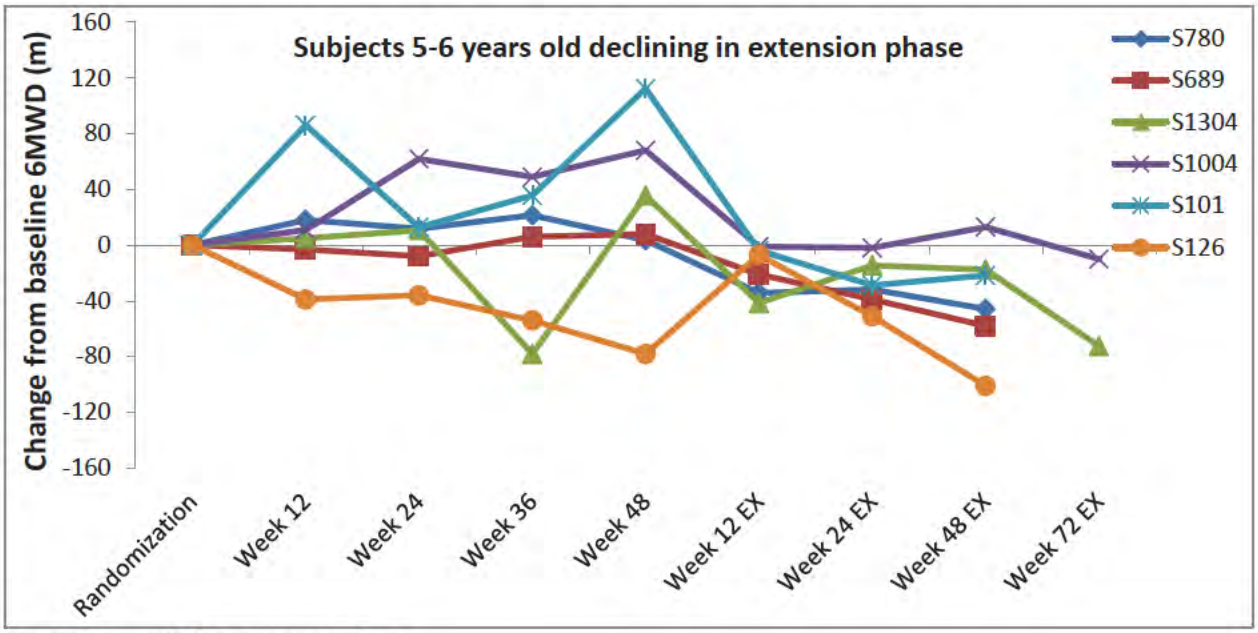
Table 54: Pooled 6MWD analysis by age for the 48 week studies in subjects with Rise from floor ≤7s

Age	Drisapersen N	Placebo N	Mean Treatment Difference
5 years	12	10	67 m
6 years	23	11	28 m
7 years	14	12	12 m
8 years	14	6	2 m
9 years	7	5	31 m
10 years	2	1	-67 m
11 years	2	1	32 m

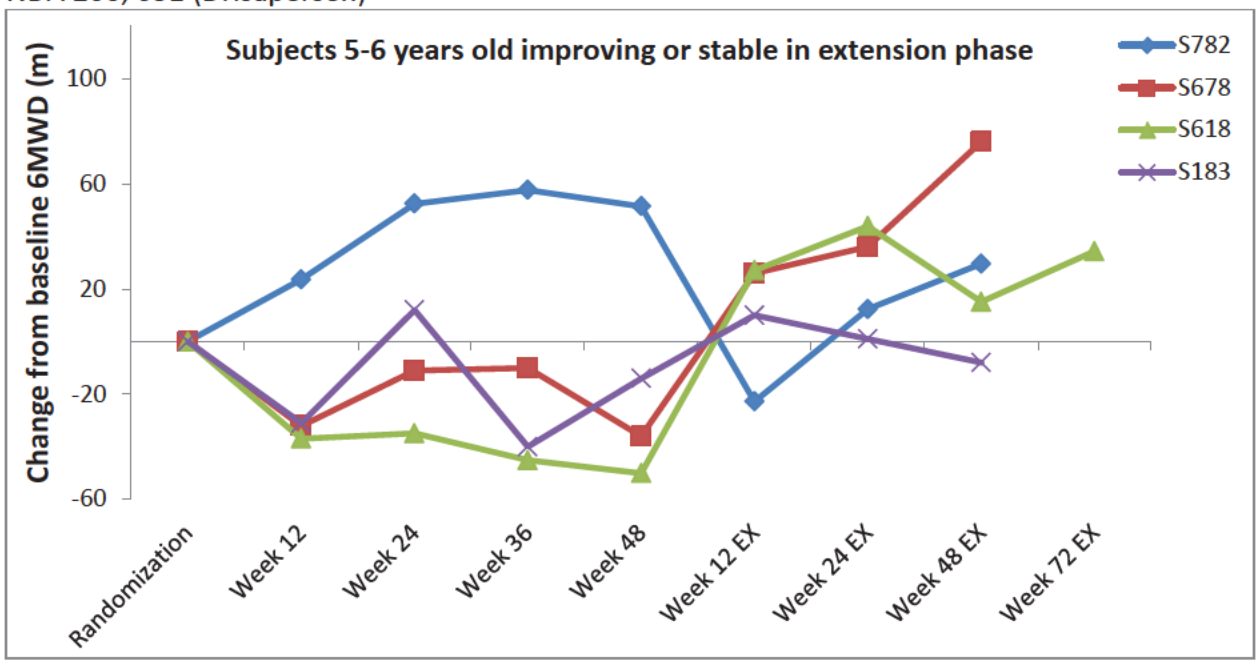
Note: The baseline 6MWD was lower by 8-20m in the drisapersen arm in the ages 5-7 years

Experts have suggested that viable muscle fibers may be required to restore dystrophin and hence would seem logical to start treatment early for a treatment benefit in the long-term. Keeping this hypothesis in mind, I looked at the 6MWD time course in the open label extension phase for the 5-6 year old from Study DMD114044. There were 10 subjects on treatment of the ages 5-6 years that had ≥2 year data from the open label extension phase. Of these 10 subjects, 6 tended to decline in the 2nd year of treatment with continuous 6mg/kg/week drisapersen, 4 tended to improve. The data from a few of these 10 subjects could be considered random noise in the measurement. For ease of visual representation these 6MWD time course of these subjects have been categorized as those improving and declining in the extension phase (Figure 32). These figures suggest that some subjects tend to decline in the 2nd year of treatment with 6mg/kg/week drisapersen. Given the variability in the progression, it is difficult to get an estimate of the time to loss of ambulation in these young subjects.

Figure 32 Two year time course of 6MWD of 5-6 year old subjects from Study DMD114044



Note: EX stands for extension phase



Overall, there is no compelling evidence that the subjects ≤ 7 years of age may benefit treatment with drisapersen, but a plausible biological argument can be logical.

Ethnicity and Race:
There is insufficient data on ethnic and race comparisons for efficacy. A total of 82-95% of the subjects were Not-Hispanic/Latino across the three studies. No interpretable differences can be obtained between ethnic groups. A total of 76% of the subjects were White Caucasian/European in Study DMD114044 and 88% in Studies DMD114117 and DMD114876. Due to the small number of subjects of the non –Caucasians, no interpretable treatment difference based on race can be obtained.

Country:
No reliable conclusions can be drawn with respect to efficacy in different countries. A Total of 23 countries participated in the placebo controlled studies. The number of subjects per treatment group was small in these countries. Only Canada, France, Italy, Germany and United States had more than 15 subjects.

7.1.4. Dose and Dose-Response

An initial loading dose of 6mg/kg drisapersen twice weekly for 3 weeks, followed by maintenance treatment with 6 mg/kg administered weekly is proposed. The loading regimen was evaluated in Study DMD114117, in which a statistical significant difference from placebo was observed for the primary endpoint 6MWD. Given, the long half-life of drisapersen (29 days), it is acceptable to initiate treatment with a loading dose for three weeks, as proposed by the Applicant.

6 mg/kg was considered to be the maximum tolerated dose by Applicant due to signs of potential subclinical nephrotoxicity (mild proteinuria) in the extension study DMD114673 after 6 months of

treatment and due to preclinical pro-inflammatory findings (including the recent preliminary data from the 39 week monkey study). Drisapersen doses 0.5, 2, 4 and 6 mg/kg were evaluated in a 5 week proof of concept Study PRO-051. Due to the lack of significant safety findings and a pharmacodynamics effect (increase in dystrophin expression) with 5 weeks of dosing, the 6 mg/kg/week dose was given the open label extension Study DMD114673. A single dose of 9 mg/kg administered to 3 non-ambulant subjects was associated with renal toxicity and inflammatory reactions and self-limiting pyrexia and flu-like symptoms in all subjects. Doses higher than 6mg/kg/week were not evaluated in any other study.

A lower 3 mg/kg dose was evaluated in Study DMD114876 in only 18 subjects. In this study:

- 6 mg/kg/week was superior than placebo (27m)
- 3 mg/kg/week was worse than placebo (-9 m)

Given the variability in the assessment of 6MWD, this difference from placebo is not substantial and could considered as indistinguishable from placebo at Week 24. The sample size of this study is small, hence difficult to conclude that 3 mg/kg/week as an ineffective dose. No clear dose-response was established.

7.1.5. Onset, Duration, and Durability of Efficacy Effects

Pharmacokinetic studies have shown that drisapersen concentrations reach steady state at 24-36 weeks and also likely the time it would take for dystrophin to accumulate in the muscles. None of the placebo controlled studies have shown any dystrophin expression at Week 12, but an exploratory published 5 week study showed an increase in dystrophin after 5 weeks of dosing. In many subjects a change from baseline in 6MWD of similar magnitude was observed both at Week 13 and Week 24. This could be due to the variability in the assessment of 6MWD.

The applicant asserts that there was maintenance of effect after further 24 weeks off treatment. In Study DMD114876, in which treatment was administered for only 24 weeks, change from baseline in 6MWD at Week 24 was 16 m and 15 m at Week 48, after a drug free period. This would suggest that treatment effect is maintained. Given the turnover half-life of dystrophin this may be physiologically possible as well. In this study the treatment difference from also remained similar (27m) at Week 24 and 48. This is because the placebo group also did not decline in the 48 week period. Similar treatment effect was also observed at Week 12, but not certain if the effect at Week 12 is due to increase in dystrophin as no dystrophin was measurable at week 12 in any study. Therefore the maintenance of change from baseline in the 6MWD at Week 48 appears less convincing of a persistence of effect from this study.

The evidence for durability of response is unclear. In Study DMD114117, a mean change from baseline in 6MWD was 31 m at Week 24 and 11 m at Week 48, suggesting a decline in 6MWD of about 20 m. A treatment difference from placebo of 35 m was observed on both Week 24 and Week 48. This probably was due to a decline in the placebo group of about 20 m in this study at Week 48, driven by a few subjects in the study.

The applicant asserts that a difference of ~30 m was observed in subjects who received continuous 6

mg/kg/week for 96 weeks in the extension study of DMD114044 compared to those subjects that received placebo in the parent study for 48 weeks followed by 48 weeks of continuous 6 mg/kg/week regimen. The sample size was reduced to almost 50% of the parent study in each arm; hence the observed difference of ~30 m is uninterpretable, even though it is plausible.

7.2. Additional Efficacy Considerations

7.2.1. Considerations on Benefit in the Postmarket Setting

Drisapersen could be used in the ambulant patients with exon-51 skip amenable DMD, if efficacy were to be established. Effectiveness of drisapersen in non-ambulant patients has not been evaluated. Postmarketting considerations are premature at this time.

7.2.2. Other Relevant Benefits

There are no other relevant benefits at this time.

7.3. Integrated Assessment of Effectiveness

In this section, I discuss the various options of regulatory pathways for drisapersen.

Drisapersen development program has 3 adequate placebo controlled studies with a single primary endpoint and several secondary clinical endpoints.

There is no substantial evidence of efficacy for drisapersen from the adequate and well controlled studies based on a clinical endpoint, change from baseline 6MWD.

There was a statistically significant treatment difference for drisapersen 6 mg/kg/week over placebo (p=0.01) at week 25 for the primary endpoint change from baseline 6MWD in only one small Phase 2 Study DMD114117. The change from baseline in 6MWD observed at Week 25 does not appear sustained at Week 49. An intermittent regimen of the same 6mg/kg dose with identical plasma exposure showed no statistically significant (p=0.80) difference over placebo in this study. The known and unknown biases from this study such as unblinding due to injection site skin reactions and baseline imbalances in prognostic factors due to chance alone could be mitigated if these findings are replicated in independent studies. It is disappointing that there is no independent substantiation of these results from the second Phase 2 Study DMD114876 (p=0.07) and the large Phase 3 DMD114044 (p=0.42) to rule out the possibility of a false positive finding due to chance alone. Removing one placebo subject from the analysis of the Phase 2 Study DMD114876 because the subject was unblinded increased the p-value to 0.21, further weakening the confidence in the evidence towards efficacy from Study DMD114117. Applicant's argument of a more functionally impaired population of the Phase 3 study DMD114044 contributing to a lack of treatment response was not substantiated by a FDA post-hoc analysis of subjects with similar baseline age, 6MWD and rise from floor time as in the Phase 2 studies. There was no statistically significant treatment difference (p=0.71) or even a larger numeric treatment

difference in this subgroup of patients, suggesting that the disease severity of the subjects was not the reason for the negative results from Study DMD114044.

Although a numeric advantage for drisapersen over placebo was observed in the three placebo controlled studies, a precise estimate of possible effect size cannot be established for drisapersen. An aggregate of the data across studies and all post-hoc analyses suggest that if it were to be concluded that the result was due to drug, there was a numerical advantage with drisapersen of approximate 10-20 m over placebo over 24-48 weeks. The clinical meaningfulness of this short term numeric advantage with drisapersen in the overall prognosis remains uncertain.

A number of secondary endpoints were assessed without control for multiplicity. None of the secondary endpoints were nominally positive in any study. Secondary endpoints such as Timed Function Tests (rise from floor, 10m walk/run, 4-stair climb/ascent –descent) and NSAA are also measures of lower limb strength like the 6MWD and are highly correlated to each other. These secondary endpoints were in the same direction as the primary endpoint in only Study DMD114117. There were no consistent trends in favor of drisapersen in other studies. The differences in the Timed Function Tests were mostly <1 second between treatment and placebo in all three studies. Muscle strength measure by myometry and pulmonary function tests did not favor drisapersen. The changes were small across treatment groups. These secondary endpoints analyses do not contribute to the assessment of efficacy.

Even if one were to consider the results of Study DMD114117 bereft of uncertainties, there is no independent substantiation of these findings. A single study approval could be argued for a rare disease with no approved treatments. Single study approvals are generally limited to situations in which the study has demonstrated a clinically meaningful effect on mortality or irreversible morbidity and in which a second trail would be ethically impossible. For this application, we have 2 other adequate controlled studies which cannot be ignored and the endpoint is not mortality or irreversible morbidity. In addition, the study was smaller than a large well powered study that failed to show effectiveness. Given the above concerns from the placebo controlled studies, I do not recommend standard full approval of drisapersen in the treatment of exon 51-skip amenable DMD.

The law under 21CFR 314.500 further provides the regulations for approval under Subpart H-Accelerated approval of new drugs for serious and life threatening disease. DMD is a severe disease in which progressive loss of muscles lead to loss of ambulation, respiratory and cardiac complications and ultimately death. There are no approved treatments of DMD in the United States.

Under Subpart H, approval can be based on either on a “surrogate endpoint” that is reasonably likely based on epidemiologic, therapeutic, pathophysiologic or other evidence to predict clinical benefit at some later time OR on an “intermediate clinical endpoint” that can be measured earlier than an effect on irreversible morbidity or mortality that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit.

I will first consider 6MWD as an “intermediate clinical endpoint”. 6MWD is a clinically meaningful

endpoint in DMD as it is a measure of how a patient functions. It measures how much a subject can walk (in meters) in a fixed time of 6 minutes. While being able to walk more is certainly meaningful in the day-to day activities of the patient, an improvement in measure of a distance could predict an effect on irreversible morbidity or mortality, which is time to loss of ambulation or death, i.e. could affect the ultimate rate of decline. If we were to consider 6MWD as an intermediate clinical endpoint, it would be on the basis of the short-term benefit of walking more reasonably likely to have an effect on the rate of decline of walking performance and thus influence the time to loss of ambulation. Drugs granted accelerated approval MUST meet the same statutory standards of effectiveness and safety as those granted traditional approval. As discussed in the previous paragraphs, the clinical evidence of efficacy for drisapersen does not meet the statutory standard and remains inconclusive based on 6MWD and other clinical secondary endpoints. An aggregate of the data across studies and all post-hoc analyses suggest a relatively short-term numerical advantage with drisapersen of approximately 10-20m over placebo. These differences were not statistically significant, hence no persuasive treatment effect size can established between drisapersen and placebo. Based on natural history studies in DMD, a 10-20m treatment difference between drisapersen and placebo is also not likely to have a large effect on delaying the time to loss of ambulation (Natural history studies have suggested decline in 6MWD of 22-58 m in a year). The open label studies in the application, though not completely interpretable, do not suggest that the rate of decline in the walking performance was different from that of natural history. Hence, based on the unpersuasive results of 6MWD from studies presented in this application, it is uncertain if 6MWD can serve as an intermediate clinical endpoint reasonably likely to predict an effect on irreversible morbidity for this application.

Therefore, the threshold for accelerated approval based on 6MWD as an intermediate clinical endpoint reasonable likely to predict benefit on time to loss of ambulation or the rate of decline appears unmet.

Lastly, I will discuss the regulatory pathway of accelerated approval based on a surrogate endpoint reasonable likely to predict clinical benefit. Creatine kinase, a marker of muscle cell integrity at a molecular level could serve as a surrogate endpoint reasonably likely to predict clinical benefit. CK was reduced by 30-40% across the three studies based on percent change from baseline with nominal p-values of 0.08, 0.53 and <0.001 for the Studies DMD114117, DMD 114876 and DMD114044 respectively, suggesting a plausible improvement in muscle cell integrity. While the reduction CK was consistent across studies, the effect of confounders such as inactivity, reduced muscle mass, steroids use and inflammatory processes cannot be completely understood from the data. There was obviously no correlation of reduced CK to the clinical endpoint 6MWD based on the studies in the Application. A statistical significant ($p<0.001$) reduction in CK was observed despite of no difference in walking ability between drisapersen and placebo ($p=0.42$). Given this, the clinical meaningfulness of the observed reduction in CK is uncertain from the studies in this application. Hence, the reasonably likelihood of a reduction in serum creatine kinase to predict a clinical benefit in function or time to loss of ambulation appears uncertain. An increase in dystrophin protein expression could reflect biological activity and can be considered a surrogate endpoint. It is disappointing that the dystrophin protein expression data were equivocal. The changes in dystrophin were minimal and reliable only in one study.

Therefore, the threshold for accelerated approval based on creatine kinase or dystrophin as a

surrogate endpoint reasonable likely to predict clinical benefit of drisapersen of this application appears unmet as well.

One can argue a numerical advantage on the subjective endpoint 6MWD and on an objective endpoint such as CK in favor of drisapersen in all studies is suggestive that drisapersen is better than placebo. Although, these two together also do not meet the threshold for being reasonably likely to predict an effect on irreversible morbidity or mortality.

The development program for drisapersen is extensive for a rare disease and exemplary. It is very disappointing that that both clinical and biomarker data for drisapersen are inconclusive at this time.

8 Review of Safety

Safety Review Approach

The safety of drisapersen is reviewed by Dr. Evelyn Mentari, MD in a separate review.

9 Advisory Committee Meeting and Other External Consultations

An Advisory Committee Meeting is scheduled for November 24th, 2015.

10 Labeling Recommendations

Labeling recommendations are deferred until Advisory Committee meeting.

11 Risk Evaluation and Mitigation Strategies (REMS)

REMS are not proposed for this application. The reader is referred to Dr. Mentari's safety review.

12 Postmarketing Requirements and Commitments

Postmarketting requirements are deferred until Advisory Committee meeting.

13 Appendices

13.1. References

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13.2. Financial Disclosure

Covered Studies: 3

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 78		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0 Note: There are 33 sub-investigators whose financial disclosure information could not be obtained despite due diligence efforts by the sponsor. These sub-investigators could not be located.		
If there are investigators with disclosable financial interests/arrangements, identify the number 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: NA Significant payments of other sorts: NA Proprietary interest in the product tested held by investigator: NA Significant equity interest held by investigator in S Sponsor of covered study: NA		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) _____		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

Appendix A

North Star Ambulatory Assessment Scale

Activity	2	1	0	Comments
1. Stand	Stands upright, still and symmetrically, without compensation (with heels flat and legs in neutral) for minimum count of 3 seconds	Stands still but with some degree of compensation (e.g. on toes or with legs abducted or with bottom stuck out) for minimum count of 3 seconds	Cannot stand still or independently, needs support (even minimal)	
2. Walk	Walks with heel-toe or flat-footed gait pattern	Persistent or habitual toe walker, unable to heel-toe consistently	Loss of independent ambulation – may use KAFOs or walk short distances with assistance	
3. Stand up from chair	Keeping arms folded Starting position 90º hips and knees, feet on floor/supported on a box step.	With help from thighs or push on chair or prone turn	Unable	
4. Stand on one leg - right	Able to stand in a relaxed manner (no fixation) for count of 3 seconds	Stands but either momentarily or needs a lot of fixation e.g. by knees tightly adducted or other trick	Unable	
5. Stand on one leg – left	Able to stand in a relaxed manner (no fixation) for count of 3 seconds	Stands but either momentarily or needs a lot of fixation e.g. by knees tightly adducted or other trick	Unable	
6. Climb box step – right	Faces step – no support needed	Goes up sideways or needs support	Unable	
7. Climb box step – left	Faces step – no support needed	Goes up sideways or needs support	Unable	
8. Descend box step -right	Faces forward, climbs down controlling weight bearing leg. No support needed	Sideways, skips down or needs support	Unable	
9. Descend box step –left	Faces forward, climbs down controlling weight bearing leg. No support needed	Sideways, skips down or needs support	Unable	
10. Gets to sitting	Starts in supine – may use one hand to assist	Self assistance e.g. – pulls on legs or uses head-on-hands or head flexed to floor	Unable	

Activity	2	1	0	Comments
11. Rise from floor	From supine – no evidence of Gowers' manoeuvre*	Gowers' evident	(a) NEEDS to use external support object e.g. chair OR (b) Unable	Time (00.0s)
12. Lifts head	In supine, head must be lifted in mid-line. Chin moves towards chest	Head is lifted but through side flexion or with no neck flexion	Unable	
13. Stands on heels	Both feet at the same time, clearly standing on heels only (acceptable to move a few steps to keep balance) for count of 3	Flexes hip and only raises forefoot	Unable	
14. Jump	Both feet at the same time, clear the ground simultaneously	One foot after the other (skip)	Unable	
15. Hop right leg	Clears forefoot and heel off floor	Able bend knee and raise heel, no floor clearance	Unable	
16. Hop left leg	Clears forefoot and heel off floor	Able bend knee and raise heel, no floor clearance	Unable	
17. Run (10m)	Both feet off the ground (no double stance phase during running)	'Duchenne jog'	Walk	Time (00.0s).....
				TOTAL= /34

Grading for Timed Function Tests

Grading of rising from floor (supine to stand)

1. Unable to stand from supine, even with use of a chair.
2. Assisted Gower`s – requires furniture for assist in arising from supine to full upright posture.
3. Full Gowers – rolls over, stands up with both hands "climbing up" the legs to achieve full upright posture.
4. Half Gowers – rolls over, stands up with one hand support on leg.
5. Rolls to the side and/or stands up with hand one or both hands on the floor to start to rise but does not touch legs.
6. Stands up without rolling over or using hands.

Grading of 10 m walk/run test

1. Unable to walk independently.
2. Unable to walk independently, but can walk with full leg calipers (KAFOs) or with support from a person.
3. Highly adapted wide based lordotic gait. Cannot increase walking speed.
4. Moderately adapted gait. Can pick up speed but cannot run.
5. Able to pick up speed, but runs with a double stance phase, i.e. cannot achieve both feet off the ground.
6. Runs and gets off both feet off the ground (with no double stance phase).

Grading of 4-stair climb

1. Unable to climb 4 standard stairs
2. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step). Uses both arms on one or both handrails.
3. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step). Using one arm on one handrail.
4. Climbs 4 standard stairs "marking time" (climbs one foot at a time, with both feet on a step before moving to next step). Not needing handrail.
5. Climbs 4 standard stairs alternating feet, needs handrail for support.
6. Climbs 4 standard stairs alternating feet, not needing handrail support.

Grading of 4-stair descend

1. Unable to descend 4 standard stairs.
2. Descends 4 standard stairs "marking time" (descends one foot at a time, with both feet on a step before moving to next step). Requires both arms on one or both handrails.
3. Descends 4 standard stairs "marking time" (descends one foot at a time, with both feet on a step before moving to next step). Requires one arm on one handrail.
4. Descends 4 standard stairs "marking time" (descends one foot at a time, with both feet on a step before moving to next step). Not needing handrail.
5. Descends 4 standard stairs alternating feet in both directions, needs handrail for support.
6. Descends 4 standard stairs alternating feet, not needing handrail support.

Appendix B [Additional Dystrophin bioassay-related reviewer comments]

RT-PCR measurement of dystrophin exon 51-skipped mRNA:

While the exact cause for these spontaneous exon skipping is unknown, alternative splicing events and skip frame-shifting that restore the open reading frame (ORF) for dystrophin might result in the spontaneous internally-deleted dystrophin in these fibers. No comprehensive study in boys with DMD has been conducted to characterize the extent of baseline revertant dystrophin but smaller studies have suggested that at least 50-62% of DMD cases show some baseline dystrophin-positive fibers and that the percentage of revertants or trace dystrophin fibers within these samples ranged from 0.01 to 25%. The reason for these baseline exon skipped mRNA and their correlation to protein levels after treatment has not yet been clearly defined in published studies. Emerging literature suggests that the *stability* of the transcript (skipped mRNA) is an important factor for determining ultimate dystrophin protein expression, rather than the *amount* of transcript. As their proposed mechanism of action, exon 51 skipping therapies should result in an increase in overall percentage of fibers with stable exon 51 skipped products when compared to a patient-matched baseline sample from the same muscle sub-group. The data is not adequate to demonstrate an increase in skipped product because it is a qualitative assay with no internal controls for reference. An intensity measurement of the PCR fragment(s) at multiple time-points could add some confidence, as attempted by the applicant with the nested RT-PCR approach.

The stability of the exon skipped transcript detected is also not apparent from the nested/lab-on-chip analysis. Any proposed correlation between dystrophin mRNA and protein levels is complicated by the known instability of the mRNA. Spitali et al (2013) have reported that Becker patients and mdx mice show significant transcript instability that obscures a clear correlation between transcript and protein levels. Anthony et al (2014) reported transcript instability in DMD patient samples with out-of-frame deletions compared to in-frame deletions. They suggest that measuring transcript stability by covering multiple exon junctions for dystrophin might indicate stability, which may be more important for predicting protein levels than measuring amounts of transcript. By using a nested PCR approach, the applicant may have hypothetically enhanced specificity for the target sequences on dystrophin but their exact primers were not described or whether their method captures transcript stability. Specifically, the applicant did not use multiple primers to cover additional regions of the target dystrophin skipped product to be able to predict stability of the transcript, as suggested by the literature cited above, which may be a better predictor of pharmacodynamic activity. While their lab-on-chip capillary electrophoresis/nested PCR method is a reasonable qualitative method for detecting dystrophin transcript, it may not provide the most accurate representation of exon skip that would be predictive of dystrophin protein expression. Additionally, no reference standard or calibration curve was used so it is not possible to interpret the applicant's data as reflective of absolute copy numbers of dystrophin transcript.

Assay cut-offs and scoring approach for the IFA and WB methods:

According to the applicant, for IFA, an increase in dystrophin was defined as an increase in mean membrane intensity of more than 4% at week 25 compared to pre-treatment biopsy. A strong increase in dystrophin was defined an increase in dystrophin intensity by ≥9% for the mean or ≥4% for the mean accompanied by an increase of ≥15% for the 10th quartile of most intensive pixels and confirmed by visual inspection of the images. A decrease was ≤ –4% for the mean membrane intensity. For WB, an increase was defined as a >30% increase in densitometric value of the ~427 kDa dystrophin band (post treatment –pre-treatment/pre-treatment)*100.

The 4% assay cutoff for an IFA score of "increase" and >9% for "strong increase" appears reasonable from a purely analytical standpoint because the inter- and intra-assay variability observed by the applicant is between 2-5% for this immunofluoresence assay. The Applicant also cited an mdx mouse model study where motor function was improved when levels of dystrophin were >4% compared to healthy control muscle. However, no

correlative human endpoint data (e.g. muscle function or DAPC protein co-localization) has been presented to support the biological significance of this scoring. Therefore, it cannot be concluded that the applicant's assay assay cut-offs are biologically meaningful.

Correlation between IFA, WB, and RT-PCR methods:
A clear, consistent, and positive correlation between all three assays - IFA, WB, and Exon skip -has not been established by the Applicant or published literature in the field using appropriate positive/negative controls (e.g. with BMD, DMD, healthy samples in the linear range). As presented by the applicant, the WB data is likely to be most reliable because a serial dilution with a healthy positive control was used for comparison and pre-treatment and post-treatment samples were run on the same gel in most instances. The IFA can suggest protein localization but is likely to be less meaningful for protein level quantitation.

Taylor et al have reported a correlation between immunofluorescence-based intensity ratios of dystrophin/spectrin and dystrophin protein levels measured by Western blotting and normalized to actin. In the article, data have been presented, suggesting a strong correlation between Western blotting and IFA (Anthony 2014 and Kevin Flanagin, Nationwide Children's Hospital, Columbus, OH, at the FDA-NIH Dystrophin Workshop 2015). However, the data presented also had an inter-laboratory variability CV of 22-67% for IFA, which lowers the confidence in the quantitative abilities of IFA and reproducibility of the findings.

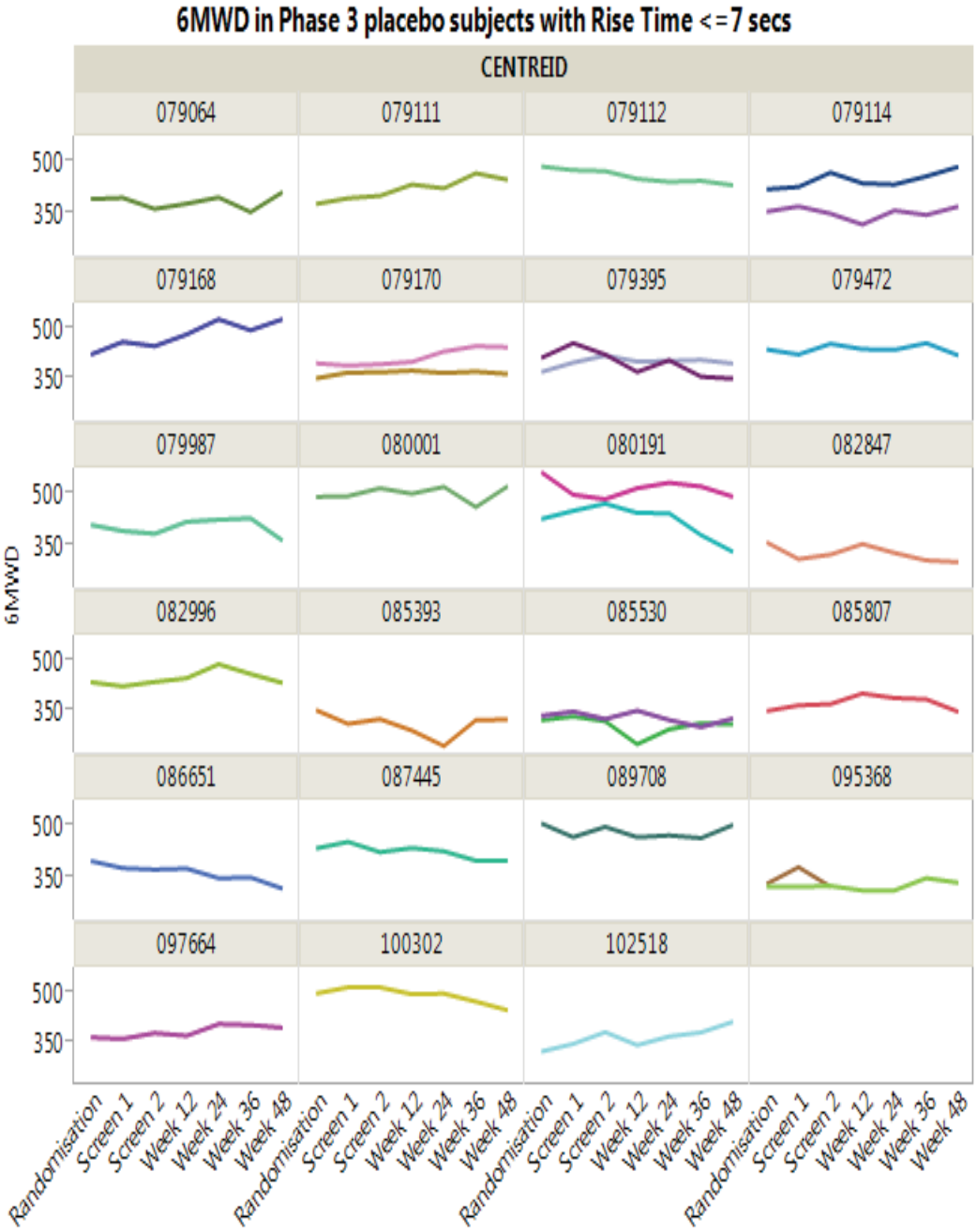
The correlation between IFA and WB methodologies likely depends on several factors including, but not limited to, (1) the differences in the basic assay methodologies (e.g. single cell-based microscopy versus homogenized lysate-based WB), (2) the antibodies used, the epitopes being targeted, as well as the exposure of those dystrophin epitopes to the antibodies in its intracellular or lysate state (3) the staining controls used (e.g. spectrin versus actin), (4) the measurement controls used (e.g. a negative DMD or positive healthy sample), (5) operator bias in the absence of automated image capture or analyses, (6) distribution and localization of dystrophin within the muscle fiber, (7) heterogeneity in the levels of revertant dystrophin between and within individual DMD patients, (8) sensitivity of the detection systems (e.g. fluorescence-tagged antibodies or densitometer instrument used), and (9) the limits of detection and quantitation for either methods. While it may be challenging to establish a strong quantitative correlation between WB and IFA, the protein levels observed the two methods for an appropriately designed experiment should trend in the same direction.

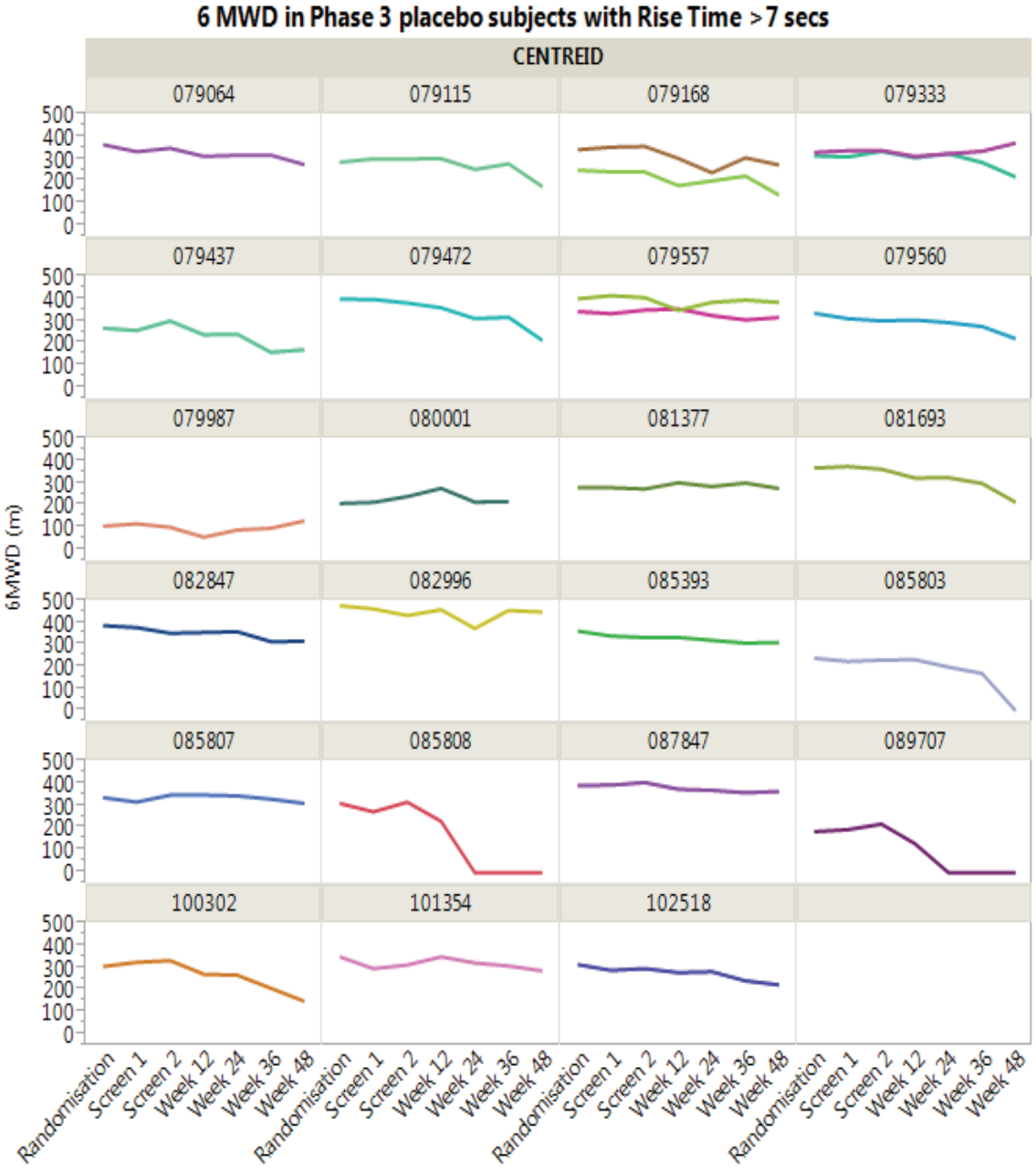
Additional comments on the deficiencies with the IFA methodology used in study 114876:
The applicant states that the inter-assay reproducibility of their IFA assay between experiments for placebo and drisapersen-treated subjects combined was 5% and ranged between 0-16% for the study. It is not clear if the mean values reported in Table 26 that are below 5% represent biologically-relevant responses or expected assay variability. There also appear to be several critical deficiencies in the applicant's analytical approach that preclude an interpretable assessment of dystrophin increase.

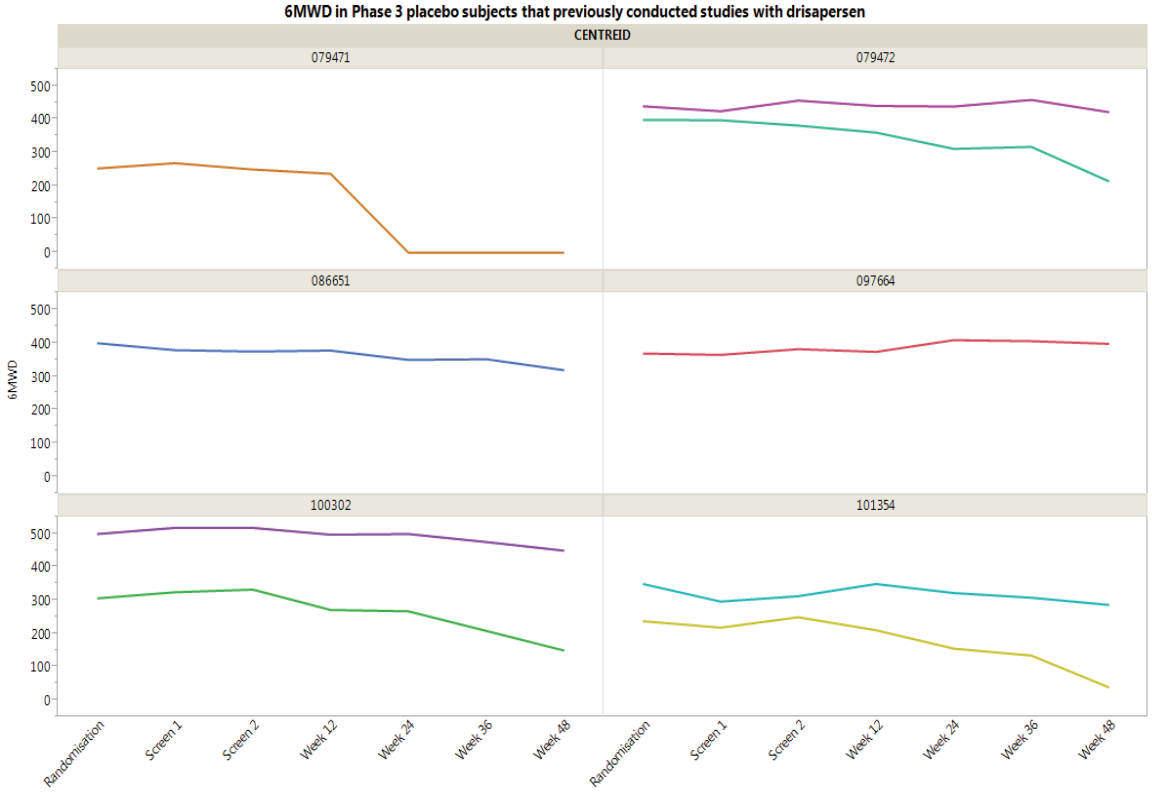
The Applicant states that a large change in spectrin (>20%) was observed between Week 24 and baseline biopsies, it is not clear whether and how this impacted dystrophin intensity measurements. However, it may suggest that either spectrin or the way spectrin was analyzed was not suitable for the intended purpose of being a muscle fiber co-stain. This could have been addressed by conducting adequate assay validation prior to clinical sample testing.

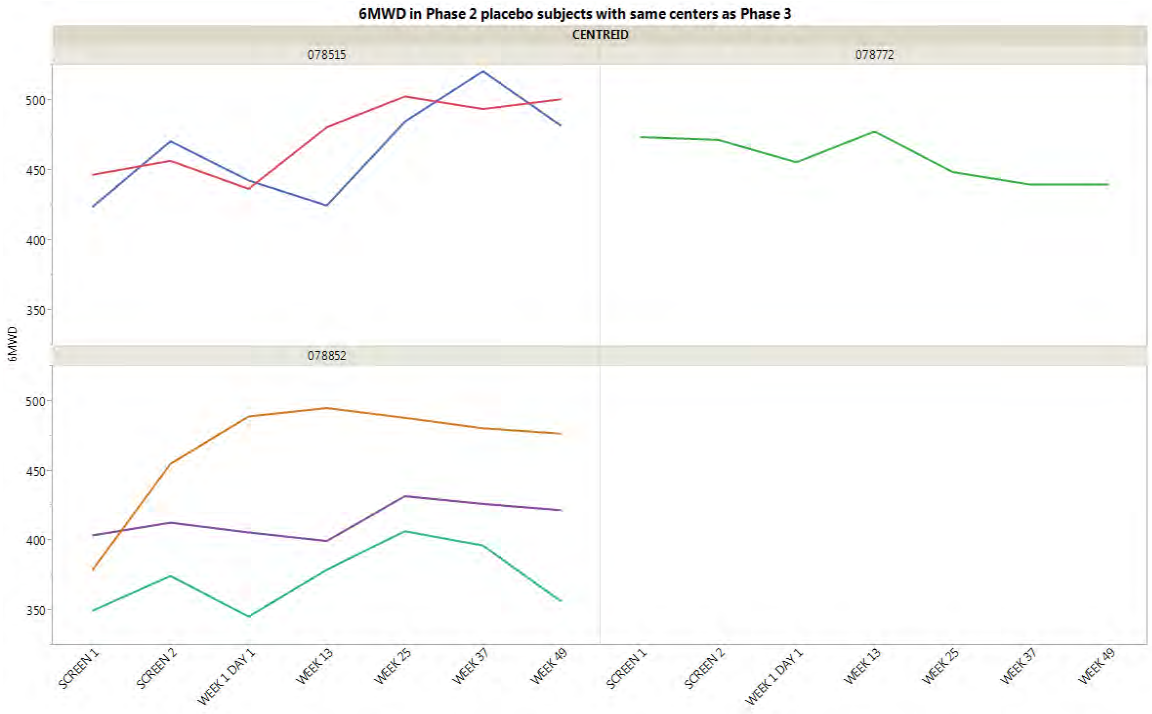
The number of subjects with sufficient quality, size, and muscle fiber content for each group was low (12, 11, and 13 for placebo, Week 24-3 mg/kg, and wk24- 6 mg/kg).

Appendix C


6MWD in Phase 3 placebo subjects with Rise Time <=7 secs
CENTREID
079064
079111
079112
079114
079168
079170
079395
079472
079987
080001
080191
082847
082996
085393
085530
085807
086651
087445
089708
095368
097664
100302
102518
6MWD
500
350
Randomisation
Screen 1
Screen 2
Week 12
Week 24
Week 36
Week 48


6 MWD in Phase 3 placebo subjects with Rise Time >7 secs
CENTREID
079064
079115
079168
079333
079437
079472
079557
079560
079987
080001
081377
081693
082847
082996
085393
085803
085807
085808
087847
089707
100302
101354
102518
6MWD (m)
0
100
200
300
400
500
Randomisation
Screen 1
Screen 2
Week 12
Week 24
Week 36
Week 48


6MWD in Phase 3 placebo subjects that previously conducted studies with drisapersen
CENTREID
079471
079472
086651
097664
100302
101354
6MWD
500
400
300
200
100
0
Randomisation
Screen 1
Screen 2
Week 12
Week 24
Week 36
Week 48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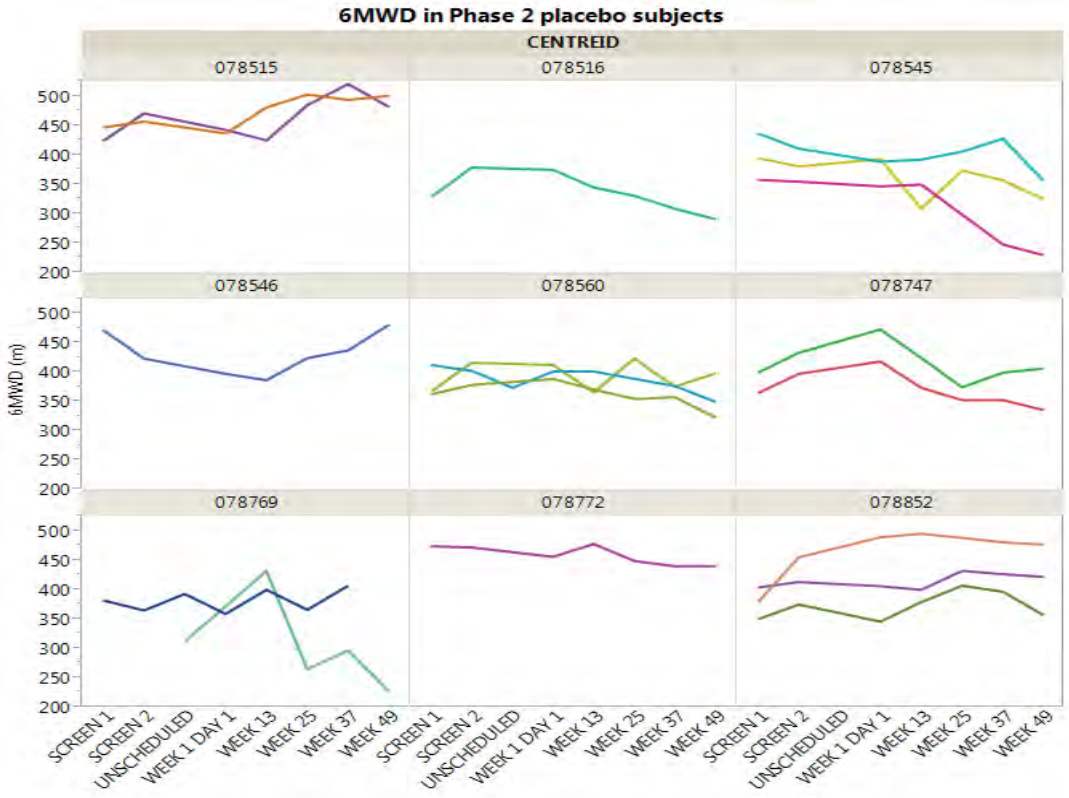
SCREEN 1
SCREEN 2
WEEK 1 DAY 1
WEEK 13
WEEK 25
WEEK 37
WEEK 49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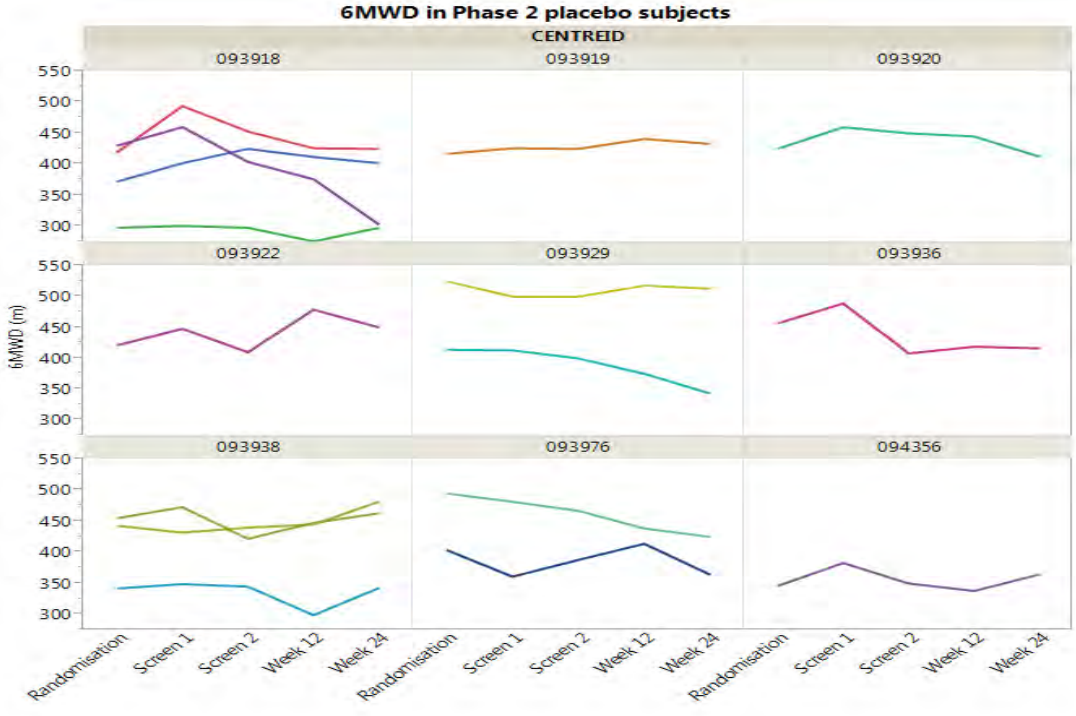
6MWD (m)
SCREEN 1
SCREEN 2
UNSCHEDULED
WEEK 1 DAY 1
WEEK 13
WEEK 25
WEEK 37
WEEK 49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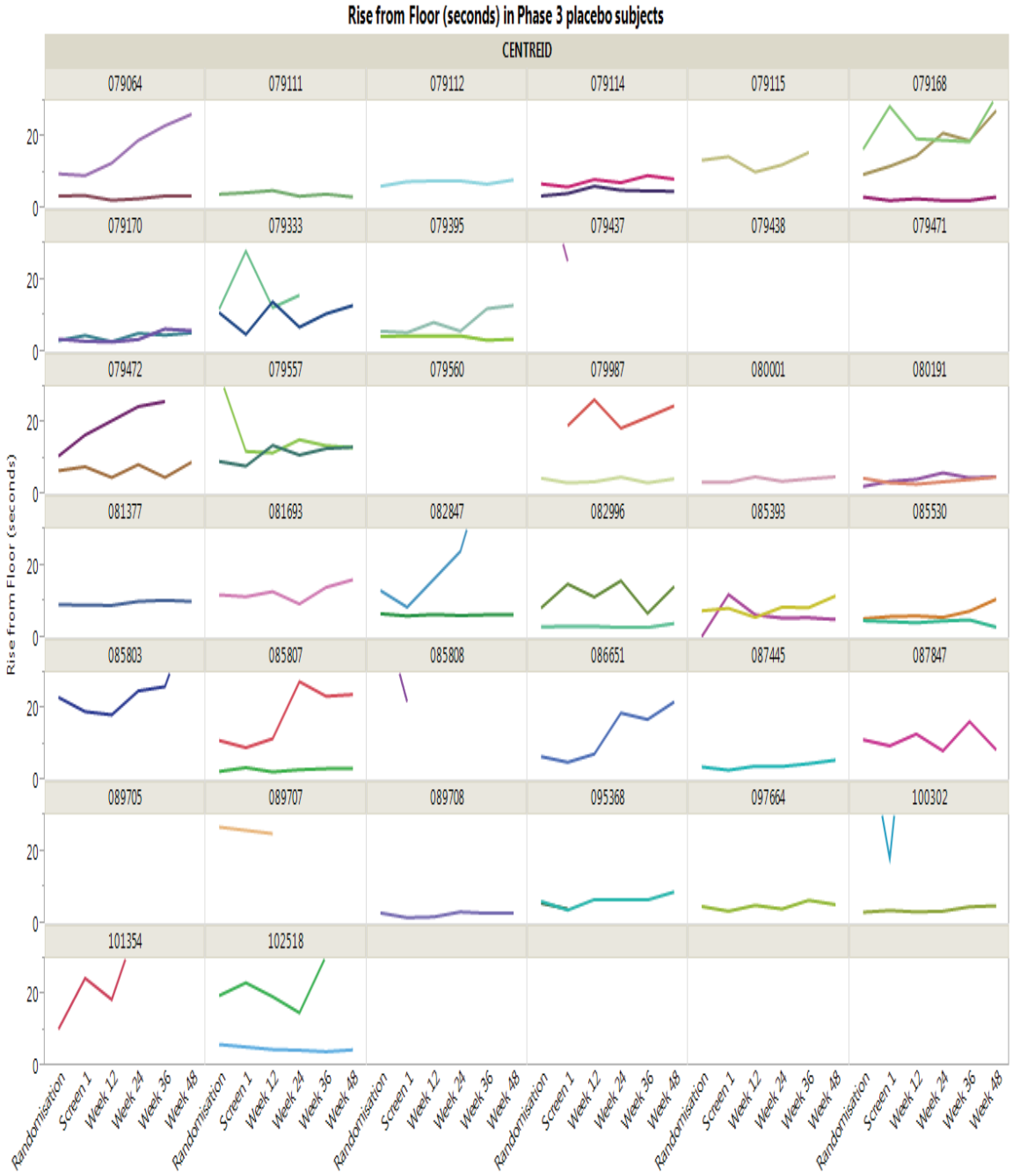
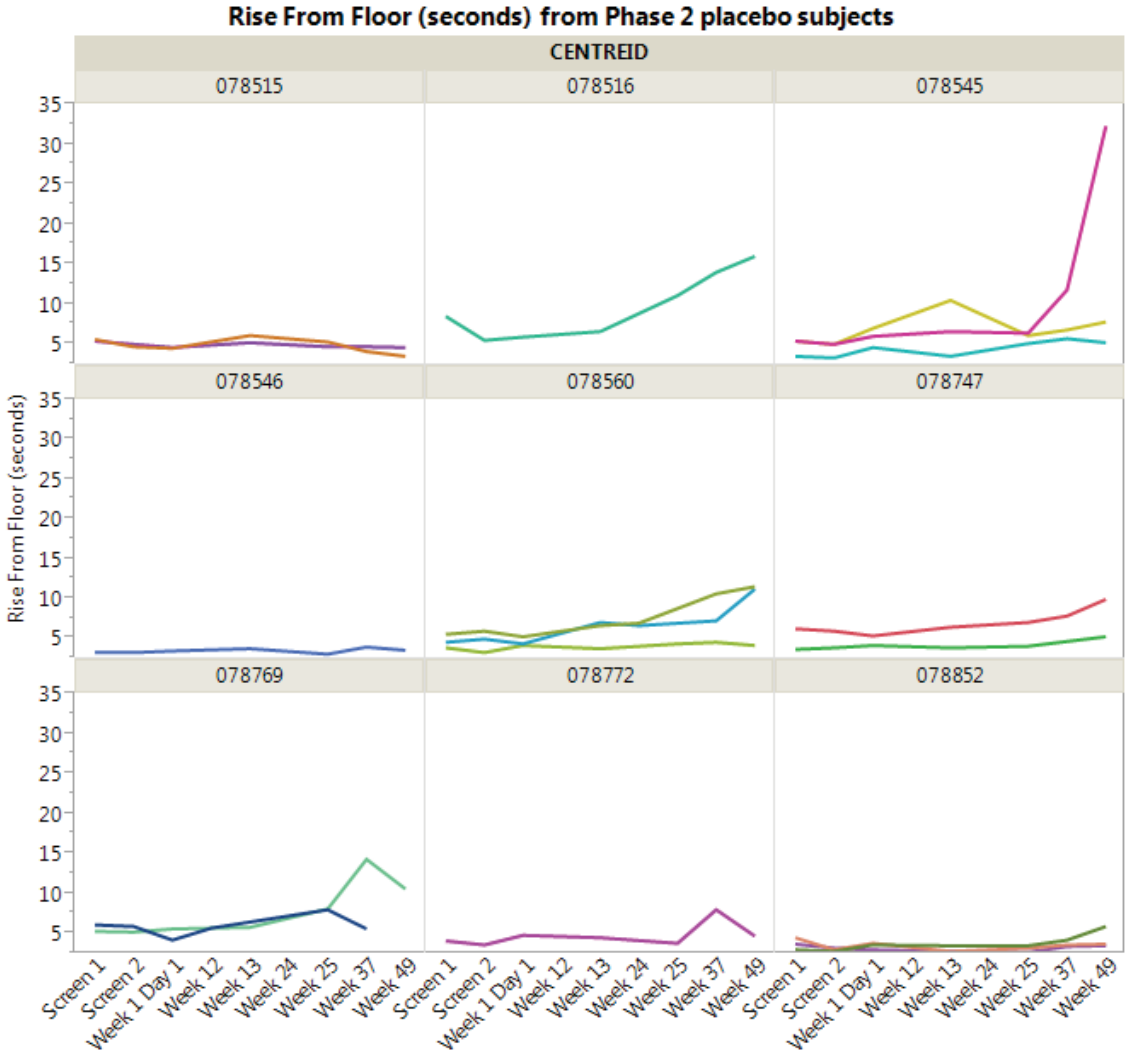
6MWD (m)
Randomisation
Screen 1
Screen 2
Week 12
Week 24



Note: The Y-axis has been truncated to 30 seconds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Rise From Floor (seconds)
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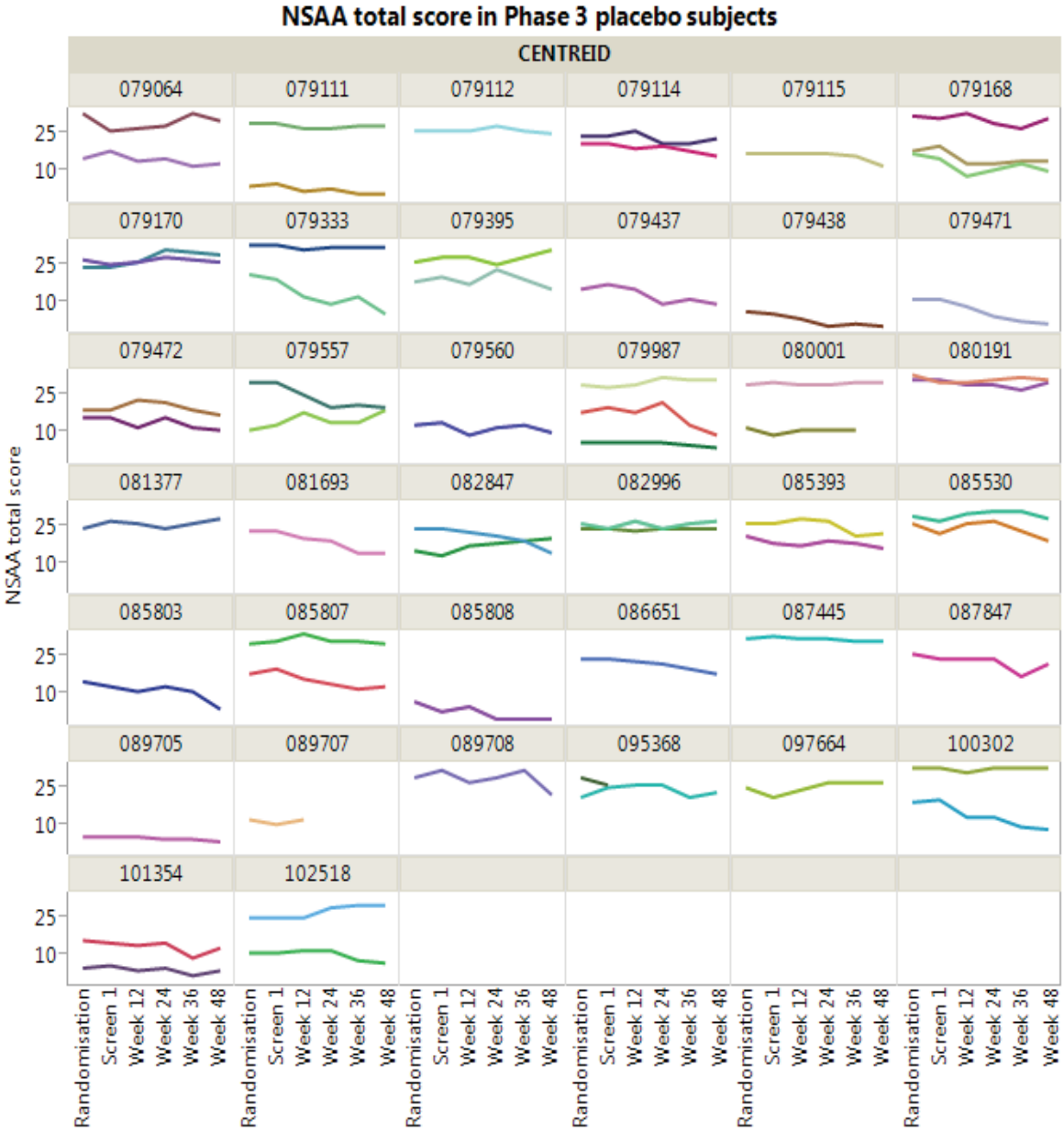
30
25
20
15
10
5
Screen 1
Screen 2
Week 1 Day 1
Week 12
Week 13
Week 24
Week 25
Week 37
Week 49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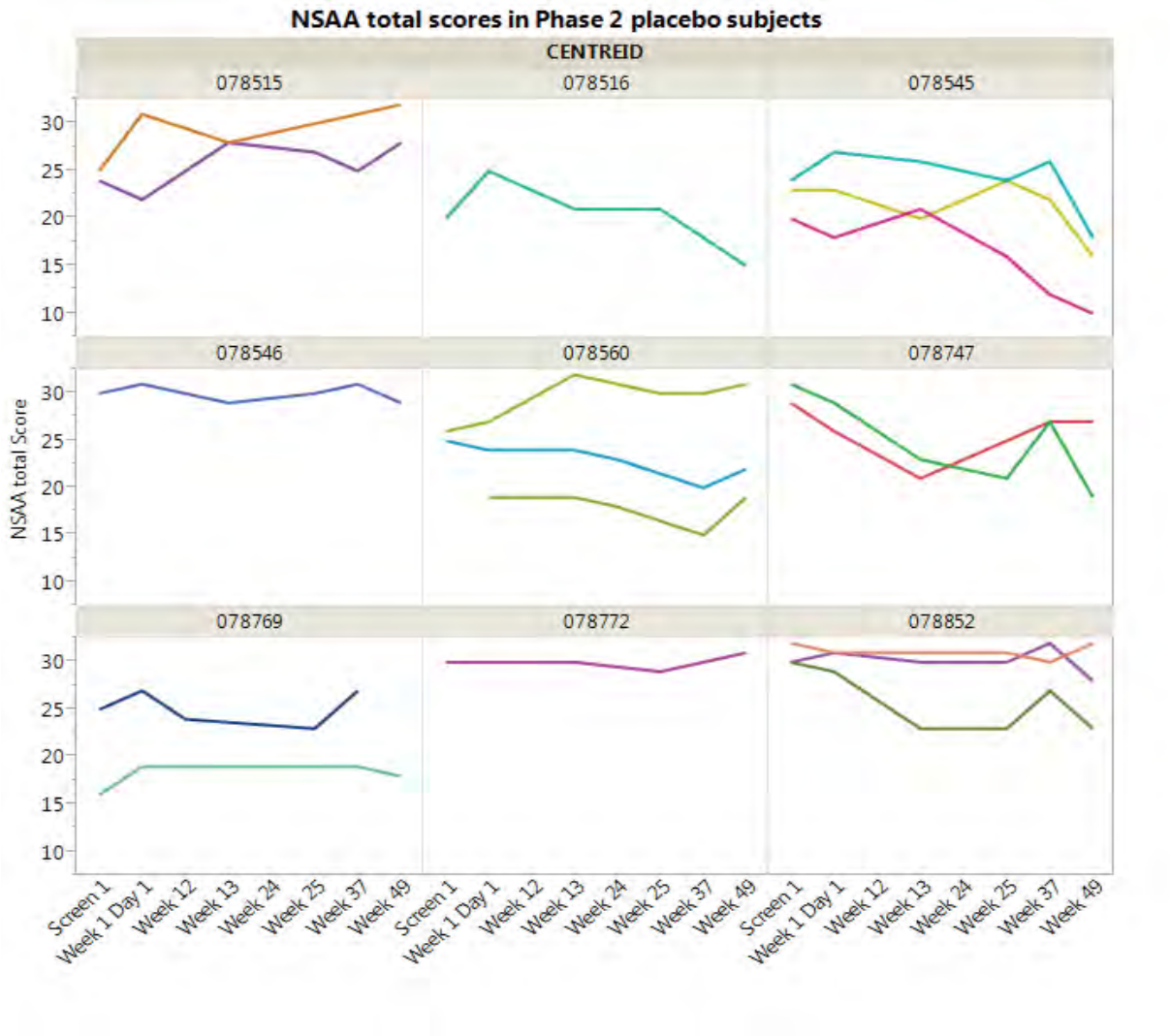
Randomisation
Screen 1
Week 12
Week 24
Week 36
Week 48


NSAA total scores in Phase 2 placebo subjects
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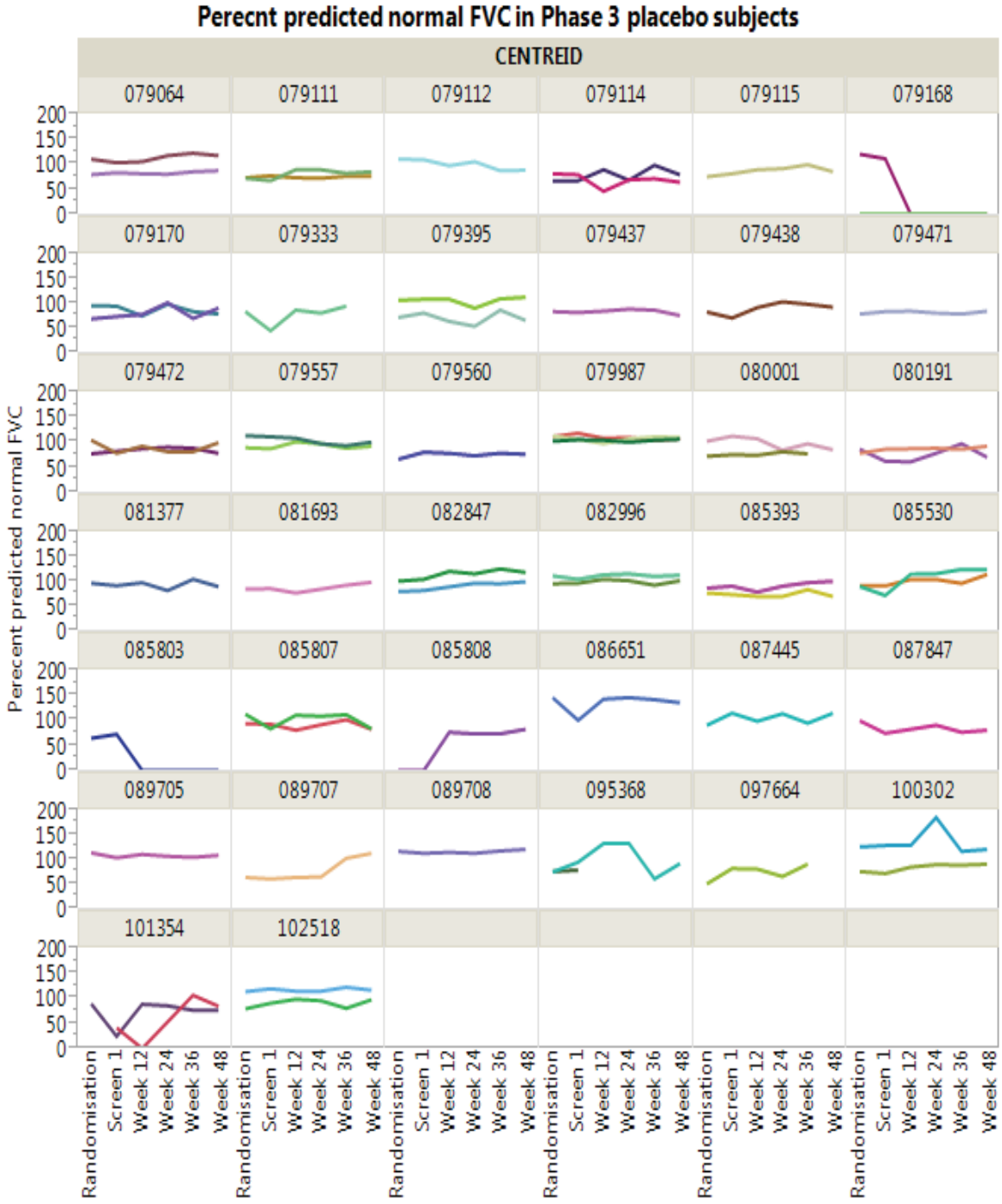
Week 1 Day 1
Week 12
Week 13
Week 24
Week 25
Week 37
Week 49


Perecnt predicted normal FVC in Phase 3 placebo subjects
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081377
081693
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085808
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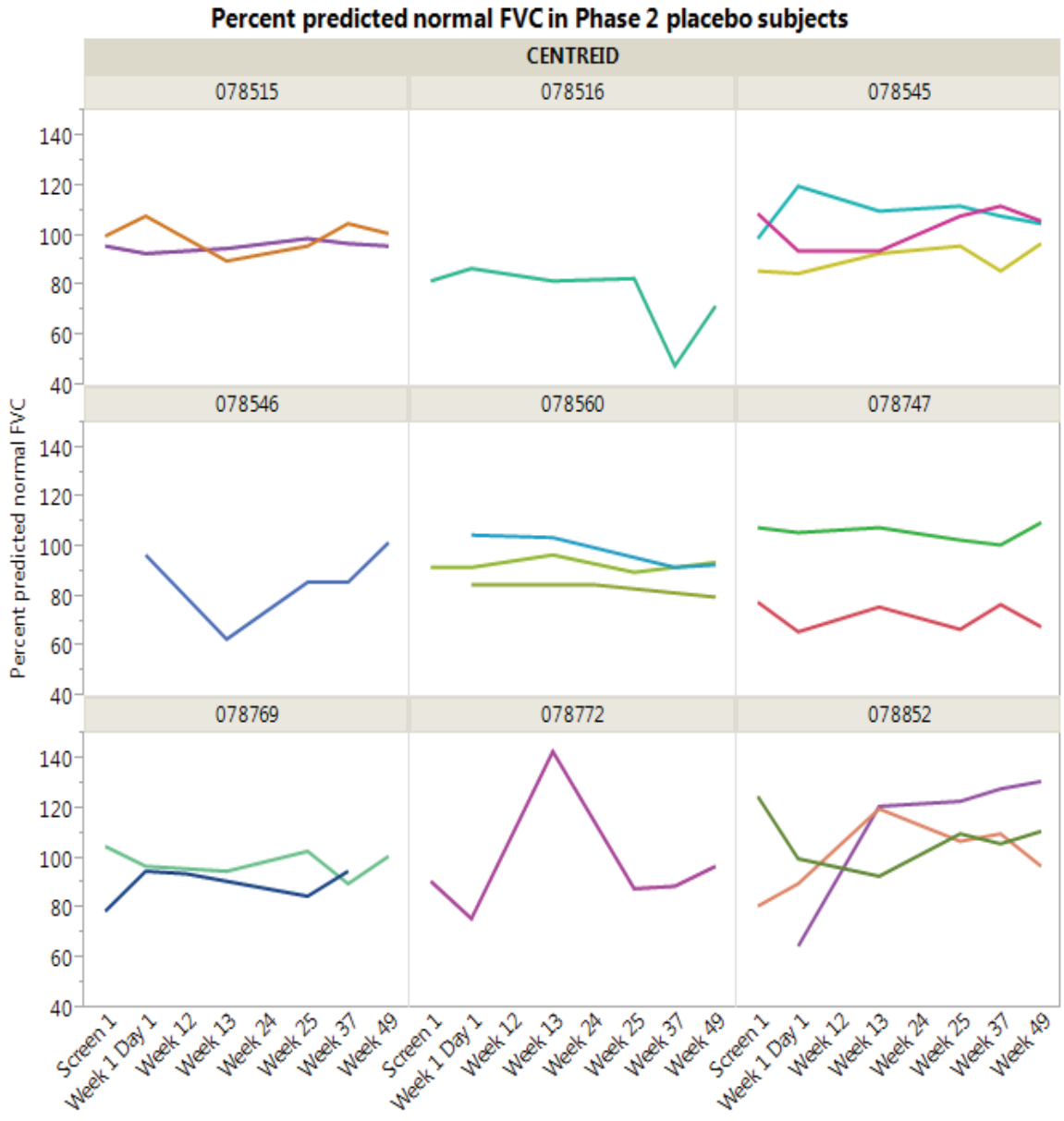
087847
089705
089707
089708
095368
097664
100302
101354
102518
Perecent predicted normal FVC
200
150
100
50
0
Randomisation
Screen 1
Week 12
Week 24
Week 36
Week 48


Percent predicted normal FVC in Phase 2 placebo subjects
CENTREID
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078769
078772
078852
Percent predicted normal FVC
140
120
100
80
60
40
Screen 1
Week 1 Day 1
Week 12
Week 13
Week 24
Week 25
Week 37
Week 49

IV. Clinical Safety Review

CLINICAL REVIEW

Application Type	NDA
Application Number(s)	206031
Priority or Standard	Priority
Submit Date(s)	April 27, 2015
Received Date(s)	April 27, 2015
PDUFA Goal Date	December 27, 2015
Division/Office	Division of Neurology Products / Office of New Drugs
Reviewer Name(s)	Evelyn Mentari, M.D., M.S.
Review Completion Date	September 29, 2015
Established Name	Drisapersen
(Proposed) Trade Name	Kyndrisa
Applicant	BioMarin
Formulation(s)	Subcutaneous
Dosing Regimen	Loading dose: Initiate with 6 mg/kg twice weekly for the first 3 weeks of treatment Maintenance dose: 6 mg/kg once weekly (2.1)
Proposed Indication(s)	Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing
Intended Population(s)	Patients with DMD (children and adults)
Recommendation on Regulatory Action	If the benefits outweigh the risks, then we recommend approval with labeling language including a boxed warning and a medication guide to mitigate the risks.

CDER Clinical Review Template Version date: April 9, 2015

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Glossary

AE	adverse event
aPTT	activated partial thromboplastin time
bpm	beats per minute
CHOP	Children's Hospital of Philadelphia
CIP	chronic intestinal pseudo-obstruction
CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
DIC	disseminated intravascular coagulation
DMD	Duchenne muscular dystrophy
ECG	electrocardiogram
FDA	Food and Drug Administration
GSK	GlaxoSmithKline
hERG	human ether-a-go-go-related gene
hsCRP	high sensitivity C-reactive protein
IDIC	independent data monitoring committee
INR	international normalized ratio
IND	investigational new drug application
ISS	integrated Summary of Safety
IU	International units
L	liter
LLN	lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
MCP-1	monocyte chemoattractant protein-1
msec	milliseconds
NDA	new drug application
OSI	Office of Scientific Investigations
OSE	Office of Surveillance and Epidemiology
PT	MedDRA Preferred Term
QTcB	QT interval value corrected according to Bazett's formula
QTcF	QT interval value corrected according to Friedericia's formula
REMS	risk evaluation and mitigation strategy
SAE	serious Adverse Event
SCS	Summary of Clinical Safety
SOC	MedDRA System Organ Class
ULN	Upper limit of normal

1 Executive Summary

1.1. Product Introduction

Drisapersen is a 2'-O-methyl-phosphorothioate oligonucleotide designed to skip exon 51 in dystrophin pre-mRNA to restore the reading frame of the mRNA. The proposed proprietary name is Kyndrisa. The proposed indication is the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing. Drisapersen is a new molecular entity.

The Sponsor's proposed loading dose is 6 mg/kg twice weekly for 3 weeks with a maintenance dose of 6 mg/kg once weekly. The route of administration is subcutaneous injection.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The reader is referred to the review of clinical efficacy by Dr. Veneeta Tandon.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Drisapersen is proposed to be used for treatment of Duchenne muscular dystrophy (DMD) in patients 5 years and older with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing. This review evaluates the safety of drisapersen. If efficacy is demonstrated and the benefits of drisapersen outweigh the risks, then we recommend approval with labeling language including a boxed warning and a medication guide to mitigate the risks.

This document reviews the risk profile of drisapersen. Please refer to Dr. Veneeta Tandon's review for discussion of Analysis of Condition and Current Treatment Options and Benefit.

Risk:

Drisapersen is associated with severe and potentially life-threatening adverse effects. Drisapersen causes immune thrombocytopenia, renal toxicity, and skin injury at injection sites.

- Six drisapersen subjects (2%) had thrombocytopenia $<20 \times 10^9$/L, levels at which patients are at risk potentially fatal complications, including spontaneous intracranial or intrapulmonary hemorrhage. Most of these patients had confirmed anti-platelet antibodies. These cases occurred 14-26 months after the first dose of drisapersen, suggesting that risk increases with duration of exposure. Platelet monitoring every 2 weeks, patient education regarding the signs and symptoms of thrombocytopenia, and facilitating prompt medical assessment and treatment can mitigate this risk. However, the decrease in platelets occurred precipitously and unpredictably, so that even with intensive monitoring, the risk remains. Concomitant use of antiplatelet, thrombolytic, or anticoagulant drugs is not recommended.
- Renal toxicity was reported in 61% of drisapersen 6 mg/kg/week subjects, compared to 34% of placebo subjects. Proteinuria was the most common renal toxicity and occurred in 44% of drisapersen 6 mg/kg/week subjects, compared to 23% of placebo subjects. One patient developed life-threatening thromboses with bilateral pulmonary emboli in the setting of glomerulonephritis with nephrotic syndrome. Renal laboratory monitoring every 2 weeks and cessation of drisapersen according to recommended laboratory criteria can mitigate this risk but will not eliminate the risk of severe and potentially fatal renal toxicity.
- Injection site reactions occurred in 79% of drisapersen patients and included ulceration, irreversible scarring, and atrophy. The risk for first injection site reaction occurred throughout the first 72 weeks of exposure. 21% of reactions were not resolved by the end of the studies. Reactions known to resolve lasted for a mean of 58 days and up to 1217 days.

Injection site reactions occurred despite administration by a medical professional and rotation of injection sites. No other strategies to mitigate the risk of injection site reactions are known.

- Drisapersen also has pro-inflammatory effects. It is not known how these effects or any other mechanism may have contributed to serious adverse events of myocarditis, myocardial ischemia, convulsion, intracranial venous sinus thrombosis, and small intestinal obstruction. The utility of monitoring inflammatory markers has not been evaluated.
- Phosphorothioate oligonucleotides are known to accumulate in the liver, and hepatic adverse events occurred in 10.5% subjects treated with drisapersen 6 mg/kg/week. Monitoring of liver function tests, including GGT, bilirubin, and INR, monthly will mitigate the risk of hepatic toxicity.

Paragraph #5

I recommend a patient registry as a post-marketing requirement to evaluate the main safety risks of drisapersen in the post-marketing setting. I recommend a boxed warning with recommendations for monitoring and administration to mitigate the risks of renal adverse events, thrombocytopenia, and injection site reactions and I recommend a Medication Guide to education patients about these risks.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Please refer to Dr. Tandon's review of clinical efficacy.	
Current Treatment Options	• Please refer to Dr. Tandon's review of clinical efficacy.	
Benefit	• Please refer to Dr. Tandon's review of clinical efficacy.	

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk	• The safety database for drisapersen includes all patients from the 3 Phase 2 and Phase 3 placebo controlled trials and from the 3 open label studies. Drug exposure is adequate and reflects the intended population for use. • The most common AEs were: Proteinuria (60%); Injection site erythema (52%); Injection site discoloration (49%). • Six drisapersen subjects (2%) had thrombocytopenia <20 x 10^9/L, levels at which patients are at risk potentially fatal complications, including spontaneous intracranial or intrapulmonary hemorrhage. These cases occurred 14-26 months after the first dose of drisapersen. Despite routine monitoring of platelets every 2 weeks, thrombocytopenia occurred precipitously in some cases. • Renal toxicity occurred in 61% of patients taking drisapersen, and the risk for a renal adverse event existed throughout 72 weeks of exposure. Proteinuria occurred in 44% of drisapersen 6 mg/kg/week patients, compared to 23% of placebo patients. Proteinuria was generally reversible on discontinuation, although one patient had life-threatening glomerulonephritis with nephrotic syndrome and renal vein and inferior vena cava thrombi with bilateral pulmonary emboli. • Injection site reactions, including discoloration, induration, pain, pruritus, bruising, atrophy, hematoma, and swelling, occurred in 79% of drisapersen patients. Chronic skin damage and ulceration occurred in 18% and 7% drisapersen subjects, respectively. The risk for first injection site reaction occurred throughout the first 72 weeks of	Major safety issues of thrombocytopenia, renal toxicity, and injection site reactions, occur at the proposed dose of drisapersen. Hepatic accumulation is a class effect and hepatic adverse events occurred in the clinical trials. The safety issues can have life-threatening outcomes; the adverse reactions can be mitigated but not completely prevented with monitoring. The magnitude of the potential for serious harm after approval is unknown. Adherence to monitoring of platelets and renal laboratory parameters every two weeks is necessary, and failure to adequately monitor, recognize signs and symptoms, and provide prompt medical treatment in the postmarketing setting would increase the risk of adverse and potentially life-threatening outcomes. Injection site reactions occurred despite administration by a medical professional and rotation of injection sites. No other strategies to mitigate the risk of injection site reactions are known. Based on adverse events in nonclinical studies and because of limitations due to

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	exposure. 21% of reactions were not resolved by the end of the studies. Reactions known to resolve lasted for a mean of 58 days and up to 1217 days. • Phosphorothioate oligonucleotides are known to accumulate in the liver, and hepatic adverse events occurred in 10.5% subjects treated with drisapersen 6 mg/kg/week. The most common hepatic adverse event was increased glutamate dehydrogenase, which occurred in 5% of drisapersen 6 mg/kg/week patients in repeat dose studies and 2.5% for drisapersen 6 mg/kg/week in placebo-controlled studies, compared to 0% for placebo. • Concomitant use with antiplatelet, thrombolytic, or anticoagulant drugs is not recommended. Patients taking these drugs were excluded from clinical studies. • Safety in the postmarketing setting: Laboratory values as markers of renal, hepatic, and thrombocytopenia adverse events were closely monitored during the clinical studies, and close monitoring will be necessary in the postmarket setting if the drug is approved. • Other uncertainties: The clinical effects of anti-drisapersen antibodies are not known. The clinical effects of pro-inflammatory activity and whether these effects could have contributed to serious adverse events of myocarditis, myocardial ischemia, convulsion, intracranial venous sinus thrombosis, and small intestinal obstruction are not known. The utility of monitoring inflammatory markers has not been evaluated.	the small number of patients exposed and duration of exposure in the clinical trials, and the uncertainty related to clinical impact of pro-inflammatory effects, it is likely that adverse reactions not identified to date will occur in the postmarketing setting.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk Management	• A patient registry as a post-marketing requirement will help to evaluate the main safety risks of drisapersen in the postmarketing setting. • Strong product labeling including a boxed warning and a Medication Guide with recommendations for monitoring of laboratory parameters and for rotation of injection sites is necessary to mitigate the risks of renal and thrombocytopenia adverse events and of injection site reactions. However, even with adequate monitoring, some patients will likely experience serious adverse events.	A patient registry as a post-marketing requirement will help to evaluate the main safety risks of drisapersen in the post-marketing setting. A boxed warning should be included in labeling to describe the risks of renal adverse events and thrombocytopenia and to provide recommendations for monitoring and to warning of the risk for injection site reactions and provide recommendations for rotation of injection sites. A medication guide should be required to describe these risks and symptoms of concern, and to highlight the need for prompt medical attention.

2 Therapeutic Context

2.1. Analysis of Condition

DMD is a severe, progressive, fatal pediatric neuromuscular disorder for which there is no available therapy. The disorder is caused by the absence of dystrophin protein due to mutations of the dystrophin gene. Dystrophin has a structural role as a cytoskeletal stabilization protein and protects muscle fibers against contraction-induced damage.[1] The disease occurs almost exclusively in males (X-linked recessive disorder) with an incidence of 1 in 3500 male births worldwide. Exon 51-skipping amenable mutations occur in approximately 13% of boys with DMD.

2.2. Analysis of Current Treatment Options

There are no FDA approved treatments for DMD. Corticosteroids are the standard of care.

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Drisapersen is a new molecular entity, and it is not currently marketed in the U.S.

3.2. Summary of Presubmission/Submission Regulatory Activity

Summary of changes of sponsorship:

- Pre-IND sponsorship transferred from Prosensa to GlaxoSmithKline (GSK) on 10/6/2009
- IND sponsorship transferred from GSK back to Prosensa on 2/18/14
- BioMarin acquired Prosensa, including all subsidiaries, on 1/16/2015

Summary of designations:

- 8/25/09: Orphan designation granted
- 12/6/10: Fast Track designation granted
- 6/27/13: Breakthrough Therapy designation granted

[1] Rybakova,IN, et al. "The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin." The Journal of cell biology 150.5 (2000): 1209-1214.

Regarding drug safety in humans, at a pre-IND meeting on July 8, 2009, the Agency expressed concern regarding hematological reactions and their reversibility. The Agency also provided input on the proposed renal and hepatic monitoring. Also in 2009, the Netherlands Medicine Evaluation Board and the sponsor agreed that thrombocytopenia is a class effect deemed important for monitoring in clinical studies.

On June 6, 2010, the IND was placed on Partial Clinical Hold, because of inadequate plans for safety monitoring. Increased laboratory monitoring, as well as study exclusion criteria based on platelet counts, coagulation and disseminated intravascular coagulation (DIC) laboratory tests, were added to clinical studies. The partial clinical hold was removed on May 4, 2011.

At an End-of Phase II meeting on May 23, 2013, the Agency agreed that the safety database was appropriate for NDA filing. At the pre-NDA meeting on January 27, 2015, the Agency indicated the additional analyses that were to be part of the NDA.

3.3. Foreign Regulatory Actions and Marketing History

There is no foreign marketing experience. A Marketing Authorization Application has been submitted to the European Medicines Agency with an opinion expected in 2016.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

The reader is referred to the OSI review.

4.2. Product Quality

The reader is referred to the Office of Product Quality review.

4.3. Clinical Microbiology

Not applicable.

4.4. Nonclinical Pharmacology/Toxicology

The reader is referred to the pharmacology/toxicology review.

4.5. Clinical Pharmacology

All clinical studies have been performed in subjects suffering from Duchenne muscular dystrophy (DMD), none in healthy volunteers. In theory, administration of drisapersen to healthy volunteers, and thereby skipping exon 51 of the DMD gene, could potentially alter a functional dystrophin protein into a non-functional form and was therefore considered unethical by the Sponsor. Production of a non-functional protein could induce side effects that would not be applicable to the subject population, where administration of the drug induces an in-frame transcript and a functional protein.

A human mass balance study using radiolabeled drisapersen (to determine mass balance, routes of excretion, identify and quantitate metabolites, etc.) has not been conducted. No human excretion data, including human urine measurements, have been evaluated with drisapersen.[2]

For additional information, the reader is referred to the clinical pharmacology review.

4.5.1. Mechanism of Action

Drisapersen is a 2'-O-methyl-phosphorothioate oligonucleotide designed to skip exon 51 in dystrophin pre-mRNA to restore the reading frame of the mRNA. Restoring the reading frame of the dystrophin gene may result in the expression of a truncated but functional dystrophin protein.

4.5.2. Pharmacodynamics

The reader is referred to the review of clinical efficacy for analyses of muscle biopsy and biomarker results.

4.5.3. Pharmacokinetics

The plasma concentrations of drisapersen increased rapidly after drug administration and, for the majority of subjects, reached maximum plasma concentrations 2 and 3 hours post-dose. Thereafter, drisapersen was rapidly distributed to tissues with a decline in plasma levels to about 18% of the Cmax at 24 hours post-dose and to about 0.6% of Cmax at the end of the dosing interval.

In Study DMD114673, muscle concentrations of drisapersen determined in muscle biopsies obtained after 5 weeks (Visit 8), 6 months (Visit 37) and after 1.5 years (Visit 81) of subcutaneous administrations of 6 mg/kg were detected in all samples analysed (see Table 15). A variance between individual subjects is found, ranging from 3.3 to 9.9 µg/g after 5 weeks, from 5.8 to 28.4 µg/g after 6 months and from 8.7 to 39.1 µg/g after 1.5 years. Overall, muscle tissue concentrations of drisapersen increased between 5 weeks and 6 months of drisapersen

[2] P. 50-51 Summary of Clinical Pharmacology. Submitted to NDA206031 on 4/27/2015.

treatment, and for four subjects between 6 months and 1.5 years of drisapersen treatment.

In study DMD114876, drisapersen treatment duration was 24 weeks; beyond this time frame drisapersen levels were maintained and decreased only slowly. The mean level of drisapersen in tissue homogenates at Week 36, 12 weeks after stopping treatment, had declined by 41%. Both the slow accumulation and the slow elimination from tibialis anterior muscle tissue suggest a tissue half-life for drisapersen in muscle in the range of 2-3 months.

4.6. Devices and Companion Diagnostic Issues

Not applicable.

4.7. Consumer Study Reviews

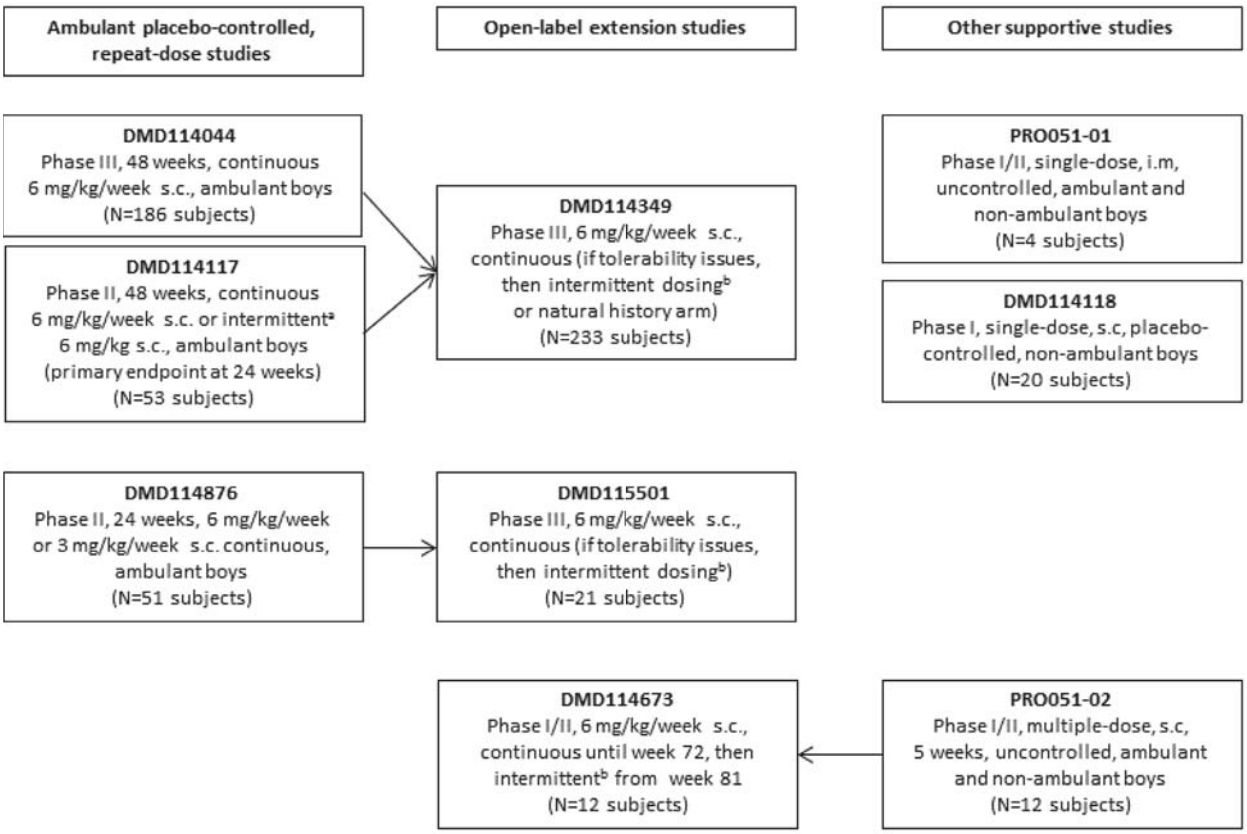
Not applicable.

5 Sources of Clinical Data and Review Strategy

5.1. Table of Clinical Studies

The figure below provides an overview of the drisapersen clinical development program. The table below summarizes clinical studies supporting safety in NDA 206031.

Figure 1. Overview of the drisapersen clinical program



Source: Sponsor Figure 2. P. 16 Summary of Clinical Safety. Submitted to NDA 206031 on 4/27/2015.
a Intermittent dosing in DMD114117 - alternating 6mg/kg/week twice weekly and 6 mg/kg/week for 6 weeks followed by 4 week off-dose period
b Intermittent dosing in DMD114349, DMD114673,and DMD115501- 6 mg/kg/week for 8 weeks followed by 4 weeks off-dose

Table 1. Listing of clinical studies to support safety in NDA 206031

Trial Identity	Trial Design	Regimen/ schedule/ route	Treatment Duration	No. of Subjects	Study Population	Countries
Placebo-controlled studies						
DMD114044	Randomized, double-blind, placebo-controlled	Drisapersen, solution for injection, s.c.6 mg/kg/week Dose-matched placebo	48 weeks	Total: 186 6mg/kg/week: 125 Placebo: 61	Ambulant boys with DMD	Argentina, Belgium, Brazil, Canada, Chile, Czech Republic, Denmark, France, Germany, Italy, Japan, Republic of Korea, Netherlands, Norway, Poland, Russian Federation, Spain, Taiwan, Turkey
DMD114117	Randomized, double-blind, placebo-controlled	Drisapersen, solution for injection, s.c. 6 mg/kg twice weekly for 3 weeks (loading dose) then either: Continuous: 6 mg/kg/week or Intermittent: 6 mg/kg twice weekly on 1st, 3rd and 5th weeks, once weekly on 2nd, 4th and	48 weeks	Total: 53 6 mg/kg/week:18 Intermittent 6 mg/kg: 17 Placebo: 18	Ambulant boys with DMD	Australia, Belgium, France, Germany, Israel, Netherlands, Spain, Turkey, UK

Trial Identity	Trial Design	Regimen/ schedule/ route	Treatment Duration	No. of Subjects	Study Population	Countries
		6th weeks, and no active drug on 7th to 10th week of each 10 week cycle. Placebo, dose–matched placebo twice weekly for 3 weeks (loading dose) then weekly				
DMD114876	Randomized, double-blind, placebo-controlled	Drisapersen 3 mg/kg, drisapersen 6 mg/kg, given s.c. once a week. Dose-matched placebo for both active arms	24 weeks	Total: 51 3mg/kg/week: 17 6mg/kg/week: 18 Placebo: 16	Ambulant boys with DMD	USA
Other repeat dose study						
PRO051-02	Randomized open label	Drisapersen, solution for injection, s.c. 0.5 mg/kg/week 2.0 mg/kg/week 4.0 mg/kg/week 6.0 mg/kg/week	5 weeks	Total: 12 3 subjects in each treatment arm	Ambulant and non-ambulant boys with DMD	Belgium, Sweden
Open-label extension studies						
DMD114349 (extension study to DMD114117 & DMD114044)	Open label	Drisapersen, solution for injection, s.c.6 mg/kg/week Subjects who met laboratory or follow-up stopping criteria or with tolerability issues had option to enter intermittent arm of 6 mg/kg/week for 8 weeks followed by 4 weeks off dose.	Minimum 104 weeks of treatment	233	Boys with DMD ambulant at the start of the parent study	Argentina, Australia, Belgium, Brazil, Bulgaria, Canada, Chile, Czech Republic, Denmark, France, Germany,

Trial Identity	Trial Design	Regimen/ schedule/ route	Treatment Duration	No. of Subjects	Study Population	Countries
		Subjects who did not wish to receive drisapersen or who had to withdraw from both active arms during the study had the option to go into a natural history observation arm				Hungary, Israel, Italy, Japan, Republic of Korea, Netherlands, Norway, Poland, Russian Federation, Spain, Taiwan, Turkey, UK.
DMD114673 (extension to PRO051-02)	Open label	Drisapersen solution for Injection 6 mg/kg s.c. for 72 weeks. After an interval off drug of 8 weeks (Weeks 73 – 80), all subjects restarted an intermittent treatment regimen of 6 mg/kg/week for 8 weeks, followed by 4 weeks off treatment = 12 weeks per cycle up to 188 weeks.	188 weeks s.c. in all 12 subjects. Subjects then received iv (5 doses), s.c. or iv (5 doses) and s.c. for a further 27 weeks until dosing was halted.	12	Subjects with DMD, ambulant and non-ambulant boys who completed the initial study	Belgium, Sweden
DMD115501 (extension to DMD114876)	Open label	Drisapersen, solution for injection, s.c.6 mg/kg/week Subjects who met laboratory or follow-up stopping criteria or with	Treatment until withdrawal criteria met or	Study in progress at the time of NDA submission. Aims to enroll about 72 subjects	Boys with DMD ambulant at the start of the	USA

Trial Identity	Trial Design	Regimen/ schedule/ route	Treatment Duration	No. of Subjects	Study Population	Countries
		tolerability issues had option to enter intermittent arm of 6 mg/kg/week for 8 weeks followed by 4 weeks off dose.	sponsor stops the study.		parent study	

Abbreviations: DMD=Duchenne Muscular Dystrophy; iv=intravenous; s.c.=subcutaneous.

5.2. Review Strategy

The clinical review of NDA 206031 is divided into a review of clinical efficacy (by Dr. Veneeta Tandon) and this review of clinical safety.

Information submitted as part of NDA 206031, as well as published information related to the oligonucleotides as a pharmacologic class and other relevant published literature, are discussed in this review.

6 Review of Relevant Individual Trials Used to Support Efficacy

Not applicable to the review of clinical safety. The reader is referred to the review of clinical efficacy by Dr. Veneeta Tandon.

7 Integrated Review of Effectiveness

Not applicable to the review of clinical safety. The reader is referred to the review of clinical efficacy by Dr. Veneeta Tandon.

8 Review of Safety

Safety Review Approach

Two main safety subject pools were used in the analyses of drisapersen clinical safety.

1. Placebo-controlled studies:

- DMD114044 (Phase 3)
- DMD114117 (Phase 2)
- DMD114876 (Phase 2)

2. Repeat dose studies (6 studies in total), which includes the 3 placebo-controlled studies, as well as 3 open label studies:

- PRO051-02
- DMD114349 (Extension study of DMD114044 and DMD114117)
- DMD114673 (Extension study of PRO051-02)

For additional details on these studies, the reader is referred to Section 5.1.

Safety issues of interest identified during drug development included:

- Thrombocytopenia
- Renal toxicity
- Injection site reactions
- Inflammation
- Coagulation disorders
- Hepatic disorders

8.2. Review of the Safety Database

8.2.1. Overall Exposure

The tables below describe the size and subject duration of exposure for the drisapersen safety population.

Table 2. Drisapersen Safety Population. Size and Denominators

Drisapersen Safety Database for treatment of Duchenne Muscular Dystrophy (DMD) (N=312)			
Clinical Trial Groups	Drisapersen (n= 312)	Active Control (n=0)	Placebo (n= 95)
Normal Volunteers	0	0	0
Controlled trials conducted for DMD indication	195	0	95
All other than controlled trials conducted for DMD indication	117	0	0
Controlled trials conducted for other indications	0	0	0

Source: Sponsor Tables 12 and 13. Summary of Clinical Safety.

Table 3. Drisapersen Safety Population. Duration of Exposure

Number of patients exposed to the study drug:			
≥24 weeks	≥48 weeks	≥72 weeks	≥96
N= 271	N= 223	N=192	N=122

Source: Sponsor Table 15. Summary of Clinical Safety.

When compared to International Conference on Harmonisation (ICH) guidelines,[3] the overall number of exposed subjects is less than the usual recommendation. However, because DMD is a rare disease, there is no specific minimum number of patients that should be studied to establish clinical safety. The number of subjects exposed ≥6 months nearly meets the ICH recommendation, and the number of subjects exposed ≥ 1 year exceeds the recommendation.

8.2.2. Relevant characteristics of the safety population:

Demographics

The table below displays demographics for subjects in all repeat-dose studies. The median age in drisapersen 6 mg/kg/week subjects was 8 years, compared to 7 years in placebo subjects. There were a total of 51 subjects from the United States.[4]

[3] 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. (ICH E-1)
[4] Data from dataset ADSL2. Submitted to NDA 206031 on June 5, 2015.

Table 4. Summary of demographic characteristics (repeat dose studies)

	Placebo N=95	Drisapersen 3mg/kg/wk N=17	Drisapersen 6mg/kg/wk N=267	Drisapersen 6mg/kg intermittent [a] N=17	Drisapersen 6mg/kg intermittent [a] N=24	Drisapersen all regimens [c] N=285
Age[d] group, n (%)						
≤7 years	48 (50.5)	8 (47.1)	123 (46.1)	7 (41.2)	5 (20.8)	132 (46.3)
>7 years	47 (49.5)	9 (52.9)	144 (53.9)	10 (58.8)	19 (79.2)	153 (53.7)
Age[d], years						
Mean (SD)	7.8 (2.13)	7.8 (1.91)	8.3 (2.25)	7.7 (1.49)	9.3 (2.07)	8.3 (2.22)
Median	7.0	8.0	8.0	8.0	9.0	8.0
Min, max	5, 16	5, 11	5, 16	5, 10	5, 14	5, 16
Ethnicity, n (%)						
Hispanic/Latino	14 (14.7)	1 (5.9)	34 (12.7)	1 (5.9)	2 (8.3)	35 (12.3)
Non-Hispanic/Latino	79 (83.2)	16 (94.1)	216 (80.9)	14 (82.4)	10 (41.7)	233 (81.8)
Missing	2 (2.1)	0	17 (6.4)	2 (11.8)	12 (50.0)	17 (6.0)
Race, n (%)						
African American/African	2 (2.1)	1 (5.9)	2 (0.7)	0	0	3 (1.1)
American Indian/Alaskan native	0	0	1 (0.4)	1 (5.9)	0	1 (0.4)
Asian – central//South	1 (1.1)	0	4 (1.5)	0	0	4 (1.4)
Asian - East	3 (3.2)	0	10 (3.7)	0	0	10 (3.5)
Asian - Japanese	5 (5.3)	0	14 (5.2)	0	1 (4.2)	14 (4.9)
Asian - South-East	1 (1.1)	0	4 (1.5)	0	1 (4.2)	4 (1.4)
Native Hawaiian, other Pacific islander	1 (1.1)	0	1 (0.4)	0	0	1 (0.4)
White – arabic/North African	4 (4.2)	0	11 (4.1)	0	1 (4.2)	11 (3.9)
White – Caucasian/European	73 (76.8)	16 (94.1)	208 (77.9)	14 (82.4)	20 (83.3)	225 (78.9)
Mixed race	3 (3.2)	0	7 (2.6)	0	1 (4.2)	7 (2.5)
Missing	2 (2.1)	0	5 (1.9)	2 (11.8)	0	5 (1.8)
Region, n (%)						
Europe	47 (49.5)	0	149 (55.8)	12 (70.6)	19 (79.2)	149 (52.3)
North America	23 (24.2)	17 (100)	38 (14.2)	0	1 (4.2)	55 (19.3)
Asia	7 (7.4)	0	23 (8.6)	0	1 (4.2)	23 (8.1)
Rest of World	18 (18.9)	0	57 (21.3)	5 (29.4)	3 (12.5)	58 (20.4)

Source: Sponsor Table 23. Summary of Clinical Safety p. 56.
Table includes data from studies DMD114117, DMD114044, DMD114876, DMD114349, PRO051-02, and DMD114673.

Inclusion and Exclusion Criteria

The study inclusion and exclusion criteria are summarized in Summary of Clinical Safety Appendix 8.1.

All studies [except for open label study PRO051-02 (N=12)] included only ambulant subjects. The clinical study findings may not fully represent drisapersen clinical safety in the setting of more advanced DMD. Also, the pharmacokinetics of drisapersen may be different in the non-ambulant population, because of differences in muscle mass.

Drisapersen clinical studies excluded patients with current or a history of liver or renal disease.

8.2.3. Adequacy of the safety database

Because DMD is a rare disease, the overall subject exposure in the drisapersen clinical development program is adequate. Duration of treatment and patient demographics are acceptable.

8.3. Adequacy of Applicant's Clinical Safety Assessments

8.3.1. Issues Regarding Data Integrity and Submission Quality

The NDA submission was well-organized. Requests for additional information were handled promptly by the Sponsor.

8.3.2. Categorization of Adverse Events

The Sponsor's process for recording AEs was appropriate. The Sponsor's coding resulted in appropriate translation of verbatim terms to preferred terms. However, AEs were often coded to multiple different equivalent Preferred Terms, which resulted in splitting of adverse events across multiple Preferred Term categories. For example, in placebo-controlled studies, proteinuria (including adverse events with PTs Proteinuria, Protein urine present, and Protein urine) occurred in 44% drisapersen 6 mg/kg/week subjects, compared to the table listing of 29%, which only included adverse events coded to the PT Proteinuria.

The Sponsor categorized adverse events as mild, moderate, or severe. Adverse events were coded to MedDRA 16.1 in the integrated summary of safety.

Adverse events with onset after the first dose up to 28 days after the last dose were considered on-treatment AEs. Those occurring from day 29 after the last dose were considered follow-up AEs. Treatment-emergent AEs were composed of on-treatment and follow-up AEs.[5]

8.3.3. Routine Clinical Tests

The laboratory assessment schedule in the drisapersen clinical development program is summarized in Appendix Section 13.3.[6] Most laboratory measurements related to adverse events of special interest (e.g., renal monitoring and platelet counts) were performed every 2 weeks.

Reviewer comment: In the opinion of this reviewer, the safety assessment methods in drisapersen clinical studies were acceptable.

8.4. Safety Results

[5] P. 26 Summary of Clinical Safety.
[6] Summary of Clinical Safety section 8.3.

8.4.1. Deaths

No subjects died during the drisapersen clinical development program.

8.4.2. Serious Adverse Events

Serious adverse events (SAEs) from the Integrated Summary of Safety (ISS) pool[7] of all repeat dose studies are summarized in the table below. Forty six of 285 (16.1%) drisapersen subjects (all regimens) had at least 1 SAE.

Table 5. Summary of All Serious Adverse Events by System Organ Class, Ordered by Decreasing Frequency (Safety): Repeat-Dose studies

System Organ Class:	Placebo (n=95) n (%)	Drisapersen 6mg/kg (n=267) n (%)	Drisapersen 6mg/kg Intermittent (n=38) n (%)	Drisapersen All Regimens (n=285) n (%)
Any SAE	9 (9.5)	44 (16.5)	4 (10.5)	46 (16.1)
Injury, Poisoning And Procedural Complications	3 (3.2)	12 (4.5)	1 (2.6)	12 (4.2)
Blood And Lymphatic System Disorders	0	9 (3.4)	0	9 (3.2)
Cardiac Disorders	0	4 (1.5)	1 (2.6)	5 (1.8)
Gastrointestinal Disorders	1 (1.1)	4 (1.5)	0	4 (1.4)
Musculoskeletal And Connective Tissue Disorders	0	4 (1.5)	0	4 (1.4)
Nervous System Disorders	1 (1.1)	4 (1.5)	0	4 (1.4)
Renal And Urinary Disorders	0	3 (1.1)	1 (2.6)	4 (1.4)
General Disorders And Administration Site Conditions	0	3 (1.1)	0	3 (1.1)
Infections And Infestations	4 (4.2)	3 (1.1)	0	3 (1.1)
Eye Disorders	0	2 (0.7)	0	2 (0.7)
Hepatobiliary Disorders	0	2 (0.7)	0	2 (0.7)
Investigations	0	2 (0.7)	0	2 (0.7)
Metabolism And Nutrition Disorders	0	2 (0.7)	0	2 (0.7)
Ear And Labyrinth Disorders	0	0	1 (2.6)	1 (0.4)
Reproductive System And Breast Disorders	0	1 (0.4)	0	1 (0.4)
Surgical And Medical Procedures	0	1 (0.4)	0	1 (0.4)

Source: Table 5. ISS addendum. Section 5.3.5.3 Sponsor Response submitted to NDA 206031 on 7/24/2015.
Subjects are counted once in each treatment group they were dosed in and once in the 'All regimens' group.

Reviewer comment: I reviewed subject narratives, as well as other documents as necessary, in the assessment of the clinical study SAEs. There were no adverse events of aplastic anemia,

[7] Study 115501 (extension study to DMD114876) (N=21) was not included in the ISS pool of all repeat dose studies, because it was ongoing at the NDA data cut-off date. Data from Study 115501 was subsequently requested and reviewed. There were two SAEs from this study (PTs Appendicitis and Femur fracture). The overall drisapersen safety profile in Study 115501 was similar to other studies in the drisapersen clinical development program. There were no additional Serious adverse events reported during the incremental 120-day safety update period. (P. 52 of the 120-Day Safety Update Report. Submitted to NDA 206031 on 8/24/2015.)

pancytopenia, acute pancreatitis, Stevens Johnson Syndrome, toxic epidermal necrolysis, or drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome reported in the drisapersen clinical development program.

Injury, Poisoning, and Procedural Complications SOC

Twelve of 285 (4.2%) subjects in repeat dose studies had SAEs coded to the Injury, Poisoning, and Procedural Complications SOC (see table below).

Table 6. Serious Adverse Events. Injury, Poisoning, and Procedural Complications SOC. Repeat dose studies. Integrated Summary of Safety analysis.

System Organ Class Preferred Term	Placebo (n = 95)	Drisapersen 6mg/kg (n = 267)	Drisapersen 6mg/kg Intermittent (n = 38)	Drisapersen All Regimens (n = 285)
Injury, Poisoning And Procedural Complications	3 (3.2%)	12 (4.5%)	1 (2.6%)	12 (4.2%)
Femur Fracture	0	6 (2.2%)	1 (2.6%)	7 (2.5%)
Tibia Fracture	0	3 (1.1%)	0	3 (1.1%)
Ankle Fracture	0	1 (0.4%)	0	1 (0.4%)
Fall	0	1 (0.4%)	0	1 (0.4%)
Head Injury	1 (1.1%)	1 (0.4%)	0	1 (0.4%)
Lumbar Vertebral Fracture	0	1 (0.4%)	0	1 (0.4%)
Wound Dehiscence	0	1 (0.4%)	0	1 (0.4%)
Avulsion Fracture	1 (1.1%)	0	0	0
Toxicity To Various Agents	1 (1.1%)	0	0	0

Source: Table 6. ISS addendum. Section 5.3.5.3 Sponsor Response submitted to NDA 206031 on 7/24/2015.

Fractures

In placebo-controlled studies, 12 of 195 (6.2%)[8] drisapersen subjects had a fracture AE, compared to 5 of 95 (5.3%) placebo subjects. In placebo-controlled studies, 4[9] of 195 (2.1%) drisapersen subjects had a fracture SAE, compared to 1[10] of 95 (1.1%) placebo subjects.

In extension studies, an additional 6 drisapersen subjects had a fracture SAE.[11]

[8] Drisapersen fracture count includes 11 cases listed in the table, as well 1 additional fracture described in an SAE narrative (DMD114044 Subject 678; PT Head injury).
Source: Table 11a. P. 4 ISS addendum. Submitted to NDA 206031 on August 20, 2015.
[9] DMD114044 Subjects 184 (femur fracture), 512 (femur fracture, 598 (tibia and lumbar vertebral fracture), and 678 [skull trauma with a linear skull fracture after a fall while playing (PT Head injury)]
[10] DMD114044 Subject 1367 (Avulsion fracture of the right patella)
[11] DMD114673 Subject 103 (femur fracture and tibia fracture); DMD114349 Subject 1302 (femur fracture); DMD114349 Subject 1305 (femur fracture); DMD114349 Subject 1307 (femur fracture); DMD114349 Subject 1367

Reviewer comment: In placebo-controlled studies, the incidence of fractures was similar in drisapersen and placebo groups. These SAEs are likely related to DMD. Fractures are a significant problem in the DMD population. In a retrospective study of 378 DMD patients (median age 12 years, range 1-25 years), 20.9% had experienced at least one fracture. [12] *There are a number of mechanisms involved, including reduced muscle tension on bone, chronic corticosteroid use, and altered calcium and Vitamin D homeostasis.* [13]

Other Injury, Poisoning, and Procedural Complications SOC SAEs

In repeat dose studies, there were 4 other SAEs in this SOC (2 in drisapersen subjects and 2 in placebo subjects):

- DMD114044 Subject 526 (drisapersen 6 mg/kg/week): accidental fall that ruptured recent muscle biopsy sutures (PT Fall) (*Unrelated to drisapersen)*
- DMD114044 Subject 638 (drisapersen 6 mg/kg/week): wound dehiscence of a muscle biopsy site (PT Wound dehiscence) (*Unrelated to drisapersen)*
- DMD114117 Subject 2078 (placebo): head injury while playing (PT Head injury)
- DMD114044 Subject 504 (placebo): drug toxicity from clonazepam (PT Toxicity to various agents)

Blood and lymphatic system disorders SOC

In placebo-controlled studies, there were no SAEs coded to the Blood and lymphatic system disorders SOC. Nine of 285 (3.2%) drisapersen-treated subjects in repeat-dose studies had SAEs in this SOC:

- Eight subjects had SAEs with the PT Thrombocytopenia;[14] Details of these SAEs are included in the evaluation of thrombocytopenia in Section 8.5.1

- DMD114349 subject 516, a 13 year old male from France, had SAEs coded to the PTs Haemolytic anemia and Hepatocellular injury in the setting of a mycoplasma infection.[15] On (b) (6), 957 days after the start of drisapersen treatment and 5 days after the most recent dose, he was hospitalized with asthenia, jaundice, and

(femur fracture and ankle fracture);and DMD114349 Subject 2202 (tibia fracture). Note: Subject 1367 also had an avulsion fracture while randomized to placebo in Study 114044.

[12] McDonald DGM, et al. "Fracture prevalence in Duchenne muscular Dystrophy." *Developmental Medicine & Child Neurology* 2002, 44: 695–698.

[13] Morgenroth VH, et al. "Insights into bone health in Duchenne muscular dystrophy." *BoneKEy Reports* 1, Article number: 9 (2012).

[14] DMD114349 subjects 505, 677, 687, 1122, 1176, 1202, 2000, and 3052.

[15] Narrative on ISS p. 3821; Section 5.3.5.3 of the April 27, 2015 submission to NDA 206031.

gastroenteritis for the preceding 48 hours, with abdominal pain, diarrhea, fever (38°C), mucosal pallor, and dark urine. Hemoglobin was 5.1 g/dL on hospital admission. Other laboratory assessments included ALT 367 U/L and AST 233 U/L. (In Study DMD114044 on August 11, 2011, the subject had an elevated GGT of 169 IU/L with ALT 800 IU/L and AST 453 IU/L; no adverse event was reported related to these laboratory measurements on 8/11/2011.) No GGT or bilirubin measurements from hospital admission were provided by the Sponsor. On (b) (6) GGT was 254 IU/L (normal range 0-65 IU/L). Mycoplasma pneumoniae IgM was positive, according to the hospital report of (b) (6) (no baseline value was available).[16] He was treated with josamycin. The event of hepatocellular injury was considered resolved on (b) (6), and the event of hemolytic anemia was considered resolved on (b) (6). Treatment with drisapersen was discontinued at the start of this SAE and was not resumed, because all dosing in the study was suspended at the time the events resolved.

Reviewer comment: This subject was Coombs test positive, and mycoplasma pneumonia IgM was positive. This is consistent with autoimmune hemolytic anemia related to mycoplasma infection.[17] Hepatic abnormalities can occur with mycoplasma infection. It is unclear whether hepatic toxicity related to drisapersen contributed to his hepatic abnormalities.

Cardiac Disorders SOC

Five of 285 (1.8%) subjects in repeat dose studies had SAEs coded to the Cardiac Disorders SOC (see table below).

Table 7. Serious Adverse Events. Cardiac Disorders SOC. Repeat dose studies. Integrated Summary of Safety analysis.

System Organ Class Preferred Term	Placebo (n = 95)	Drisapersen 6mg/kg (n = 267)	Drisapersen 6mg/kg Intermittent (n = 38)	Drisapersen All Regimens (n = 285)
Cardiac Disorders	0	4 (1.5%)	1 (2.6%)	5 (1.8%)
Cyanosis	0	2 (0.7%)	0	2 (0.7%)
Cardiac Fibrillation	0	1 (0.4%)	0	1 (0.4%)
Myocardial Ischaemia	0	1 (0.4%)	0	1 (0.4%)
Myocarditis	0	0	1 (2.6%)	1 (0.4%)

Source: Table 6. ISS addendum. Section 5.3.5.3 Sponsor Response submitted to NDA 206031 on 7/24/2015.

[16] P. 1373 Study 114349 subject 516 case report form. Submitted to NDA 206031 on 4/27/2015.
[17] *Mycoplasma pneumoniae* and Its Role as a Human Pathogen. Waites KB, et al. Clin. Microbiol. Rev. October 2004 vol. 17 no. 4 697-728.

In placebo-controlled studies, 3 of 195 (1.5%) drisapersen subjects had an SAE in the Cardiac Disorders SOC, compared to 0 of 95 placebo subjects:

- DMD114044 Subject 1111,[18] a 6 year old male from Chile with no previous cardiac medical history and no relevant concomitant medications, had an SAE coded to the PT Myocardial ischemia. He had acute precordial chest pain, and ECG was reported as consistent with subendocardial ischemia. Cardiac enzymes were not performed. Echocardiogram was normal. ECG changes and chest pain resolved on the same day after a period of observation. Drisapersen was withheld for 4 weeks.
  Reviewer comment: This SAE is possibly related to drisapersen. Myocardial ischemia is rare in children. No structural cardiac abnormalities were reported on echocardiogram. There is evidence of increased inflammation with drisapersen, and vascular inflammation is a possible drug-related cause for this SAE. He tested positive for anti-drisapersen antibodies.[19] Inflammatory markers at the time of the event (April 20-25, 2012) were not measured. On May 9, 2012 he had an elevated sensitivity C-reactive protein (hsCRP) level of 7.9 mg/L.[20] (It is unclear to what degree this laboratory value is drug related.) Prior to the SAE, this subject's hsCRP levels were 0.2-0.6 mg/L.

- DMD114117 Subject 2132, a 6 year old boy from Spain, had an SAE coded to the PT Myocarditis that occurred on December 2, 2011, approximately 2 months after starting drisapersen. Serology results for coxsackie virus from December 16, 2011 include: coxsackie virus IgG 395 U/mL (normal range: 80 – 100); and coxsackie virus IgM: 45 U/mL (normal range: 30 – 50).[21] No endomyocardial biopsy was performed. No action was taken with drisapersen in response to this myocarditis event. He had no pericarditis. The event was reported to be resolved with sequelae. (Sequelae were not reported.)[22]
  Reviewer comment: This subject had an SAE of myocarditis in the setting of a positive coxsackie virus serology. Myocarditis is a rare event in children. This event may be an event of viral myocarditis. The diagnosis of myocarditis is dependent in large part on clinical suspicion rather than definitive diagnostic tests.[23] Coxsackie virus is the virus most often associated with myocarditis.[24] An inflammatory drug effect is also possible. The event was reported as resolved despite continued drisapersen treatment.

- DMD114044 Subject 505, a 6 year old male from France, had an SAE coded to the PT Cardiac fibrillation at the end of anesthesia while undergoing elective surgery for transtympanic aerators with sevoflurane as a general anesthetic. He received cardio-

[18] Narrative on ISS p. 3821; Section 5.3.5.3 of the April 27, 2015 submission to NDA 206031.
[19] P. 48 of the STD 2015-012 study report. Link located on p. 72 of the Summary of Clinical Pharmacology.
[20] Subject profile submitted to NDA 206031 on 4/27/2015. No laboratory range of normal values was provided.
[21] Sponsor IR response submitted to NDA 206031 on 10/5/2015.
[22] Sponsor IR response submitted to NDA 206031 on 9/22/2015.
[23] Feldman AM, et al. *N Engl J Med* 2000; 343:1388-1398
[24] Kearny MT, et al. *Postgrad Med J* 2001;77:4-10

respiratory arrest treatment and resuscitation. The event resolved the same day and did not recur. The patient continued treatment with drisapersen for approximately 1 more year.
Reviewer comment: This SAE is unrelated to drisapersen. Cardiac arrhythmias are a known effect of sevoflurane.

Two drisapersen-treated extension study (114349) subjects had SAEs in the Cardiac Disorders SOC. Subjects 597 and 598 both had SAEs coded to the PT Cyanosis.
Reviewer comment: For both Subjects 597 and 598, cyanosis was related to obstructive sleep apnea (related to DMD) and not related to drisapersen.

Gastrointestinal disorders SOC

Four of 285 (1.4%) drisapersen subjects in repeat dose studies had SAEs coded to the Gastrointestinal Disorders SOC (see table below).

Table 8. Serious Adverse Events. Gastrointestinal Disorders SOC. Repeat dose studies. Integrated Summary of Safety analysis.

System Organ Class Preferred Term	Placebo (n = 95)	Drisapersen 6mg/kg (n = 267)	Drisapersen 6mg/kg Intermittent (n = 38)	Drisapersen All Regimens (n = 285)
Gastrointestinal Disorders	1 (1.1%)	4 (1.5%)	0	4 (1.4%)
Diarrhoea	0	1 (0.4%)	0	1 (0.4%)
Enteritis	0	1 (0.4%)	0	1 (0.4%)
Small Intestinal Obstruction	0	1 (0.4%)	0	1 (0.4%)
Vomiting	0	1 (0.4%)	0	1 (0.4%)
Glossitis	1 (1.1%)	0	0	0

Source: Table 6. ISS addendum. Section 5.3.5.3 Sponsor Response submitted to NDA 206031 on 7/24/2015.

In placebo-controlled studies, 2 of 195 (1.0%) drisapersen subjects had an SAE in the Gastrointestinal Disorders SOC, compared to 1 of 95 placebo subjects:[25]

- DMD114044 Subject 1601, a 5 year old male from Turkey, had an SAE coded to the PT Enteritis with nausea and vomiting that started approximately 4 hours after drisapersen dosing. He was hospitalized and given intravenous fluid replacement. The event resolved 7 days later.
  Reviewer comment: This event is possibly related to drisapersen. In placebo-controlled studies, the frequencies of common gastrointestinal and infection-related AEs were generally similar in drisapersen and placebo subjects, except gastroenteritis occurred

[25] Study 114117 placebo subject 2002 had an SAE of glossitis 3 days after the last dose of placebo.

more commonly in drisapersen treated subjects. However, this subject had no gastrointestinal symptoms with other drisapersen doses.

Table 9. On-treatment adverse events (by SOC and preferred term) that occurred in at least 5% of subjects in placebo or drisapersen 6 mg/kg/wk group (placebo-controlled studies)

Adverse event System organ class Preferred term	Placebo N=95 n (%)	Drisapersen 6mg/kg/wk N=161 n (%)
Infections and infestations	**70 (73.7)**	**110 (68.3)**
Nasopharyngitis	30 (31.6)	51 (31.7)
Upper respiratory tract infection	18 (18.9)	20 (12.4)
Gastroenteritis	6 (6.3)	19 (11.8)
Rhinitis	9 (9.5)	15 (9.3)
Influenza	5 (5.3)	10 (6.2)
Pharyngitis	5 (5.3)	6 (3.7)
Ear infection	6 (6.3)	6 (3.7)
Gastrointestinal disorders	**48 (50.5)**	**85 (52.8)**
Vomiting	23 (24.2)	38 (23.6)
Diarrhoea	15 (15.8)	35 (21.7)
Abdominal pain	10 (10.5)	21 (13.0)
Abdominal pain upper	4 (4.2)	12 (7.5)
Constipation	6 (6.3)	10 (6.2)
Nausea	7 (7.4)	8 (5.0)

Source: Sponsor Table 36. Summary of Clinical Safety.

- DMD114117 Subject 3001, a 6 year old male from Australia, had SAEs coded to the PTs Vomiting, Pain in extremity, and Oedema peripheral. After a party in a water park, he had red, swollen and painful calves, vomiting, elevated body temperature of 37.9°C (100.2 °F), and a heart rate of 160 beats per minute (bpm). He received acetaminophen and ondansetron treatment, and all 3 events resolved on the same day.
  Reviewer comment: These SAEs are consistent with heat exhaustion and are unrelated to drisapersen.

Two additional extension study (114349) subjects had SAEs in the Gastrointestinal Disorders SOC:

- Subject 1310, an 8 year old male from Canada, had an SAE coded to the PT Small intestinal obstruction. First drisapersen dose in Study 114044 was August 3, 2011. On Oct. 10, 2011 he had an AE of abdominal pain. He had gastrointestinal AEs intermittently throughout Study 114044 (e.g., abdominal pain, vomiting diarrhea). In Study 114349 (June 10, 2013) he had a partial small bowel obstruction. He underwent endoscopy and colonoscopy under general anesthesia. Biopsies of the duodenum, stomach, distal esophagus, and colon showed non-specific inflammatory changes in the duodenum, possibly drug-related or infectious in nature. No inflammatory changes were noted in the large bowel. Endoscopy revealed evidence of mild esophagitis, gastritis associated with ulceration and erosions, and duodenitis. The gastric erosions were suggested to be secondary to steroid use. There was evidence of moderate patchy colitis in about 1/3 of the colon, suggestive of a diffuse inflammatory or infective process. No evidence of mycoplasmal or mycobacterial infection was noted.

Reviewer comment: This SAE of small bowel obstruction with inflammatory changes is possibly related to drisapersen. There is evidence of increased inflammation with drisapersen in animal studies, as well as in the clinical laboratory data.

- Subject 598, a 17 year old male from Germany who started drisapersen 6 mg/kg/week in March 2011, had SAEs coded to the PTs Diarrhoea and Hypotonia. No history of gastrointestinal symptoms before drisapersen treatment were reported. Twenty eight months after starting drisapersen, he had intermittent, moderate diarrhea for 2 months. Drisapersen was stopped in response to these events. Treatment included loperamide and dimenhydrinate. The events of diarrhea and hypotonia were considered resolved as of 23 August 2013. He did not restart drisapersen, because of proteinuria (8/28/2013 – 9/17/2013). In October 2009, dosing was stopped in all Study DMD114349 subjects.[26] No specific details were provided regarding this subject's hypotonia.
  Reviewer comment: The cause of this subject's diarrhea is unclear. Colonoscopy and gastroscopy, stool cultures, and abdominal ultrasound were negative. No gastrointestinal biopsy results were reported. This case may be consistent with DMD-related chronic intestinal pseudo-obstruction (CIP), which involves fibrosis of the gastrointestinal smooth muscle and can cause episodic gastrointestinal symptoms. Other evidence of DMD-related muscle fibrosis in this subject included cardiac akinesia with MRI suggestive of heart muscle fibrosis in a pattern typical of that seen in DMD (March 2013). In placebo-controlled studies, diarrhea occurred in 22% of drisapersen 6 mg/kg/week subjects, compared to 16% of placebo subjects.[27] A role of drisapersen in this subject's diarrhea is possible.

Nervous System Disorders

Four of 285 (1.4%) subjects in repeat dose studies had SAEs coded to the Nervous System Disorders SOC (see table below).

[26] P. 5 DMD114349 subject 598 subject profile. Submitted to NDA 206031 on 4/27/2015.
[27] Table 36 Summary of Clinical Safety

Table 10. Serious Adverse Events. Nervous System Disorders SOC. Repeat dose studies. Integrated Summary of Safety analysis.

System Organ Class Preferred Term	Placebo (n = 95)	Drisapersen 6mg/kg (n = 267)	Drisapersen 6mg/kg Intermittent (n = 38)	Drisapersen All Regimens (n = 285)
Nervous System Disorders	1 (1.1%)	4 (1.5%)	0	4 (1.4%)
Benign Intracranial Hypertension	0	1 (0.4%)	0	1 (0.4%)
Convulsion	0	1 (0.4%)	0	1 (0.4%)
Hypotonia	0	1 (0.4%)	0	1 (0.4%)
Intracranial Venous Sinus Thrombosis	0	1 (0.4%)	0	1 (0.4%)
Intercostal Neuralgia	1 (1.1%)	0	0	0

Source: Table 6. ISS addendum. Section 5.3.5.3 Sponsor Response submitted to NDA 206031 on 7/24/2015.

In placebo-controlled studies, 2 of 195 (1.0%) drisapersen subjects had an SAE in the Nervous System Disorders SOC, compared to 1 of 95 (1.0%) placebo subjects:

- DMD114044 Subject 1270, a 7 year old male from Brazil had SAEs coded to PTs Intracranial venous sinus thrombosis and Spinal pain. On 12/4/2012, high sensitivity C-reactive protein (hsCRP) was elevated at 9.5 mg/L. On Dec. 11, 2012 (6 months after his first dose of drisapersen), he developed a headache. Over the next few days, he developed seizures, strabismus, and severe thoraco-lumbar pain. A head CT showed hyperattenuating content partially filling the superior sagittal sinus and the straight sinus. Neurological assessment confirmed paralysis of cranial nerve VI (abducens) and signs of thrombosis of venous sinuses. The event of spinal pain was considered resolved on 1 February 2013, and the event of intracranial venous sinus thrombosis was considered resolved with sequelae (paralysis of the VI cranial nerve) on that same date. *Reviewer comment: The cause of this SAE is unclear. Coagulation abnormalities have been reported with DMD.[28] Fibrinogen, aPTT, INR, platelet count, and hemoglobin were normal. Conclusive anti-drisapersen antibody testing was not available.[29] High sensitivity C-reactive protein was elevated 1 week prior to the onset of headache. It is unclear whether drug-related inflammation may have contributed to this event.*

- DMD114044 Subject 576, a 14 year old male from Germany treated with drisapersen 6 mg/kg/week since January 2011, had an SAE coded to the PT Benign intracranial hypertension in July 2015, 2011. After starting drisapersen, he had 6 adverse events of headache (May – July 2011).[30] He had taken deflazacort since 2005. After the diagnosis

[28] Toshio Saito (2014). Coagulation and Fibrinolysis Abnormalities in Patients with Muscular Dystrophy, Fibrinolysis and Thrombolysis, Dr. Krasimir Kolev (Ed.), ISBN: 978-953-51-1265-5, InTech, DOI: 10.5772/57411. Available from: http://www.intechopen.com/books/fibrinolysis-and-thrombolysis/coagulation-and-fibrinolysis-abnormalities-in-patients-with-muscular-dystrophy
[29] P. 48 of the STD 2015-012 study report. Link located on p. 72 of the Summary of Clinical Pharmacology.
[30] Sponsor IR response submitted to NDA 206031 on 9/18/2015.

of intracranial hypertension, he continued drisapersen until the end of Study DMD114349 (last dose January 3, 2014). The event of benign intracranial hypertension ended on April 5, 2013.
Reviewer comment: This SAE may be related to treatment with deflazacort, which he received since 2005. There is a published report of a 9 year old U.S. DMD patient who developed idiopathic intracranial hypertension after starting deflazacort treatment.[31] *However, a contribution of drisapersen to this SAE cannot be ruled out.*

In the extension studies, there were 2 additional drisapersen subjects who had SAEs:

- DMD114673 Subject 105, an 8 year old male from Belgium, who had a seizure (PT Convulsion). On (b) (6), 2 days after his latest dose of drisapersen, he developed a fever of 39.5°C. Four hours later he had a generalized seizure, which lasted 20 minutes. He was hospitalized. Viral swab was positive for H1N1 influenza A. No action was taken with drisapersen treatment.
  Reviewer comment: This subject's seizure is likely related to his fever and H1N1 influenza A infection, which can lead to seizures (with or without fever).[32] *The seizure is consistent with a complex febrile seizure, because of the subject's age and the long seizure duration of 20 minutes. An inflammatory effect (e.g., cerebral vasculitis) of drisapersen contributing to this seizure is possible. Two seizures occurred in drisapersen nonclinical studies.*
- DMD114349 Subject 598, a 17 year old male from Germany, had SAEs coded to the PTs Diarrhoea and Hypotonia.
  Reviewer comment: The reader is referred to the Gastointestinal disorders SOC section of Section 8.4.2 for details of this case.

Musculoskeletal and Connective Tissues Disorders SOC

Four of 285 (1.4%) subjects in repeat dose studies had SAEs coded to the Musculoskeletal and Connective Tissues Disorders SOC (see table below).

[31] Weig SG, et al.. "Idiopathic Intracranial Hypertension in a Child with Duchenne Muscular Dystrophy." Pediatric neurology 45.6 (2011): 406-408.
[32] Pinki,S, et al. "Neurological complications of pandemic influenza A H1N1 2009 infection: European case series and review." European journal of pediatrics 170.8 (2011): 1007-1015.

Table 11. Serious Adverse Events. Musculoskeletal and Connective Tissues Disorders SOC. Repeat dose studies. Integrated Summary of Safety analysis.

System Organ Class Preferred Term	Placebo (n = 95)	Drisapersen 6mg/kg (n = 267)	Drisapersen 6mg/kg Intermittent (n = 38)	Drisapersen All Regimens (n = 285)
Musculoskeletal And Connective Tissue Disorders	0	4 (1.5%)	0	4 (1.4%)
Pain In Extremity	0	1 (0.4%)	0	1 (0.4%)
Scoliosis	0	1 (0.4%)	0	1 (0.4%)
Spinal Pain	0	1 (0.4%)	0	1 (0.4%)
Tendinous Contracture	0	1 (0.4%)	0	1 (0.4%)

Source: Table 6. ISS addendum. Section 5.3.5.3 Sponsor Response submitted to NDA 206031 on 7/24/2015.

In placebo-controlled studies, 2 of 195 (1.0%) drisapersen subjects had an SAE in the Disorders Musculoskeletal and Connective Tissues Disorders SOC, compared to 0 of 95 placebo subjects:

- DMD114117 Subject 3001, a 6 year old male from Australia, had SAEs coded to the PTs Vomiting, Pain in extremity, and Oedema peripheral. (Previously discussed in the Gastrointestinal disorders SOC section.)
  Reviewer comment: These SAEs are consistent with heat exhaustion and are unrelated to drisapersen.

- DMD114044 Subject 1270, a 7 year old male from Brazil had SAEs coded to PTs Intracranial venous sinus thrombosis and Spinal pain.
  Reviewer comment: The reader is referred to the Nervous System Disorders SOC section for discussion of this SAE.

Two extension study (114673) drisapersen subjects had SAEs related to DMD and not related to drisapersen:

- Subject 201 (SAE PT Scoliosis) underwent surgical scoliosis correction.
- Subject 106 (SAE PT Tendinous contracture) underwent tendon retraction release surgery.

Renal and Urinary Disorders SOC

Details of the 4 SAEs coded to the Renal and urinary disorders SOC are discussed in the evaluation of renal toxicity in Section 8.5.2.

General Disorders and Administration Site Conditions SOC

Three of 285 (1.1%) subjects in repeat dose studies had SAEs coded to the General Disorders and Administration Site Conditions SOC (see table below).

Table 12. Serious Adverse Events. General Disorders and Administration Site Conditions SOC. Repeat dose studies. Integrated Summary of Safety analysis.

System Organ Class Preferred Term	Placebo (n = 95)	Drisapersen 6mg/kg (n = 267)	Drisapersen 6mg/kg Intermittent (n = 38)	Drisapersen All Regimens (n = 285)
General Disorders And Administration Site Conditions	0	3 (1.1%)	0	3 (1.1%)
Injection Site Oedema	0	2 (0.7%)	0	2 (0.7%)
Oedema Peripheral	0	1 (0.4%)	0	1 (0.4%)
Pyrexia	0	1 (0.4%)	0	1 (0.4%)

Source: Table 6. ISS addendum. Section 5.3.5.3 Sponsor Response submitted to NDA 206031 on 7/24/2015.

In placebo-controlled studies, there was 1 SAE in this SOC. Study 114117 Subject 3001 had SAEs coded to the PTs Vomiting, Pain in extremity, and Oedema peripheral. (Previously discussed in the Gastrointestinal disorders SOC section.)

In extension study 114349, Subject 125 had SAEs coded to PTs Injection site oedema and Pyrexia, and Subject 511 had an SAE coded to the PT Injection site oedema. These SAEs are discussed in detail in the evaluation of injection site reactions in Section 8.5.3.

8.4.3. Dropouts and/or Discontinuations Due to Adverse Effects

A total of 13 subjects reported AEs that led to permanent treatment discontinuation during the repeat-dose studies, all 13 (4.9%) treated with drisapersen 6 mg/kg/week (see table below). There were no withdrawals in any other treatment groups.

Table 13. Summary of adverse events leading to permanent treatment discontinuation (repeat dose studies)

Adverse event preferred term	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent N=38 n (%)	Drisapersen all regimens N=285 n (%)
Any AE leading to withdrawal	**0**	**0**	**13 (4.9)**	**0**	**13 (4.6)**
Thrombocytopaenia	0	0	8 (3.0)	0	8 (2.9)
Asthenia	0	0	1 (0.4)	0	1 (0.4)
Glomerulonephritis	0	0	1 (0.4)	0	1 (0.4)
Injection site oedema	0	0	1 (0.4)	0	1 (0.4)
Intracranial venous sinus thrombosis	0	0	1 (0.4)	0	1 (0.4)
Proteinuria	0	0	1 (0.4)	0	1 (0.4)
Spinal pain	0	0	1 (0.4)	0	1 (0.4)

Source: Sponsor Table 44

All of the adverse events leading to permanent treatment discontinuation are SAEs discussed elsewhere in this review, except for the AE coded to the PT Asthenia in Study DMD 114349 Subject 520. This subject started treatment in Study 114044 on 5/9/2011. Asthenia was first documented as an AE on 3/28/2012 and was eventually described as severe in August 2012. His

last dose was on 11/14/2012. Additional reasons for discontinuation in this subject were "fear of relapse"…"myalgia, abdominal pain, and lack of efficacy."[33]

I have reviewed the clinical study criteria for stopping treatment.[34] In the opinion of this reviewer, the criteria were appropriate. Additional details will be discussed in the relevant review sections.

8.4.4. Significant Adverse Events

The Sponsor categorized clinical study adverse events by severity (mild, moderate, or severe) in the integrated summary of safety datasets. Most adverse events categorized as severe (and not already included in the serious adverse event assessment) are discussed in Section 8.5 Submission-Specific Safety Issues.

One severe adverse event not discussed elsewhere in this review occurred in Study DMD114876 Subject 203,[35] who had a severe adverse event of a full body rash and hives (PT Urticaria). He started treatment on Aug 1, 2012. He had no prior history of urticaria or medication allergies. He had a severe event of full body rash and hives on Oct. 4, 2012, which was 1 day after drisapersen dosing. No respiratory impairment, cough, wheezing, or angioedema was reported. He was treated with diphenhydramine, and the event was considered resolved on Oct. 9, 2012. He had 2 additional mild episodes of urticaria, which occurred 6 and 165 days after his latest dose of drisapersen. He enrolled in extension study DMD115501 and received 13 doses of drisapersen in April – August 2015 without reported urticaria.
Reviewer comment: This subject had 1 severe episode of urticaria 1 day after drisapersen treatment. He had 2 additional mild episodes of urticaria, which occurred 6 and 165 days after his latest dose of drisapersen. These events are possibly related to drisapersen. It is unclear whether, after the first urticaria event, he received preventive antihistamine medication prior to drisapersen dosing.

8.4.5. Treatment Emergent Adverse Events and Adverse Reactions

Adverse events that occurred in at least 5% of drisapersen subjects in repeat dose studies and more frequently than placebo are summarized in the table below.

[33] Subject 520 narrative. P. 3925 Integrated Summary of Safety.
[34] Summary of Clinical Safety Appendix 8.2.
[35] Narrative submitted to NDA 206031 on 9/21/2015.

Table 14. Summary of adverse events (by system organ class and preferred term) that occurred in at least 5% of drisapersen 6 mg/kg/week subjects (placebo-controlled studies) and greater than placebo

Adverse event System organ class Preferred term	Placebo N=95 n (%)	Drisapersen 6mg/kg/wk N=161 n (%)
Any adverse event	89 (93.7)	158 (98.1)
General disorders and administration site conditions	**44 (46.3)**	**138 (85.7)**
Injection site erythema	8 (8.4)	85 (52.8)
Injection site discolouration	5 (5.3)	56 (34.8)
Pyrexia	22 (23.2)	41 (25.5)
Injection site reaction	2 (2.1)	29 (18.0)
Injection site pain	5 (5.3)	26 (16.1)
Injection site pruritus	1 (1.1)	26 (16.1)
Injection site bruising	9 (9.5)	19 (11.8)
Injection site haematoma	6 (6.3)	14 (8.7)
Injection site induration	1 (1.1)	17 (10.6)
Injection site swelling	0	11 (6.8)
Injection site atrophy	0	9 (5.6)
Injection site urticaria	0	10 (6.2)
Infections and infestations	**70 (73.7)**	**110 (68.3)**
Gastroenteritis	6 (6.3)	19 (11.8)
Influenza	5 (5.3)	10 (6.2)
Gastrointestinal disorders	**48 (50.5)**	**85 (52.8)**
Diarrhoea	15 (15.8)	35 (21.7)
Abdominal pain	10 (10.5)	21 (13.0)
Abdominal pain upper	4 (4.2)	12 (7.5)
Investigations	**28 (29.5)**	**76 (47.2)**
Protein urine present	6 (6.3)	20 (12.4)
Cystatin C increased	4 (4.2)	17 (10.6)
Urine protein/creatinine ratio increased	4 (4.2)	14 (8.7)
Red blood cells urine positive	5 (5.3)	15 (9.3)
Red blood cells urine	5 (5.3)	13 (8.1)
Protein urine	0	8 (5.0)
Injury, poisoning and procedural complications	**47 (49.5)**	**71 (44.1)**
Fall	19 (20.0)	35 (21.7)
Renal and urinary disorders	**26 (27.4)**	**66 (41.0)**
Proteinuria	16 (16.8)	47 (29.2)
Haematuria	5 (5.3)	24 (14.9)
Respiratory, thoracic and mediastinal disorders	**32 (33.7)**	**52 (32.3)**
Epistaxis	6 (6.3)	12 (7.5)
Nasal congestion	3 (3.2)	9 (5.6)
Rhinorrhoea	3 (3.2)	9 (5.6)
Nervous system disorders	**26 (27.4)**	**49 (30.4)**
Headache	20 (21.1)	43 (26.7)
Musculoskeletal and connective tissue disorders	**28 (29.5)**	**38 (23.6)**
Arthralgia	1 (1.1)	11 (6.8)

Source: Sponsor Table 36. Summary of Clinical Safety.

Reviewer comment: I reviewed the adverse events in the drisapersen 3 mg/k/week (N=17) and drisapersen 6 mg/kg/week intermittent (N=38) dose groups. While data is limited because of the small sample sizes in these groups, adverse event frequencies were generally similar in the drisapersen 6 mg/kg/week and 6 mg/kg /week intermittent dose groups. Adverse event frequencies were generally smaller in the drisapersen 3 mg/kg/week group.

Adverse events coded to the SOCs Eye disorders, Psychiatric disorders, Metabolism and nutrition disorders, Ear and labyrinth disorders occurred less frequently in drisapersen subjects, compared to placebo subjects.

Analyses were performed to combine the frequencies of split terms related to renal toxicity.[36] In placebo-controlled studies, proteinuria[37] occurred in 70 of 161 (43.5%) drisapersen 6 mg/kg/week subjects, compared to 22 of 95 (23.2%) placebo subjects. Hematuria[38] occurred in 26 of 161 (16.1%) drisapersen 6 mg/kg/week subjects, compared to 10 of 95 (10.5%) placebo subjects.

Analyses were performed to combine the frequencies of split terms for injection site reactions.[39] In placebo-controlled studies, skin discoloration[40] occurred in 58 of 161 (36.0%) drisapersen 6 mg/kg/week subjects, compared to 7 of 95 (7.4%) placebo subjects. Chronic skin damage[41] occurred in 19 of 161 (11.8%) drisapersen 6 mg/kg/week subjects, compared to 1 of 95 (1.1%) placebo subjects. Ulceration[42] occurred in 5 of 161 (3.7%) drisapersen 6 mg/kg/week subjects, compared to 0 of 95 placebo subjects. Injection site hair growth[43] occurred in 10 of 161 (6.2%) drisapersen 6 mg/kg/week subjects, compared to 0 of 95 placebo subjects.

The following categories of common events are discussed in detail in Section 8.5 Analysis of Submission-Specific Safety Issues, including thrombocytopenia, renal toxicity, injection site reactions, and inflammatory biomarkers.

Arthralgia was reported in 11 of 161 (6.8%) drisapersen 6 mg/kg/week subjects, compared to 1 of 95 (1.1%) placebo subjects.

In repeat dose studies, alopecia was reported in 13 out of 285 (4.6%) drisapersen subjects.

[36] Table 1. ISS addendum submitted to NDA 206031 on 09/25/2015.
[37] Adverse events with PTs Proteinuria, Protein urine present, and Protein urine were combined. Subjects with adverse events coded to more than 1 of the 3 terms and were counted once.
[38] Hematuria-- Subjects had an adverse event coded to at least one of these Preferred Terms: Red blood cells urine positive, or Red blood cells urine
[39] Table 2. ISS addendum submitted to NDA 206031 on 09/25/2015.
[40] Skin discoloration -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Injection site discoloration, Pigmentation disorder, Skin hyperpigmentation, or Skin discoloration.
[41] Chronic skin damage -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Atrophy, Fat tissue decreased, Injection site nodule, Hypertrophy, Plaque, Calcification, Scar, Mass, Acquired lipodystrophy, or Skin fibrosis.
[42] Ulceration -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Injection site vesicles, Application site vesicles, Injection site erosion, Injection site ulcer, or Injection site scab
[43] Hair growth -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Hair growth, Hypertrichosis, or Hirsutism at injection site.

The sponsor proposes to include a table in the label in which AEs for drisapersen occurred in at least 5% of subjects and at least twice the placebo rate.

Reviewer comment: In the opinion of this reviewer, the Sponsor's plan to include a table in the label in which AEs for drisapersen occurred in at least 5% of subjects and at least twice the placebo rate is acceptable. This threshold includes the common adverse events of clinical importance.

8.4.6. **Laboratory Findings**

Hematology

Mean baseline values and mean changes at Weeks 24 and 48 in placebo-controlled studies are summarized in the table below. Decreases in hemoglobin, hematocrit, leukocytes, neutrophils, erythrocytes and reticulocytes were greater with drisapersen than with placebo; however, the size of these decreases was small.

At 48 weeks, the mean change in platelet count was -67.1 x 10^9/L in drisapersen 6 mg/kg/week subjects, compared to -8.7 x 10^9/L in placebo subjects.

Reviewer comment: No cases of immune thrombocytopenia were diagnosed in placebo-controlled studies. No post-treatment platelet levels < 75 x 10^9/L occurred in placebo-controlled studies. *The decreases in platelet count seen in placebo-controlled studies may have occurred via a different mechanism, which is unclear at this time.*

One subject, treated with drisapersen 6 mg/kg/week intermittent had a shift from Grade 1 to Grade 2 for decreased hemoglobin[44] at Weeks 12 and 24. No other shifts to Grade 2, 3 or 4 were observed for hemoglobin. One subject, treated with drisapersen 3 mg/kg/week had a decrease in leukocytes[45] from normal to Grade 2 at Week 24. No other shifts to Grade 2, 3 or 4 were observed for leukocytes. The percentages of subjects with shifts in lymphocytes and neutrophils were similar for placebo and drisapersen.

[44] Reported as anaemia in ISS Table 11.81.
Hemoglobin CTCAE Grade Ranges in G/L Units: 0: ≥LLN; 1: ≥100 - <LLN; 2: ≥ 80 - <100; 3: ≥65 - <80; 4: <65.
[45] Leukocytes CTCAE Grade Ranges in x 10^9/L Units: 0: ≥LLN; 1: ≥3.0 - <LLN; 2: ≥ 2.0 - < 3.0; 3: ≥ 2.0 - < 1.0; 4: <1.0.

Table 15. Summary of hematology parameters: Baseline mean (SD) and mean (SD) changes from baseline at Weeks 24 and 48 (placebo-controlled studies)

	Placebo N=95		Drisapersen 3 mg/kg/wk N=17		Drisapersen 6 mg/kg/wk N=161		Drisapersen 6 mg/kg intermittent [a] N=17		Drisapersen all regimens N=195	
Haemoglobin, g/L										
Baseline mean (SD)	n=95	136.8 (11.48)	n=17	138.9 (11.77)	n=161	137.5 (9.34)	n=17	134.5 (12.06)	n=195	137.4 (9.82)
Week 24: Mean (SD) change	n=93	-1.0 (7.57)	n=15	-7.3 (7.02)	n=157	-3.8 (7.75)	n=16	-6.9 (7.84)	n=188	-4.3 (7.77)
Week 48: Mean (SD) change	n=76	0.4 (7.72)	NA	NA	n=137	-5.2 (7.67)	n=14	-3.4 (12.08)	n=151	-5.0 (8.22)
Haematocrit, ratio										
Baseline mean (SD)	n=95	0.4121 (0.03533)	n=17	0.4161 (0.03897)	n=161	0.4144 (0.02988)	n=17	0.4062 (0.03441)	n=195	0.4139 (0.03107)
Week 24: Mean (SD) change	n=93	-0.0037 (0.02554)	n=15	-0.0220 (0.02802)	n=157	-0.0161 (0.02604)	n=16	-0.0234 (0.02404)	n=188	-0.0172 (0.02602)
Week 48: Mean (SD) change	n=76	0.0029 (0.02405)	NA	NA	137	-0.0187 (0.02656)	n=14	-0.0186 (0.03587)	n=151	-0.0186 (0.02741)
Lymphocyte count, GI/L										
Baseline mean (SD)	n=95	2.666 (1.3183)	n=17	2.257 (1.0164)	n=161	2.888 (1.2599)	n=17	2.434 (1.1758)	n=195	2.793 (1.2460)
Week 24: Mean (SD) change	n=92	0.077 (1.4650)	n=15	0.282 (1.2126)	n=156	-0.507 (1.2663)	n=15	-0.421 (1.5440)	n=186	-0.437 (1.2966)
Week 48: Mean (SD) change	n=75	0.009 (1.13169)	NA	NA	n=136	-0.552 (1.1630)	n=14	-0.215 (1.0104)	n=150	-0.520 (1.1507)
Neutrophil count, GI/L										
Baseline mean (SD)	n=95	5.580 (2.8535)	n=17	6.620 (2.4867)	n=161	5.459 (2.6390)	n=17	5.154 (2.3110)	n=195	5.534 (2.6105)
Week 24: Mean (SD) change	n=92	-0.513 (3.1363)	n=15	-1.966 (2.6005)	n=156	-1.149 (2.5595)	n=15	-1.099 (2.3155)	n=186	-1.211 (2.5410)
Week 48: Mean (SD) change	n=75	-0.614 (2.8493)	NA	NA	n=136	-1.314 (2.5129)	n=14	-1.300 (3.2791)	n=150	-1.313 (2.5806)
Thrombocyte count, GI/L										
Baseline mean (SD)	n=95	306.4 (64.78)	n=17	311.5 (64.52)	n=161	309.5 (68.75)	n=17	277.5 (95.28)	n=195	306.9 (71.23)
Week 24: Mean (SD) change	n=92	-1.3 (49.62)	n=16	-44.8 (44.94)	n=157	-49.8 (52.89)	n=17	-27.8 (115.75)	n=190	-47.4 (60.36)
Week 48: Mean (SD) change	n=73	-8.7 (37.61)	NA	NA	n=136	-67.1 (54.58)	n=14	-20.0 (100.22)	n=150	-62.7 (61.35)
Erythrocyte count, TI/L										
Baseline mean (SD)	n=95	4.78 (0.389)	n=17	4.72 (0.388)	n=161	4.76 (0.338)	n=17	4.71 (0.364)	n=195	4.75 (0.343)
Week 24: Mean (SD) change	n=93	0.00 (0.291)	n=15	-0.21 (0.260)	n=157	-0.16 (0.279)	n=16	-0.22 (0.254)	n=188	-0.17 (0.275)
Week 48: Mean (SD) change	n=76	0.05 (0.284)	NA	NA	n=137	-0.27 (0.290)	n=14	-0.26 (0.388)	n=151	-0.27 (0.299)
Reticulocyte count, TI/L										
Baseline mean (SD)	n=95	0.06821 (0.032167)	n=17	0.08152 (0.033182)	n=161	0.06667 (0.029584)	n=17	0.06632 (0.33939)	n=195	0.06793 (0.030420)
Week 24: Mean (SD) change	n=92	-0.00508 (0.028806)	n=15	-0.01903 (0.026295)	n=156	-0.01299 (0.028599)	n=16	-0.01431 (0.032650)	n=187	-0.01359 (0.028677)
Week 48: Mean (SD) change	n=76	-0.00742 (0.026653)	NA	NA	n=137	-0.016667 (0.025619)	n=14	-0.01773 (0.034990)	n=151	-0.01677 (0.026481)
Leukocyte count, GI/L										
Baseline mean (SD)	n=95	8.69 (2.782)	n=17	9.31 (2.252)	n=161	8.82 (2.663)	n=17	8.06 (2.334)	n=195	8.80 (2.606)
Week 24: Mean (SD) change	n=92	-0.39 (3.157)	n=15	-1.69 (2.803)	n=156	-1.61 (2.700)	n=15	-1.55 (2.665)	n=186	-1.61 (2.691)
Week 48: Mean (SD) change	n=75	-0.59 (2.647)	NA	NA	n=136	-1.83 (2.613)	n=14	-1.42 (3.435)	n=150	-1.79 (2.689)

Clinical chemistry laboratory results

In placebo-controlled studies, data were available for the following electrolytes: sodium, phosphate, calcium, and potassium. Mean changes in these electrolytes were similar in drisapersen and placebo subjects.

Reviewer comment: No serum bicarbonate measurements were available for analysis. This laboratory parameter is of interest, because of drisapersen accumulation in the proximal tubule and the renal toxicity associated with drisapersen. There were no blood acid-base disorders reported as adverse events in the clinical development program.

In placebo-controlled studies, there were no shifts from CTCAE[46] Grade 0 or Grade 1 at baseline to Grade 2, 3 or 4 for hyponatraemia,[47] hyperkalaemia,[48] hypophosphataemia,[49] hypocalcemia,[50] and hypercalcaemia.[51]

Similar frequencies of hypernatremia, categorized by CTCAE grade, occurred in drisapersen and placebo subjects (see table below).

Table 16. Hypernatremia. Shifts from baseline to worst post-treatment value in hypernatremia by CTCAE grade. Placebo-controlled studies.

	Baseline CTCAE Grade					
Laboratory Test Name / Treatment Group / Worst Post-Treatment CTCAE Grade	0	1	2	3	4	Total
Hypernatremia (sodium)						
Placebo (n=95)						
0	83 (87.4%)	0	0	0	0	83
1	9 (9.5%)	0	0	0	0	9
2	1 (1.1%)	0	0	0	0	1
3	2 (2.1%)	0	0	0	0	2
4	0	0	0	0	0	0
Total	95	0	0	0	0	95
6 mg/kg/week Drisapersen (n=162)						
0	135 (83.3%)	0	0	0	0	135
1	12 (7.4%)	1 (0.6%)	0	0	0	13
2	8 (4.9%)	1 (0.6%)	1 (0.6%)	0	0	10
3	3 (1.9%)	0	0	0	0	3
4	1 (0.6%)	0	0	0	0	1
Total	159	2	1	0	0	162

Source: Sponsor Table 10.1. ISS addendum submitted to NDA 206031 on July 20, 2015.

Reviewer comment: The etiology of hypernatremia cases was not discussed in the NDA submission. Hypernatremia can occur with DMD-associated exertional rhabdomyolysis.[52]

[46] Common Terminology Criteria for Adverse Events (CTCAE) v4.0. NIH publication # 09-7473. May 29, 2009.
[47] Hyponatremia CTCAE Grade Ranges in mmol/L Units: 0: ≥LLN; 1: ≥130 - <LLN; 2:N/A; 3: ≥120 - <130; 4: <120.
[48] Hyperkalemia CTCAE Grade Ranges in mmol/L Units: 0: <ULN; 1: >ULN - 5.5; 2: >5.5 – 6.0; 3: > 6.0 – 7.0; 4:> 7.0.
[49] Hypophosphatemia CTCAE Grade Ranges in mmol/L Units: 0: ≥LLN; 1: ≥0.8 - <LLN; 2:≥0.6 – <0.8; 3: ≥0.3 - <0.6; 4: <0.3.
[50] Hypocalcemia CTCAE Grade Ranges in mmol/L Units: 0: ≥LLN; 1: ≥2.0 - <LLN; 2:≥1.75 – <2.0; 3: ≥1.5 - <1.75; 4: <1.5.
[51] Hypercalcemia CTCAE Grade Ranges in mmol/L Units: 0: <ULN; 1: >ULN – 2.9; 2: >2.9 – 3.1; 3: > 3.1 – 3.4; 4:> 3.4.

Laboratory findings related to the following adverse events of special interest are discussed in Section 8.5:

- Renal toxicity
- Thrombocytopenia
- Hepatic toxicity
- Coagulation abnormalities
- Inflammation

Reviewer comment: Apart from results associated with drisapersen adverse events of special interest (listed above), laboratory results were generally similar for drisapersen and placebo subjects.

8.4.7. **Vital Signs**

In placebo-controlled studies, categorical analyses of changes in vital signs from baseline were calculated at Weeks 12, 24, 36, and 48.[53] Results were similar for drisapersen and placebo subjects at each time point. The table below displays changes in vital signs from baseline to Week 48. Analyses of baseline vital signs data and changes from baseline at Weeks 12, 24, 36, and 48 were also similar for drisapersen and placebo subjects.

[52] Figarella-Branger, D., et al. "Exertional rhabdomyolysis and exercise intolerance revealing dystrophinopathies." *Acta neuropathologica* 94.1 (1997): 48-53.

[53] ISS Table 11.84

Table 17. Frequency of categorical changes in vital signs from baseline to Week 48. Placebo-controlled studies.

Study Visit Vital Signs, unit Changes Compared to Baseline	Placebo (N=95)	3 mg/kg/week Drisapersen (N=17)	6 mg/kg/week Drisapersen (N=161)	6 mg/kg Drisapersen Intermittent - 9 doses in 10 week cycle (N=17)	Any Drisapersen (N=195)
Week 48					
Systolic Blood Pressure (SBP), mm Hg					
n	78	N/A	137	15	152
SBP increment > 20 mm Hg	5 (6.4%)		7 (5.1%)	1 (6.7%)	8 (5.3%)
SBP increment > 40 mm Hg	0		1 (0.7%)	0	1 (0.7%)
SBP decrement > 20 mm Hg	2 (2.6%)		6 (4.4%)	1 (6.7%)	7 (4.6%)
SBP decrement > 40 mm Hg	0		0	0	0
Diastolic Blood Pressure (DBP), mm Hg					
n	78	N/A	137	15	152
DBP increment > 10 mm Hg	11 (14.1%)		18 (13.1%)	3 (20.0%)	21 (13.8%)
DBP increment > 20 mm Hg	4 (5.1%)		5 (3.6%)	0	5 (3.3%)
DBP decrement > 10 mm Hg	9 (11.5%)		21 (15.3%)	1 (6.7%)	22 (14.5%)
DBP decrement > 20 mm Hg	1 (1.3%)		3 (2.2%)	0	3 (2.0%)
Heart Rate (HR), bpm					
n	78	N/A	137	15	152
HR increment > 15 bpm	12 (15.4%)		19 (13.9%)	1 (6.7%)	20 (13.2%)
HR increment > 30 bpm	3 (3.8%)		5 (3.6%)	0	5 (3.3%)
HR decrement > 15 bpm	12 (15.4%)		13 (9.5%)	3 (20.0%)	16 (10.5%)
HR decrement > 30 bpm	2 (2.6%)		1 (0.7%)	1 (6.7%)	2 (1.3%)
Temperature, °C					
n	60	N/A	119	N/A	119
Temperature > 38.0 C	0		0		0

Source: ISS Table 11.84

8.4.8. **Electrocardiograms (ECGs)**

Characteristics of ECG testing in the drisapersen clinical development program are summarized in the table below.

Table 18. Characteristics of ECG testing in the drisapersen clinical development program

Study	Number of subjects with at least 1 post-treatment ECG	Schedule of ECG Assessment (baseline and post-treatment)	Number of scheduled ECG assessments	ECG readers blinded to treatment assignment (Y/N/NA)	ECG reading method (e.g., cardiologist, automatic reading)
DMD114044	186	Week 0(baseline), 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48	13	Y	Automatic reader was used unless values were reported as 'abnormal' at which point the ECG was read by a cardiologist or a person trained in assessments of ECGs
DMD114117	53	Week 1(baseline), 3, 5, 7, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49	15	Y	Same
DMD114876	51	Week 0(baseline), 4, 8, 12, 24	5	Y	Same
PRO-051-02	12	Week 1 (baseline), 5, 18	3	N	Same
DMD114673	12	Treatment beyond core study period: Every 4 weeks for the first 85 weeks, and then, every cycle consisting of 4 weeks, 5 weeks, and 3 weeks until week 193, and Week 191, 194, 196, 197, 199, 200, 202, 203, 205, 209, 214, 217.		N	Same
DMD114349	233	Week 0 (baseline), 24, 48, 72, 104	5	N	Same

Source: P. 6 Sponsor submission to NDA206031 on 9/25/2015

The table below summarizes QT values corrected according to Bazett's formula[54] (QTcB) in placebo-controlled studies. Thirty of 161 (18.6%) of drisapersen 6 mg/kg/week subjects had a maximum change in QTcB from baseline >30 to ≤ 60 milliseconds (msec), compared to 16 of 95 (16.8%) placebo subjects. Eighteen of 161 (11.2%) of drisapersen 6 mg/kg/week subjects had a maximum change in QTcB from baseline >60 msec, compared to 9 of 95 (9.5%) placebo subjects. An automatic reader was used, unless values were reported as 'abnormal,' at which point the ECG was read by a cardiologist or a person trained in assessments of ECGs.

[54] Post-treatment changes in heart rate from baseline were similar in drisapersen subjects, compared to placebo subjects.

Table 19. Summary of graded QTcB and change in QTcB (placebo-controlled studies)

Timepoint	Range (msec)	Placebo N=95 n (%)	Drisapersen 6 mg/kg/wk N=161 n (%)
QTcB value			
Baseline	n	95 (100)	159 (98.8)
	≤450	93 (97.9)	155 (96.3)
	>450 and ≤480	2 (2.1)	2 (1.2)
	>480 and ≤500	0	2 (1.2)
	>500	0	0
Maximum on treatment	n	95 (100)	161 (100)
	≤450	77 (81.1)	124 (77.0)
	>450 and ≤480	15 (15.8)	34 (21.1)
	>480 and ≤500	3 (3.2)	3 (1.9)
	>500	0	0
Increase in QTcB from baseline			
Maximum on treatment	n	80 (84.2)	143 (88.8)
	0 to ≤30	55 (57.9)	95 (59.0)
	>30 to ≤60	16 (16.8)	30 (18.6)
	>60	9 (9.5)	18 (11.2)

Source: Summary of Clinical Safety Table 91

The table below summarizes QT values corrected according to Friedericia's formula (QTcF) in placebo-controlled studies. Thirty one of 161 (19.3%) of drisapersen 6 mg/kg/week subjects had a maximum change in QTcB from baseline >30 to ≤ 60 milliseconds (msec), compared to 13 of 95 (13.7%) placebo subjects. Fourteen of 161 (8.7%) of drisapersen 6 mg/kg/week subjects had a maximum change in QTcB from baseline >60 msec, compared to 7 of 95 (7.4%) placebo subjects.

Table 20. Summary of change in QTcF (placebo-controlled studies)

	Range (msec)	Placebo (N=95) n (%)	6 mg/kg/wk (N=161) n (%)
Maximum on-treatment value [1]	n	82 (86.3)	144 (89.4)
	0 - <=30	62 (65.3)	99 (61.5)
	>30 and <=60	13 (13.7)	31 (19.3)
	>60	7 (7.4)	14 (8.7)

Source: ISS Table 11.68

In Study DMD114876, 12-lead Holter monitoring was performed on all subjects, and Holter ECGs were read by a central cardiologist blinded to study treatment. Evaluation of QTcB showed that mean changes were small for all treatment groups (drisapersen 3 mg/kg/week, drisapersen 6 mg/kg/week, and placebo), and there was no clear dose response relationship and no clear difference from placebo. Outlier analyses at Week 24 showed no subjects with QTcB >480 msec in any group and no subjects with QTcB >450 msec in the drisapersen 6 mg/kg/week group. There were no changes from baseline of >60 msec and one change of 30 to 60 msec in the group receiving drisapersen 6 mg/kg/week (see table below).

Table 21. Summary of Outlier Analysis of Holter ECG Data from Study DMD114876

	Number (%) of Subjects		
	Placebo (combined) (N=16)	Drisapersen 3mg/kg/week (N=17)	Drisapersen 6mg/kg/week (N=18)
n [a]	15	15	18
Any event	**4 (27)**	**3 (20)**	**4 (22)**
Heart rate <50 bpm and ≥25% decrease from baseline	0	0	0
Heart rate >100 bpm and ≥25% increase from baseline	3 (20)	2 (13)	3 (17)
PR interval >200 msec and ≥25% increase from baseline	0	0	0
QRS interval >100 msec and ≥25% increase from baseline	0	0	0
QT interval >500 msec and baseline QT interval ≤500 msec	0	0	0
QTcF interval >500 msec and baseline QTcF interval ≤500 msec	0	0	0
QTcF interval >480 msec and baseline QTcF interval ≤480 msec	0	0	0
QTcF interval >450 msec and baseline QTcF interval ≤450 msec	0	0	0
QTcF interval change from baseline 30-60 msec	0	1 (7)	1 (6)
QTcF interval change from baseline >60 msec	0	0	0
QTcB interval >500 msec and baseline QTcB interval ≤500 msec	0	0	0
QTcB interval >480 msec and baseline QTcB interval ≤480 msec	0	0	0
QTcB interval >450 msec and baseline QTcB interval ≤450 msec	1 (7)	1 (7)	0
QTcB interval change from baseline 30-60 msec	0	0	1 (6)
QTcB interval change from baseline >60 msec	0	0	0

Source: Table 53 Study DMD114876 Clinical Study Report
a. Subjects with a baseline assessment and at least one on-treatment assessment.
For Heart Rate, baseline assessments were those within a 30 minute time period from, and closest in time to the corresponding Week 23 assessments (regardless of heart rate).
For PR, QRS, QT, QTcF and QTcB, baseline assessments were those within a 30 minute time period from, and with the closest heart rate to, and within 10 BPM of the corresponding Week 23 assessments.

There were no reports of torsade de pointes or ventricular tachycardia in the drisapersen clinical development program. One case of cardiac fibrillation (DMD114044 Subject 505) occurred while undergoing elective surgery for transtympanic aerators with sevoflurane as a general anesthetic; no QT prolongation was reported in this case. DMD114673 Subject 105 had a seizure in the setting of H1N1 influenza A infection and fever; no QT prolongation was reported in this case.

8.4.9. QT

No thorough QT study has been performed with drisapersen.

Reviewer's comment: In ECG measurements (read by an automatic reader unless reported as 'abnormal'), increases in QTcB and QTcF from baseline were more frequent in drisapersen subjects, compared to placebo subjects. However, in Study DMD114876 QTcB readings from Holter ECGs (read by a central cardiologist blinded to treatment) showed no clear dose response relationship and no clear difference from placebo. In nonclinical studies, drisapersen did not affect the potassium outward current in Chinese hamster ovary (CHO) cells transfected with

hERG cDNA.[55] This is consistent with the lack of alteration of ion channel function seen with other members of the phosphorothioate oligonucleotide class.[56] Given that oligonucleotides are polyanionic molecules of large molecular weight, alteration of ion channel function is less likely. At this time, there is no nonclinical or strong clinical evidence of an adverse effect on the QT interval. The Division of Cardiovascular and Renal Products will be consulted.

8.4.10. **Immunogenicity**

Plasma samples obtained for pharmacokinetic measurements in clinical study DMD114044 were analyzed for anti-drug antibody (ADA) presence (titled Study 2015-012). A total of 109 subjects treated with drisapersen and 50 subjects who received placebo could be conclusively analyzed.

Reviewer comment: Subjects with no week 47/48 plasma sample available for ADA testing were categorized as Anti-drug antibody (ADA) inconclusive (14 drisapersen subjects and 8 placebo subjects). Subjects 527 (SAE Glomerulonephritis) and 1270 (SAE Intracranial venous sinus thrombosis) discontinued Study 114044 early and had inconclusive ADA results (i.e., no positive ADA test and no testing at Week 47/48).[57]

In 29.4% (32 out of 109 subjects) of the treated evaluable subjects, ADAs were detected.[58] Overall, median titers increased with prolonged treatment. Median titers ranging from 50–300 at Weeks 8 to 24, and were 1000 and 800 at Weeks 36 and 48, respectively.[59] All placebo samples were negative, except for one subject who was considered a likely false positive.[60]

Reviewer comment: Overall, median titers increased with longer durations of exposure. There may be an increasing risk of adverse events related to immunogenicity with longer exposure to

[55] P. 12 Nonclinical Overview. Submitted to NDA 206031 on 10/10/2014.
[56] Henry SP, et al. "Toxicologic properties of 2′ O-methoxyethyl chimeric antisense inhibitors in animals and man." Antisense drug technology: principles, strategies and applications. CRC, Boca Raton, FL (2007): 327-364.
[57] According to the Sponsor (submitted 9/21/2015): "There were no positive anti-drisapersen antibody test results for DMD114044 Subject 527 or Subject 1270. Testing was performed at Week 24 for Subject 527 and at Week 8, 12, and 24 for Subject 1270. The SAE Glomerulonephritis for Subject 527 occurred 29 weeks after start of the treatment and SAE Intracranial venous sinus thrombosis for Subject 1270 occurred 25 weeks after start of treatment."
[58] Section 4.1.1. Summary of Clinical Pharmacology. Submitted to NDA 206031 on 4/27/2015.
[59] Sponsor Table 12. P. 40 Summary of Clinical Pharmacology.
[60] For one placebo subject the first sample obtained was positive (titer of 200) at Week 0, whereas all subsequent samples from this subject were negative, indicating a likely false positive.

drisapersen. Immunogenicity data from extension study DMD114349 have been collected, but Sponsor analyses were ongoing at the time of this review.[61]

Study 2015-012 did not demonstrate a difference between ADA positive and ADA negative subjects with regard to demographics or rates of SAEs, adverse events of special interest (AESIs) and AEs that occurred in at least 5% of subjects) and laboratory parameters (thrombocyte count, high sensitivity C-reactive protein (hsCRP), urine protein excretion, serum cystatin C, urine cystatin C, alanine transferase (ALT), glutamate dehydrogenase (GLDH) and total bilirubin) and muscle distribution.

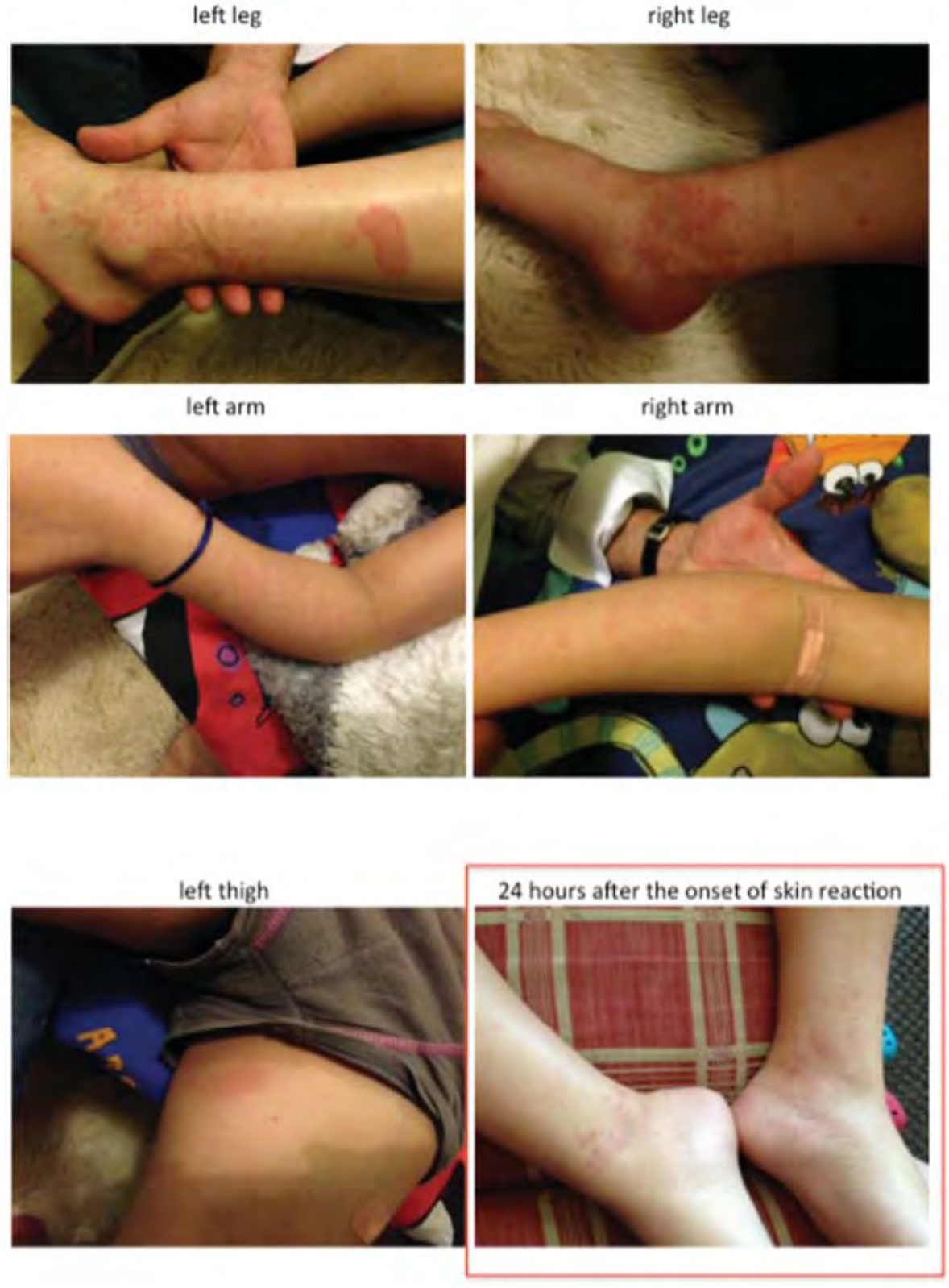
Reviewer comment: Study 2015-012 did not have an adequate number of subjects to make conclusions regarding rare adverse events and serious adverse events. The study analysis included 3 SAEs in drisapersen 6 mg/kg/week subjects and 4 in placebo subjects.

Study DMD114349 subject 2026, a 6 year old male from France, had an adverse event coded to PT Henoch-Schonlein purpura with concomitant positive anti-drisapersen IgG antibodies.[62] He started treatment with 6 mg/kg/week drisapersen in Study DMD114349 on November 2, 2011. Starting on October 2, 2012 (Week 48), the subject tested positive for anti-drisapersen IgG; an additional positive test for IgG occurred on 22 January 2013 (Week 64).

Starting in April 2013, he had multiple episodes of fatigue, weakness, feeling cold, and pallor approximately 1 hour after drisapersen dosing. These episodes resolved spontaneously after about 1 hour. On June 11, 2013, he had a reaction similar to his previous post-administration symptoms. He had an elevated pulse (112 bpm) and normal blood pressure and temperature. He complained of pain in the left thigh, above the injection site. Six hours after drisapersen administration, he developed a skin reaction on his legs, thighs, arms, and abdomen. Photographs of the rash 6 hours (the first 5 pictures) and 24 hours (the 6th picture) after the June 11, 2013 injection are provided below.

[61] Sponsor response submitted to NDA 206031 on 9/21/2015.
[62] ISS addendum. Submitted to NDA 206031 on 8/29/2015.

Figure 2. DMD114349 Subject 2026. AE PT Henoch-Schonlein purpura. Rash 6 and 24 hours after June 11, 2013 Injection



On June 16, 2013 he saw his pediatrician, who reported a Henoch-Schönlein-like purpural rash on the lower limbs, along with mild pharyngitis and conjunctivitis. No events of abdominal pain, arthritis, or arthralgia were reported with the rash. His next dose, on (b) (6), was administered during an overnight hospitalization, because his post-administration symptoms had become worse. He developed a rash on his lower extremities, 3 days after dosing, which he had (b) (6). He received no additional drisapersen doses. According to the Sponsor, all dosing in Study DMD114349 was put on hold in September 2013.

Laboratory results during the study were as follows:

Table 22. DMD114349 Subject 2026. Laboratory results.

Date	Complement C3	C-reactive protein	Platelets	Cystatin C	Creatinine	Urine Protein	Urine RBC
	0.9-1.8 g/L	mg/L	130-400 GI/L	0.6-0.8 mg/L	33.6-64.5 µmol/L	mg/L	
2 November 2011 (Baseline)	1.41	0.3	371	0.6	14.7	83	None
20 March 2012	1.29	0.3	416	0.6	NR	99	None
2 October 2012	1.3	0.5	317	0.8	21.5	122	None
19 March 2013	1.44	0.6	338	0.9	20.4	52	None
14 May 2013	0.97	1.1	352	0.9	16.8	208	5-10
9 July 2013	0.93	1.2	231	0.9	18.5	51	None
3 September 2013	0.83	2.2	277	0.8	20.2	119	None
29 October 2013	1.21	0.4	293	0.8	17.5	50	None

Source: ISS addendum. Submitted to NDA 206031 on 8/29/2015.

No biopsy data related to this AE is available.

Reviewer comment: This AE coded to the PT Henoch-Schonlein purpura, an antibody-related disease, is related to drisapersen. This subject had concurrent positive anti-drisapersen IgG antibodies. No biopsy data are available.

Oligonucleotides are designed to be structurally related to nucleic acids, especially DNA. Thus, antibodies to an oligonucleotide can cross-react with endogenous DNA, especially circulating DNA. DNA is present in the blood of normal individuals, and levels can rise in disease states characterized by inflammation or cell injury and death, such as DMD. One potential mechanism of pathogenesis with anti-oligonucleotide antibodies, such as anti-drisapersen antibodies, is the

formation of immune complexes which can deposit into tissues such as the kidney or blood vessels.[63]

Reviewer conclusion: Immunogenicity occurred in 29.4% of drisapersen subjects with evaluable plasma samples. The currently available information is insufficient to describe the effects of anti-drisapersen antibodies, especially in regards to rare adverse events and serious adverse events.

8.5. Analysis of Submission-Specific Safety Issues

8.5.1. Thrombocytopenia

In placebo-controlled studies, mild thrombocytopenia occurred more frequently in drisapersen subjects, compared to placebo subjects. In placebo-controlled studies, 16 of 161 (9.9%) drisapersen 6 mg/kg/week subjects had a platelet count below the lower limit of normal and ≥ 75 x 10^9/L (the level below which primary hemostasis is generally considered to be impaired), compared to 3 of 95 (3.2%) placebo subjects. No subjects (in any treatment group) had treatment-emergent platelet levels < 75 x 10^9/L in placebo-controlled studies.[64]

While only mild thrombocytopenia occurred in placebo-controlled studies, serious thrombocytopenia occurred with drisapersen in the extension studies. In extension studies, eight of 267 (3.0%) drisapersen 6 mg/kg/week subjects had a thrombocytopenia serious adverse event (see table below).

Table 23. Summary of on-treatment thrombocytopenia events (repeat dose studies)

Adverse event	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent[a] N=38 n (%)	Drisapersen all regimens[b] N=285 n (%)
Any thrombocytopaenia event	0	0	19 (7.1)	2 (5.3)	20 (7.0)
Any thrombocytopaenia SAE	0	0	8 (3.0)	0	8 (2.8)
Any severe thrombocytopaenia event	0	0	7 (2.6)	0	7 (2.5)

Source: Sponsor Table 71. Summary of Clinical Safety p. 145.
[a] Intermittent includes subjects dosed with 9 doses in a 10-week cycle
[b] Subjects treated with more than one drisapersen regimen are only counted once in this group.
Table includes studies DMD114117, DMD114044, DMD114876, DMD114349, PRO051-02, and DMD114673.

Six subjects had thrombocytopenia <20 x 10^9/L. Drisapersen subjects had symptoms of bleeding that included hematemesis, epistaxis, petechiae, and gingival bleeding. Patients with

[63] Wang, Jian, et al. "Oligonucleotide-Based Drug Development Considerations for Clinical Pharmacology and Immunogenicity." Therapeutic Innovation & Regulatory Science (2015).
[64] P. 80 ISS addendum submitted to NDA 206031 on July 20, 2015.

thrombocytopenia <20 x 10^9/L are at risk for potentially fatal complications, including spontaneous intracranial and intrapulmonary hemorrhage.[65]

In 5 out of 8 patients with thrombocytopenia SAEs, the presence of anti-platelet antibodies was confirmed; this is consistent with immune thrombocytopenia. The table below summarizes the anti-platelet antibody data in drisapersen subjects.

Table 24. Anti-platelet antibody data in drisapersen subjects

Subject ID	Anti-platelet antibodies (as reported)
000505	Antibodies against IIb and IIIa glycoproteins positive. Not positive against glycoprotein complex Ia, IIa, Ib and IX. No positive antibodies in serum against the different part of the glycoprotein complex.
000677	Platelet antibody test result: direct anti-GPIIb/IIa slightly positive: 1.2 (normal value less than 1.0); anti-GBIb/IX direct and indirect both negative. Anti-platelet antibodies which showed an increase: direct method (antibody anti-GPIIb/IIIa 5.7, anti-GP1a/IIa 2.8, anti-GPIb/IX 2.2). Indirect antibodies persistently negative.
001025	Slightly positive
001122	Blood circulating anti-platelet antibody result: positive
001176	Marked decrease in number of anti-platelet antibodies: negative.
001202	Subject's anti-platelet antibody (not further specified): positive
002000	Anti-platelet antibodies were reported as positive and broadly reactive, mainly with IIb/IIIa and Ia/IIa.

Source: Table 1, p. 4 BioMarin response to FDA information request. Submitted to NDA 206031 on July 20, 2015.
a thrombocytopenia serious adverse event <50 x 10^9/L, except for Subject 1025, who had a nadir platelet count of 69 x 10^9/L.

Reviewer comment: Anti-platelet antibody testing was not performed in Subjects 687 and 3052. Anti-platelet antibody testing was negative in Subject 1176. Tests for drug-dependent antibodies can be negative in patients with probable drug-induced thrombocytopenia, because assay methods may be insufficiently sensitive to detect some antibodies.[66]

[65] Aster RH. et al. *N Engl J Med* 2007; 357:580-587
[66] George, James N., and Richard H. Aster. "Drug-induced thrombocytopenia: pathogenesis, evaluation, and management." ASH Education Program Book 2009.1 (2009): 153-158.

Table 25. Thrombocytopenia Serious Adverse Events

Subject Age Country	Time from first drisapersen to SAE start (months)	Anti-platelet antibody positive	Nadir platelet count (x 10^9/L)	Description/comment
505 7 France	14	Y	14	Platelet counts were generally normal,[a] including a platelet count of 198 x 10^9/L on March 19, 2015, until a platelet count of 18 x 10^9/L was detected with routine laboratory measurement on April 2, 2013 (when he received his last drisapersen dose). The subject had no symptoms of thrombocytopenia. Drisapersen was stopped. antibodies against thrombocytes were positive (against IIb and IIIb glycoproteins). He received no other treatment for thrombocytopenia was reported. His platelet count returned to normal on June 10, 2013 and remained normal.
677 11 Italy	18	Y	8	Platelet counts were normal, including a platelet count of 138 x 10^9/L on Dec. 7, 2012, until a platelet count of 38 x 10^9/L was detected with routine laboratory measurement on December 20, 2012. Drisapersen was stopped after the dose given on January 4, 2013. On January 8, 2013, he was treated with tranexamic acid, i.v. immunoglobulin, and prednisone. On January 15, the platelet count had decreased to 15 x 10^9/L. He was hospitalized on (b) (6) for a second i.v. immunoglobulin infusion (0.8 g/kg) and tranexamic acid was restarted. Before the second immunoglobulin infusion, anti-thrombocyte antibodies showed an increased response (direct method: AB anti GPIIb / IIIa 5.7, anti GPIa / IIa 2.8, anti GPIb / IX 2.2; while indirect Ab were persistently negative'). The subject had epistaxis on (b) (6) He was discharged on (b) (6) (platelet count was 105 x 10^9/L). On January 22, 2013 he developed diarrhea, hematemesis, and epistaxis. Platelet count was 24 x 10^9/L on Jan. 31, 2013. Prednisone was re-started. On Feb. 6, 2013 his platelet count was 210 x 10^9/L.
687 12 Italy	17	Not tested	5	Platelet counts were normal, including a platelet count of 161 x 10^9/L on Nov. 27, 2012, until a platelet count of 56 x 10^9/L was detected with routine laboratory measurement on December 11, 2012. Last dose of drisapersen was administered on Dec. 11, 2012. Nadir platelet count was 5 x 10^9/L on (b) (6) He was hospitalized and had easy bruising. He was treated with tranexamic acid. His platelet count was normal on Jan. 22, 2013 and Feb. 4, 2013.
1122 10 Chile	26	Y	9	Platelet counts were normal, including a platelet count of 227 x 10^9/L on Sept. 4, 2013, until a platelet count of 9 x 10^9/L was detected with routine laboratory measurement on (b) (6) (date of the last drisapersen dose). He had epistaxis, gingival bleeding, and ecchymoses. He was hospitalized for platelet transfusion, and intravenous immunoglobulin. Anti-platelet antibodies were reported as 'positive.'[67] His platelet count was normal on Oct. 9, 2013 and Jan. 22, 2014.[68]

[67] Table 1, p. 4 BioMarin response to FDA information request. Submitted to NDA 206031 on July 20, 2015.

1176 8 Rep. of Korea	14	N	8	He received drisapersen 6 mg/kg/week in Study 114044 from March 11, 2011 to Feb. 1, 2012. First low platelet count was 78 x 10^9/L on Feb. 7, 2012. On March 21, 2012 (after 7 weeks without drisapersen), his platelet count was 144 x 10^9/L. He restarted drisapersen at the beginning of Study 114349 on March 28, 2012. After 7 weekly injections, his platelet count was 83 x 10^9/L on May 8, 2012. He had petechiae on (b) (6), and drisapersen was discontinued. Platelet count was 17 x 10^9/L, and he was hospitalized for platelet transfusion. He received 2 2-day courses of immunoglobulin therapy (May 17, 2012 and June 7, 2012), as well as steroids. A bone marrow examination performed on May 18, 2012 showed an "adequate number of megakaryocytes with normocellular marrow." Platelet count was normal on June 12, 2012 and remained normal in July 2012. *Reviewer comment: The onset of thrombocytopenia was more gradual, and anti-platelet antibody testing was negative. This patient's thrombocytopenia happened to coincide with an 8 week break from drisapersen at the end of Study 114044, which likely affected the course of his thrombocytopenia.. In this reviewer's opinion, this case is related to drisapersen. He had thrombocytopenia in Study 114044, improved while off of drisapersen treatment for 8 weeks after the end of Study 114044 (positive dechallenge), and had recurrence of thrombocytopenia after restarting drisapersen in extension study 114349.*
1202 7 Taiwan	18	Y	3	Platelet counts were generally normal,[b] including a platelet count of 132 x 10^9/L on Dec. 14, 2015, until a platelet count of 23 x 10^9/L was detected with routine laboratory measurement on Dec. 28, 2013 (when he received his last drisapersen dose). On January 2, 2013 he had bruising and petechiae. He was hospitalized on (b) (6), when his platelet count was 3 x 10^9/L. He was treated with platelet transfusion intravenous immunoglobulin, and prednisolone. On January 12, 2013, his anti-platelet antibodies were reported as 'positive.' By January 18, 2013, platelet count was normal and remained normal at follow-ups in Feb., May, and Nov. 2013.
2000 10 Belgium	29	Y	35	Platelet counts were normal, until he had a low platelet count of 85 x 10^9/L on Dec. 12, 2012. Treatment with drisapersen was interrupted for 1 week and treatment was re-started as the platelet count recovered. On Feb. 14, 2013, his platelet count dropped to 35 x 10^9/L. The subject was withdrawn from the study. The subject did not have symptoms. Anti-platelet antibody tests were positive, mainly reactive with gp IIb/IIIa and gp Ia/IIa. Platelet counts were normal on March 27, 2013 and April 3, 2013.
3052 7 Turkey	10	Not tested	26	Platelet counts were normal, until they decreased gradually: 129 x 10^9/L on April 3, 2013, 104 x 10^9/L on April 17, 2013, 71 x 10^9/L on April 30, 2013 (date of last drisapersen), and 26 x 10^9/L on May 8, 2013. He was hospitalized and treated with intravenous immunoglobulin. His platelet count improved to 171 x10^9/L on May 15, 2013.

[68] Dataset ADLB. Submitted to NDA 206031 on April 27, 2015.

All thrombocytopenia SAEs occurred in extension study DMD114349 while receiving drisapersen 6 mg/kg/week.
Source: Narratives, patient profiles, Sponsor IR responses, and dataset PLTTHR submitted to NDA 206031 on August 20, 2015.
[a] Subject 505 had 2 platelet counts of 128 x 10^9/L on 12/24/2012 and 1/7/2013. (LLN = 130 x 10^9/L)
[b] Subject 1202 had 1 platelet count of 121 x 10^9/L on 6/29/2012.(LLN = 130 x 10^9/L)

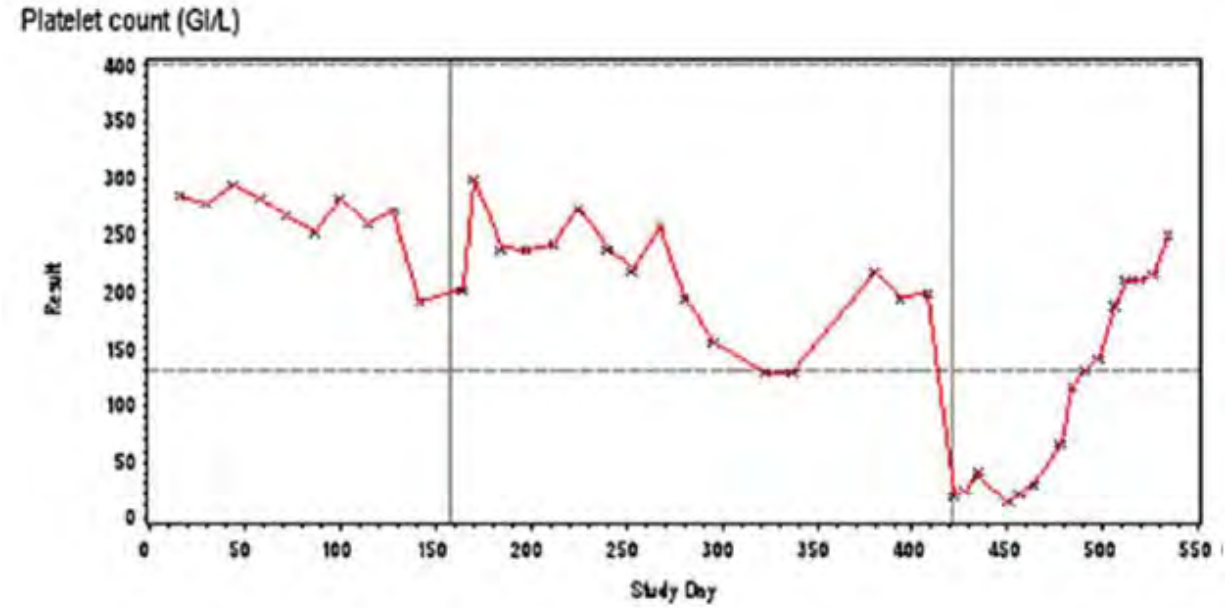
Systematic assessments of anti-platelet antibodies were included in neither the placebo-controlled studies[69] nor the main open-label extension study.[70-71] Other cases of immune thrombocytopenia may have occurred but may not have been detected.

Cases of immune thrombocytopenia with drisapersen were not reported until the open-label extension studies. The reason for this time course for immune thrombocytopenia with drisapersen is unclear. There are no known factors that increase the risk of thrombocytopenia in certain patient subgroups.

The onset of severe immune thrombocytopenia with drisapersen is frequently precipitous and unpredictable. The figures below show the platelet counts by study day for Study 114349 Subjects 505 and 1122, both of whom had treatment-emergent anti-platelet antibodies and a nadir platelet count <20 x 10^9/L. Prior to developing thrombocytopenia, both of these subjects had consistently normal platelet counts, including a normal platelet count within 2 weeks of having a platelet count <20 x 10^9/L.

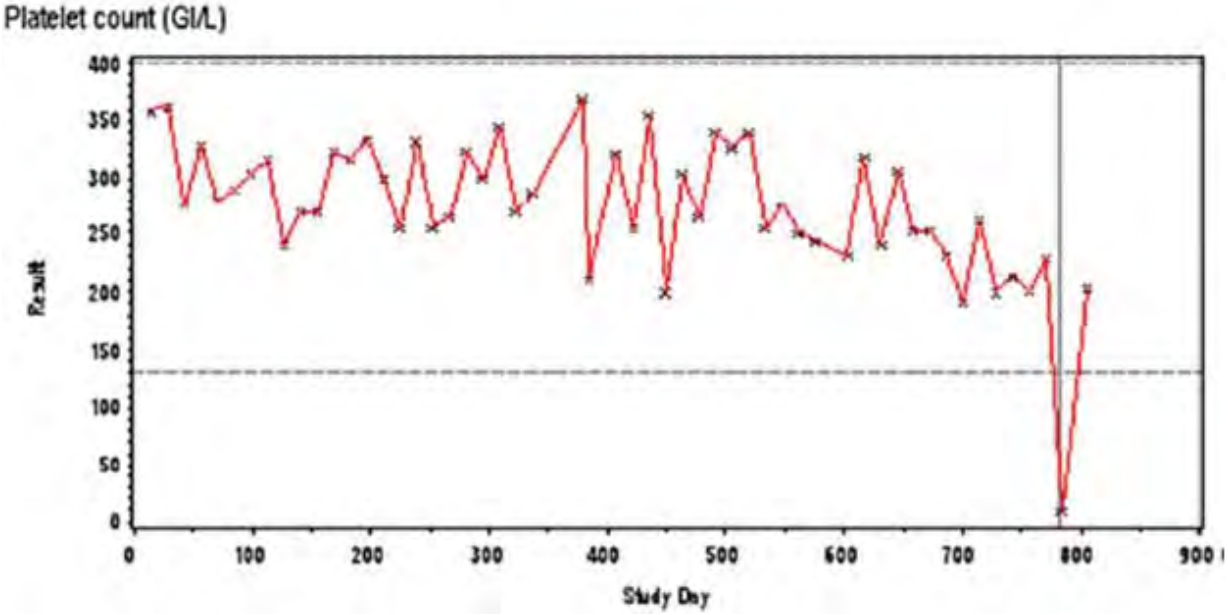
[69] DMD114044, DMD114117, and DMD114876
[70] DMD114349
[71] In the ongoing open-label extension studies DMD115501 (N=21) and DMD114673 (N=12), study protocols were amended to include analysis of clinical samples for any subject who has a confirmed platelet count below 75 x 10^9/L
at a specialist center for analysis of anti-platelet antibodies and platelet function.
At the time of the 120-day safety update report, 1 subject (Study 114673 Subject 105 with nadir platelet count 47 x 10^9/L) received specialist center analysis for antiplatelet antibodies. This subject did not have evidence of antiplatelet antibodies, and the etiology of thrombocytopenia in this subject is unclear.

Figure 3. Subject 505: Platelet counts



Source: Sponsor Figure 12. Summary of Clinical Safety p. 147.

Figure 4. Subject 1122: Platelet counts



Source: Sponsor Figure 15. Summary of Clinical Safety p. 150.

None of the 8 drisapersen subjects who had a thrombocytopenia SAE was rechallenged with drisapersen.[72] According to a review on drug-induced immune thrombocytopenia by Aster:[73] "Once established, drug sensitivity probably persists indefinitely. Therefore, patients should be advised to avoid permanently the medication thought to be the cause of thrombocytopenia."

The Division of Neurology Products consulted the Division of Hematology Products regarding thrombocytopenia with drisapersen.[74] They had the following recommendations:

- Due to the potential increased risk of bleeding in patients with DMD due to abnormally functioning platelets, monitoring and dose adjustment as per protocol DMD114044 should be incorporated into product labeling to limit potential major bleeding and severe thrombocytopenia. Rates of thrombocytopenia and bleeding in clinical practice may differ from the rates seen in clinical studies.

- Drisapersen should not be restarted in patients with thrombocytopenia that recovered after drisapersen discontinuation unless the benefit of therapy outweighs the risk of thrombocytopenia and potential bleeding.

Reviewer comment: This agrees with the recommendations provided by the Division of Hematology Products. Platelet counts were measured every two weeks in clinical studies; this frequency of platelet monitoring will be necessary in the postmarketing setting.
Patients will need to be educated about the signs and symptoms of bleeding related to thrombocytopenia, in order to facilitate prompt diagnosis and treatment.

When considering the use of antiplatelet (e.g., aspirin, adenosine diphosphate receptor inhibitors), thrombolytic (e.g., tissue plasminogen activator, streptokinase), or anticoagulant drugs (e.g., heparin, warfarin) concomitantly with drisapersen, I recommend consideration of the risk of potential bleeding from thrombocytopenia. Patients taking these drugs were excluded from clinical studies.

8.5.2. **Renal Toxicity**

Renal toxicity: Adverse events

The kidney is a target organ for drisapersen with drug accumulating in the proximal tubule.[75] Oligonucleotides are filtered at the glomerulus and reabsorbed by proximal tubule

[72] P. 6. BioMarin response to FDA information request. Submitted to NDA 206031 on July 20, 2015.
[73] Aster RH. et al. N Engl J Med 2007; 357:580-587
[74] 8/12/2015

epithelium.[76] In nonclinical studies, dose-related accumulation of drisapersen occurred in the renal tubule epithelial cells. In placebo-controlled studies in humans, 60.9% of drisapersen 6 mg/kg/week subjects had a renal toxicity adverse event, compared to 33.7% of placebo subjects (see table below).[77]

Table 26. Summary of on-treatment renal toxicity adverse events by preferred term (placebo-controlled studies)

Adverse event System organ class Preferred term	Placebo N=95 n (%)	Drisapersen 6mg/kg/wk N=161 n (%)
Any renal abnormality	32 (33.7)	98 (60.9)
Proteinuria	16 (16.8)	47 (29.2)
Haematuria	5 (5.3)	24 (14.9)
Protein urine present	6 (6.3)	20 (12.4)
Cystatin C increased	4 (4.2)	17 (10.6)
Red blood cells urine positive	4 (4.2)	15 (9.3)
Urine protein/creatinine ratio increased	4 (4.2)	14 (8.7)
Red blood cells urine	5 (5.3)	13 (8.1)
Protein urine	0	8 (5.0)
Urine analysis abnormal	0	3 (1.9)
Urinary sediment present	1 (1.1)	2 (1.2)
Nephropathy toxic	0	2 (1.2)
Albuminuria	0	2 (1.2)
Urine protein/creatinine ratio	0	2 (1.2)
Cystatin C	1 (1.1)	1 (0.6)
White blood cells urine positive	0	1 (0.6)
Protein total increased	0	1 (0.6)
Red blood cell count increased	0	1 (0.6)
Creatinine renal clearance decreased	0	1 (0.6)
Urine leukocyte esterase positive	0	1 (0.6)
Urine protein/creatinine ratio abnormal	0	1 (0.6)
White blood cell count	0	1 (0.6)
Chromaturia	2 (2.1)	1 (0.6)
Renal impairment	1 (1.1)	1 (0.6)
Glomerulonephritis	0	1 (0.6)
Myoglobinuria	0	1 (0.6)
Red blood cell abnormality	0	1 (0.6)
Glycosuria	1 (1.1)	0
Nephrolithiasis	1 (1.1)	0
Urine abnormality	1 (1.1)	0

Source: Sponsor Table 54 on Summary of Clinical Safety p. 113.
Table includes data from studies DMD114117, DMD114044, and DMD114876.

Reviewer comment: Analyses were performed to combine the frequencies of split terms related to renal toxicity.[78] In placebo-controlled studies, proteinuria[79] occurred in 70 of 161 (43.5%)

[75] P. 122. Summary of Clinical Safety; Section 2.7.4 of the April 27, 2015 submission to NDA 206031.
[76] P. 22 Nonclinical Overview. Submitted to NDA 206031 on 10/10/2014.
[77] In placebo-controlled studies, 70.6% and 11.8% of drisapersen subjects had a renal toxicity adverse event in the 6 mg/kg/week intermittent and 3 mg/kg/week dose groups, respectively. These results are limited by small sample sizes (17 subjects in each group). (ISS Table 11.63.1 on ISS p. 2589-2590)
[78] Table 1. ISS addendum submitted to NDA 206031 on 09/25/2015.

drisapersen 6 mg/kg/week subjects, compared to 22 of 95 (23.2%) placebo subjects. Hematuria[80] occurred in 26 of 161 (16.1%) drisapersen 6 mg/kg/week subjects, compared to 10 of 95 (10.5%) placebo subjects.

In repeat-dose studies, 71.5% of drisapersen 6 mg/kg/week subjects had a renal toxicity adverse event (see table below).

[79] Adverse events with PTs Proteinuria, Protein urine present, and Protein urine were combined. Subjects with adverse events coded to more than 1 of the 3 terms and were counted once.
[80] Hematuria-- Subjects had an adverse event coded to at least one of these Preferred Terms: Red blood cells urine positive, or Red blood cells urine

Table 27. Summary of on-treatment renal toxicity adverse events by preferred term (repeat dose studies)

Adverse event preferred term	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent[a] N=38 n (%)	Drisapersen all regimens[b] N=285 n (%)
Any renal abnormality AE	**32 (33.7)**	**2 (11.8)**	**191 (71.5)**	**29 (76.3)**	**194 (68.1)**
Proteinuria	16 (16.8)	1 (5.9)	115 (43.1)	12 (31.6)	118 (41.4)
Protein urine present	6 (6.3)	0	42 (15.7)	7 (18.4)	47 (16.5)
Haematuria	5 (5.3)	0	43 (16.1)	4 (10.5)	44 (15.4)
Cystatin C increased	4 (4.2)	0	33 (12.4)	11 (28.9)	40 (14.0)
Red blood cells urine positive	4 (4.2)	0	36 (13.5)	3 (7.9)	39 (13.7)
Urine protein/creatinine ratio increased	4 (4.2)	0	30 (11.2)	7 (18.4)	36 (12.6)
Red blood cells urine	5 (5.3)	1 (5.9)	24 (9.0)	3 (7.9)	27 (9.5)
Albuminuria	0	0	9 (3.4)	12 (31.6)	16 (5.6)
Protein urine	0	0	13 (4.9)	2 (5.3)	15 (5.3)
Alpha 1 microglobulin urine increased	0	0	11 (4.1)	7 (18.4)	11 (3.9)
White blood cells urine positive	0	0	5 (1.9)	3 (7.9)	8 (2.8)
Urinary sediment present	1 (1.1)	0	6 (2.2)	0	6 (2.1)
Urinary sediment abnormal	0	0	5 (1.9)	4 (10.5)	5 (1.8)
Red blood cell count increased	0	0	4 (1.5)	1 (2.6)	5 (1.8)
Nephropathy toxic	0	0	5 (1.9)	0	5 (1.8)
Urinary casts	0	0	5 (1.9)	0	5 (1.8)
Urine analysis abnormal	0	0	4 (1.5)	1 (2.6)	5 (1.8)
Albumin urine present	0	0	3 (1.1)	0	3 (1.1)
Renal impairment	1 (1.1)	0	3 (1.1)	0	3 (1.1)
Chromaturia	2 (2.1)	1 (5.9)	1 (0.4)	0	2 (0.7)
Myoglobinuria	0	0	2 (0.7)	0	2 (0.7)
Blood urine present	0	0	2 (0.7)	0	2 (0.7)
Creatinine renal clearance decreased	0	0	2 (0.7)	0	2 (0.7)
Protein total increased	0	0	1 (0.4)	1 (2.6)	2 (0.7)
Urine protein/creatinine ratio	0	0	2 (0.7)	0	2 (0.7)
Urine /protein/creatinine ratio abnormal	0	0	2 (0.7)	0	2 (0.7)
White blood cells urine	0	0	0	2 (5.3)	2 (0.7)
Cystatin C	1 (1.1)	0	1 (0.4)	0	1 (0.4)
Glycosuria	1 (1.1)	0	1 (0.4)	0	1 (0.4)
Glomerulonephritis	0	0	1 (0.4)	0	1 (0.4)
Haemoglobinuria	0	0	1 (0.4)	0	1 (0.4)
Leukocyturia	0	0	1 (0.4)	0	1 (0.4)
Albumin globulin ratio increased	0	0	1 (0.4)	0	1 (0.4)
Albumin urine	0	0	1 (0.4)	0	1 (0.4)
Alpha 1 microglobulin	0	0	0	1 (2.6)	1 (0.4)
Alpha-2 macroglobulin increased [c]	0	0	1 (0.4)	0	1 (0.4)
Creatine urine increased	0	0	1 (0.4)	0	1 (0.4)
Light chain analysis increased	0	0	0	1 (2.6)	1 (0.4)
Protein albumin ratio increased	0	0	0	1 (2.6)	1 (0.4)
Urine leukocyte esterase positive	0	0	1 (0.4)	0	1 (0.4)
White blood cell count	0	0	1 (0.4)	0	1 (0.4)
pH urine increased	0	0	1 (0.4)	0	1 (0.4)
Red blood cell abnormality	0	0	1 (0.4)	0	1 (0.4)
Nephrolithiasis	1 (1.1)	0	0	0	0
Urine abnormality	1 (1.1)	0	0	0	0

Source: Sponsor Table 53 on Summary of Clinical Safety p. 111.

[a] intermittent includes subjects dosed with 9 doses in a 10-week cycle

[b] Subjects treated with more than one drisapersen regimen are only counted once in this group.

[c] The verbatim text for this preferred term was 'periods of elevated alpha 1 microglobuline 45.2 mg/L'.

Reviewer comment: This adverse event appears to be miscoded and would underestimate the occurrence of the event. If the alpha 1 microglobulin measurement is from the serum, the measurements are mildly elevated. The normal range for serum alpha 1 microglobulin has been reported as 20-42 mg/L (Weber MH. Klin Wochenschr. 1985 Aug 1;63(15):711-7.)

Table includes data from studies DMD114117, DMD114044, DMD114876, DMD114349, PRO051-02, and DMD114673.

Reviewer comment: In analyses of repeat dose studies performed to combine the frequencies of split terms,[81] proteinuria[82] occurred in 161 of 267 (60.3%) of drisapersen 60 mg/kg/week subjects. Hematuria[83] occurred in 53 of 267 (19.9%) drisapersen 6 mg/kg/week subjects.

In placebo-controlled studies, 2 of 195 (1%) drisapersen-treated subjects had a renal SAE, compared to 0 of 95 placebo-treated subjects:

- **PT Glomerulonephritis**

  DMD114044 Subject 527,[84] a 10 year old boy from France, had no history of kidney disease or kidney disease risk factors prior to study entry. He started drisapersen 6 mg/kg/week on April 6, 2011. Baseline spot urine protein (March 15, 2011) was 0.06 g/L. On September 21, 2011, spot urine protein rose to 0.5 g/L. Spot urine protein was 1.1 g/L on October 5, 2011 and 1.6 g/L on October 21, 2011.

  The study's monitoring criteria required a 24-hour urinalysis after 2 consecutive urine protein ≥ 0.2 g/L on 2 consecutive weekly samples). However, this subject did not have a 24-hour urinalysis until one month after the first spot urine protein ≥ 0.2 g/L .

  On October 26, 2015, 5.8 g of protein was measured in a 24 hour urine collection. (Nephrotic range proteinuria is defined as 1 g/day in children.) The last drisapersen dose was administered on (b) (6).

  After cessation of drisapersen treatment, spot urine protein measurements increased to 8.05 and 8.96 g/L on November 2 and 9, 2011, respectively. On (b) (6), (26 days after last drisapersen dose), the subject was hospitalized with left sided back pain, and developed tachycardia and tachypnea. Bilateral pulmonary emboli were diagnosed (left pulmonary artery and right middle lobe). On (b) (6), CT scan showed thrombosis of the inferior vena cava and right renal vein with infarction of the right kidney.[85] Kidney biopsy obtained on (b) (6), showed type 1 grade 2 (moderate) membranous glomerulonephritis.

  The subject was treated with anticoagulation. On December 12, 2011 spot urine protein was 1.1 g/L. Doppler ultrasound in June 2012 showed no evidence of thrombus the

[81] Table 1. ISS addendum submitted to NDA 206031 on 09/25/2015.
[82] Proteinuria -- Subjects had an adverse event coded to at least one of these Preferred Terms: Proteinuria, Protein urine present, Protein urine, or Albuminuria.
[83] Hematuria-- Subjects had an adverse event coded to at least one of these Preferred Terms: Red blood cells urine positive, or Red blood cells urine

[85] Safety report submitted to IND 067476 on December 23, 2011.

renal vein or inferior vena cava. His proteinuria eventually improved to 0.11 g /day on 24 hour urinalysis. (Follow-up date June 25, 2102; test date not reported.)

Serum cystatin C was 0.7mg/L at screening (normal range 0.6 - 0.8 mg/L). At study withdrawal (12/12/2011), serum cystatin C was 1.0 mg/L. No serum electrolyte abnormalities were reported.

This event is likely related to drisapersen. Membranous glomerulonephritis is a rare disease in children.[86-87] Membranous glomerulonephritis is related to immune deposits in the kidney. It is unclear whether anti-drisapersen antibodies contributed to this case. Subject's kidney biopsy sample was not tested for anti-drisapersen antibodies, and conclusive plasma anti-drisapersen antibody testing was not performed in this subject.

- **PT Haematuria** – DMD114117 Subject 3000:[88]

  Hours after having a protocol-required muscle biopsy while under general anesthesia, this 7 year old subject developed frank hematuria. Urine myoglobin was 182 mg/L (normal = ≤0.1 mg/L). Electrolytes, renal function tests, coagulation tests, and renal ultrasound were normal. The event resolved after 6 days.

  Reviewer comment: This event is unrelated to drisapersen. The subject had anesthesia-associated rhabdomyolysis, which has been reported in children with congenital muscle disease, including DMD.[89]

In extension studies there were 2 renal SAEs in drisapersen-treated subjects:

- **PT Proteinuria** – DMD144044 / DMD114349 Subject 1124:[90]

  This 8 year old boy received all planned drisapersen doses in Study 114044 (starting on August 10, 2011), in which his peak 24 hour urinalysis measurement was 0.2 g/24 hours.[91] He was first dosed in Study DMD114349 on August 8, 2012. His spot urine

[86] Eddy AA, et al. Nephrotic syndrome in childhood. Lancet 2003; 362: 629–39.
[87] Nephrotic syndrome in children: Prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. International Study of Kidney Disease in Children. Kidney International, Vol. 13 (1978), pp. 159 —165.
[88] Narrative on p. 3817 Integrated Summary of Safety (ISS); Section 5.3.5.3 of the April 27, 2015 submission to NDA 206031.
[89] Pedrozzi, NE. Rhabdomyolysis and anesthesia: a report of two cases and review of the literature. *Pediatric neurology.* 15.3 (1996): 254-257.
[90] Narrative on p. 4349 Integrated Summary of Safety (ISS); Section 5.3.5.3 of the April 27, 2015 submission to NDA 206031.
[91] The treatment stopping criterion for protein in a 24 hour urine sample was >0.3 g/day and double the baseline value, according to the document titled "January 6, 2012 Discussion"; Section 1.11.4 of the 1/11/2012 submission to IND 105284.

protein on 8/8/2012 was 0.2 g/L. Two weeks later on 8/22/2012, his spot urine protein was 5.2 g/L. He continued to receive drisapersen, on August 29, 2012 and September 5, 2012. On September 5, 2012, 24 hour urine protein was 11.0 g/day. Drisapersen treatment was discontinued. Urine protein decreased after discontinuation of drisapersen (see table below).

Table 28. Subject 1124: 24-hour protein measurements

Date of measurement	24-hour urine protein measurement (g/day)
9/5/2012	11.0
9/11/2012	6.8
9/19/2012	4.5
10/2/2012	2.8
10/23/2012	0.3
11/13/2012	0.1
5/14/2013	0.1

Source: Dataset ADLB2349[92]

There was no frank edema or hypoalbuminemia reported.

Reviewer comment: In the opinion of this reviewer, this case is likely related to drisapersen.

- **PT Renal impairment** -- DMD144044 / DMD114349 Subject 1002:[93]

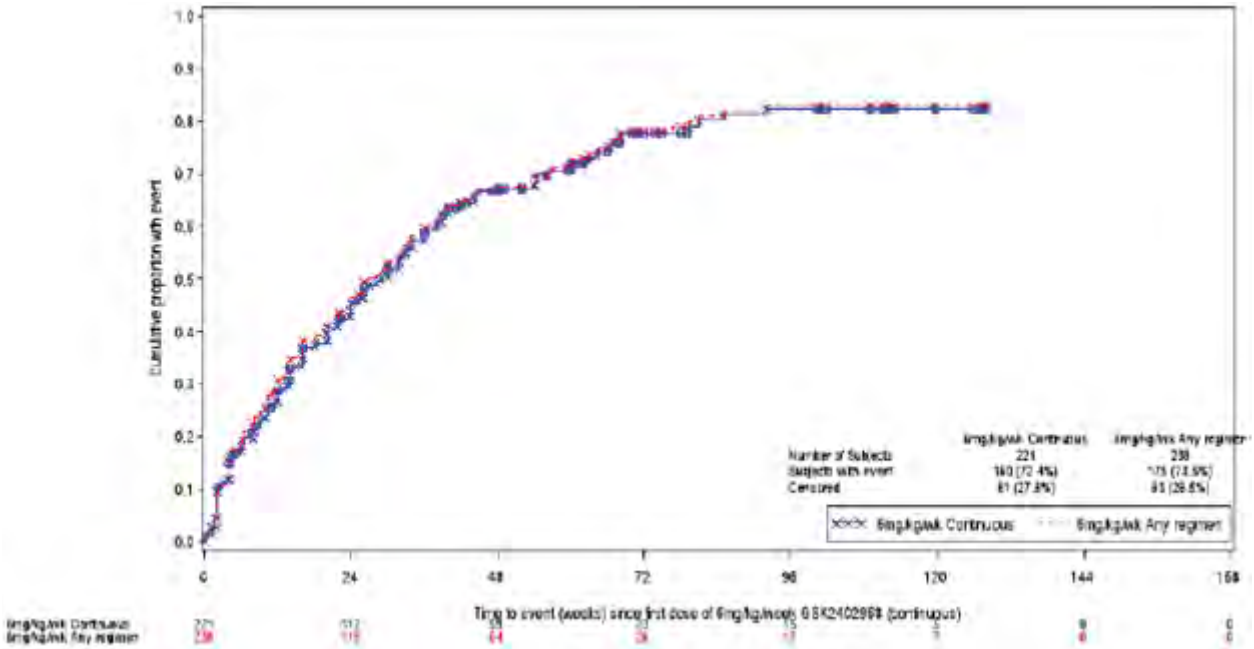
  Twenty three months after his first drisapersen dose on Study 114044, this 9 year old boy presented with acute renal failure (increased BUN and creatinine) in the setting of a viral infection, diarrhea, and volume depletion. He received fluid and electrolyte replacement. The event was resolved after 8 days.

  Reviewer comment: This event is related to volume depletion in the setting of a viral infection and diarrhea and is unrelated to drisapersen.

The figure below shows the time to first renal toxicity in drisapersen Study DMD114349 subjects.

[92] Section 5.3.5.2 of the 7/29/15 submission to NDA 206031.
[93] Narrative on p. 3828 Integrated Summary of Safety (ISS); Section 5.3.5.3 of the April 27, 2015 submission to NDA 206031.

Figure 5. Cumulative distribution of time to first renal toxicity adverse event for subjects receiving drisapersen 6 mg/kg/week in DMD114349, by treatment in the parent study (DMD114349 and parent studies – long-term safety)



Source: SCS Figure 7 p. 119.
Figure includes data from studies DMD114117, DMD114044, and DMD114349.

In all repeat dose studies, 36 of 1183 (3.2%) renal toxicity adverse events were unresolved at the end of the study. In the drisapersen 6 mg/kg/week group, events that were unresolved were proteinuria (13 events), cystatin C increased (14 events), protein urine present (2 events), urinary sediment present (2 events), urinary casts (1 event) and glomerulonephritis (1 event). The median duration of renal adverse events in drisapersen 6 mg/kg/week subjects was 41 days.

Renal toxicity: Laboratory data

Urinalysis results for the ambulant placebo-controlled studies showed mean increases in urine protein that were greater with drisapersen 6 mg/kg/week than with placebo. In placebo-controlled studies, mean changes from baseline for drisapersen 6 mg/kg/week, were 47 mg/L at Week 12, 70 mg/L at Week 24, 72 mg/L at Week 36, and 64 mg/L at Week 48 compared with mean changes of 3, 5, 8, and 6 mg/L, respectively for placebo. Thirty percent of drisapersen 6 mg/kg/week subjects had a high (≥0.15 g/ day) 24 hour urine protein, compared to 4% of placebo subjects.[94]

[94] Table 10.2 on p. 149 of the ISS addendum submitted on 7/20/2015 to NDA 206031.

There were no clinically significant changes in urine red blood cells, white blood cells or casts in drisapersen subjects, compared to placebo.

Creatinine as a marker of renal function has limited value in Duchenne muscular dystrophy (DMD), because of reduced muscle mass. Cystatin C has been evaluated as a biomarker for monitoring renal function in DMD,[95] and it was measured in drisapersen clinical studies. The range of normal values for cystatin C is 0.6-0.8 mg/L. In placebo-controlled studies, 58% of drisapersen 6 mg/kg/week subjects went from a normal cystatin C at baseline to a high value post-treatment, compared to 27% of placebo subjects.[96] In placebo-controlled studies, the median maximum post-baseline cystatin C level in drisapersen 6 mg/kg/week subjects was 0.9 mg/L (interquartile range 0.8-1.0), compared to 0.8 mg/L for placebo (interquartile range 0.7-0.9) (see table below).

Table 29. Summary of maximum post-baseline cystatin C (mg/L). Placebo-controlled studies.

		Drisapersen			
	Placebo (N=95)	3mg/kg/week (N=17)	6mg/kg/week (N=161)	6mg/kg Intermittent (N=17)	All Regimens (N=195)
n	95	17	161	17	195
Mean (SD)	0.81 (0.108)	0.83 (0.110)	0.94 (0.145)	0.94 (0.154)	0.93 (0.146)
Median	0.80	0.80	0.90	0.90	0.90
Q1, Q3	0.7, 0.9	0.7, 0.9	0.8, 1.0	0.9, 1.0	0.8, 1.0
Min, Max	0.6, 1.1	0.7, 1.0	0.7, 1.5	0.7, 1.3	0.7, 1.5

Source: P. 11 Section 1.11.4 of the 7/29/2015 submission to NDA 206031

Reviewer comment: While treatment-emergent increases in cystatin C were more frequent in drisapersen subjects, the changes were small and not sustained. The interquartile ranges for maximum post-baseline cystatin C were overlapping for the drisapersen 6 mg/kg/week and placebo groups. The highest cystatin C measurement of 1.5 mg/L (in drisapersen 6 mg/kg/week subject 235) was not treatment-emergent. Subject 235's baseline cystatin C was 1.2 mg/L for unclear reasons.

Placebo-controlled studies did not indicate evidence of blood electrolyte abnormalities, which can occur with renal tubular dysfunction (e.g., Fanconi syndrome). (See Section 8.4.6 Laboratory Findings)

[95] Violett L. Utility of Cystatin C to monitor renal function in Duchenne muscular dystrophy. *Muscle Nerve*. 2009 September ; 40(3): 438–442. doi:10.1002/mus.21420.
[96] P. 96 of the ISS addendum in Section 5.3.5.2 of the 7/20/2015 submission to NDA 206031.

Renal toxicity: Reviewer discussion and recommendations

In the submitted product label, the Sponsor proposes renal monitoring and stopping criteria as follows: "Monitor for urine protein by urine dipstick analysis once a month during [TRADENAME] treatment. Patients with a dipstick of 3+ for protein should undergo a 24 hour urine collection. Suspend [TRADENAME] treatment when urine protein is >1 gram per 24 hours. Treatment may be resumed when urine protein is ≤ 1 gram per 24 hours and based on individual risk-benefit assessment. Discontinue treatment if patient develops glomerulonephritis."[97]

In the opinion of this reviewer, Sponsor's proposed method (urine dipstick testing) and frequency of renal monitoring, as well as the proposed renal criterion for stopping drisapersen treatment, are not acceptable.

In repeat dose studies of drisapersen, 2 of 285 (0.7%)[98] of subjects had a renal toxicity SAE that was related to drisapersen treatment. With each of these SAEs, there was a rapid progression of proteinuria severity, with urine spot protein increasing at least 3 fold within a month. In the membranous glomerulonephritis SAE (Subject 527), potentially fatal thromboses with bilateral pulmonary emboli ensued. This subject's proteinuria and clinical condition worsened for about 1 month after drisapersen was discontinued.

In the postmarketing setting, baseline renal testing and frequent renal monitoring will be necessary, especially because of the potential for: a) rapid progression of renal toxicity; b) a time period of worsening renal toxicity after drisapersen treatment discontinuation; and c) serious and potentially fatal consequences of renal toxicity.

In clinical studies, subjects had scheduled quantitative urine protein testing every 2 weeks. Drisapersen treatment stopping criteria included:[99]

- Urinary protein concentration ≥0.2 g/L and <0.45g/L on two consecutive weekly samples;

(If urinary protein concentration was ≥0.45 g/L on urinalysis, study drug was stopped and the urinary protein analysis was repeated. If the repeat urinary protein value was ≥0.2 g/L, investigators were advised to continue to hold the study drug and perform a 24 hour urine test. If the repeat urinary protein value was <0.2 g/L, the study drug could be restarted.)

- Protein/creatinine ratio in the morning sample is >0.5 on two consecutive samples;
- Serum concentration of cystatin C is above the normal range and 50%above the baseline value.
- If the results of the 24 hour urine test triggered by any of the above do not meet criteria defined below, study drug may be restarted

[97] Monitoring to Assess Safety Section 2.2. Sponsor proposed product label. Submitted to NDA 206031 on 4/27/2015.
[98] Subject 527 (PT Glomerulonephritis) and Subject 1124 (PT Proteinuria).
[99] Latest iteration of renal treatment stopping criteria (circa April – June 2012). P. 219 of the Summary of Clinical Safety.

- Proteinuria in 24-hour urine sample is >300 mg/day and double the baseline value.

The clinical study stopping and follow-up parameters were reviewed and agreed upon by (b) (4) served on the GlaxoSmithKline Independent Data Monitoring Committee (IDMC), which periodically reviewed unblinded safety data from placebo-controlled studies.

The sponsor's proposal to use a urine dipstick of 3+ for protein as a screening test for significant proteinuria is inadequate. Urine dipstick testing was not used in clinical studies. In a published study of children known to have nephrotic syndrome, urine dipstick of 3+ or 4+ had only a 70% sensitivity to detect a 24 hour urine protein excretion of >1g.[100]

Reviewer conclusion: In the postmarketing setting, baseline renal testing and renal monitoring every 2 weeks will necessary. Criteria for discontinuing drisapersen treatment based on renal testing should be similar to stopping criteria used in clinical studies.

8.5.3. Injection Site Reactions

In repeat dose studies, 210 of 267 (78.7%) drisapersen 6 mg/kg/week subjects reported at least 1 injection site reaction (see table below).

Table 30. Summary of injection site reactions (repeat dose studies)

Adverse event	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent[a] N=38 n (%)	Drisapersen all regimens[b] N=285 n (%)
Any injection site reaction	21 (22.1)	11 (64.7)	210 (78.7)	31 (81.6)	224 (78.6)
Any injection site reaction SAE	0	0	2 (0.7)	0	2 (0.7)
Any severe injection site reaction	0	0	9 (3.4)	0	10 (3.5)

Source: Sponsor Table 47. Summary of Clinical Safety
Includes data from studies DMD114117, DMD114044, DMD114876, DMD114349, PRO051-02, and DMD114673.

Two drisapersen subjects (both receiving 6 mg/kg/week) had injection site reaction SAEs:

- DMD114349 Subject 511, a 9 year old subject from France, started drisapersen in March 2011. On (b) (6), he received his dose and developed severe injection site edema on the back of his upper arm. Ultrasound showed edema and infiltration of subcutaneous tissues. He was hospitalized and treated with paracetamol. The event resolved after 7 days. Treatment with drisapersen was discontinued.

[100] Abitbol, Carolyn, et al. "Quantitation of proteinuria with urinary protein/creatinine ratios and random testing with dipsticks in nephrotic children." The Journal of pediatrics 116.2 (1990): 243-247.

- DMD114349 Subject 126, a 6 year old subject from Poland, started drisapersen in July 2011. On (b) (6), he developed severe injection site edema in his upper arm. The next day he developed fever and was hospitalized and treated with prednisone. The event resolved after 2 days. He continued drisapersen treatment.

Ten of 285 (3.5%) drisapersen subjects reported severe injection site reactions, including injection site atrophy, injection site pain, injection site induration, injection site discoloration, and injection site edema.

The table below summarizes injection site reactions in repeat dose studies by MedDRA Preferred Term. The most common injection site reaction Preferred Terms were Injection site erythema and Injection site discolouration, reported in 52.1% and 47.2% of drisapersen 6 mg/kg/week subjects, respectively. Other injection site reaction PTs reported in at least 10% of drisapersen 6 mg/kg/week subjects included: Injection site induration (29.6%), Injection site pain (19.5%), Injection site reaction (18.4%), Injection site pruritus (16.9%), Injection site bruising (13.1%), Injection site atrophy (12.0%), Injection site haematoma (12.0%), and Injection site swelling (10.1%).

Table 31. Summary of on-treatment injection site reactions by preferred terms (repeat dose studies)

Adverse event preferred term	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent[a] N=38 n (%)	Drisapersen all regimens[b] N=285 n (%)
Any on-treatment injection site reaction AE	21 (22.1)	11 (64.7)	210 (78.7)	31 (81.6)	224 (78.6)
Injection site erythema	8 (8.4)	10 (58.8)	139 (52.1)	16 (42.1)	157 (55.1)
Injection site discolouration	5 (5.3)	5 (29.4)	126 (47.2)	18 (47.4)	133 (46.7)
Injection site induration	1 (1.1)	0	79 (29.6)	14 (36.8)	81 (28.4)
Injection site pain	5 (5.3)	0	52 (19.5)	12 (31.6)	58 (20.4)
Injection site pruritus	1 (1.1)	2 (11.8)	45 (16.9)	7 (18.4)	54 (18.9)
Injection site reaction	1 (1.1)	0	49 (18.4)	5 (13.2)	52 (18.2)
Injection site atrophy	0	0	32 (12.0)	10 (26.3)	42 (14.7)
Injection site bruising	9 (9.5)	2 (11.8)	35 (13.1)	3 (7.9)	41 (14.4)
Injection site haematoma	5 (5.3)	0	32 (12.0)	12 (31.6)	39 (13.7)
Injection site swelling	0	1 (5.9)	27 (10.1)	3 (7.9)	31 (10.9)
Injection site inflammation	0	0	13 (4.9)	2 (5.3)	13 (4.6)
Injection site urticaria	0	0	13 (4.9)	1 (2.6)	13 (4.6)
Injection site rash	2 (2.1)	0	10 (3.7)	1 (2.6)	11 (3.9)
Injection site oedema	0	0	11 (4.1)	0	11 (3.9)
Injection site vesicles	0	0	10 (3.7)	1 (2.6)	11 (3.9)
Injection site dryness	0	0	10 (3.7)	1 (2.6)	10 (3.5)
Injection site macule	1 (1.1)	0	7 (2.6)	2 (5.3)	9 (3.2)
Injection site warmth	0	0	7 (2.6)	0	7 (2.5)
Injection site scab	0	0	5 (1.9)	0	5 (1.8)
Injection site haemorrhage	0	0	4 (1.5)	1 (2.6)	4 (1.4)
Fat tissue decreased	0	0	4 (1.5)	0	4 (1.4)
Injection site irritation	0	0	3 (1.1)	1 (2.6)	4 (1.4)
Application site vesicles	0	0	3 (1.1)	0	3 (1.1)
Injection site anaesthesia	0	0	3 (1.1)	0	3 (1.1)
Injection site erosion	0	0	3 (1.1)	0	3 (1.1)
Injection site exfoliation	0	0	3 (1.1)	0	3 (1.1)
Injection site mass	0	0	3 (1.1)	0	3 (1.1)
Injection site nodule	0	0	3 (1.1)	0	3 (1.1)
Injection site ulcer	0	0	0	3 (7.9)	3 (1.1)
Injection site extravasation	0	0	2 (0.7)	0	2 (0.7)
Injection site hypersensitivity	0	0	2 (0.7)	0	2 (0.7)
Injection site hypertrophy	0	0	2 (0.7)	0	2 (0.7)
Injection site movement impairment	0	0	2 (0.7)	0	2 (0.7)
Injection site plaque	0	0	2 (0.7)	0	2 (0.7)
Infusion site bruising	0	0	1 (0.4)	0	1 (0.4)
Injection site calcification	0	0	0	1 (2.6)	1 (0.4)
Injection site dysaesthesia	0	0	1 (0.4)	0	1 (0.4)
Injection site eczema	0	0	0	1 (2.6)	1 (0.4)
Injection site hyperaesthesia	0	0	1 (0.4)	0	1 (0.4)
Injection site injury	0	0	1 (0.4)	0	1 (0.4)
Injection site paraesthesia	0	0	1 (0.4)	0	1 (0.4)
Injection site scar	0	0	1 (0.4)	0	1 (0.4)
Mass	0	0	1 (0.4)	0	1 (0.4)
Infusion site haematoma	1 (1.1)	0	0	0	0
Injection site papule	1 (1.1)	0	0	0	0
Lipodystrophy acquired	0	0	4 (1.5)	0	4 (1.4)
Pigmentation disorder	1 (1.1)	0	3 (1.1)	0	3 (1.1)
Erythema	0	0	2 (0.7)	1 (2.6)	3 (1.1)
Macule	0	0	1 (0.4)	1 (2.6)	2 (0.7)
Skin hyperpigmentation	0	0	2 (0.7)	0	2 (0.7)
Pain of skin	0	0	1 (0.4)	0	1 (0.4)
Pruritus generalised	0	0	1 (0.4)	0	1 (0.4)
Rash macular	0	0	1 (0.4)	0	1 (0.4)
Skin discolouration	0	0	1 (0.4)	0	1 (0.4)

Adverse event preferred term	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent[a] N=38 n (%)	Drisapersen all regimens[b] N=285 n (%)
Skin fibrosis	0	0	1 (0.4)	0	1 (0.4)
Injection related reaction	0	0	10 (3.7)	1 (2.6)	10 (3.5)
Contusion	1 (1.1)	0	3 (1.1)	0	3 (1.1)
Post procedural complication	0	0	1 (0.4)	0	1 (0.4)
Skin injury	0	0	1 (0.4)	0	1 (0.4)
Hyperaemia	0	0	3 (1.1)	0	3 (1.1)
Haematoma	0	0	2 (0.7)	0	2 (0.7)
Injection site cellulitis	0	0	2 (0.7)	0	2 (0.7)

Source: Sponsor Table 48. Summary of Clinical Safety.
Includes data from studies DMD114117, DMD114044, DMD114876, DMD114349, PRO051-02, and DMD114673.

Analyses were performed to combine the frequencies of split terms for injection site reactions.[101] In placebo-controlled studies, skin discoloration[102] occurred in 58 of 161 (36.0%) drisapersen 6 mg/kg/week subjects, compared to 7 of 95 (7.4%) placebo subjects. Chronic skin damage[103] occurred in 19 of 161 (11.8%) drisapersen 6 mg/kg/week subjects, compared to 1 of 95 (1.1%) placebo subjects. Ulceration[104] occurred in 5 of 161 (3.7%) drisapersen 6 mg/kg/week subjects, compared to 0 of 95 placebo subjects. Injection site hair growth[105] occurred in 10 of 161 (6.2%) drisapersen 6 mg/kg/week subjects, compared to 0 of 95 placebo subjects.

In repeat dose studies, skin discoloration[106] occurred in 130 of 267 (48.7%) drisapersen 6 mg/kg/week subjects. Chronic skin damage[107] occurred in 49 of 267 (18.4%) drisapersen 6 mg/kg/week subjects. Ulceration[108] occurred in 19 of 267 (7.1%) drisapersen 6 mg/kg/week

[101] Table 2. ISS addendum submitted to NDA 206031 on 09/25/2015.
[102] Skin discoloration -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Injection site discoloration, Pigmentation disorder, Skin hyperpigmentation, or Skin discoloration.
[103] Chronic skin damage -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Atrophy, Fat tissue decreased, Injection site nodule, Hypertrophy, Plaque, Calcification, Scar, Mass, Acquired lipodystrophy, or Skin fibrosis.
[104] Ulceration -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Injection site vesicles, Application site vesicles, Injection site erosion, Injection site ulcer, or Injection site scab
[105] Hair growth -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Hair growth, Hypertrichosis, or Hirsutism at injection site.
[106] Skin discoloration -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Injection site discoloration, Pigmentation disorder, Skin hyperpigmentation, or Skin discoloration.
[107] Chronic skin damage -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Atrophy, Fat tissue decreased, Injection site nodule, Hypertrophy, Plaque, Calcification, Scar, Mass, Acquired lipodystrophy, or Skin fibrosis.
[108] Ulceration -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Injection site vesicles, Application site vesicles, Injection site erosion, Injection site ulcer, or Injection site scab

subjects. Injection site hair growth[109] occurred in 13 of 267 (4.9%) drisapersen 6 mg/kg/week subjects.

At the time of this review, skin biopsy results were available for 2 subjects:

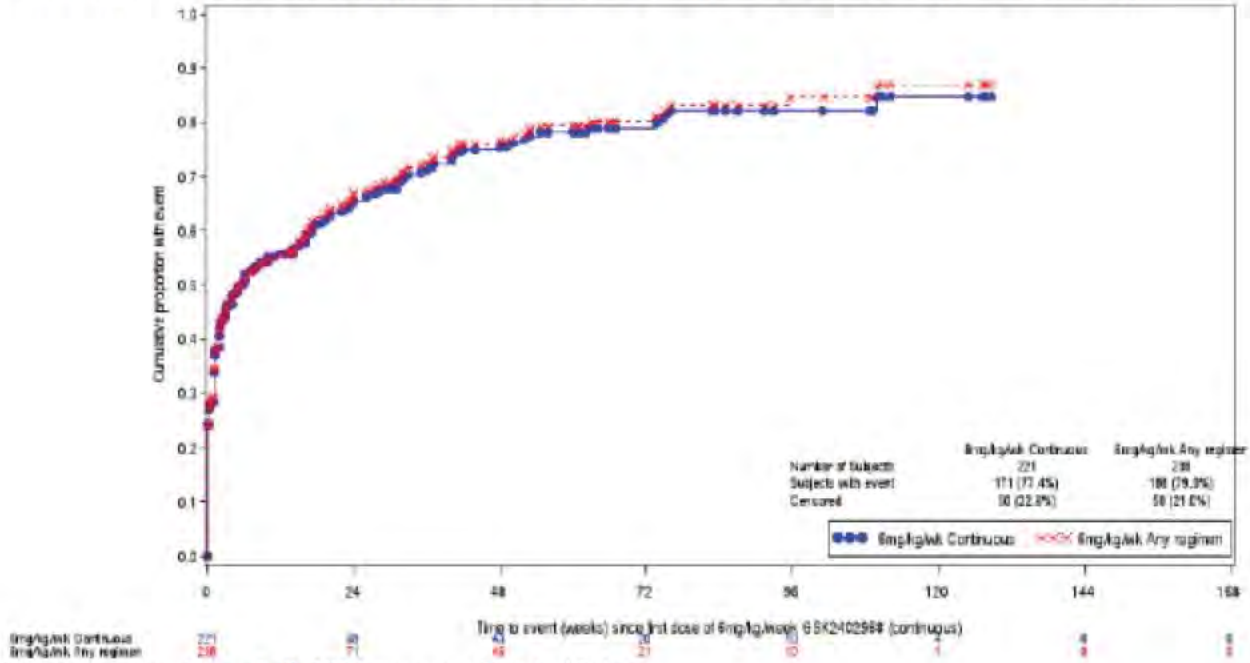
- DMD114349 Subject 576,[110] a 14 year old male from Germany treated with drisapersen 6 mg/kg/week from January 2011 to September 2013. While receiving drisapersen, moderate injections site discoloration and severe injection site induration of skin on his abdomen and thigh were reported. In March 2014 he had severe pain at the injection site. He was seen by a dermatologist in October 2014, who reported "irritating thickening mainly on the abdomen which increased in severity." Histological assessment on September 19, 2014 (1 year after cessation of drisapersen) reported "calcinosis of the skin with pathologic changes at the border of the biopsy tissue sample (obtained from the left upper arm). Treatment with pamidronate 30 mg i.v. was recommended by the dermatologist and it was also noted in the report that 'prior to this, treatment with diltiazem should be assessed'."
  Reviewer comment: This subject experienced severe injection site pain and skin thickening that was worsening 1 year after the cessation of drisapersen. Pathologic calcinosis was found on skin biopsy. No biopsy was performed at the main site of this subject's symptoms (abdomen).

- Study DMD114673) Subject 101,[111] a 9 year old male from the Netherlands, received 5 subcutaneous injections of drisapersen 0.5 mg/kg/week in Study PRO051-02 from May 5 to June 2, 2008. In extension study DMD114673, he received drisapersen 6 mg/kg via various dosing schedules starting in July 2009. Mild injection site reactions of erythema, hematoma, and induration were reported.
  In February 2011 a skin biopsy taken from a chronic injection site reaction revealed findings consistent with fibrosis and chronic inflammation described as "scleroderma-like reaction."[112] Immunohistochemical evaluation of the biopsy showed septal fibrosis and a predominantly mononuclear inflammatory infiltrate, again suggesting chronic inflammation.

The time to first injection site reaction is displayed in the figure below.

[109] Hair growth -- Subjects had an injection site reaction adverse event coded to at least one of these Preferred Terms: Hair growth, Hypertrichosis, or Hirsutism at injection site.
[110] Narrative on p. 4176-4178 120-day safety update report. Submitted to NDA 206031 on August 24, 2015.
[111] Narrative on p. 4192-4196 120-day safety update report. Submitted to NDA 206031 on August 24, 2015.
[112] Skin biopsy report. Submitted in Module 5.3.5.2 on 8/24/2015 to NDA 206031.

Figure 6. Cumulative distribution of time to first injection site reaction for subjects receiving drisapersen 6 mg/kg/week in DMD114349 (DMD114349 and parent studies)



Source: Sponsor Figure 5. P. 102 Summary of Clinical Safety

The table below summarizes the outcome and duration of injection site reactions in repeat dose studies. Of the 3872 injection site reaction events reported during drisapersen treatment, 795 (20.9%) events were reported as unresolved by the end of the studies. For resolved events for which the duration could be calculated, the mean duration of injection site reactions was 57.7 days (maximum duration 1217 days).

Table 32. Summary of outcome and duration of on-treatment injection site reaction events (repeat dose studies)

	Placebo N= 95	Drisapersen 3mg/kg/week N=17	Drisapersen 6mg/kg/week N=267	Drisapersen 6mg/kg intermittent N=38	Drisapersen all regimens N=285
Number of subjects (%)	21 (22.1)	11 (64.7)	210 (78.7)	31 (81.6)	224 (78.6)
Number of events	64	98	3477	261	3872
Outcomes, n (%)					
N	64	98	3451	224	3809
Recovered/resolved	58 (90.6)	66 (67.3)	2023 (58.6)	152 (67.9)	2275 (59.7)
Recovering/resolving	1 (1.6)	11 (11.2)	122 (3.5)	23 (10.3)	156 (4.1)
Not recovered/not resolved	1 (1.6)	5 (5.1)	739 (21.4)	49 (21.9)	795 (20.9)
Recovered/resolved with sequelae	4 (6.3)	16 (16.3)	567 (16.4)	0	583 (15.3)
Duration (days)					
N	61	82	2586	154	2855
n with missing data (including ongoing AEs)	3	16	891	107	1017
Mean (SD)	12.6 (9.18)	27.0 (22.89)	57.9 (128.99)	81.6 (176.86)	57.7 (129.81)
Median	13.0	21.5	14.0	10.0	14.0
Min, max	1, 57	3, 119	1, 1217	1, 801	1, 1217

Source: Sponsor Table 51. Summary of Clinical Safety

In the drisapersen 6 mg/kg/week group, AEs reported at least 5 times that were most frequently unresolved were: lipodystrophy acquired (81.0% unresolved), injection site atrophy (75.0% unresolved), injection site induration (56.9% unresolved), hyperaemia (50.0% unresolved), injection site nodule (45.5% unresolved), pigmentation disorder (42.9% unresolved), injection site discolouration (42.8% unresolved), and injection site reaction (25.5% unresolved).

For resolved events in the drisapersen 6 mg/kg/week group, the injection site reactions that had the longest mean durations included: fat tissue decreased (86.5 days; n=4), injection site erythema (57.8 days; n=953), injection site pain (59.6 days; n=173), injection site nodule (84.0 days; n=12), injection site discolouration (86.0 days; n=309), erythema (91.3 days; n=3), injection site reaction (116.6 days; n=195), injection site induration (143.7 days; n=76), injection site atrophy (270.3 days; n=12), lipodystrophy acquired (311.0 days; n=4), and injection site hypertrophy (323.0 days; n=3).

Medical photography is a standard method for documenting dermatologic conditions. No photographs of injection site reactions were prospectively collected and documented in any of the drisapersen studies included in the NDA. The only available photographs documenting injection site reactions were from 12 subjects in a DMD114673 substudy, who had a period of relatively intense subcutaneous administration exclusively in the abdomen from between approximately 50 and 72 weeks of weekly treatment.[113] These photographs are accompanied by limited documentation. The photographs shown below document injection site reactions at sites other than the abdomen, which were not subjected to relatively intense subcutaneous administration.

Reviewer comment: To maintain subject privacy, subject numbers and potentially identifying information (e.g., age, country of origin) are not included with these figures.

[113] Submitted to NDA 206031 on 6/19/2015.

Figure 7. Injection site reaction: Leg ulcer

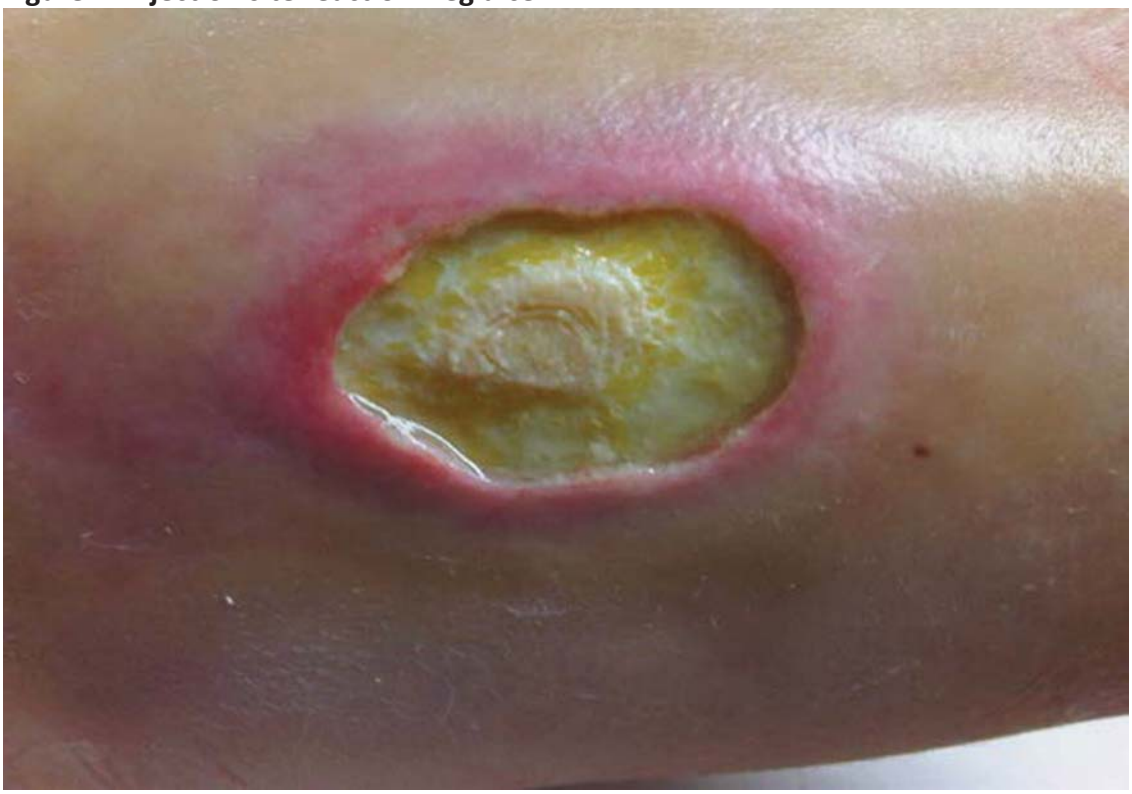


Figure 8. Injection site discoloration

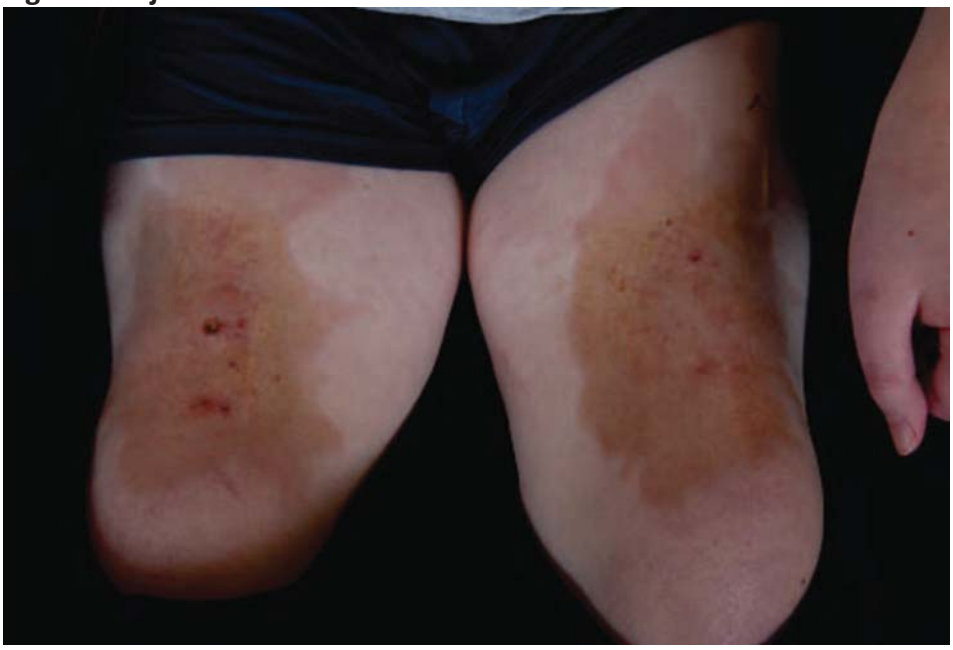
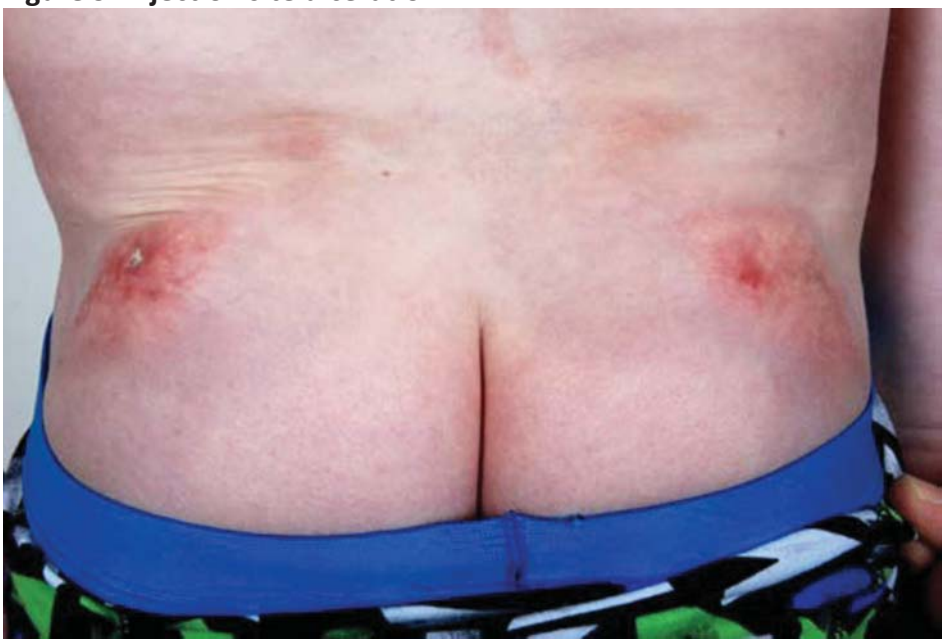


Figure 9. Injection site ulceration



Reviewer comment: At the time of this review, well-documented photographs of drisapersen injection site reactions are not available. The Division has asked the Sponsor to include medical photography as a part of standard documentation of moderate to severe injection site reactions in the limited number of subjects participating in ongoing studies. A report with this information is pending at the time of this review.

Reviewer conclusion

To facilitate proper administration of drisapersen, the Sponsor advises that dosing be performed by a medical professional. The Sponsor's proposed label provides detailed instructions, including guidelines for injection site rotation. Injection site rotation sites were added twice in the clinical program. The label administration instructions are the same as the last version of instructions in clinical studies. It is unclear whether there are any measures that can decrease the frequency or severity of injection site reactions with drisapersen.

8.5.4. **Inflammation**

In nonclinical studies, inflammatory effects of drisapersen were evident in mice and monkeys, impacting a multitude of organs and tissues and implicated in the majority of premature sacrifices or deaths. Inflammatory effects were evident at all dose levels in all species and were characterized by a dose related increase in (basophilic) granular/ vacuolated macrophages,

enlargement of lymphoid organs (spleen, lymph nodes) associated with lymphoid hyperplasia, and lymphocytic cell infiltrates in multiple tissues including at the site of injections. Changes were generally not associated with fibrosis, except at the injection sites following chronic dosing in mice and monkeys, and in the salivary glands in the monkey. Details of the mechanism of these inflammatory changes are not understood.

In three monkeys treated with drisapersen, high grade vascular changes consistent with those expected from complement-mediated effects were associated with thrombus formation resulting in myocardial infarction and early termination in two of these three monkeys.

The following laboratory markers of inflammation were measured in clinical studies: complement factor C3, haptoglobin, fibrinogen, high sensitivity C-reactive protein (hsCRP), immunoglobulin IgG, and Monocyte chemoattractant protein-1 (MCP-1). Inflammatory marker changes in placebo-controlled studies are summarized in the table below.

Table 33. Summary of inflammatory markers: baseline mean (SD) and mean (SD) changes from baseline at Weeks 24 and 48 (placebo-controlled studies)

	Placebo N=95		Drisapersen 3mg/kg/wk N=17		Drisapersen 6mg/kg/wk N=161		Drisapersen 6mg/kg intermittent [a] N=17		Drisapersen all regimens N=195	
Complement factor C3 (g/L)										
Baseline mean (SD)	n=95	1.235 (0.1982)	n=17	1.182 (0.2434)	n=161	1.269 (0.2223)	n=17	1.225 (0.2879)	n=195	1.257 (0.2306)
Week 24: Mean (SD) change	n=94	0.004 (0.2116)	n=15	-0.043 (0.1642)	n=159	-0.075 (0.2165)	n=17	-0.050 (0.1507)	n=191	-0.070 (0.2073)
Week 48: Mean (SD) change	n=77	-0.025 (0.1686)	NA	NA	n=137	-0.085 (0.2374)	n=15	-0.008 (0.1873)	n=152	-0.077 (0.2335)
Haptoglobin (g/L)										
Baseline mean (SD)	n=95	1.274 (0.3965)	n=17	1.182 (0.3762)	n=161	1.278 (0.4223)	n=17	1.205 (0.4406)	n=195	1.264 (0.4193)
Week 24: Mean (SD) change	n=93	0.016 (0.4068)	n=15	-0.087 (0.2973)	n=158	0.035 (0.3922)	n=17	-0.012 (0.3944)	n=190	0.021 (0.3856)
Week 48: Mean (SD) change	n=77	-0.005 (0.3901)	NA	NA	n=135	0.101 (0.4763)	n=15	0.103 (0.5553)	n=150	0.101 (0.4827)
Fibrinogen (g/L)										
Baseline mean (SD)	n=95	1.889 (0.4234)	n=17	1.714 (0.4989)	n=160	1.970 (0.4794)	n=17	2.004 (0.5379)	n=194	1.951 (0.4893)
Week 24: Mean (SD) change	n=91	0.044 (0.5656)	n=15	0.027 (0.5094)	n=155	0.118 (0.4987)	n=16	-0.106 (0.5457)	n=186	0.091 (0.5051)
Week 48: Mean (SD) change	n=78	0.041 (0.4662)	NA	NA	n=127	0.289 (0.6240)	n=15	0.140 (0.5060)	n=142	0.273 (0.6128)
hsCRP (mg/L)										
Baseline mean (SD)	n=95	0.51 (0.609)	n=17	0.58 (1.133)	n=161	0.82 (3.610)	n=17	3.55 (11.948)	n=195	1.03 (4.821)
Week 24: Mean (SD) change	n=93	0.39 (2.377)	n=15	-0.01 (1.068)	n=158	0.55 (4.593)	n=17	-2.85 (12.088)	n=190	0.20 (5.561)
Week 48: Mean (SD) change	n=78	0.48 (2.961)	NA	NA	n=135	0.83 (2.868)	n=15	-0.34 (14.353)	n=150	0.72 (5.185)
Immunoglobulin IgG (g/L)										
Baseline mean (SD)	n=95	7.948 (1.9785)	n=17	6.826 (1.8668)	n=161	7.664 (1.6923)	n=17	6.669 (1.6714)	n=195	7.504 (1.7327)
Week 24: Mean (SD) change	n=94	-0.170 (1.1140)	n=14	-0.315 (0.8670)	n=159	0.878 (1.4884)	n=17	0.636 (1.2840)	n=190	0.768 (1.4635)
Week 48: Mean (SD) change	n=78	-0.198 (1.3426)	NA	NA	n=136	1.786 (1.6481)	n=15	1.257 (1.8415)	n=151	1.734 (1.6692)
MCP-1 (ng/L)										
Baseline mean (SD)	n=82	805.12 (445.186)	n=17	931.18 (506.123)	n=148	915.62 (487.629)	n=4	703.55 (280.693)	n=169	912.16 (484.712)
Week 24: Mean (SD) change	n=80	18.21 (605.336)	n=15	-187.41 (556.790)	n=146	162.80 (623.632)	n=4	32.03 (340.773)	n=165	127.80 (618.750)
Week 48: Mean (SD) change	N=65	-87.83 (575.384)	NA	NA	n=123	305.08 (1073.622)	n=4	544.13 (660.044)	n=127	312.61 (1062.168)

Source: Table 84. Summary of Clinical Safety. Submitted to NDA 206031 on 4/27/2015.
Table includes data from studies DMD114117, DMD114044, and DMD114876 (first 24 weeks only).

Mean changes in complement C3 levels and haptoglobin were similar between drisapersen 6 mg/kg/week and placebo subjects. Mean values for fibrinogen were similar for drisapersen 6 mg/kg/week and placebo at baseline and at Week 12 and Week 24. At Week 48, mean change in fibrinogen was slightly higher at Week 48 in drisapersen subjects (0.289 g/L), compared to placebo subjects (0.04 g/L). Similar results were seen for immunoglobulin IgG.

For MCP-1, mean baseline values were similar for drisapersen 6 mg/kg/week and placebo. At Week 24 and Week 48, mean increases in this parameter were observed in subjects treated with drisapersen 6 mg/kg/week that were not seen in placebo-treated subjects. Mean baseline values for hsCRP were higher for drisapersen 6 mg/kg/week than for placebo. Mean increases were observed in both treatment groups at Week 12 that were larger for placebo than for drisapersen. At Week 24, the mean increases were similar in the two treatment groups and at Week 48, the mean increase with placebo was smaller than that seen with drisapersen.

The percentages of subjects with shifts from normal to high or low in complement C3 at each time point in the studies were similar for placebo and drisapersen at Week 12 and Week 24.[114] At Week 48, the percentage of subjects shifting from normal to low complement C3 was higher for drisapersen than for placebo (16 [11.7%] subjects versus 1 [1.3%] subject, respectively). At Week 48, there were 10 (8.1%) subjects in the drisapersen 6 mg/kg/week group with a shift from normal to high for MCP-1 compared with 1 (1.5%) subject in the placebo group.

In placebo-controlled studies inflammation adverse events occurred at similar rates in drisapersen 6 mg/kg/week subjects (29.8%), compared to placebo (27.4%) (see table below).

Table 34. Summary of on-treatment inflammation events by Preferred Terms (placebo-controlled studies)

Adverse event Preferred term	Placebo N=95 n (%)	Drisapersen 6mg/kg/wk N=161 n (%)
Any inflammation event	26 (27.4)	48 (29.8)
Pyrexia	21 (22.1)	41 (25.5)
Influenza like illness	2 (2.1)	2 (1.2)
Blood fibrinogen increased	1 (1.1)	2 (1.2)
Complement factor C3 decreased	0	2 (1.2)
Haptoglobin increased	3 (3.2)	1 (0.6)
Haptoglobin abnormal	0	1 (0.6)
Immunology test abnormal	0	1 (0.6)
Neutrophil toxic granulation present	1 (1.1)	0

Source: Sponsor Table 61. P. 125 Summary of Clinical Safety. Submitted to NDA 206031 on 4/27/2015.

Reviewer comment: There was 1 SAE in DMD114044 Subject 126 that was categorized as an inflammation event, coded to a PT 'Pyrexia.' The SAE occurred with an injection site reaction, as opposed to a more general process of inflammation.

Adverse event coding usually does not reflect a possible underlying inflammatory process. Thus, it is difficult to identify events through a search of specific MedDRA terms. This reviewer evaluated drisapersen adverse events for a possible inflammatory etiology.

[114] ISS Table 11.82

SAEs in drisapersen subjects that had or may have had an inflammatory etiology include the following:

- DMD114044 Subject 1111: PT Myocardial ischemia (possibly related to inflammation)
  This 6 year old male from Chile with no previous cardiac medical history and no relevant concomitant medications, had an SAE coded to the PT Myocardial ischemia. He had acute precordial chest pain, and ECG was reported as consistent with subendocardial ischemia. Cardiac enzymes were not performed. Echocardiogram was normal. ECG changes and chest pain resolved on the same day after a period of observation. Drisapersen was withheld for 4 weeks.
  Reviewer comment: This SAE is possibly related to drisapersen. Myocardial ischemia is rare in children. No structural cardiac abnormalities were reported on echocardiogram. He tested positive for anti-drisapersen antibodies.[115] *Inflammatory markers at the time of the event (April 20-25, 2012) were not measured. On May 9, 2012 he had an elevated sensitivity C-reactive protein (hsCRP) level of 7.9 mg/L.*[116- 117] *(It is unclear to what degree this laboratory value is drug related.) Prior to the SAE, this subject's hsCRP levels were 0.2-0.6 mg/L.*

- DMD114044 Subject 127: PT Intracranial venous sinus thrombosis (possibly related to inflammation)
  This event occurred in a 7 year old boy from Brazil. On 12/4/2012, high sensitivity C-reactive protein (hsCRP) was elevated at 9.5 mg/L. One week later, on Dec. 11, 2012 (6 months after his first dose of drisapersen), he developed a headache. Over the next few days, he developed seizures, strabismus, and severe thoraco-lumbar pain. A head CT showed hyperattenuating content partially filling the superior sagittal sinus and the straight sinus. Neurological assessment confirmed paralysis of cranial nerve VI (abducens) and signs of thrombosis of venous sinuses. The event of spinal pain was considered resolved on 1 February 2013, and the event of intracranial venous sinus thrombosis was considered resolved with sequelae (paralysis of the VI cranial nerve) on that same date.
  Reviewer comment: The cause of this SAE is unclear. Coagulation abnormalities have been reported with DMD.[118] *Fibrinogen, aPTT, INR, platelet count, and hemoglobin were normal. Conclusive anti-drisapersen antibody testing was not available.*[119] *High*

[115] P. 48 of the STD 2015-012 study report. Link located on p. 72 of the Summary of Clinical Pharmacology.
[116] Subject profile submitted to NDA 206031 on 4/27/2015. No laboratory range of normal values was provided.
[117] For all subjects, the Sponsor provided a normal range of 0-3 mg/L for hsCRP. This is an adult reference range, because a reference range for ages 0-17 has not been established (Sponsor 9/21/2015 submission to NDA 206031).
[118] Toshio Saito (2014). Coagulation and Fibrinolysis Abnormalities in Patients with Muscular Dystrophy, Fibrinolysis and Thrombolysis, Dr. Krasimir Kolev (Ed.), ISBN: 978-953-51-1265-5, InTech, DOI: 10.5772/57411. Available from: http://www.intechopen.com/books/fibrinolysis-and-thrombolysis/coagulation-and-fibrinolysis-abnormalities-in-patients-with-muscular-dystrophy
[119] P. 48 of the STD 2015-012 study report. Link located on p. 72 of the Summary of Clinical Pharmacology.

sensitivity C-reactive protein was elevated 1 week prior to the onset of headache. It is unclear whether drug-related inflammation may have contributed to this event.

- DMD 114349 Subject 1310: PT Small intestinal obstruction (inflammatory changes seen in the duodenum and colon)
  The first drisapersen dose for this 8 year old boy from Canada was August 3, 2011 in Study 114044. On Oct. 10, 2011 he had an AE of abdominal pain. He had gastrointestinal AEs intermittently throughout Study 114044 (e.g., abdominal pain, vomiting diarrhea). In Study 114349 (June 10, 2013), he had a partial small bowel obstruction. He underwent endoscopy and colonoscopy under general anesthesia. Biopsies of the duodenum, stomach, distal esophagus, and colon showed non-specific inflammatory changes in the duodenum, possibly drug-related or infectious in nature. No inflammatory changes were noted in the large bowel. Endoscopy revealed evidence of mild esophagitis, gastritis associated with ulceration and erosions, and duodenitis. The gastric erosions were suggested to be secondary to steroid use. There was evidence of moderate patchy colitis in about 1/3 of the colon, suggestive of a diffuse inflammatory or infective process. No evidence of mycoplasmal or mycobacterial infection was noted. *Haptoglobin results were normal in this subject. On the measurement prior to the event (May 8, 2013; 33 days prior), High sensitivity C-Reactive protein (hsCRP) was 2.3 mg/L, which was an increase from previous values which ranged from 0.2 – 0.9 mg/L. No complement factor C3, IgG, and MCP-1 results were provided for this subject.*

- DMD114673 Subject 105: PT Convulsion (possible contribution of drug-related inflammation)
  On (b) (6), 2 days after his latest dose of drisapersen, this 8 year old male from Belgium developed a fever of 39.5°C. Four hours later he had a generalized seizure, which lasted 20 minutes. He was hospitalized. Viral swab was positive for H1N1 influenza A. No action was taken with drisapersen treatment.
  Reviewer comment: This subject's seizure is likely related to his fever and H1N1 influenza A infection, which can lead to seizures (with or without fever).[120-121] *An inflammatory effect (e.g., cerebral vasculitis) of drisapersen contributing to this seizure is possible. Clonic seizures occurred in nonclinical studies.*

- DMD114117 Subject 2132: PT Myocarditis. (possible contribution of drug-related inflammation)
  In November 2011, this 6 year old boy from Spain, had chest pain and was diagnosed with myocarditis. Serology results for coxsackie virus, performed on December 16, 2011, were: coxsackie virus IgG 396 U/mL (normal range: 80 – 100); and coxsackie virus IgM

[120] Pinki,S, et al. "Neurological complications of pandemic influenza A H1N1 2009 infection: European case series and review." European journal of pediatrics 170.8 (2011): 1007-1015.
[121] The event is consistent with a complex febrile seizure, given the subject's age and the long seizure duration of 20 minutes.

45 U/mL (normal range: 30 – 50).[122] No action was taken with drisapersen in response to this myocarditis event. The event was reported to be resolved with sequelae. (Sequelae were not reported.)[123]
Reviewer comment: This subject had an SAE of myocarditis in the setting of a positive coxsackie virus serology. Myocarditis is a rare event in children. This event may be an event of viral myocarditis. An inflammatory drug effect is also a possible cause. The event was reported as resolved while the patient continued drisapersen treatment.

Reviewer conclusion:

Larger changes in inflammatory markers MCP-1, complement C3, and hsCRP occurred in drisapersen subjects, compared to placebo. Some SAEs in drisapersen subjects had or may have had an inflammatory etiology. Elevations in hsCRP were associated with some events. However, hsCRP is a nonspecific marker of inflammation and can be elevated for a variety of reasons. The utility of hsCRP in predicting drug-related inflammatory events is unclear.

This reviewer supports describing preclinical and clinical inflammation findings in the product label. If a patient has inflammatory changes after drisapersen treatment, withholding treatment may be considered if the risk outweighs the benefit.

8.5.5. **Coagulation disorders**

In drisapersen preclinical studies, vascular thrombosis and inflammation occurred in some animals that died prematurely.

Sheehan and Lan[124] published a study that demonstrated aPTT prolongation (using in vitro coagulation assays in human plasma and purified enzyme systems) with ISIS 2302, which, like drisapersen, is a phosphorothioate oligonucleotide. In this study, ISIS 2302 showed partial inhibition of intrinsic tenase activity, which was oligonucleotide sequence–independent but required the phosphorothioate backbone. The authors suggested that inhibition of intrinsic tenase is a general property of phosphorothioate oligonucleotides.

[122] Sponsor IR response submitted to NDA 206031 on 10/5/2015.
[123] Sponsor IR response submitted to NDA 206031 on 9/22/2015.
[124] Sheehan JP1, Lan HC. Phosphorothioate oligonucleotides inhibit the intrinsic tenase complex. *Blood.* 1998 Sep 1;92(5):1617-25.

Based on this preclinical and in vitro data, coagulation disorders have been an area of clinical safety concern during the drisapersen clinical development program. Coagulation abnormalities have been reported with DMD.[125]

In placebo-controlled studies, coagulation abnormalities were reported in 13 of 161 (8.1%) drisapersen subjects, compared to 14 of 95 (14.7%) placebo subjects. In repeat dose studies, coagulation abnormalities were reported in 33 of 285 (11.6%) drisapersen subjects. Two (0.7%) drisapersen subjects had coagulation abnormality SAEs, and 2 (0.7%) subjects experienced severe coagulation abnormality AEs; these AEs were related to laboratory abnormalities and are described below.

Table 35. Summary of coagulation abnormality adverse events (repeat dose studies)

Adverse event	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent N=38 n (%)	Drisapersen all regimens N=285 n (%)
Any coagulation abnormality	14 (14.7)	0	32 (12.0)	1 (2.6)	33 (11.6)
Any coagulation abnormality SAE	0	0	2 (0.7)	0	2 (0.7)
Any severe coagulation abnormality	0	0	2 (0.7)	0	2 (0.7)

Source: Sponsor Table 63. Summary of Clinical Safety

The most commonly reported coagulation abnormality adverse events were International normalized ratio increased, Blood fibrinogen decreased, Prothrombin time prolonged, Activated partial thromboplastin time prolonged, and Fibrin D dimer increased (see table below).

Table 36. Summary of coagulation abnormality adverse events by Preferred Term (repeat dose studies)

Adverse event preferred term	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent[a] N=38 n (%)	Drisapersen all regimens[b] N=285 n (%)
Any coagulation AE	**14 (14.7)**	**0**	**32 (12.0)**	**1 (2.6)**	**33 (11.6)**
International normalised ratio increased	4 (4.2)	0	11 (4.1)	1 (2.6)	12 (4.2)
Blood fibrinogen decreased	7 (7.4)	0	11 (4.1)	0	11 (3.9)
Prothrombin time prolonged	4 (4.2)	0	7 (2.6)	0	7 (2.5)
Activated partial thromboplastin time prolonged	3 (3.2)	0	6 (2.2)	1 (2.6)	7 (2.5)
Fibrin D dimer increased	2 (2.1)	0	6 (2.2)	0	6 (2.1)
Fibrin degradation products increased	0	0	2 (0.7)	0	2 (0.7)
Coagulation time prolonged	2 (2.1)	0	1 (0.4)	0	1 (0.4)
Activated partial thromboplastin time abnormal	1 (1.1)	0	1 (0.4)	0	1 (0.4)
Activated partial thromboplastin time	0	0	1 (0.4)	0	1 (0.4)
Bleeding time prolonged	0	0	1 (0.4)	0	1 (0.4)
Blood test abnormal	0	0	1 (0.4)	0	1 (0.4)

[125] Toshio Saito (2014). Coagulation and Fibrinolysis Abnormalities in Patients with Muscular Dystrophy, Fibrinolysis and Thrombolysis, Dr. Krasimir Kolev (Ed.), ISBN: 978-953-51-1265-5, InTech, DOI: 10.5772/57411. Accessed on 09/25/2015 at : http://www.intechopen.com/books/fibrinolysis-and-thrombolysis/coagulation-and-fibrinolysis-abnormalities-in-patients-with-muscular-dystrophy

Coagulation test abnormal	0	0	1 (0.4)	0	1 (0.4)
Blood fibrinogen	1 (1.1)	0	0	0	0
Fibrin degradation products	1 (1.1)	0	0	0	0
Hypofibrinogenaemia	0	0	1 (0.4)	0	1 (0.4)

Source: Sponsor Table 64. Summary of Clinical Safety

Standardised MedDRA Queries (SMQs)

In a search of repeat dose studies using the MedDRA Embolic and Thrombotic SMQ, 1 subject had an AE; this subject (Study 114044 subject 1270, treated with drisapersen 6 mg/kg/week) experienced an SAE of Intracranial venous sinus thrombosis (discussed in detail in Section 8.4.2). The subject was withdrawn from study treatment.

Reviewer comment: The mechanism for this subject's intracranial venous sinus thrombosis is unclear. Fibrinogen, aPTT, INR, platelet count, and hemoglobin were normal. Conclusive anti-drisapersen antibody testing was not available.[126] High sensitivity C-reactive protein was elevated 1 week prior to the onset of headache. It is unclear whether drug-related inflammation may have contributed to this event.

DMD114044 subject 527 experienced glomerulonephritis with renal venous thrombosis and pulmonary emboli. Renal vein thrombosis and pulmonary emboli were not coded separately from his overall diagnosis of glomerulonephritis, so the events were not detected by the SMQ search. (See Section 8.5.2for a detailed description of this subject.)

In a search of repeat dose studies using the MedDRA Haemorrhages SMQ, events were reported for 154 of 285 (56.8%) drisapersen 6 mg/kg/week subjects, compared to 40 of 95 (42.1%) placebo subjects. Preferred terms with an increased frequency in drisapersen subjects compared to placebo included Haematuria and Injection site haematoma; after adjusting for treatment exposure, the incidence rate of these Preferred Terms per 100 subject-years remained increased in drisapersen subjects.[127] Preferred terms Injection site bruising and Epistaxis were also more frequent in drisapersen subjects compared to placebo subjects; however, after adjusting for treatment exposure, incidence rates for these PTs were similar in drisapersen and placebo subjects. The rates of other Preferred Terms in the MedDRA Haemorrhages SMQ were similar between drisapersen and placebo subjects.

The only hemorrhage SMQ AE categorized as a Serious occurred in drisapersen-treated Subject 3000 (PT Haematuria). This SAE was related to anesthesia-associated rhabdomyolysis and was not drisapersen-related. (See Renal Toxicity Section 8.5.2 for additional details.)

Some hemorrhage SMQ adverse events in drisapersen subjects occurred as part of SAEs of immune thrombocytopenia. However, they were not categorized as SAEs themselves.

[126] P. 48 of the STD 2015-012 study report. Link located on p. 72 of the Summary of Clinical Pharmacology.
[127] Sponsor Table 35. Summary of Clinical Safety p. 72-73.

Reviewer comment:

MedDRA Haemorrhages SMQ Preferred Terms with an increased frequency in drisapersen subjects compared to placebo, after adjusting for treatment exposure, were Haematuria and Injection site haematoma. These events were associated with drisapersen safety issues of renal toxicity and injection site reactions. In addition, there were some bleeding adverse events associated with SAEs of immune thrombocytopenia. The frequencies of other hemorrhage SMQ events were similar in drisapersen and placebo subjects.

This reviewer concludes that increased frequency of hemorrhage SMQ events with drisapersen subjects occurred with drisapersen safety issues, including renal toxicity, injection site reactions, and thrombocytopenia.

Laboratory data

In analyses of changes from baseline to Week 48, mean changes in aPTT and INR[128] were seen which were similar for drisapersen and placebo.[129] Mean change from baseline to Week 48 in aPTT was -2.5 seconds in drisapersen 6 mg/kg/week subjects, compared to 0.3 seconds in placebo subjects. Mean change from baseline to Week 48 in INR was -0.1 in drisapersen 6 mg/kg/week subjects, compared to 0.1 in placebo subjects.

Shifts from baseline to worst post-treatment value[130] were similar in drisapersen and placebo subjects (see table below).

[128] Integrated Summary of Safety documents and datasets included the following terminology: "PTT (INR)", "PTT (INR) ratio", "Activated Partial Thromboplastin Time ratio (INR)" or "Activated Partial Thromboplastin Time ratio." In its response to an FDA information request (submitted to NDA 206031 on September 10, 2015) BioMarin confirmed that such terminology "all refer exclusively to INR calculated from prothrombin time (PT)."
[129] ISS Table 11.77
[130] Categorized according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0. NIH publication # 09-7473. May 29, 2009.

Table 37. Shift from baseline to worst post-treatment aPTT measurement based on CTCAE grade. Placebo-Controlled Studies

Laboratory Test Name / Treatment Group / Worst Post-Treatment CTCAE Grade	Baseline CTCAE Grade 0	1	2	3	4	Total
aPTT increased						
Placebo (n=95)						
0	42 (44.2%)	0	0	0	0	42
1	37 (38.9%)	8 (8.4%)	0	0	0	45
2	3 (3.2%)	3 (3.2%)	0	0	0	6
3	1 (1.1%)	0	0	1 (1.1%)	0	2
4	0	0	0	0	0	0
Total	83	11	0	1	0	95
6 mg/kg/week Drisapersen (n=162)						
0	85 (52.5%)	2 (1.2%)	0	0	0	87
1	51 (31.5%)	15 (9.3%)	0	0	0	66
2	4 (2.5%)	2 (1.2%)	1 (0.6%)	0	0	7
3	1 (0.6%)	1 (0.6%)	0	0	0	2
4	0	0	0	0	0	0
Total	141	20	1	0	0	162
INR						
Placebo (n=95)						
0	10 (10.5%)	0	0	0	0	10
1	54 (56.8%)	20 (21.1%)	1 (1.1%)	0	0	75
2	3 (3.2%)	1 (1.1%)	0	0	0	4
3	3 (3.2%)	3 (3.2%)	0	0	0	6
4	0	0	0	0	0	0
Total	70	24	1	0	0	95
6 mg/kg/week Drisapersen (n=162)						
0	8 (4.9%)	0	0	0	0	8
1	110 (67.9%)	20 (12.3%)	0	1 (0.6%)	0	131
2	8 (4.9%)	5 (3.1%)	3 (1.9%)	0	0	16
3	4 (2.5%)	2 (1.2%)	0	1 (0.6%)	0	7
4	0	0	0	0	0	0
Total	130	27	3	2	0	162

Source: Sponsor Table 10.1. Submitted to NDA 206031 on July 20, 2015.

Three subjects (Study DMD114349 Subjects 505, 687 and 2000, all treated with drisapersen 6 mg/kg/week) met the stopping criteria for disseminated intravascular coagulation (DIC); these subjects had a thrombocyte count <75 x 10^9/L and either fibrin split product test or D-dimer above the upper limit of the normal range. Disseminated intravascular coagulation was not confirmed in any of these subjects. These subjects are described in detail in the Thrombocytopenia Section 8.5.1. Anti-platelet antibodies were confirmed in Subjects 505 and 2000.

Reviewer comment: Testing for disseminated intravascular coagulation (DIC) was performed to evaluate for potential causes for thrombotic lesions in drisapersen preclinical studies.[131] *No cases of DIC were identified in preclinical studies of drisapersen. The drisapersen clinical studies do not provide evidence of DIC with drisapersen.*

Two (0.7%) drisapersen 6 mg/kg/week subjects experienced coagulation abnormality events that were SAEs:

[131] Consult review by Dr. Shashaty 05/05/2010.

- DMD114349 Subject 624 had an SAE with a PT Alanine aminotransferase increased. AST and ALT were not significantly different from baseline. GGT and bilirubin measurements were normal. He had one INR measurement of 6.2 on September 11, 2013. According to the narrative, the lab reported that the elevated INR may have resulted from clinical or sample integrity problems. Other INR values were 1.2, which was his baseline value. *There were no clinically significant changes in hepatic laboratory measurements, except for one increased INR measurement. The increased INR measurement resolved without treatment. The patient had no symptoms of coagulopathy.*

- DMD114044 Subject 37 had an SAE with a PT Alanine aminotransferase increased. [132] He had no change in clinical status. His laboratory measurements are summarized in the table below:

Table 38. DMD114044 Subject 37 Hepatic laboratory measurements

Date	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	LDH (IU/L)	Total Bilirubin (µmol/L)	Total Protein (g/L)	INR
Normal	*0-45*	*0-42*	*60-415*	*0-65*	*0-250*	*0-22*	*55-80*	*0.9-1.1*
29 Sep 2011 (Baseline)	425	323	124	11	1195	4	65	1.1
13 Oct 2011	**627**	**270**	**123**	**10**	**1521**	**5**	**68**	**2.2**
27 Oct 2011	**426**	**181**	**120**	**9**	**918**	**4**	**67**	**2.4**
8 Nov 2011	467	437	108	-	-	5	-	-
10 Nov 2011	449	208	113	10	1056	5	71	1.0
	466	213	114			4		
24 Nov 2011	304	168	116	10	781	5	67	1.2

Reviewer comment: The subject had increased ALT and INR without clinical symptoms. GGT remained normal.

Two (0.7%) drisapersen 6 mg/kg/week subjects experienced coagulation abnormality events that were categorized as severe:

- DMD114349 Subject 526, an 8 year old boy from France, who had severe adverse events of aPTT prolonged, International normalised ratio increased, and Prothrombin time prolonged. He had an elevated INR (1.4; normal range 0.9 – 1.1) and a normal aPTT pre-treatment at screening.
  He was treated with drisapersen 6 mg/kg/week in study DMD114044 from January 3, 2011 to November 28, 2011. He received open-label drisapersen at 6 mg/kg/week from January 3, 2012 in study DMD114349. On July 19, 2013, INR was 5.5 and aPTT was 54

[132] ISS p. 3824.

seconds. Dosing with drisapersen was interrupted. On 7/23/2013, INR and aPTT were similar to his baseline levels (see table below).

Table 39. Subject 526 aPTT and INR measurements

Test date	INR	aPTT	Notes
12/7/2010	1.4	29	Screening
1/3/2011	1.3	28	Baseline
1/17/2011	1.7	31	
2/28/2011	1.4	28	
3/28/2011	1.5	30	
4/26/2011	1.8	44	
5/9/2011	1.4	30	
6/21/2011	1.5	29	
8/16/2011	1.5	25	
10/10/2011	1.5	29	
12/6/2011	1.5	31	
1/3/2012	1.5	30	
2/27/2012	1.4	27	
3/26/2012	1.4	28	
4/23/2012	1.3	28	
5/21/2012	1.3	27	
6/19/2012	1.4	27	
8/13/2012	1.3	28	
10/8/2012	1.4	26	
12/4/2012	1.5	28	
1/28/2013	1.5	27	
3/25/2013	1.4	28	
5/21/2013	1.5	27	
7/15/2013	5.5	54	Measurements corresponding to the reported Severe adverse events
7/23/2013	1.5	30	
7/30/2013	1.6	29	
8/6/2013	1.7	40	
9/10/2013	1.6	32	

Source: ISS datasets
Normal laboratory range INR: 0.9-1.1
Normal laboratory range aPTT: 22-34 seconds

Reviewer comment: The cause of this subject's elevations in aPTT and INR in July 2013 is unclear. There were no adverse events that provide a possible explanation for the aPTT and INR results.

- Study DMD114349 Subject 000511, a 9 year old boy from France, had severe adverse events of Fibrin degradation products increased and Fibrin D-dimer increased. He started treatment with drisapersen 6 mg/kg/week in study DMD114044 on March 24, 2011. He started open-label drisapersen in study DMD114349 at 6 mg/kg weekly on March 2012. He was hospitalized for severe injection site edema on the back of his upper arm on (b) (6). On September 4, 2013, he developed a severe AE coded as 'Fibrin degradation products increased,' and on September 19, 2013, he had an increase in fibrin D-dimer. The events were not resolved by the end of the study. *Reviewer comment: It is possible that this subject's increase in fibrin degradation products, including D-dimer, were related to drisapersen. No baseline values were provided, so the change after starting drisapersen treatment could not be assessed.*

Reviewer conclusion and recommendations:

Sheehan and Lan[133] reported inhibition of intrinsic tenase and aPTT prolongation (using in vitro coagulation assays in human plasma and purified enzyme systems) with ISIS 2302, which, like drisapersen, is a phosphorothioate oligonucleotide. In clinical studies, some drisapersen subjects had increases in aPTT and INR without a clear cause. However, the frequencies of abnormal aPTT and PT measurements were similar in placebo and drisapersen subjects. An increased frequency of hemorrhage SMQ events with drisapersen subjects occurred with drisapersen safety issues, including renal toxicity, injection site reactions, and thrombocytopenia.

In drisapersen preclinical studies, vascular thrombosis and inflammation occurred in some animals that died prematurely. In clinical studies, the 2 cases of thrombosis (1 case of intracranial venous sinus thrombosis with normal aPTT and INR measurements and one case of thromboemboli related to glomerulonephritis and nephrotic syndrome) were not accompanied by changes in coagulation factors.

If drisapersen is approved, we will continue to evaluate cases of thrombosis, as well as related safety issues, including inflammation, renal toxicity, and immunogenicity. This reviewer supports describing the preclinical findings of vascular thrombosis and inflammation, as well as the case of intracranial venous sinus thrombosis in the Warnings and Precautions section of the prescribing information.

[133] Sheehan JP1, Lan HC. Phosphorothioate oligonucleotides inhibit the intrinsic tenase complex. *Blood.* 1998 Sep 1;92(5):1617-25.

8.5.6. Hepatic toxicity

The liver is considered a target organ of drisapersen, because most antisense oligonucleotides accumulate in the liver. Because it is deposited in the liver, drisapersen has the potential for hepatotoxicity.

Liver function tests that are most frequently evaluated (AST, ALT, and LDH) are commonly elevated in patients with DMD. With muscle degeneration, substantial quantities of these enzymes can be released from cardiac and skeletal muscle.[134] Liver enzyme elevations are often greater than 5x ULN, persist for years, and tend to be highest at a younger age in DMD patients. Gamma-glutamyl transferase (GGT) is a membrane bound enzyme produced primarily in the liver, with little or none produced from human skeletal muscle. GGT has been shown to be normal in DMD patients. In addition, the total bilirubin levels remain normal in DMD patients.

In repeat dose studies, hepatic toxicity adverse events were reported for 28 (10.5%) subjects treated with drisapersen 6 mg/kg/week (see tables below). The most commonly reported hepatic toxicity AE was glutamate dehydrogenase increased. In placebo controlled studies, the most commonly reported hepatic toxicity AE was also glutamate dehydrogenase increase (2.5% for drisapersen vs 0% for placebo).

Table 40. Summary of hepatic toxicity adverse events (on treatment in repeat dose studies)

Adverse event	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent N=38 n (%)	Drisapersen all regimens N=285 n (%)
Any hepatic abnormality	2 (2.1)	0	28 (10.5)	7 (18.4)	31 (10.9)
Any hepatic abnormality SAE	0	0	4 (1.5)	0	4 (1.4)
Any severe hepatic abnormality	0	0	1 (0.4)	0	1 (0.4)
Any hepatic abnormality leading to withdrawal	0	0	0	0	0

Source: Summary of Clinical Safety Table 67
Includes data from studies DMD114117, DMD114044, DMD114876, DMD114349, PRO051-02, and DMD114673.

[134] Koronoes D, Brown,M, Palis M, "'Liver Function Tests' Are Not Always Tests of Liver Function," *American Journal of Hematology*, Vol. 66 (2001): 46-48.

Table 41. Hepatic toxicity adverse event by MedDRA Preferred Term (on treatment in repeat dose studies)

Adverse event preferred term	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent N=38 n (%)	Drisapersen all regimens N=285 n (%)
Any hepatic abnormality AE	**2 (2.1)**	**0**	**28 (10.5)**	**7 (18.4)**	**31 (10.9)**
Glutamate dehydrogenase increased	0	0	13 (4.9)	5 (13.2)	16 (5.6)
Alanine aminotransferase increased	2 (2.1)	0	7 (2.6)	0	7 (2.5)
Gamma-glutamyltransferase increased	0	0	7 (2.6)	2 (5.3)	7 (2.5)
Alanine aminotransferase	0	0	1 (0.4)	0	1 (0.4)
Aspartate aminotransferase increased	0	0	1 (0.4)	0	1 (0.4)
Transaminases increased	0	0	1 (0.4)	0	1 (0.4)
Ultrasound liver abnormal	0	0	1 (0.4)	0	1 (0.4)
Hepatic function abnormal	0	0	1 (0.4)	0	1 (0.4)
Hepatic steatosis	0	0	1 (0.4)	0	1 (0.4)
Hepatocellular injury	0	0	1 (0.4)	0	1 (0.4)
Hepatomegaly	0	0	1 (0.4)	0	1 (0.4)
Hepatotoxicity	0	0	1 (0.4)	0	1 (0.4)
Liver disorder	0	0	1 (0.4)	0	1 (0.4)
Hepatitis	1 (1.1)	0	0	0	0

Source: Summary of Clinical Safety Table 68
Includes data from studies DMD114117, DMD114044, DMD114876, DMD114349, PRO051-02, and DMD114673.

Hepatic laboratory data

Mean baseline values for GGT were similar for drisapersen 6 mg/kg/week and placebo. Mean increases from baseline in GGT were larger for drisapersen than for placebo. For drisapersen 6 mg/kg/week subjects, the mean change in GGT from baseline was 2.9 IU/L at Week 12, 8.2 IU/L at Week 24, and 16.6 IU/L at Week 48, compared -0.1, -0.2, and 0.9 IU/L, respectively, in placebo subjects. In placebo-controlled studies, Grade 1[135] and Grade 2 increases in GGT occurred in 10 (5.1%) and 7 (3.6%) drisapersen subjects,[136] respectively. All subjects had normal GGT values at baseline. None of the 95 placebo subjects had any abnormal GGT values.

Mean increases in glutamate dehydrogenase (GLDH) from baseline were larger for drisapersen than for placebo. For drisapersen 6 mg/kg/week the mean change from baseline was 1.29 U/L at Week 12, 2.14 U/L at Week 24, and 3.59 U/L at Week 48, compared to 0.22, 0.09, and -0.04 U/L, respectively, for placebo. In placebo-controlled studies, 89 of 139 (54.9%) drisapersen 6 mg/kg/week subject had a shift from baseline normal to post-treatment high GLDH, compared to 26 of 87 (27.4%) placebo subjects.[137]

Only small mean changes in bilirubin values (total and direct) were observed for both

[135] CTCAE Grade 1 = >ULN - 2.5 x ULN; CTCAE Grade 2 = >2.5 - 5.0 x ULN
[136] P. 62-63 ISS addendum submitted to NDA 206031 on 7/20/2015.
[137] P. 83-84 ISS addendum submitted to NDA 206031 on 7/20/2015.

drisapersen 6 mg/kg/week and for placebo. In placebo-controlled studies, 2 of 194 (1.0%) drisapersen subject and 1 of 94 (1.1%) placebo subject had a shift from baseline normal to Grade 1 increased blood bilirubin (>ULN - 1.5 x ULN).[138] No subjects in any group had a shift in blood bilirubin greater than Grade 1.

There were no cases of Hy's law drug-induced liver injury (ALT increases ≥3 x ULN with concomitant elevations in total bilirubin ≥2 x ULN) during treatment in the drisapersen clinical program.

There were 4 hepatic SAEs in drisapersen subjects:

- DMD114349 Subject 624 had an SAE with a PT Alanine aminotransferase increased. AST and ALT were not significantly different from baseline. GGT and bilirubin measurements were normal. He had one INR measurement of 6.2 on September 11, 2013. According to the narrative, the lab reported that the elevated INR may have resulted from clinical or sample integrity problems. Other INR values were 1.2, which was his baseline value. *ad no symptoms of coagulopathy.*
- DMD114044 Subject 37 had an SAE with a PT Alanine aminotransferase increased. [139] He had no change in clinical status. His laboratory measurements are summarized in the table below:

Table 42. DMD114044 Subject 37 hepatic laboratory measurements

Date	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	LDH (IU/L)	Total Bilirubin (µmol/L)	Total Protein (g/L)	INR
Normal	*0-45*	*0-42*	*60-415*	*0-65*	*0-250*	*0-22*	*55-80*	*0.9-1.1*
29 Sep 2011 (Baseline)	425	323	124	11	1195	4	65	1.1
13 Oct 2011	627	270	123	10	1521	5	68	2.2
27 Oct 2011	426	181	120	9	918	4	67	2.4
8 Nov 2011	467	437	108	-	-	5	-	-
10 Nov 2011	449	208	113	10	1056	5	71	1.0
	466	213	114			4		
24 Nov 2011	304	168	116	10	781	5	67	1.2

Reviewer comment: The subject had increased ALT and INR without clinical symptoms. GGT remained normal.

[138] P. 59-60 ISS addendum submitted to NDA 206031 on 7/20/2015.
[139] ISS p. 3824.

- DMD114349 Subject 516 had an SAE with a PT Hepatocellular injury. He had hemolytic anemia and hepatocellular injury with mycoplasma infection.
  Reviewer comment: Hepatic abnormalities can occur with mycoplasma infection. It is unclear whether hepatic toxicity related to drisapersen contributed to his hepatic abnormalities. For details on this case, see Section 8.4.2.

- DMD114349 Subject 1281 had an SAE with a PT Hepatic abnormality. This 8-year-old subject was treated with placebo from 11 June 2012 to 09 May 2013 in study DMD114044. He started treatment with open-label drisapersen 6 mg/kg/week from 11 June 2013. On 25 June 2013, 14 days after the start of drisapersen, the subject developed grade 2 or moderate liver toxicity. The subject presented with international normalized ratio greater than 1.5 and ALT greater than 8xULN. Other relevant tests included an abdominal ultrasound which showed no change. Treatment with drisapersen was interrupted on 25 June 2013. The event resolved on 22 July 2013. Treatment with drisapersen was re-started on 29 July 2013. The event did not recur.
  Reviewer comment: No alternate etiology for this subject's liver toxicity was reported. Considering the accumulation of drisapersen in liver tissue, it is possible that this event is related to drisapersen.

Conclusion and monitoring recommendation

Phosphorothioate oligonucleotides are known to accumulate in the liver, and hepatic toxicity occurred in 10.5% subjects treated with drisapersen 6 mg/kg/week.
The Sponsor's proposed labeling related to hepatic laboratory monitoring and treatment stopping criteria is displayed below:

Label Section 2.2 Monitoring to Assess Safety

Elevations in Liver Enzymes [see Adverse Reactions (6.1)]
Conduct liver function tests prior to initiating [TRADENAME] treatment to assess baseline levels. Monitoring of liver function tests once every 6 months is recommended during [TRADENAME] treatment. Interrupt [TRADENAME] treatment if one or more of the following abnormalities are observed:

- Bilirubin 2 x ULN
- INR >1.5
- GGT >2 x ULN
- Symptoms of hepatitis (e.g., onset or worsening of nausea, anorexia, jaundice or abdominal pain) or hypersensitivity (fever, rash, eosinophilia)

Reviewer comment: This reviewer supports measurement of liver function tests, including GGT, bilirubin, and INR monthly, instead of every 6 months as proposed by the Sponsor. For most of the Phase 3 study, liver function tests were monitored monthly. This reviewer agrees with the Sponsor's proposed treatment stopping criteria based on liver function tests, which were used in clinical studies.

8.6. Specific Safety Studies/Clinical Trials

No specific safety studies were performed in the drisapersen clinical development program.

8.7. Additional Safety Explorations

8.7.1. Human Carcinogenicity or Tumor Development

Other than adverse events of Skin papilloma, no neoplasms were reported in the NDA submission.

8.7.2. Human Reproduction and Pregnancy

Not applicable. Subjects were boys between the ages of 5 to 16.

8.7.3. Pediatrics and Assessment of Effects on Growth

In placebo-controlled studies, changes in height, weight, and body mass index were similar in drisapersen 6 mg/kg/week and placebo subjects in up to 1 year of treatment.[140]

8.7.4. Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Overdose

There were no adverse events of overdose in the clinical program.

Drug abuse potential

In a consult regarding NDA 206031, the FDA Controlled Substance Staff found no issues related to drug abuse potential and provided the following conclusions:

> "1. No oligonucleotide is scheduled under the Controlled Substance Act and we are unaware of any instance(s) of abuse for oligonucleotides as a therapeutic class.
> 2. Drisapersen did not show any neurobehavioral changes in pre-clinical tests (Irwin test) and we are unaware of any pharmacological interaction with known CNS receptors of abuse (opioid, dopamine, etc). *Accordingly, the Sponsor's proposed label does not include a Section 9 (Abuse and Dependence).*
> 3. Drisapersen has no structural similarities between the chemical structure known drugs or abuse such as amphetamine, cocaine, benzodiazepines, opioids, LSD, MDMA, PCP, and cannabinoid agonists.
> 4. Drisapersen is not a prodrug of a known drug of abuse."

[140] Table 11.72 on ISS p. 3033-3037.

Reviewer comment: This reviewer agrees with the Controlled Substance Staff conclusions.

Withdrawal and rebound

This reviewer preformed a search using the MedDRA Drug Withdrawal SMQ. No drug withdrawal or rebound adverse events were found in drisapersen clinical studies.

8.8. Safety in the Postmarket Setting

8.8.1. Safety Concerns Identified Through Postmarket Experience

Not applicable. There is no previous postmarket experience.

8.8.2. Expectations on Safety in the Postmarket Setting

The clinical study findings may not fully represent drisapersen clinical safety in the setting of more advanced DMD. All studies [except for open label study PRO051-02 (N=12)] included only ambulant subjects. Also, the pharmacokinetics of drisapersen may be different in the non-ambulant population, because of differences in muscle mass.

If it is approved, drisapersen will be administered by a health professional, in a way similar to administration in clinical studies. Because its mechanism of action is specific to the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping, we do not anticipate significant off-label use of drisapersen.

8.9. Additional Safety Issues From Other Disciplines

The reader is referred to Section 4 of this review.

8.10. Integrated Assessment of Safety

The main safety concerns with drisapersen are drug-induced immune thrombocytopenia, renal toxicity, and injection site reactions, and inflammation. Hepatic accumulation is a class effect and hepatic adverse events occurred in the clinical trials.

Six drisapersen subjects (2%) had thrombocytopenia <20 x 10^9/L, levels at which patients are at risk potentially fatal complications, including spontaneous intracranial or intrapulmonary hemorrhage. Most of these patients had confirmed anti-platelet antibodies. These cases occurred 14-26 months after the first dose of drisapersen. Platelet monitoring every 2 weeks, patient education regarding the signs and symptoms of thrombocytopenia, and facilitating prompt medical assessment and treatment can mitigate the risk of clinically significant

bleeding. Concomitant use of drisapersen with antiplatelet, thrombolytic, or anticoagulant drugs is not recommended. Patients taking these drugs were excluded from clinical studies.

Renal toxicity was reported in 61% of drisapersen 6 mg/kg/week subjects, compared to 34% of placebo subjects. Proteinuria was the most common renal toxicity reported in 44% of drisapersen 6 mg/kg/week subjects compared to 23% of placebo subjects. One drisapersen subject developed multiple life-threatening thromboses with bilateral pulmonary emboli in the setting of glomerulonephritis with nephrotic syndrome. Renal laboratory monitoring every 2 weeks and cessation of drisapersen according to recommended laboratory criteria can mitigate the risk of glomerulonephritis.

Injection site reactions including discoloration, induration, pain, pruritus, bruising, atrophy, hematoma, and swelling, occurred in 79% of drisapersen patients. The risk for first injection site reaction occurred throughout the first 72 weeks of exposure. 21% of reactions were not resolved by the end of the studies. Reactions known to resolve lasted for a mean of 58 days and up to 1217 days. Injection site reactions occurred despite administration by a medical professional and rotation of injection sites. No other strategies to mitigate the risk of injection site reactions are known.

In nonclinical studies, inflammatory effects of drisapersen were evident in mice and monkeys, impacting a multitude of organs and tissues and implicated in the majority of premature sacrifices or deaths. In clinical studies, larger changes in inflammatory markers MCP-1, complement C3, and hsCRP occurred in drisapersen subjects, compared to placebo. Serious adverse events in drisapersen subjects had or may have had an inflammatory etiology included myocardial ischemia, intracranial venous sinus thrombosis, and small intestinal obstruction with inflammatory changes. Describing preclinical and clinical inflammation findings in the product label will educate prescribers about this issue. If a patient has inflammatory changes after drisapersen treatment, withholding treatment may be considered if the risk outweighs the benefit.

Phosphorothioate oligonucleotides are known to accumulate in the liver, and hepatic toxicity AEs occurred in 10.5% subjects treated with drisapersen 6 mg/kg/week. Monitoring of liver function tests, including GGT, bilirubin, and INR, monthly will mitigate the risk of hepatic toxicity.

I recommend a patient registry as a post-marketing requirement to evaluate the main safety risks of drisapersen in the post-marketing setting. I recommend a boxed warning with recommendations for monitoring and administration to mitigate the risks of renal adverse events, thrombocytopenia, and injection site reactions and I recommend a Medication Guide to educate patients about these risks.

9 Advisory Committee Meeting and Other External Consultations

An advisory committee meeting is scheduled. At the time of this review, it has not yet been held.

10 Labeling Recommendations

10.1. Prescribing Information

This reviewer recommends a boxed warning to describe the thrombocytopenia, renal toxicity, and injection site reactions with drisapersen. Recommendations for laboratory monitoring will be necessary for thrombocytopenia, renal toxicity, and hepatic toxicity. This reviewer also recommends a Medication Guide (see Section 10.2).

10.2. Patient Labeling

This reviewer recommends development of a Medication Guide. It will be an important tool in educating patients and caregivers about the symptoms of severe thrombocytopenia (e.g., petechiae, bruising, bleeding) to facilitate prompt recognition and treatment.

10.3. Non-Prescription Labeling

Not applicable.

11 Risk Evaluation and Mitigation Strategies (REMS)

11.1. Safety Issue(s) that Warrant Consideration of a REMS

Safety issues that warrant consideration of a REMS include:

- Thrombocytopenia
- Renal toxicity
- Injection site reactions

11.2. Conditions of Use to Address Safety Issue(s)

Conditions of use to address the safety issues listed in Section 11.1 are described below.

Thrombocytopenia

Laboratory monitoring every 2 weeks will be necessary to mitigate the risk of complications related to thrombocytopenia with drisapersen. Patients would see a medical professional

weekly for administration of drisapersen. This weekly contact with a medical professional will facilitate laboratory monitoring.

Educating patients and caregivers about the symptoms of severe thrombocytopenia (e.g., petechiae, bruising, bleeding) will be necessary to mitigate the risk of complications related to thrombocytopenia with drisapersen. A Medication Guide can be used as an education tool.

Renal toxicity

Quantitative urine testing every 2 weeks will be necessary to mitigate the risks of renal toxicity with drisapersen. With abnormal results, weekly quantitative urine testing may be necessary. Patients would see a medical professional weekly for administration of drisapersen. This weekly contact with a medical professional will facilitate quantitative urine testing.

Injection Site Reactions

Proper administration technique and knowledge about injection site reactions caused by drisapersen are necessary for safe use. Patients would see a medical professional weekly for administration of drisapersen, which will facilitate risk mitigation.

11.3. Recommendations on REMS

At the time of this review, the Division of Neurology Products and the Division of Risk Management (DRISK) in the Office of Surveillance and Epidemiology do not recommend a REMS for this NDA. Factors influencing this decision include:

- Duchenne muscular dystrophy (DMD) is mainly treated in specialty centers with detailed knowledge of DMD and its treatment.
- Patients would see a medical professional weekly for administration of drisapersen, which will facilitate risk mitigation.
- The DMD community is active in educating patients and families regarding DMD treatment.

12 Postmarketing Requirements and Commitments

I recommend a patient registry as a post-marketing requirement to evaluate the main safety risks of drisapersen in the post-marketing setting.

13 Appendices

13.1. References

References are included as footnotes throughout this review document.

13.2. Financial Disclosure

The reader is referred to the review of clinical efficacy by Dr. Veneeta Tandon.

13.3. Frequency of laboratory measurements

Phase I/II Studies								
Phase I/II Studies								
PRO051-02 - 5 escalating doses) (Study duration of 18 weeks (up to Visit 12)								
Parameter	Screening	Day of dosing	+ 1 day (+24 hrs)	+ 7 days	+ 14 days	+21 days	+ 28 days (1 Mth)	Follow-up (no treatment)
Haematology	S1 + S2	X (pre-dose t=-3 to 0)		X (pre-dose t=-3 to 0 and +24±3 hrs post-dose)	X pre-dose t=-3 to 0 and +24±3 hrs post-dose)	X pre-dose t=-3 to 0 and +24±3 hrs post-dose)	X (pre-dose t=-3 to 0 and +24±3 hrs post-dose)	+ 1 week, + 1 week, +3 weeks and + 3 weeks
Biochemistry	S1 + S2	X (pre-dose t=-3 to 0)						
Urinalysis	X	X (pre-dose t=-3 to 0)						
aPTT		X (pre-dose t=-3 to 0)	X (+3hrs, +6hrs, +24hr post-dose)				X (t=-3 to 0 pre-dose, + 3 hrs, + 6 hrs, +24 hrs post-dose)	
Complement split products		X (pre-dose t=-3 to 0)	X (+3hrs, +6hrs, +24hr post-dose)					
Inflammatory markers		X (pre-dose t=-3 to 0)	X (+3hrs, +6hrs, +24hr post-dose)					
Dystrophin AB		X (pre-dose t=-3 to 0)						+6 weeks and + 4 weeks
Haematology Parameters: haemoglobin, MCV, erythrocyte count, haematocrit, MCH, MCHC, reticulocyte count, thrombocyte count, leukocyte count, leukocyte differentiation (neutrophils, eosinophils, basophils, monocytes, lymphocytes) Biochemistry Parameters: sodium, potassium, calcium, BUN, creatinine, AST, ALT, GGT, LDH, alkaline phosphatase, bilirubin, CK, amylase, total protein, albumin, glucose, cholesterol, fatty acids (only for original protocol and Amendment 1) Urinalysis (dipstick): leukocytes, blood, protein, ketones, glucose, nitrite, pH, urine sediment, protein electrophoresis. Quantitative urinalysis (urine protein to creatinine ratio): protein, creatinine Complement Factors: complement factor C3, complement split products (C3a, SC5b-9, Bb) Inflammatory Factors:IL-6, TNF-α, MCP-1 HIV/HBV/HCV will be performed at screening visit Abbreviations: AB = Antibodies, aPTT = Activated Partial Thromboplastin Time, t = time								
DMD114673 (extension study of PRO051-02 - weekly dosing for 72 weeks, intermittent dosing[a] for 96 weeks with a sub-group testing IV dosing)								
Parameter	Frequency							
Haematology (excl Thrombocyte count)	every 4 weeks for 40 weeks, +8 weeks, every 4 weeks for 40 weeks, 9 cycles of +5 weeks, +3 weeks, +4 weeks + 5 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose, followed by 4 cycles of +1 week, +2 weeks (IV dosing subset only: pre-dose and +24hrs post-dose) + 1 week, + 2 weeks, + 4 weeks, + 5 weeks, + 3 weeks							
Biochemistry	every 4 weeks for 40 weeks, +8 weeks, every 4 weeks for 40 weeks, 9 cycles of +5 weeks, +3 weeks, +4 weeks + 5 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose, followed by 4 cycles of +1 week, +2 weeks (IV dosing subset only: pre-dose and +24hrs post-dose)							
Thrombocyte Count	every 2 weeks (for when study drug is administered)							
Urinalysis	weekly for 16 weeks and every 2 weeks for 20 weeks, + 4 weeks, + 8 weeks, + 4 weeks for 20 weeks, + 4 weeks, + 4 weeks, + 2							

Phase I/II Studies	
	weeks (for 8 weeks), 8 cycles of + 5 weeks, +1 week, + 2 weeks + 2 weeks, + 2 weeks, + 5 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose and metabolite recovery from 0-24 hours) and 4 cycles of +1 week, + 2 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose and metabolite recovery from 0-24 hours), + 1 week, + 2 weeks (for 6 weeks), + 5 weeks, + 1 week, + 2 weeks
Urine α1-microglobulin	every 12 weeks for 48 weeks, every 4 weeks for 40 weeks, intermittent dosing schedule cycle: + 5 weeks, +3 weeks, + 4 weeks (for 96 weeks), + 5 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose) and 4 cycles of +1 week, + 2 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose),+ 1 week, +2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
Urine cystatin C	every 4 weeks for 20 weeks (starting from Week 68), intermittent dosing schedule cycle: + 5 weeks, +3 weeks, + 4 weeks (for 96 weeks), + 5 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose) and 4 cycles of +1 week, + 2 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose), + 1 week, +2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
Complement split products	every 4 weeks for 88 weeks, intermittent dosing schedule cycle: + 5 weeks, +3 weeks, + 4 weeks (for 96 weeks), + 5 weeks (IV dosing subset only: pre-dose, + 2 hours, + 4 hours, + 6 hours, +24 hrs post-dose) and 4 cycles of +1 week, + 2 weeks (IV dosing subset only: pre-dose, + 2 hours, + 4 hours, + 6 hours, +24 hrs post-dose)+ 1 week, +2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
Troponin	every 4 weeks for 56 weeks only
Fibrinogen	every 4 weeks for 88 weeks, intermittent dosing schedule cycle: + 5 weeks, +3 weeks, + 4 weeks (for 96 weeks), + 5 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose, followed by 4 cycles of +1 week, +2 weeks (IV dosing subset only: pre-dose and +24hrs post-dose) , + 1 week, +2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
Haptoglobin	every 4 weeks for 88 weeks, intermittent dosing schedule cycle: + 5 weeks, +3 weeks, + 4 weeks (for 96 weeks), + 5 weeks (IV dosing subset only: pre-dose and +24 hrs post-dose, followed by 4 cycles of +1 week, +2 weeks (IV dosing subset only: pre-dose and +24hrs post-dose), +1 week, +2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
C-Reactive Protein	every 4 weeks for 88 weeks, intermittent dosing schedule cycle: + 5 weeks, +3 weeks, + 4 weeks (for 96 weeks), + 5 weeks (IV dosing subset only: pre-dose, + 2 hours, + 4 hours, + 6 hours, +24 hrs post-dose) and 4 cycles of +1 week, + 2 weeks (IV dosing subset only: pre-dose, + 2 hours, + 4 hours, + 6 hours, +24 hrs post-dose), +1 week, +2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
Inflammatory markers (IL-6, TNF-α, MCP-1)	MCP-1 as per biochemistry schedule IL-6/TNF-α - IV dosing subset only: pre-dose, + 2 hours, + 4 hours, + 6 hours, +24 hrs post-dose
Cystatin C	every 12 weeks for 96 weeks, 7 cycles of +4 weeks, + 5 weeks, + 3 weeks, then + 4 weeks, IV dosing: pre-dose and +24 hrs, followed by 4 cycles of +1 week, +2 weeks (pre-dose and +24hrs post-dose), +1 week, , + 2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
Coagulation Parameters (blood)	every 4 weeks for 88 weeks, intermittent dosing schedule cycle: + 5 weeks, +3 weeks, + 4 weeks (for 96 weeks), + 5 weeks (IV dosing subset only: pre-dose, + 2 hours, + 4 hours, + 6 hours, +24 hrs post-dose) and 4 cycles of +1 week, + 2 weeks (IV dosing subset only: pre-dose, + 2 hours, + 4 hours, + 6 hours, +24 hrs post-dose),+ 1 week, +2 weeks, + 4 weeks, + 5 weeks, + 3 weeks
Dystrophin AB	every 12 weeks for 24 week and then every 24 weeks thereafter

a. Intermittent dosing = 8 weeks of weekly treatment, 4 weeks off treatment (1 cycle)
Haematology: haemoglobin, haematocrit, MCV, MCH, MCHC, erythrocyte count, reticulocyte count, leukocyte count, leukocyte differential count (neutrophils, eosinophils, basophils, monocytes, lymphocytes), thrombocyte count, blood smear for schistocytes
Biochemistry Parameters: sodium, potassium, calcium, BUN, creatinine, creatinine clearance (calculated), cystatin C, AST, ALT, GGT, LDH, GLDH, alkaline phosphatase, bilirubin, amylases, CK, total protein, albumin, albumin/globulin ratio, glucose, cholesterol, cystatin C, MCP-1
Urinalysis: glucose, albumin, protein, creatinine, KIM-1, protein/creatinine ratio, microscopy of urine sediment for erythrocytes, leukocytes and casts, protein electrophoresis
Coagulation Parameters (blood): aPTT, PTT (INR)
HIV/HBV/HCV will be performed at screening visit

Phase I/II Studies			
Phase II Studies			
Parameter	Screening	Day of First Dose	Frequency
DMD114117 (48 Weeks Treatment Duration)			
Haematology (excl Thrombocyte count)	S1 + S2	X (pre-dose)	+2 weeks, + 2 weeks and every 4 weeks up to Week 25, and every 8 weeks thereafter (all samples pre-dose)
Biochemistry	S1 + S2	X (pre-dose)	+2 weeks, + 2 weeks and every 4 weeks up to Week 25, and every 8 weeks thereafter (all samples pre-dose)
Thrombocyte Count	S1 + S2	X (pre-dose)	Required every 2 weeks (all samples pre-dose)
Urinalysis	S1 + S2	X (pre-dose)	Every 2 weeks (all samples pre-dose)
24-hour Urine		X (pre-dose)	To be performed if stopping criteria met
Dystrophin AB		X (pre-dose)	+12 weeks, + 8 weeks, + 12 weeks (all samples pre-dose)
Coagulation Parameters	S1 + S2	X (pre-dose)	+2 weeks, + 2 weeks and every 4 weeks up to Week 25, and every 8 weeks thereafter (all samples pre-dose)
Renal Markers (α1-microglobulin/Cystatin C/KIM-1)	S1	X (pre-dose)	Every 4 weeks (all samples pre-dose)
Haematology: haemoglobin, haematocrit, MCV, MCH, MCHC, erythrocyte count, reticulocyte count, leukocyte count, leukocyte differential count (bands forms, segment forms, neutrophils, eosinophils, basophils, monocytes, lymphocytes), blood smear for schistocytes, Biochemistry Parameters: sodium, potassium, calcium, phosphate, BUN, creatinine, creatinine clearance, cystatin C, AST, ALT, GGT, LDH, GLDH, alkaline phosphatase, bilirubin (total), bilirubin (conjugated or "direct"), CK, total protein, albumin, albumin/globulin ratio, glucose, complement factor C3, haptoglobin, fibrinogen, CRP, IgG, Dystrophin antibodies, monocyte chemotactic protein-1 (MCP-1) Urinalysis (morning spot urine): glucose, albumin, protein, creatinine, protein/creatinine ratio, microscopy of urine sediment for erythrocytes, leukocytes and casts, protein electrophoresis Coagulation Parameters (blood): aPTT, PTT (INR) HIV/HBV/HCV will be performed at screening visit 1 Abbreviations: AB = Antibodies, KIM-1 = Kidney Injury Molecule 1, S = Screening, W = Week, HIV = Human Immunodeficiency Virus, HBV = Hepatitis B Virus, HCV = Hepatitis C Virus			
DMD114876 (24 Weeks Treatment Duration and 24 Weeks Follow-up)			
Haematology (excl Thrombocyte count)	S1	X (pre-dose)	+2 weeks, + 2 weeks, + 4 weeks and every 4 weeks until Week 24 then +6 until week 36, then + 12 weeks (all samples pre-dose)
Biochemistry	S1	X (pre-dose)	+2 weeks, + 2 weeks, + 4 weeks and every 4 weeks until Week 24, then +6 weeks until week 36, then + 12 weeks (all samples pre-dose)
Thrombocyte Count	S1	X (pre-dose)	Required every 2 weeks (all samples pre-dose)
Urinalysis	S1	X (pre-dose)	Every 2 weeks for 30 weeks + 18 weeks (all samples pre-dose)
24-hour Urine	S1 or S2		
Dystrophin AB		X (pre-dose)	+12 weeks and + 12 weeks (all samples pre-dose)
Fibrin Split Products, D-dimer and Schistocytes	S1	X (pre-dose)	every 4 weeks until Week 24 (all samples pre-dose)
Coagulation Parameters	S1	X (pre-dose)	+2 weeks, + 2 weeks, + 4 weeks and every 4 weeks until Week 24 then +6 until week 36, then + 12 weeks (all samples pre-dose)
Haematology: haemoglobin, haematocrit, MCV, MCH, MCHC, erythrocyte count, reticulocyte count, leukocyte count, leukocyte differential count (bands forms, segment forms, neutrophils,			

Phase I/II Studies

eosinophils, basophils, monocytes, lymphocytes)
Biochemistry Parameters: sodium, potassium, calcium, phosphate, BUN, creatinine, creatinine clearance (calculated), cystatin C, AST, ALT, GGT, LDH, GLDH, alkaline phosphatase, bilirubin (total), bilirubin (conjugated or "direct"), CK, total protein, albumin, globulin, albumin/globulin ratio, glucose, complement factor C3, haptoglobin, fibrinogen, hsCRP, IgG, Dystrophin antibodies, MCP-1
Urinalysis (morning spot urine): glucose, albumin, protein, creatinine, α1-microglobulin, protein/creatinine ratio, urine cystatin C, KIM-1, microscopy of urine sediment for erythrocytes, leukocytes and casts
Urinalysis (24-hour urine): protein, albumin, creatinine (urine and serum), protein electrophoresis
Coagulation Parameters (blood): aPTT, PTT (INR)
HIV/HBV/HCV will be performed at screening visit
Abbreviations: AB = Antibodies, KIM-1 = Kidney Injury Molecule 1, S = Screening, W = Week

Phase III Study

Parameter	Screening	Day of First Dose	Frequency
DMD114044 (48 Weeks Treatment Duration)			
Haematology (excl Thrombocyte count)	S1	X (pre-dose)	+2 weeks, + 2 weeks, and every 4 weeks (all samples pre-dose)
Biochemistry	S1	X (pre-dose)	+2 weeks, + 2 weeks, and every 4 weeks (all samples pre-dose)
Thrombocyte Count	S1	X (pre-dose)	Required every 2 weeks (all samples pre-dose)
Urinalysis	S1	X (pre-dose)	Every 2 weeks (all samples pre-dose)
24-hour Urine	X (prior to randomisation)		To be performed if stopping criteria met
Coagulation Parameters	S1	X (pre-dose)	+2 weeks, + 2 weeks, and every 4 weeks (all samples pre-dose)
Dystrophin AB		X (at randomisation)	every 12 weeks (all samples pre-dose)

Haematology: haemoglobin, haematocrit, MCV, MCH, MCHC, erythrocyte count, reticulocyte count, leukocyte count, leukocyte differential count (bands forms, segment forms, neutrophils, eosinophils, basophils, monocytes, lymphocytes), blood smear for schistocytes
Biochemistry Parameters: sodium, potassium, calcium, phosphate, BUN, creatinine, creatinine clearance (calculated), cystatin C, AST, ALT, GGT, LDH, GLDH, alkaline phosphatase, bilirubin (total), bilirubin (conjugated or "direct"), CK, total protein, albumin, globulin, albumin/globulin ratio, glucose (fasting), complement factor C3, haptoglobin, fibrinogen, CRP, IgG, Dystrophin antibodies, MCP-1
Urinalysis: glucose, albumin, protein, creatinine, α1-microglobulin, protein/creatinine ratio, urine cystatin C, KIM-1, microscopy of urine sediment for erythrocytes, leukocytes and casts, protein electrophoresis
Coagulation Parameters (blood): aPTT, PTT (INR)
HIV/HBV/HCV will be performed at screening visit
Abbreviations: S = Screening, W = Week

Phase I/II Studies		
Extension Phase Study		
Parameter	Day of First Dose	Frequency
DMD114349 (104 Weeks Treatment Duration and 20 Weeks Follow-up)		
Haematology (excl Thrombocyte count)	X (pre-dose)	+2 weeks, + 2 weeks, and every 4 weeks up to Week 24 and 8 weekly thereafter (all samples pre-dose)
Biochemistry	X (pre-dose)	+2 weeks, + 2 weeks, and every 4 weeks up to Week 24 and 8 weekly thereafter (all samples pre-dose)
Thrombocyte Count	X (pre-dose)	Required every 2 weeks (all samples pre-dose)
Urinalysis	X (pre-dose)	Every 2 weeks (all samples pre-dose)
Coagulation Parameters	X (pre-dose)	+2 weeks, + 2 weeks, and every 4 weeks up to Week 24 and 8 weekly thereafter (all samples pre-dose)
Dystrophin AB	X (pre-dose)	+12 weeks (removed in Amendment 1), + 12 weeks (all samples pre-dose)
Haematology: haemoglobin, haematocrit, MCV, MCH, MCHC, erythrocyte count, reticulocyte count, leukocyte count, leukocyte differential count (bands forms, segment forms, neutrophils, eosinophils, basophils, monocytes, lymphocytes), blood smear for schistocytes Biochemistry Parameters: sodium, potassium, calcium, phosphate, BUN, creatinine, creatinine clearance (calculated), cystatin C, AST, ALT, GGT, LDH, alkaline phosphatase, bilirubin (total), bilirubin (conjugated or "direct"), CK, total protein, albumin, albumin/globulin ratio, glucose, complement factor C3, haptoglobin, fibrinogen, CRP, MCP-1 Urinalysis (morning spot urine): glucose, albumin, protein, creatinine, protein/creatinine ratio, microscopy of urine sediment for erythrocytes, leukocytes and casts. Urinalysis (24-hour urine): protein, albumin, creatinine (urine and serum), protein electrophoresis Coagulation Parameters (blood): aPTT, PTT (INR) HIV/HBV/HCV will be performed at screening visit Abbreviations: W = Week		

Source: P. 223-227 Summary of Clinical Safety.

V. Statistical Review

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

sNDA/BLA Serial Number:	206,031
Drug Name:	Drisapersen
Indication(s):	Duchenne Muscular Dystrophy
Applicant:	Biomarin
Date(s):	Date of Submission: April 27, 2015 PDUFA Due Date: December 27, 2015
Review Priority:	Priority Review
Biometrics Division:	Division of Biometrics I
Statistical Reviewer:	Sharon Yan, Ph.D.
Concurring Reviewers:	Kun Jin, Ph.D., Team Leader James Hung, Ph.D., Director
Medical Division:	Neurology
Clinical Team:	Veneeta Tandon, M.D., Clinical Reviewer Evelyn Mentari, M.D., Safety Reviewer Ronald Farkas, M.D., Team Leader Eric Bastings, M.D., Deputy Director Billy Dunn, M.D., Director
Project Manager:	Fannie Choy, RPh.

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1 EXECUTIVE SUMMARY

The submission of this NDA contains one phase-3 study (DMD114044) and two phase-2 studies (DMD114117 and DMD114876). All three studies were randomized, double-blind, parallel-group studies in ambulant subjects with Duchenne muscular dystrophy (DMD) resulting from a mutation thought to be corrected by exon 51 skipping induced by drisapersen.

The phase-2 study DMD114117, with 53 randomized patients, included 4 treatment arms of 6 mg/kg drisapersen continuous regimen, 6 mg/kg drisapersen intermittent regimen, placebo continuous regimen and placebo intermittent regimen. The treatment of 6 mg/kg drisapersen continuous regimen met the primary objective by showing an adjusted mean improvement of 35 meters over 24 weeks in 6-minute walking distance (6MWD) test compared to combined placebo group (p=0.014). No treatment benefit was observed for the 6 mg/kg drisapersen intermittent regimen.

The phase-2 study DMD114876, with 51 randomized patients, included 4 treatment arms of 3 mg/kg drisapersen, 6 mg/kg drisapersen, and volume matched 3 mg/kg placebo and 6 mg/kg placebo. Neither of the drisapersen doses achieved statistically significant treatment effect. A numerical difference of 27 meters (p=0.069) in favor of 6 mg/kg drisapersen compared to combined placebo was observed.

The large phase-3 study DMD114044 conducted concurrently with a total of 186 randomized patients failed to demonstrate treatment benefit of drisapersen 6 mg/kg over placebo. The adjusted mean treatment difference of 10 meters carried a p-value of 0.415.

Differences among the 3 studies, mainly between the phase-2 and phase-3 studies in demographic and baseline characteristics, were observed. Patients enrolled in the phase-2 studies were relatively younger and less impaired with higher baseline 6MWD scores compared to the phase-3 study.

2 INTRODUCTION

2.1 Overview

DMD is a severe, progressive fatal pediatric neuromuscular disorder for which there is no available therapy. It occurs almost exclusively in males.

Drisapersen was studied under IND 105284 and was granted Orphan Drug Designation, Fast Track designation, and breakthrough therapy designation.

The submission of this NDA included 3 clinical studies: DMD114044, DMD114117, and DMD114876, referred as studies 044, 117 and 876 thereafter. Study 044 was a phase-3 study and studies 117 and 876 were phase-2 studies. All three studies were randomized, double-blind, parallel-

group studies in ambulant subjects with Duchenne muscular dystrophy (DMD) resulting from a mutation thought to be corrected by exon 51 skipping induced by drisapersen.
In Study 044, subjects were randomized to receive either drisapersen 6 mg/kg or dose-matched placebo (2:1 ratio) as subcutaneous injections once a week for 48 weeks. A total of 186 patients were randomized: 125 patients to the 6 mg/kg drisapersen group and 61 patients to the placebo group. The study endpoint was change from baseline in 6-minute walking distance (6MWD) test. No statistically significant treatment difference was found (p=0.415).

Study 117 included 2 drisapersen regimens, a 6 mg/kg continuous regimen and a 6 mg/kg intermittent regimen. A total of 53 patients were randomized. Patients were treated for 48 weeks, but the efficacy was evaluated at week 24. The same study endpoint as in Study 044 was used. A statistically significant (p=0.014) treatment benefit of 35 meters in the change from baseline in 6MWD was observed for the 6 mg/kg drisapersen continuous regimen compared to placebo. No treatment benefit was observed for the 6 mg/kg intermittent regimen.

Study 876 included a 3 mg/kg drisapersen dose group, a 6 mg/kg drisapersen dose group and dose volume matched placebo groups. A total of 51 patients were randomized in the 4 treatment groups. Patients were treated for 24 weeks. A numerical treatment difference of 27 meters in the change from baseline in 6MWD favoring 6 mg/kg drisapersen did not achieve statistical significance (p=0.069). The 3 mg/kg drisapersen dose did not show treatment benefit.

A summary of the phase-2 and phase-3 studies included in this review is presented in Table 1.

Table 1 List of studies included in this review

Study	Phase and Design	Duration of treatment	Dosage/ regiment	Comparator	# of Subjects randomized
Protocol 114044	Phase 3, randomized, double-blind, PBO-controlled	48 weeks	6 mg/kg/wk	Placebo	186
Protocol 114117	Phase 2, randomized, double-blind, PBO-controlled	48 weeks	6 mg/kg/wk continuous & 6 mg/kg/wk intermittent	Placebo	53
Protocol 114876	Phase 2, randomized, double-blind, PBO-controlled	24 weeks	6 mg/kg/wk & 3 mg/kg/wk	Placebo	51

2.2 Data Sources

All documents reviewed for this NDA submission are in electronic form with eCTD format. The electronic files are compatible with eCTD viewer software Global Summit. Both raw and derived datasets are included in the submission. The SAS programs for primary and secondary analyses

are also included. The path to CDER Electronic Document Room for documents of this NDA is listed below:
\\cdsesub1\evsprod\NDA206031

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

No issues in data and analysis quality were identified.

3.2 Evaluation of Efficacy

3.2.1 Evaluation of Study 044

3.2.1.1 Study Design – Study 044

The primary objective of Study 044 was to assess the efficacy of subcutaneous 6 mg/kg drisapersen versus placebo administered weekly over 48 weeks in ambulant subjects with DMD.

Study 044 was a phase 3, randomized, double-blind, parallel-group study in ambulant subjects with DMD resulting from a mutation thought to be corrected by exon 51 skipping induced by drisapersen. The study enrolled male patients with minimum age of 5 years.

Prior to randomization, subjects had two screening visits, one at 2~4 weeks prior to the first dose and one at 1~2 weeks prior to the first dose. The 6MWD test was performed at both screening visits as well as randomization/baseline visit. Subjects had to be able to complete 6MWD test with minimal distance of 75 meters at each pre-drug visit. In addition, results of 6MWD had to be within 20% of each other at each pre-drug visit.

Subjects were randomized to receive either drisapersen 6 mg/kg or dose-matched placebo (2:1 ratio) as subcutaneous injections once a week for 48 weeks. At the end of the treatment period, subjects who completed the study had the option to enter into an open-label extension study (Study DMD114349).

Study 044 was conducted in 44 centers in 19 countries. A schematic of the study design is presented in Figure 1.

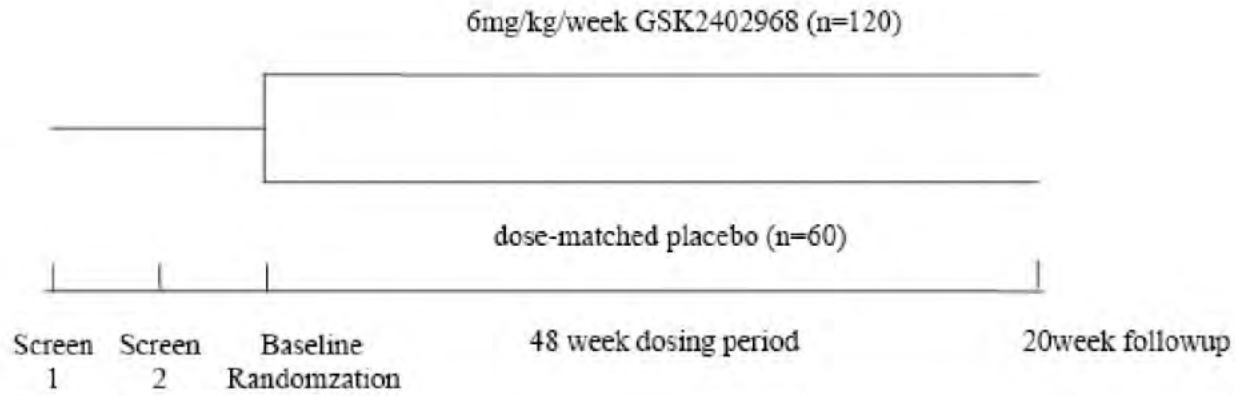

Figure 1 Study Schematic
Source: Clinical Study Report

3.2.1.2 Study Endpoints – Study 044

The primary efficacy endpoint was muscle function using 6-minute walking distance (6MWD) test assessed at Week 48.

During the 6MWD, subjects were asked to walk, at their own preferred speed, up and down a fixed distance until they were told to stop after 6 minutes. The subjects were warned of the time and were told that they could stop earlier if they felt unable to continue. The total distance walked within 6 minutes (or until the subject stopped in case of early termination of the test) was recorded in meters; any falls were also recorded.

The 6MWD was conducted at Screen 1, Screen 2, Baseline (Week 1), Weeks 12, 24, 36 and 48 or Early Withdrawal.

Key secondary endpoints (rank ordered) were:

- Change from baseline in Linearized North Star Ambulatory Assessment (NSAA) total score
- Change from baseline in 4-stair climb (ascent) velocity
- Change from baseline in the 10-meter walk/run velocity

3.2.1.3 Statistical Methodologies – Study 044

The primary population for efficacy evaluation was the ITT population, which was defined as all subjects who were randomized to the study, received at least one dose of study medication and had at least one post-baseline efficacy assessment.

Change from baseline in the 6MWD was analyzed on all available assessment data from the ITT population using a mixed model for repeated measures (MMRM) with restricted maximum likelihood estimation and an unstructured covariance matrix. The MMRM model included fixed

categorical terms for treatment, visit, treatment-by-visit interaction, country grouping, and continuous fixed covariates of baseline 6MWD and baseline 6MWD-by-visit interaction.

The primary time point of interest was the end of treatment (Week 48). Additional supportive analyses for treatment differences for Weeks 12, 24 and 36 were also estimated using the MMRM model.

If the assumptions of normality were not met, a log-transformation of the data prior to an MMRM analysis, or non-parametric analysis producing Hodges-Lehmann estimates of the Wilcoxon Rank Sum Test were to be provided. The primary inference was to be based on the MMRM models.

The following sensitivity analyses were to be performed to examine the robustness of the results in the presence of missing data, and to determine the impact of protocol violators.
1. ANCOVA utilizing the data from all observed cases (OC) for the ITT population.
2. ANCOVA utilizing last-observation-carry-forward (LOCF) data, for the ITT population.
3. ANCOVA utilizing data imputed via multiple imputation, for the ITT population.
4. MMRM analysis utilizing available assessment data from the PP population.

The ANCOVA models included fixed terms for treatment group, baseline 6MWD, and country/center grouping.

Confirmatory statistical testing was to be performed only for the primary variable and key secondary endpoints through hierarchical testing in the given order.

Each secondary endpoint was analyzed for the OC dataset using a mixed model for repeated measures (MMRM). The MMRM model for each secondary endpoint included fixed terms for treatment, visit, treatment-by-visit interaction, country/country group, baseline score, and baseline score by visit.

The study aimed to randomize 180 subjects, assuming an approximate 10% dropout, in order to recruit at least 162 evaluable subjects. The study was designed to show superiority of drisapersen compared with dose-matched placebo with 90% power to detect a difference in 6MWD between drisapersen and placebo of 30 meters, assuming a common standard deviation of 55 meters.

3.2.1.4 Study Population Results – Study 044

3.2.1.4.1 Patient Disposition

A total of 186 subjects were randomized in 44 centers in 19 countries. A total of 5 subjects consisting of 1 (2%) subject in the placebo group and 4 (3%) subjects in the drisapersen group withdrew from the study prematurely (Table 2).

Table 2 Summary of Subject Disposition (Safety Population) – Study 044

Subject Status	Number (%) of Subjects		
	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)	Total (N=186)
Completed	60 (98)	121 (97)	181 (97)
Withdrawn	1 (2)	4 (3)	5 (3)
Primary reason for study withdrawal[a]			
Adverse event[b]	0	2 (2)	2 (1)
Withdrew consent	0	2 (2)	2 (1)
Protocol deviation[c]	1 (2)	0	1 (<1)

Source: Clinical Study Report

3.2.1.4.2 Patient Demographic and Baseline Characteristics

Demographic characteristics were similar across treatment groups, though the drisapersen group had slightly higher percentage of patients in the older age group than the placebo group (Table 3).

Table 3 Summary of Demographic Characteristics (Safety Population) – Study 044

	Placebo (N=61)	Drisapersen 6 mg/kg/week (N-125)
Age (years)		
Mean (SD)	8.0 (2.37)	8.3 (2.43)
Median	8.0	8.0
$5 \leq$ age ≤ 7; n (%)	29 (47.5)	51 (40.8)
$7 <$ age ≤ 10; n (%)	25 (41.0)	53 (42.4)
Age > 10; n (%)	7 (11.5)	21 (16.8)
Sex, n (%)		
Male	61 (100)	125 (100)
Race, n (%)		
African American	1 (1.6)	0
Asian	9 (14.8)	20 (16.0)
White-Arabic/North African Heritage	4 (6.6)	5 (4.0)
White-Caucasian	46 (75.4)	95 (76.0)
Mixed	1 (1.6)	5 (4.0)

Source: Clinical Study Report

The time since first symptoms, diagnosis and first steroid use in the drisapersen group were numerically longer than the placebo group. The majority of subjects in both treatment groups (placebo: 85%; drisapersen: 86%) were on a continuous regimen of glucocorticosteroid. Mean baseline values for the 6MWD test were slightly lower in the drisapersen group (337.46 m) than in the placebo group (348.00 m). A summary of baseline disease characteristics is presented in Table 4.

Table 4 Summary of Baseline Disease Characteristics (Safety Population) – Study 044

	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)	Total (N=186)
Time Since First Symptoms (months)[a]			
n	58	122	180
Mean (SD)	66.7 (31.30)	71.8 (31.58)	70.2 (31.49)
Median	60.8	70.2	66.8
Min., Max.	11, 168	12, 176	11, 176
Time Since Diagnosis (months)[a]			
n	61	125	186
Mean (SD)	54.2 (32.84)	58.0 (35.16)	56.7 (34.37)
Median	49.8	54.5	53.1
Min., Max.	6, 148	6, 163	6, 163
Time Since First Corticosteroid Taken (months)[a]			
n	61	125	186
Mean (SD)	29.1 (25.77)	35.6 (28.99)	33.5 (28.07)
Median	18.9	26.6	25.6
Min., Max.	7, 135	6, 146	6, 146
Corticosteroid Regimen, n (%)[b]			
n	61	125	186
Continuous	52 (85)	108 (86)	160 (86)
Intermittent	9 (15)	17 (14)	26 (14)
6MWD (m)	61	125	NA
Mean (SD)	348.00 (92.153)	337.46 (95.594)	NA

Source: Clinical Study Report

3.2.1.5 Efficacy Results – Study 044

Analysis of the Primary Endpoint

Mean decreases from baseline in 6MWD (m) were observed for both the placebo and drisapersen groups, indicating a decline in ambulatory function over 48 weeks. In the primary efficacy MMRM analysis of change from baseline in 6MWD (m) at Week 48 (Table 5 and Figure 2), a 10.3 m treatment difference over placebo was observed for the drisapersen treatment group. This difference was not statistically significant (p=0.415).

Table 5 Summary of MMRM Analysis of Change from Baseline in 6MWD (ITT Population) – Study 044

	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)
Baseline		
n	61	125
Mean (SD)	348.00 (92.153)	337.46 (95.594)
Week 12		
n	58	124
Adjusted mean change (SE)	-12.80 (5.460)	-8.08 (3.877)
Adjusted mean difference vs. placebo		4.725
95% CI		(-8.081 ,17.530)
p-value		0.468
Week 24		
n	59	122
Adjusted mean change (SE)	-29.11 (8.267)	-24.34 (5.815)
Adjusted mean difference vs. placebo		4.767
95% CI		(-14.896, 24.431)
p-value		0.633
Week 36		
n	60	116
Adjusted mean change (SE)	-35.13 (9.010)	-33.24 (6.385)
Adjusted mean difference vs. placebo		1.887
95% CI		(-19.644, 23.419)
p-value		0.863
Week 48		
n	59	117
Adjusted mean change (SE)	-52.65 (10.423)	-42.32 (7.378)
Adjusted mean difference vs. placebo		10.334
95% CI		(-14.645, 35.312)
p-value		0.415

Source: Clinical Study Report

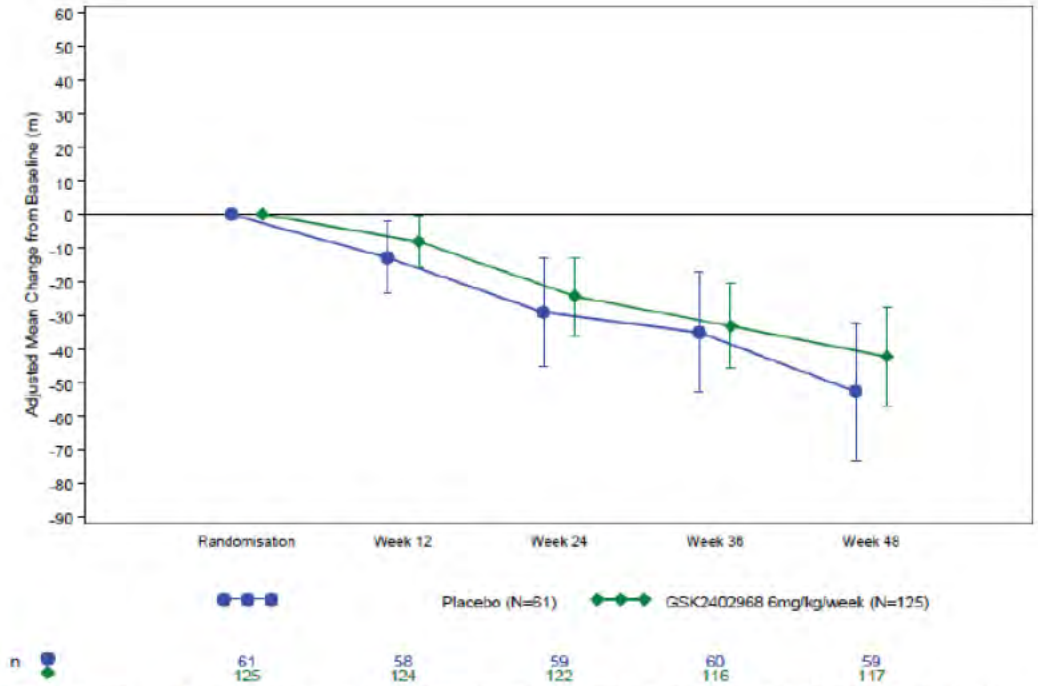

Figure 2 Adjusted Mean Change from Baseline (95% CI) in 6MWD (m) (ITT Population)
Source: Clinical Study Report

The planned sensitivity analyses (Section 3.2.1.3) were performed and the results were generally similar to the one obtained from the primary analysis (Table 6).

Table 6 Sensitivity Analysis for Change from Baseline in 6MWD (m) – Study 044

Analysis/Treatment	n	Adjusted Mean Change from Baseline (SE)	Treatment Difference[a]	95% CI	p-value
MMRM OC (ITT)[b]					
Placebo	59	-52.65 (10.423)			
Drisapersen 6 mg/kg/wk	117	-42.32 (7.378)	10.334	(-14.645, 35.312)	0.415
MMRM OC (PP)[b]					
Placebo	53	-45.23 (10.413)			
Drisapersen 6 mg/kg/wk	99	-40.36 (7.589)	4.873	(-20.313, 30.059)	0.703
ANCOVA OC (ITT)[c]					
Placebo	59	-49.97 (10.732)			
Drisapersen 6 mg/kg/wk	117	-38.38 (7.739)	11.586	(-13.684, 36.855)	0.367
ANCOVA LOCF (ITT)[c]					
Placebo	60	-49.69 (10.593)			
Drisapersen 6 mg/kg/wk	125	-38.26 (7.531)	11.430	(-13.414, 36.274)	0.365
ANCOVA Multiple Imputed (MAR approach) (ITT)[d]					
Placebo	59	-50.27 (10.585)			
Drisapersen 6 mg/kg/wk	117	-40.24 (7.669)	10.026	(-15.036, 35.088)	0.430
ANCOVA Multiple Imputed (CIR approach) (ITT)[d]					
Placebo	59	-50.32 (10.588)			
Drisapersen 6 mg/kg/wk	117	-40.51 (7.656)	9.807	(-15.244, 34.858)	0.439

Data Source: Table 2.3, Table 2.4, Table 2.5, Table 2.6, Table 2.7.

ANCOVA=Analysis of Covariance, CIR=Copy Increment from Reference, LOCF=Last Observation Carried Forward, MAR=Missing at Random, MMRM=Mixed effect Model Repeated Measure, OC=Observed Cases.

a. Note a positive difference compared to placebo represents benefit over placebo.
b. Model includes terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit.
c. Model includes terms for Treatment, Country Grouping and Baseline 6MWD.
d. Model includes terms for Treatment, Visit, Treatment by Visit, Country Grouping and Baseline 6MWD.

Source: Clinical Study Report

Some patients lost ambulation during the trial and could not perform the 6MWD. A 0 value was recorded for the visits that a subject could not perform the 6MWD. Altogether, 14 subjects in the drisapersen group and 6 subjects in the placebo group had 0 values on 6MWD at one or more visits, resulted in a total of 29 visits with 0 values for subjects in the drisapersen group and 16 visits with 0 values for subjects in the placebo group. These 0 values resulted in large negative changes from baseline for these patients. Analysis removing these visits with 0 values was performed. The adjusted mean change from baseline was changed from -42.3 to -33.1 in the drisapersen group and was changed from -52.7 to -39.4 in the placebo group (compare Table 5 to Table 7). The impact of these patients in the drisapersen group did not appear to be larger than in the placebo group.

Table 7 Number (%) of patients / visits with 0 value in 6MWD assessment – Study 044

	Placebo N=61	Drisapersen 6 mg N=125
Number of patients with 0 in 6MWD	6 (9.8%)	14 (11.2%)
Number of visits with 0 in 6MWD	16 (6.6%)	29 (5.8%)
Analysis removing visits with 0 values		
Adjusted mean change at week 48	-39.4	-33.1
Treatment difference		6.33
Nominal p-value		0.5836

Source: Reviewer's analysis

Analysis of Secondary Endpoints

No secondary endpoints showed treatment benefit of drisapersen compared to placebo.

3.2.2 Evaluation of Study 117

3.2.2.1 Study Design – Study 117

The primary objective of the study was to assess the efficacy of 2 different dosing regimens of subcutaneous drisapersen administered over 24 weeks in ambulant subjects with DMD.

This was a phase 2, double-blind, placebo-controlled, parallel-group study in ambulant male subjects at least 5 years of age with DMD resulting from a mutation that was to be corrected by exon 51 skipping induced by drisapersen.

The active doses were:

- Continuous regimen; 6 mg/kg drisapersen once weekly
- Intermittent regimen; 6 mg/kg drisapersen twice weekly on 1st, 3rd and 5th weeks, once weekly on 2nd, 4th and 6th weeks, and no active drug on 7th to 10th week of each 10 week cycle

Similar to Study 044, there were 2 screening visits and a randomization/baseline visit at which 6MWD assessments were performed. The same criteria of 6MWD were applied.

Following a 2 to 4 week screening period, eligible patients were randomized into 2 parallel cohorts (the continuous regimen cohort and intermittent regimen cohort). Each cohort included subjects on drisapersen and matched placebo in a 2:1 ratio.

All subjects received a loading dosing regimen of twice weekly dosing with 6 mg/kg drisapersen for the first 3 weeks of treatment only. The intermittent regimen cycles started after completion of the loading dosing regimen (i.e. from Week 4). Subjects were treated for 48 weeks (including the loading dose period). At the end of the treatment period, subjects who completed the study had the option to enter an open-label extension study (Study DMD114349).

The study was fully blinded with respect to active drug and placebo in each cohort. The different regimens were not fully blinded. Subjects allocated to the continuous dosing regimen received a total of 51 doses of drisapersen or placebo, whereas subjects allocated to the intermittent regimen received a total of 50 doses of drisapersen or placebo.

The study aimed to randomize 54 subjects, which would provide 48 evaluable subjects assuming a drop-out rate of approximately 10%. This study was conducted at 13 centers in 9 non-US countries.

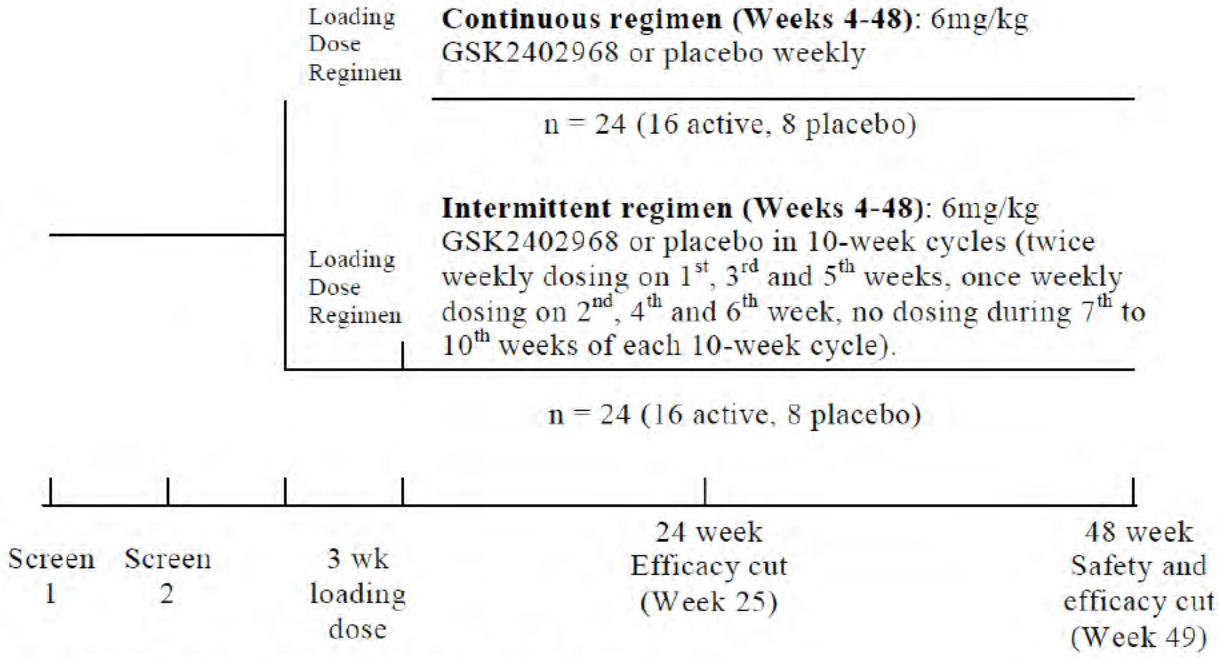

Figure 3 Study Schematic (Source: Clinical Study Report)

3.2.2.2 Study Endpoints – Study 117

The primary efficacy endpoint was muscle function using 6 minute walking distance (6MWD) test, the same endpoint used in Study 044.

The 6MWD was conducted at Screen 1, Screen 2, Baseline (Week 1), and Weeks 13, 25, 37 and 49. Refer to Section 3.2.1.2 for detailed description of 6MWD.

Secondary efficacy endpoints were:

- Timed function tests (times and grading): Grading of the 10 meter walk/run, the timed rising from floor and the 4-stair climb and descend was assessed on a 6 point scale
- Muscle strength (total score)
- North Star Ambulatory Assessment (NSAA)

- Frequency of accidental falls (during 6MWD)
- Time to loss of ambulation
- Creatine kinase serum concentrations
- Pulmonary function (FEV1, FVC, MIP, MEP, PCF and PF)
- Dystrophin expression (muscle biopsies)

3.2.2.3 Statistical Methodologies – Study 117

The primary efficacy endpoint was the change from baseline at Week 25 in the 6MWD.

The primary population for efficacy evaluation was the ITT population (see Section 3.2.1.3). The observed case (OC) data from the ITT population was analyzed using a mixed model for repeated measures (MMRM) with restricted maximum likelihood estimation and an unstructured covariance matrix. The model included treatment, visit, treatment-by-visit interaction, center/country grouping, baseline 6MWD and baseline 6MWD-by-visit interaction as fixed effects. Separate comparisons were made for each treatment regimen versus placebo. Due to the two different treatment regimens, the type 1 error rate was preserved by utilizing the Bonferroni-Holm adjustment. The p-values from the two primary analyses (6 mg/kg drisapersen continuous vs. placebo, and 6 mg/kg drisapersen intermittent vs. placebo), were ordered smallest to largest. The smallest p-value was compared to a significance level of $\alpha/2$ (0.025). If the result was demonstrated to be statistically significant at this level, i.e. $p<0.025$, then the second p-value was compared to a significance level of α (0.05). If the initial comparison showed the result not to reach statistical significance, then the second comparison was also considered not to have reached statistical significance.

The following sensitivity analyses were to be performed to examine the robustness of the results in the presence of missing data, and to determine the impact of protocol violators.

1. Analysis of covariance (ANCOVA) utilizing the OC data, for the ITT population.
2. ANCOVA utilizing last observation carried forward (LOCF) data, for the ITT population.
3. ANCOVA utilizing data imputed via multiple imputation, for the ITT population.
4. MMRM analysis utilizing the OC data for the PP population.

The ANCOVA models included fixed terms for treatment group, baseline 6MWD, and country/center grouping.

The data from all secondary endpoints were summarized. In addition, the analyses detailed below were to be performed.

Continuous Endpoints

Each secondary endpoint was analyzed for the OC dataset using a mixed model for repeated measures (MMRM). The MMRM model for each secondary endpoint included fixed terms for treatment, visit, treatment-by-visit interaction, country/country group, baseline score, and baseline score by visit.

Time to Event Endpoints

The time to loss of ambulation was to be analyzed if ≥ 4 subjects in any treatment group (placebo group combined) experienced a loss of ambulation. Kaplan-Meier estimates, their associated plot, and a Log-Rank test were to be produced.

3.2.2.4 Study Population Results – Study 117

3.2.2.4.1 Patient Disposition

A total of 53 subjects were randomized at 13 centers in 9 countries to one of the three treatment arms: 18 in drisapersen 6 mg/kg continuous group, 17 in drisapersen 6 mg/kg intermittent group, and 18 in combined placebo group. All subjects completed the study.

3.2.2.4.2 Patient Demographic and Baseline Characteristics

Subject demographics are presented in Table 8. Subjects in drisapersen 6 mg/kg intermittent group were slightly older on average than the subjects in the other two treatment groups. All patients were male, and the majority of patients were Caucasians.

Table 8 Summary of Demographic Characteristics (Safety Population) – Study 117

	Placebo (combined) N=18	Drisapersen continuous N=18	Drisapersen intermittent N=17
Age (years)			
Mean (SD)	6.9 (1.18)	7.2 (1.66)	7.7 (1.49)
Median	7.0	6.5	8.0
Min, Max	5, 9	5, 11	5, 10
Sex, n (%)			
Male	18 (100)	18 (100)	17 (100)
Race, n (%)			
White – Caucasian	13 (72)	15 (83)	14 (82)
Other	3 (17)	2 (11)	1 (6)
Not collected	2 (11)	1 (6)	2 (12)

Source: Clinical Study Report

Baseline DMD characteristics of patients are summarized in Table 9. Patients in the 6 mg/kg continuous group had their first symptom slightly more recently and had higher baseline 6MWD than the other two treatment groups.

Table 9 Summary of Baseline DMD Characteristics (Safety Population) – Study 117

	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)	Total (N=53)
Time Since First Symptoms (months)[a]				
Mean (SD)	63.5 (24.00)	61.1 (24.86)	64.5 (24.56)	63.0 (24.04)
Median	73.3	57.4	63.1	62.2
Min, Max	15, 95	27, 112	27, 105	15, 112
Time Since Diagnosis (months)[a]				
Mean (SD)	44.4 (21.61)	44.6 (27.69)	47.8 (26.48)	45.5 (24.93)
Median	35.5	41.4	47.0	43.4
Min, Max	12, 82	3, 96	3, 105	3, 105
Time Since First Corticosteroid Taken (months)[a]				
Mean (SD)	24.2 (14.02)	26.0 (21.20)	32.6 (17.04)	27.5 (17.72)
Median	22.5	13.7	33.8	25.6
Min, Max	7, 60	7, 69	7, 63	7, 69
Corticosteroid Regimen[b]				
Continuous	11 (61)	12 (67)	9 (53)	32 (60)
Intermittent	7 (39)	6 (33)	8 (47)	21 (40)
6MWD (m)				
Mean	403.18	427.61	394.57	NA
(SD)	(45.131)	(70.045)	(66.952)	

Source: Clinical Study Report

3.2.2.5 Efficacy Results – Study 117

Primary Analysis

In the primary efficacy MMRM analysis of change from baseline in 6MWD (m) at Week 25, a statistically significant treatment benefit (35.09 m; $p<0.025$) over combined placebo in 6MWD was observed for the continuous regimen (Table 10 and Figure 4). No statistically significant or clinically meaningful treatment benefit over placebo at Week 25 was observed for the intermittent regimen.

Table 10 Summary of MMRM Analysis of Change from Baseline in 6MWD – Study 117

	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)
Baseline			
n	18	18	17
Mean (SD)	403.18 (45.131)	427.61 (70.045)	394.57 (66.952)
Week 25			
n	16	16	15
Adjusted mean change (SE)	-3.6 (9.73)	31.5 (9.75)	-0.1 (10.34)
Adjusted mean difference vs. placebo		35.09	3.51
95% CI		(7.59, 62.60)	(-24.34, 31.35)
p-value		0.014	0.801

Source: Clinical Study Report

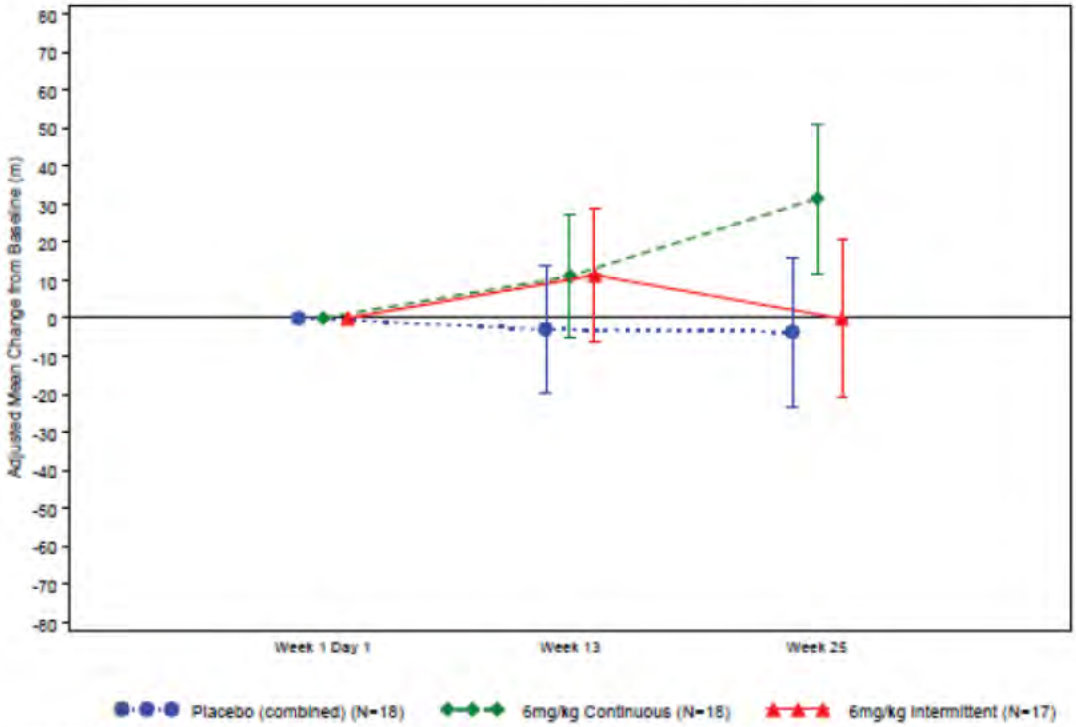

Figure 4 MMRM Analysis of Change from Baseline in 6MWD (m) at Week 25 (ITT Population)
Source: Clinical Study Report

In the sponsor's analysis reported above, 6 patients had their week 25 assessments excluded. All 6 patients completed the study and assessments. The exclusion of their data was resulted from a tight assessment window of 6 days. Five of the 6 patients (2 in each group) had their week 25 assessment performed at week 24, and one was performed at week 27. Analysis including week

25 assessments of these 6 patients resulted in a treatment difference of 31.2 m (p=0.022) between the continuous regiment and placebo and a treatment difference of 13.3 m (p=0.534) between the intermittent regiment and placebo.

The treatment difference between the continuous regiment and intermittent regiment was 22.9 m with a nominal p-value of 0.098. It was not clear what caused the discrepancy in treatment effect between the continuous regiment and intermittent regiment, as the total exposures for the two regimens were about the same. It is worth noting that leading into the week 25 assessment, there was an empty dosing period of 4 weeks from week 20 to week 23 for the drisapersen intermittent group due to the 10-week dosing cycle.

Sensitivity Analyses of the Primary Endpoint

Further comparisons of the drisapersen continuous dosing regimen with placebo for the primary efficacy endpoint at Week 25 were made by the sponsor to assess the sensitivity of the primary analysis. Results are presented in Table 11. Except for the analysis noted in footnote d (unslotted), analyses presented in Table 11 excluded 6 patients who had Week 25 visit outside the visit window.

Table 11 6 mg/kg Drisapersen Continuous Regimen Sensitivity Analyses: Change from Baseline in 6MWD (m) at Week 25 – Study 117

Analysis	Population	Dataset	Treatment Difference	95% CI	P-value
MMRM[a]	ITT	OC	35.09	(7.59, 62.60)	0.014
MMRM[a]	PP	OC	37.83	(8.31, 67.34)	0.013
ANCOVA[b]	ITT	OC	35.25	(7.41, 63.08)	0.014
ANCOVA[b]	ITT	LOCF	30.63	(3.77, 57.49)	0.026
MMRM[a]	ITT	OC (unslotted)[d]	31.19	(4.70, 57.67)	0.022
MMRM[a]	ITT	OC (exc. outlier)[c]	30.69	(3.85, 57.53)	0.026
MMRM[a], Uncombined Placebo	ITT	OC	46.78	(11.93, 81.63)	0.010

Source: Week 25 Analysis Table 2.3, Week 25 Analysis Table 2.10, Week 25 Analysis Table 2.7, Week 25 Analysis Table 2.8, Week 25 Analysis Table 2.60, Week 25 Analysis Table 2.62, and Week 25 Analysis Table 2.65.

a. Model includes terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit.
b. Model includes terms for Treatment, Country Grouping and Baseline 6MWD.
c. Subject 3002, randomised to placebo intermittent, was determined to be an outlier (6MWD of 322 m, 432.2 m and 265 m at Baseline, Week 13 and Week 25 respectively).
d. For reporting purposes, efficacy data were slotted to pre-defined visit windows, based on the time of the assessment since first dose. Where a visit was attending particularly early or late, the assessment would have fallen outside of the visit window and was thus excluded from analyses. This analysis of unslotted data, analysed the data according to the investigator recorded visit, regardless of the time at which this occurred.

Source: Clinical Study Report

Numerical differences in 6MWD between placebo continuous regimen and placebo intermittent regimen were observed. The following table presents the results where the two regimens of placebo were analyzed separately.

Table 12 Summary of MMRM Analysis of Change from Baseline in 6MWD (m) – Placebo Group Analyzed separately – Study 117

	Placebo Continuous (N=9)	Placebo Intermittent (N=9)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)
Baseline				
n	9	9	18	17
Mean (SD)	405.90 (49.365)	400.46 (43.297)	427.61 (70.045)	394.57 (66.952)
Week 25				
n	7	9	16	15
Adjusted mean change (SE)	-15.2 (14.37)	6.1 12.99	31.6 (9.72)	0.3 (10.32)
Adjusted mean difference vs. placebo			46.78	-5.81
95% CI			(11.93, 81.63)	(-38.26, 26.64)
p-value			0.010	0.720
Week 49				
n	9	8	18	15
Adjusted mean change (SE)	-35.9 (17.67)	-13.2 (18.16)	11.2 (12.65)	2.8 (13.65)
Adjusted mean difference vs. placebo			47.14	15.95
95% CI			(3.54, 90.75)	(-29.03, 60.92)
p-value			0.035	0.479

Source: Clinical Study Report

Due to the small sample size, it was difficult to determine whether or not the difference was due to reasons other than variation.

Variations among the 3 Pre-treatment Assessment Values

Large within-subject variations in 6MWD assessments were observed in the three pre-treatment visits (Screen 1, Screen 2 and Baseline). The study required that the 3 pre-treatment 6MWD had to be within 20% of each other, which could result in a difference of as much as 100 meters.

In order to examine whether the choice of baseline had an impact on the efficacy results, the average score of the 3 pre-treatment 6MWD assessments was obtained for each patient and used as the baseline in the primary analysis model. The analysis included all patients (including 6 patients with assessments outside the visit window).

As shown in the following table, the average scores of the pre-treatment 6MWDs were close to the baseline values for the placebo and drisapersen continuous groups, and average score was a little higher than the baseline for the intermittent group. The adjusted mean difference for 6

mg/kg continuous groups vs. placebo was a little smaller when average pre-treatment score was used as baseline, but findings generally agreed with the results from the primary analysis.

Table 13 Analysis of 6MWD using average of the pre-treatment assessments as baseline – Study 117

6MWD	Placebo (combined) N=18	6 mg/kg Drisapersen Continuous N=18	6 mg/kg Drisapersen Intermittent N=17
Baseline			
Mean	403.2	427.6	394.6
Median	400.0	425.0	381.0
Average of Pre-treatment			
Mean	400.4	427.6	402.4
Median	402.7	429.3	395.0
Week 25			
Adjusted mean change	-2.2	22.7	-4.4
Adjusted mean diff vs. placebo		24.9	-2.1
p-value		0.051	0.864

Source: Reviewer's analysis

Week 49 Analysis of 6MWD

An MMRM analysis was also conducted for the 6MWD including all data at Week 49. The model was analogous to that performed for the primary analysis at Week 25. Numerical treatment differences of 35.84 m and 27.08 m were observed for the continuous and intermittent groups respectively at Week 49 (Table 14 and Figure 5).

Table 14 Summary of MMRM Analysis of Change from Baseline in 6MWD (m) at Week 49 – Study 117

	Placebo (combined) (N=18)	6 mg/kg Drisapersen Continuous (N=18)	6 mg/kg Drisapersen Intermittent (N=17)
Baseline			
n	18	18	17
Mean (SD)	403.18 (45.131)	427.61 (70.045)	394.57 (66.952)
Week 25			
n	16	16	15
Adjusted mean change (SE)	-3.6 (9.73)	31.5 (9.75)	-0.1 (10.34)
Adjusted mean difference vs. placebo		35.09	3.51
95% CI		(7.59, 62.60)	(-24.34, 31.35)
p-value		0.014	0.801
Week 49			
n	17	18	15
Adjusted mean change (SE)	-24.7 (12.75)	11.2 (12.64)	2.4 (13.63)
Adjusted mean difference vs. placebo		35.84	27.08
95% CI		(-0.11, 71.78)	(-9.83, 63.99)
p-value		0.051	0.147

Source: Clinical Study Report

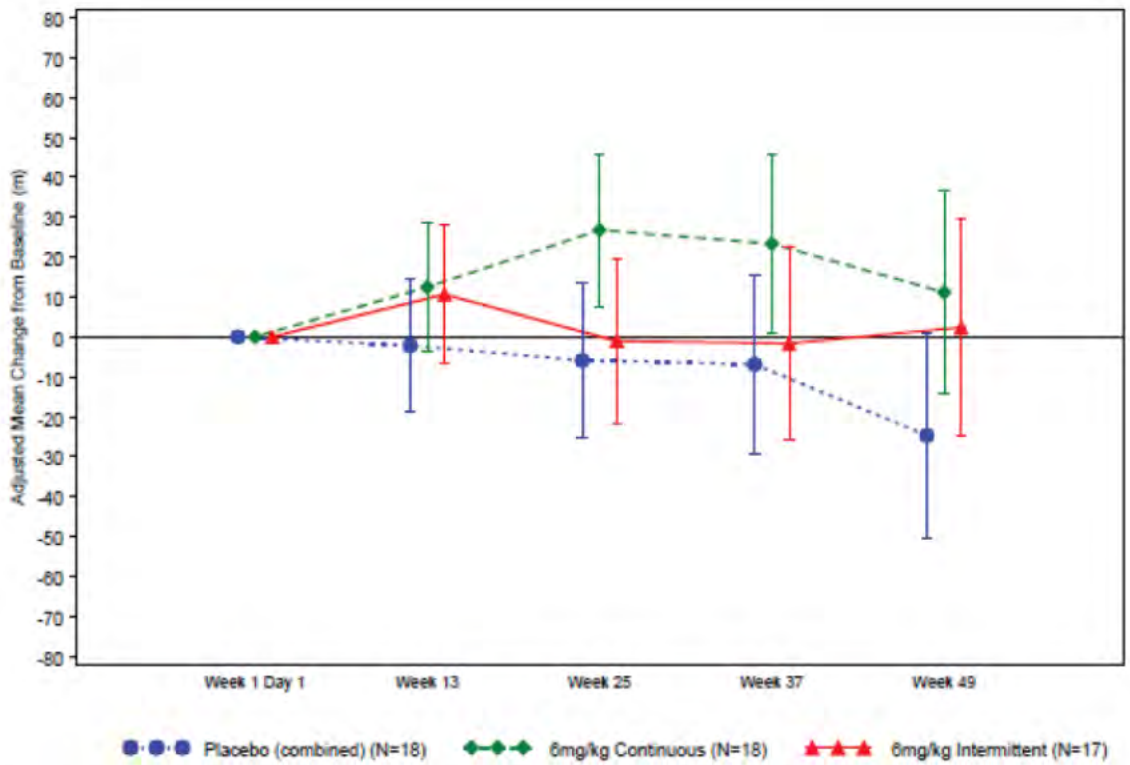

Figure 5 MMRM Analysis of Change from Baseline in 6MWD (m) at Week 49
Source: Clinical Study Report

Efficacy of Secondary Endpoints

None of the secondary endpoints reached nominal significance in treatment difference.

3.2.3 Evaluation of Study 876

3.2.3.1 Study Design – Study 876

This was a phase 2, double blind, parallel-group, placebo-controlled study in ambulant subjects with DMD resulting from a mutation that was to be corrected by exon 51 skipping induced by drisapersen.

Similar to Studies 044 and 117, there were 2 screening visits and a randomization visit at which 6MWD was performed. Subjects received drisapersen 3 mg/kg, drisapersen 6 mg/kg, drisapersen 3 mg/kg volume-matched placebo, or drisapersen 6 mg/kg volume-matched placebo (2:2:1:1 ratio) given SC once a week for 24 weeks. After the last dose of drisapersen /placebo, subjects continued into a 24 week post-treatment period. At the end of the post-treatment period, subjects who completed the study had the option to enter an open-label extension study.

A schematic of the study design is presented in Figure 6.

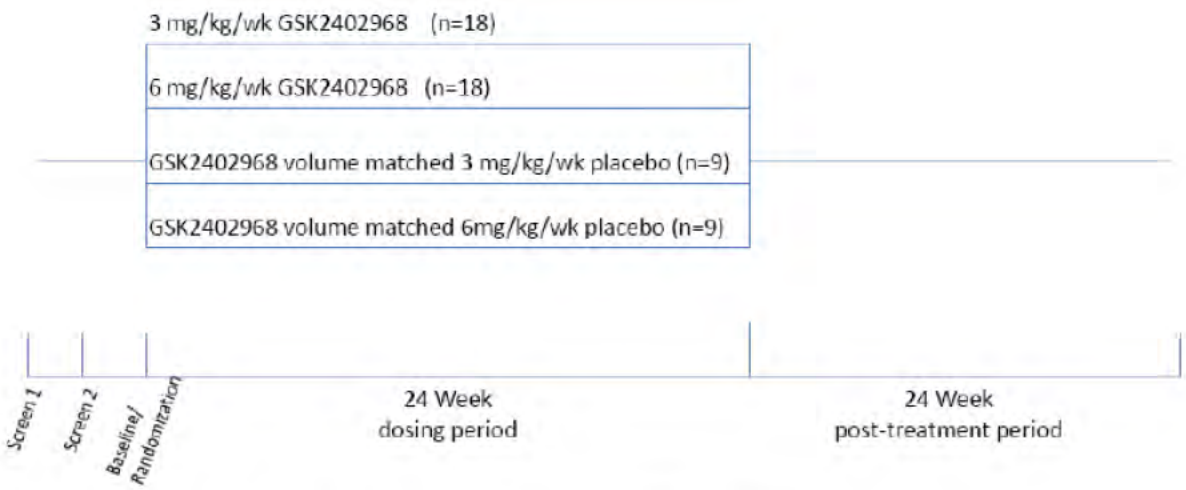

Figure 6 Study Schematic
Source: Clinical Study Report

The study was fully blinded with respect to active and placebo; however the study was not fully blinded to dose. Subjects allocated to the 3mg/kg or volume-matched placebo arms received a lower volume of injection than those allocated to the 6 mg/kg or volume-matched placebo arms.

This study was conducted in pediatric males aged 5 and up at 13 centers in the United States.

3.2.3.2 Study Endpoints – Study 876

The primary efficacy endpoint was muscle function using the 6 minute walking distance (6MWD) test. Refer to Section 3.2.1.2 for detailed description of 6MWD assessment.

A group of similar secondary endpoints as in Study 117 were included.

- Clinician Global Impression of Improvement (CGI-I) (Study 876)
- Functional Outcomes Assessments (Study 876)

3.2.3.3 Statistical Methodologies – Study 876

Change from baseline in the 6MWD was analyzed for the observed cases (OC) of ITT patient population dataset using a mixed model for repeated measures (MMRM). The MMRM model for change from baseline in 6MWD included fixed categorical terms for treatment, visit, treatment-by-visit interaction, center grouping, and continuous fixed covariates of baseline 6MWD and baseline 6MWD-by-visit interaction.

Primary inferences regarding treatment differences for the changes from baseline in the 6MWD was derived from the MMRM models at Week 24. As additional supportive information, treatment differences for Week 12 were also estimated using the MMRM models. Separate comparisons were made for each dose versus placebo. Due to the two different doses, the type 1 error rate was preserved by utilizing a hierarchical approach. The difference between the 6 mg/kg dose of drisapersen was assessed first. If a statistically significant difference was observed conclusions could be drawn regarding the significance of the 3mg/kg dose versus placebo. If no statistically significant difference was observed between the 6 mg/kg dose and placebo, then further analyses of the 3mg/kg dose were considered exploratory, and no conclusions regarding the statistical significance of the treatment difference were made.

Same sensitivity analyses as used in Study 117 were performed.

3.2.3.4 Study Population Results – Study 876

3.2.3.4.1 Patient Disposition

A total of 51 subjects were randomized at 13 centers in the United States. All subjects completed the study.

One subject in the placebo group (000158) had the treatment blind broken during Week 7.

3.2.3.4.2 Patient Demographic and Baseline Characteristics

Patient's demographic characteristics are presented in Table 15. Patients in drisapersen 6 mg/kg group had lower median age of 6.5 years but included a 13 year old patient. In comparison, the median age was 8 years with maximum age of 11 years in the other two treatment groups.

Table 15 Summary of Demographic Characteristics – Study 876

	Placebo (combined) N=16	Drisapersen 3 mg N=17	Drisapersen 6 mg N=18
Age (years)			
Mean (SD)	8.0 (1.79)	7.8 (1.91)	7.6 (2.70)
Median	8.0	8.0	6.5
Min, Max	5, 11	5, 11	5, 13
Sex, n (%)			
Male	16 (100)	17 (100)	18 (100)
Race, n (%)			
White/Caucasian/European	14 (88)	16 (94)	15 (83)
Other	2 (12)	1 (6)	3 (17)

Source: Clinical Study Report

Table 16 presents the baseline DMD characteristics of patients. The mean baseline 6MWD value was about 20 meters lower in the drisapersen 6 mg/kg group compared to the other two treatment groups.

Table 16 Summary of Muscular Dystrophy Disease Characteristics – Study 876

	Number (%) of Subjects			
	Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)	Total (N=51)
Time Since First Symptoms (months)[a]				
Mean (SD)	57.3 (29.68)	67.3 (27.13)	59.0 (29.50)	61.2 (28.55)
Median	59.6	60.4	60.0	60.4
Min., Max	13, 112	25, 124	8, 112	8, 124
Time Since Diagnosis (months)[a]				
Mean (SD)	45.5 (29.70)	47.1 (26.35)	46.5 (26.76)	46.4 (27.03)
Median	44.5	38.3	46.2	43.3
Min., Max.	9, 111	13, 108	7, 96	7, 111
Time Since First Corticosteroid Taken (months)[a]				
Mean (SD)	37.1 (24.31)	33.3 (15.98)	26.8 (22.51)	32.2 (21.21)
Median	38.9	28.0	18.5	26.9
Min., Max.	7, 85	8, 58	6, 81	6, 85
Corticosteroid Regimen[b]				
Continuous	15 (94)	15 (88)	18 (100)	48 (94)
Intermittent	1 (6)	2 (12)	0	3 (6)
6MWD (m)				
Mean	416.41	415.21	396.18	NA
(SD)	(56.988)	(58.049)	(60.662)	NA

Source: Clinical Study Report

3.2.3.5 Efficacy Results – Study 876

Analysis of the Primary Endpoint

In the primary efficacy MMRM analysis of change from baseline in 6MWD at Week 24, a numerical treatment difference of 27.10 meters over placebo was observed for the 6 mg/kg drisapersen group (Table 17 and Figure 7). This result was not statistically significant (p=0.069). The adjusted mean change at Week 24 for the drisapersen 3 mg/kg group was numerically lower than the placebo group. Results from sensitivity analyses were similar to the one from the primary analysis.

Table 17 Summary of Repeated Measures Analysis of Change from Baseline in 6MWD – Study 876

	Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)
Baseline			
n	16	17	18
Mean (SD)	416.41 (56.988)	415.21 (58.049)	396.18 (60.662)
Week 12			
n	16	17	18
Adjusted mean change (SE)	-4.70 (10.643)	-6.60 (9.941)	14.98 (9.919)
Adjusted mean difference vs. placebo	NA	-1.896	19.679
95% CI	NA	(-32.009, 28.217)	(-9.566, 48.924
p-value	NA	0.900	0.182
Week 24			
n	16	17	18
Adjusted mean change (SE)	-10.98 (10.666)	-19.93 (9.964)	16.12 (9.941)
Adjusted mean difference vs. placebo	NA	-8.946	27.099
95% CI	NA	(-39.122, 21.229)	(-2.210, 56.408)
p-value	NA	0.554	0.069

Source: Clinical Study Report

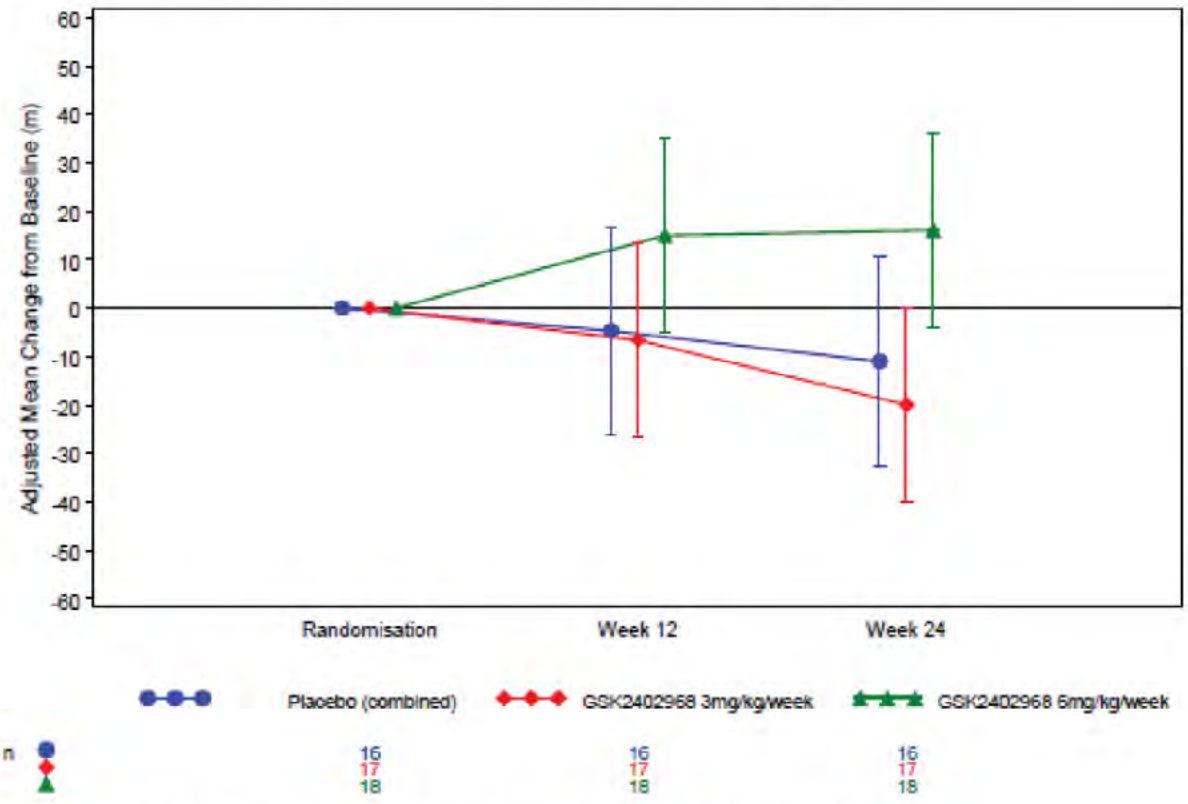

Figure 7 MMRM Analysis of Change from Baseline in 6MWD at Week 24
Source: Clinical Study Report

Sensitivity analyses similar to the ones performed in Study 117 were conducted and results were generally consistent with the one from primary analysis (Table 18).

Table 18 Sensitivity Analyses: Change from Baseline in 6MWD (m) at Week 24 – Study 876

Analysis	Population	Dataset	Treatment Difference	95% CI	P-value
Drisapersen 3 mg/kg/wk					
MMRM[a]	ITT	OC	-8.945	(-39.12, 21.23)	0.554
MMRM[a]	PP	OC	-16.43	(-49.36, 16.49)	0.319
ANCOVA[b]	ITT	OC	-9.26	(-39.92, 21.39)	0.546
ANCOVA[b]	ITT	LOCF	-9.26	(-2.72, 56.63)	0.546
Drisapersen 6 mg/kg/wk					
MMRM[a]	ITT	OC	27.10	(-2.21, 56.41)	0.069
MMRM[a]	PP	OC	19.43	(-11.58, 50.44)	0.213
ANCOVA[b]	ITT	OC	26.95	(-2.72, 56.63)	0.074
ANCOVA[b]	ITT	LOCF	26.95	(-2.72, 56.63)	0.074

Data Source: Week 24 Analysis Table 2.3, Week 24 Analysis Table 2.4, Week 24 Analysis Table 2.5, Week 24 Analysis Table 2.6

a. Model included terms for Treatment, Visit, Treatment by Visit, Baseline 6MWD and Baseline 6MWD by Visit.
b. Model included terms for Treatment and Baseline 6MWD.

Source: Clinical Study Report

Similar to Study 117, within-subject variations in 6MWD assessments were observed in the three pre-treatment visits and analysis using average score of the 3 pre-treatment 6MWD assessments as baseline was performed.

Difference between the average pre-treatment 6MWD score and baseline 6MWD was small in average, and adjusted treatment differences between each of the drisapersen dose group compared to placebo were similar to what were obtained from the primary analysis (Table 19).

Table 19 Analysis of 6MWD using average of the pre-treatment assessments as baseline – Study 876

6MWD	Placebo (combined) N=16	Drisapersen 3 mg/kg N=17	Drisapersen 6 mg/kg N=18
Baseline			
Mean	416.4	415.2	396.2
Median	420.0	403.0	396.0
Average of Pre-treatment			
Mean	419.2	414.3	400.0
Median	428.8	396.7	396.7
Week 25			
Adjusted mean change	-15.3	-19.3	13.6
Adjusted mean diff vs. placebo		-4.1	28.9
p-value		0.775	0.042

Source: Reviewer's analysis

No discrepancies were found in the two placebo matched dose groups.

An MMRM analysis was also conducted for the 6MWD including all data up to Week 48. The model was analogous to that performed for the primary analysis at Week 24. Numerical treatment differences of -24.75 m and 27.87 m were observed for the drisapersen 3 mg/kg and drisapersen 6 mg/kg groups respectively at Week 48 (Table 20).

Table 20 Summary of Repeated Measures Analysis of Change from Baseline in 6MWD – Study 876

	Placebo (combined) (N=16)	Drisapersen 3 mg/kg (N=17)	Drisapersen 6 mg/kg (N=18)
Baseline			
n	16	17	18
Mean (SD)	416.41 (56.988)	415.21 (58.049)	396.18 (60.662)
Week 12			
n	16	17	18
Adjusted mean change (SE)	-3.54 (10.617)	-7.45 (9.923)	15.92 (9.898)
Adjusted mean difference vs. placebo	NA	-3.914	19.458
95% CI	NA	(-33.933, 26.104)	(-9.709, 48.626)
p-value	NA	0.794	0.186
Week 24			
n	16	17	18
Adjusted mean change (SE)	-9.82 (10.660)	-20.79 (9.966)	17.06 (9.940)
Adjusted mean difference vs. placebo	NA	-10.965	26.879
95% CI	NA	(-41.107, 19.177)	(-2.416, 56.174)
p-value	NA	0.468	0.071
Week 36			
n	15	16	18
Adjusted mean change (SE)	-8.72 (13.114)	-26.58 (12.395)	19.41 (12.226)
Adjusted mean difference vs. Placebo	NA	-17.864	28.133
95% CI	NA	(-54.868, 19.139)	(-8.156, 64.422)
p-value	NA	0.335	0.125
Week 48 (end of post treatment period)			
n	15	17	18
Adjusted mean change (SE)	-13.17 (14.843)	-37.92 (14.059)	14.69 (13.891)
Adjusted mean difference vs. placebo	NA	-24.750	27.866
95% CI	NA	(-66.371, 16.871)	(-13.043, 68.775)
p-value	NA	0.238	0.177

Source: Clinical Study Report

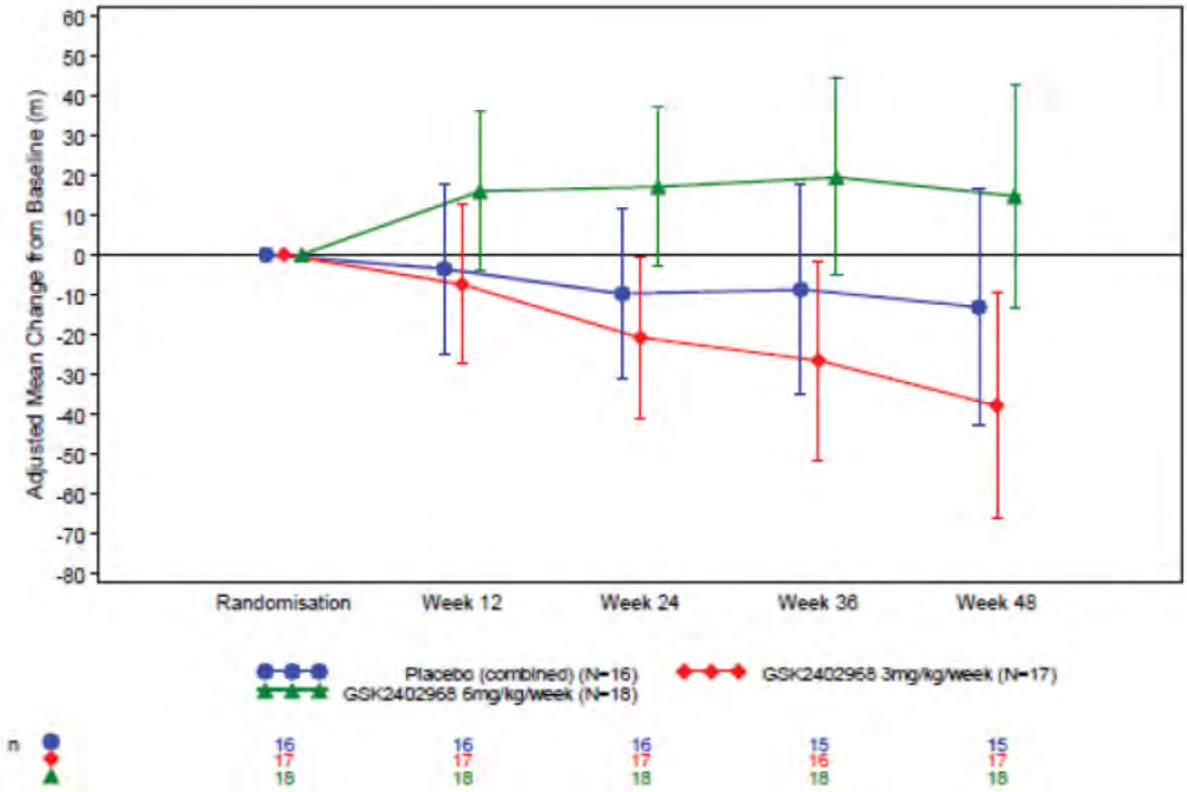

Figure 8 MMRM Analysis of Change from Baseline in 6MWD (m) at Week 48
Source: Clinical Study Report

Analysis of Secondary Endpoints

None of the secondary endpoints reached nominal significance in treatment benefit.

3.3 Evaluation of Safety

Refer to Safety Review by Dr. Evelyn Mentari and Clinical Review by Dr. Veneeta Tandon for Evaluation of Safety.

4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1 Gender, Race, Age, and Geographic Region

All subjects were male and no analysis was performed with respect to gender difference. Analysis by race was not performed either as the majority of patients were Caucasians.

It was believed that subjects at age 7 or under do markedly better than subjects older than 7 years of age generally. Therefore, subjects were grouped by ≤ 7, 7 to 10, and > 10 years in age in Study 044. Few subjects were older than 10 years of age and subjects were grouped by ≤ 7 and > 7 years in age in Studies 117 and 876.

Analysis of Efficacy by Subgroups - Study 044

In general, younger patients did markedly better than older patients as was believed. It appeared that 6 mg/kg drisapersen group had more patients in the older age group and fewer patients in the younger age group. An analysis including age group in the model was performed. The results were similar (treatment difference =11.7 meters; p=0.349) to the ones from the primary analysis in the change from baseline in 6MWD. See more discussions on age effect in the next section.

Numerically, the 6 mg/kg drisapersen group had smaller decreases (or better results) in 6MWD than the placebo group on average in the Eastern and Northern European sites, but the group did poorly (worse than placebo) in sites in Asia, Canada and Southern Europe.

Table 21 Change from Baseline in 6MWD (m) by Demographic Characteristics – Study 044

	Placebo N=60	Drisapersen 6 mg N=125
Age ≤ 7		
N (%)	29 (48.3)	51 (40.8)
Baseline mean (median)	382.8 (373.0)	367.9 (372.9)
Week 48 mean (median)	358.9 (365.1)	367.6 (384.0)
Adjusted mean change	-25.3	-3.8
7 < Age ≤ 10		
N (%)	25 (41.7)	53 (42.4)
Baseline mean (median)	320.9 (325.9)	333.4 (361.0)
Week 48 mean (median)	239.1 (224.0)	270.6 (323.8)
Adjusted mean change	-79.9	-71.7
Age > 10		
N (%)	6 (10.0%)	21 (16.8)
Baseline mean (median)	295.3 (261.1)	273.8 (261.9)
Week 48 mean (median)	219.6 (172.3)	195.6 (200.0)
Adjusted mean change	-81.7	-94.3
Asia		
N (%)	7 (11.7)	16 (12.8)
Baseline mean (median)	301.8 (285.0)	325.4 (328.0)
Week 48 mean (median)	254.6 (277.0)	279.4 (338.5)
Adjusted mean change	-39.9	-49.2
Canada		
N (%)	6 (10.0)	13 (10.4)
Baseline mean (median)	377.3 (376.5)	386.3 (383.0)
Week 48 mean (median)	380.4 (407.0)	362.2 (385.0)
Adjusted mean change	13.7	-27.1

Northern Europe		
N (%)	11 (18.3)	22 (17.6)
Baseline mean (median)	367.4 (369.0)	352.1 (358.5)
Week 48 mean (median)	285.2 (317.0)	325.7 (346.3)
Adjusted mean change	-87.0	-24.3*
Russia & Eastern Europe		
N (%)	7 (11.7)	12 (9.6)
Baseline mean (median)	353.8 (355.0)	380.5 (383.3)
Week 48 mean (median)	284.0 (311.0)	379.0 (371.4)
Adjusted mean change	-70.1	-1.3
South America		
N (%)	8 (13.3)	21 (16.8)
Baseline mean (median)	280.8 (292.1)	260.7 (278.0)
Week 48 mean (median)	220.2 (198.2)	206.7 (244.4) (n=18)
Adjusted mean change	-63.3	-59.3
Southern Europe		
N (%)	20 (33.3)	37 (29.6)
Baseline mean (median)	377.2 (374.0)	360.0 (400.0)
Week 48 mean (median)	326.7 (368.6)	295.1 (343.8)
Adjusted mean change	-54.5	-63.7

Source: Reviewer's analysis

Analysis of Efficacy by Subgroups - Study 117

Patients in Study 117 were generally younger than the patients in Study 044. All but one patient was older than 10 years of age.

It should be noted that this study had only 53 patients in total. Once divided into subgroups, variation could play a big role. Therefore, one should be cautious in interpreting the results presented in this section.

The results presented in this and the next section are different from what were obtained by the sponsor as 6 patients were excluded in the sponsor's analysis due to visit outside the window, which I believe was not appropriate.

In this study, drisapersen intermittent regimen had larger proportion of patients in the older age group compared to the other two groups. However, this group, although with lower baseline 6MWD scores, was responding to the treatment slightly better than the younger age group numerically.

The adjusted mean change from baseline for the younger age group and older age group appeared to be similar in the two drisapersen groups. However, in the placebo group, the older age subjects appeared to be doing more poorly than the younger age ones.

Compared to Study 044, the patient population in Study 117 appeared to be younger and less impaired, and age did not appear to have negatively impacted treatment effect as large treatment differences between active drug groups versus placebo were observed in the older age patients.

Table 22 Change from Baseline in 6MWD (m) by Demographic Characteristics – Study 117

	Placebo (combined) N=18	6 mg/kg Drisapersen Continuous N=18	6 mg/kg Drisapersen Intermittent N=17
Age ≤ 7			
N (%)	13 (72.2)	11 (61.1)	7 (41.2)
Mean Baseline	409.6	413.4	414.1
Adjusted Mean Change	7.0	26.7	-3.9
Adjusted diff vs. placebo		19.8	-10.9
Age > 7			
N	5 (27.8)	7 (38.9)	10 (58.8)
Mean Baseline	386.4	449.9	380.9
Adjusted Mean Change	-43.7	25.5	6.4
Adjusted diff vs. placebo		69.1	50.1
Australia, UK, Belgium, Netherlands			
N	10 (55.6)	8 (44.4)	8 (47.1)
Mean Baseline	378.7	403.9	401.0
Adjusted Mean Change	-15.4	20.3	3.8
Adjusted diff vs. placebo		35.7	19.2
Spain, France, Germany			
N		6	7
Mean Baseline	5	466.8	394.5
Adjusted Mean Change	445.8	18.3	-21.1
Adjusted diff vs. placebo	-11.1	29.4	-10.0
Turkey and Israel			
N		4	2
Mean Baseline	3	416.3	369.2
Adjusted Mean Change	413.7	36.6	14.6
Adjusted diff vs. placebo	32.5	4.0	-17.9

Source: Reviewer's analysis

Analysis of Efficacy by Subgroups - Study 876

Study 876 was conducted in US, and no analysis by region was performed.

In Study 876, there were 6 patients who were older than 10 at the baseline. The mean baseline 6MWD score for the older age group was slightly higher in 6 mg/kg drisapersen group, but was about 45 meters lower in each of the other two groups, compared to the mean baseline 6MWD in the younger age group. No large differences in the adjusted mean change in 6MWD were found between the younger age patients and older age patients in any of the treatment groups.

Table 23 Change from Baseline in 6MWD (m) by Demographic Characteristics – Study 876

	Placebo (combined) N=16	3 mg/kg Drisapersen N=17	6 mg/kg Drisapersen N=18
Age ≤ 7			
N	6	8	10
Mean Baseline	445.3	439.1	385.2
Adjusted Mean Change	-13.6	-21.5	17.1
Adjusted diff vs. placebo		-7.9	30.7
Age > 7			
N	10	9	8
Mean Baseline	399.1	393.9	409.9
Adjusted Mean Change	-16.0	-16.1	11.8
Adjusted diff vs. placebo		-0.1	27.8

Source: Reviewer's analysis

4.2 Other Special/Subgroup Populations

The sponsor submitted an Information Amendment on August 31, 2015, in which additional post-hoc analyses were presented. The sponsor stated that combined with the analyses presented in ISE in the original submission, these analyses supported the conclusion that Study 044 included a large proportion of subjects who were older and more impaired at baseline when compared with the populations in the two concurrently conducted phase-2 studies. When removing the older, more progressed subjects from the DMD114044 population, a positive treatment effect in the 3 remaining population subgroups was revealed, all of whom saw a 6MWD treatment benefit similar to or greater than the effects seen in the Phase-2 placebo-controlled studies. Using this analysis, the Phase-3 data could be seen to confirm the efficacy demonstrated in the other randomized Phase-2 studies.

In this Information Amendment, the sponsor made 3 major arguments.

1. Data displays by both age and baseline 6MWD categories (Sponsor's Table 1 below) revealed significant differences in 6MWD trajectory among subgroups, with consistent treatment effect for all subgroups except for the subgroup of older boys with low baseline walking function (age >7 and 6MWD <=330 m). However, although subgroup analyses of treatment effect taking age and baseline 6MWD into account were performed and included in the Phase-3 CSR, the analysis that considered a combination of age group and baseline 6MWD as a covariate was not included in the overall model.
2. Baseline difference between the treatment groups disadvantaged the drisapersen group. As the Phase-3 study was not designed to accommodate these subgroup differences, critical baseline differences occurred in the randomized groups. For example while 46% of placebo-treated subjects were in the young, functionally-able group at baseline, only 26% of drisapersen-treated subjects were in this category.
3. Formal statistical analyses of the residuals from the model fit confirmed a highly skewed data distribution rejecting the MMRM model assumption of normal data distribution ($p<0.0001$), different from what was observe from Phase-2 studies.

Table 24 Table 1 of Sponsor's Information Amendment – Study 044

Table 1: (Table 6 of Module 2.5): Summary of 6MWD change from Baseline to Week 48 by age and baseline categories (DMD114044)

	Age ≤ 7 years				Age > 7 years			
	6MWD ≤ 330 meters		6MWD > 330 meters		6MWD ≤ 330 meters		6MWD > 330 meters	
	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo
N	17	4	33	25	33	17	34	13
Mean	11.38	-62.38	-11.67	-17.68	-123.63	-107.96	-25.37	-51.87
SD	49.43	98.72	63.24	50.66	93.80	108.27	61.27	69.93
Median	**23.0**	**-53.8**	**-6.60**	**-26.2**	**-109.3**	**-109.5**	**-22.4**	**-55.0**
Min	-108.7	-184.0	-215.0	-96.0	-303.0	-311.0	-194.0	-185.0
Max	68.0	42.0	112.4	105.5	48.2	89.0	102.0	48.0
Difference in Medians	**+76.8**		**+19.8**		**-0.2**		**+32.6**	

Source: DMD114044 CSR Table 2.138 provided in the original NDA (27 April 2015)
Note: Difference refers to difference in 6MWD median for drisapersen-treated subjects compared with placebo

In an post-hoc analysis performed by the sponsor (Table 25 [Table 2 of Information Amendment]), the primary analysis model (MMRM) was enhanced to account for potential subgroup differences by adding the baseline age and baseline 6MWD subgroup (age<=7 & 6MWD<=330, age<=7 & 6MWD>330, age>7 & 6MWD<=330, and age>7 & 6MWD >330) and treatment by age and baseline 6MWD subgroup interaction terms to the MMRM model. This analysis yielded a treatment difference of 20 m (p = 0.118), and confirmed a significant treatment-by-subgroup interaction (p=0.047).

The only subgroup that did not show a treatment benefit was the group of older patients with reduced baseline walking function (age > 7 and 6MWD <= 330m). The enhanced MMRM model generated an average treatment benefit of 33 m with a nominal p-value of 0.012 after excluding this more severe population (Table 25).

To address the issue of non-normal data distribution, the MMRM model adjusting for subgroup difference was further enhanced by standardized rank transformation. This nonparametric analysis model confirmed a highly significant treatment-by-subgroup interaction (p=0.006), and significant treatment effect at Week 48 for the total trial population (p=0.012) (Table 25).

With the non-parametric approach, when the older, more progressed subjects were removed from the analysis, the treatment effect at Week 48 was highly significant (p=0.002). Table 25 summarizes the treatment effect based on MMRM and MMRM-Ranked modeling.

Table 25 Table 2 of Sponsor's Information Amendment – Study 044

Table 2: MMRM and MMRM RANKED Modeling: Summary of Treatment Effect

Model	Population	Placebo (adjusted mean)	6mg/kg (adjusted mean)	Treatment effect vs. Placebo	P-value of Interaction of Treatment and Subgroup	P-value of Treatment Difference	P-value of Interaction of Treatment and Subgroup (Ranked data)	P-value of Treatment Difference (Ranked data)
MMRM	Overall*	-60 m	-40m	+20 m	0.047	0.118	0.006	0.012
MMRM	Overall ** minus older/more severe group	-44 m	-11m	+33 m	0.013	0.012	0.068	0.002

Source Tables: 005_1.pdf, 005_2.pdf, 005_3.pdf, 005_4.pdf, 005_5.pdf, 005_6.pdf, 005_7.pdf, 005_8.pdf

* Overall population includes subgroups: <=7 <=330; <=7 >330; >7 <=330; >7 >330m

** Overall population minus older/more severe group: <=7 <=330; <=7 >330; >7 <=330; >7 >330m

Note: p-values included are provided for descriptive purposes

The sponsor concluded that collectively, the post hoc analyses presented in the NDA and supplemented in this document provided additional supportive evidence of clinical benefit similar to what was observed in Phase-2 studies.

Reviewer's Analysis

The results shown in sponsor's Table 1 appeared to show contradictory, or at least inconsistent, results. It showed that in the younger age group, the drug was benefiting the more impaired patients (6MWD <=330) with median treatment difference of 76.8 meters. However, in the older age group, the drug was benefiting less impaired patients (6MWD > 330) with median treatment difference of 32.6.

Putting aside the treatment comparisons, which somewhat relied on how well placebo-treated patients responded, one could simply look at each individual subgroup in the median change from baseline. The median change from baseline for the placebo-treated patients appeared to be consistent with the common belief that patients who were more impaired do worse as shown in both younger age group and older age group (-53.8 vs. -26.2 and -109.5 vs. -55.0, respectively). However, the drisapersen-treated patients again showed contradicting results as in the younger age more impaired patients were doing relatively better (23.0 vs. -6.6) and in the older age more impaired patients were doing worse (-109.3 vs. -22.4). This occurred when all subgroup had at least 17 patients.

In addition, the analysis shown in Table 1 used a specific subgrouping with age cut at 7 years and baseline 6MWD cut at 330 meters. The results presented could be sensitive to the cutoff points chosen. The following table presents a summary using baseline 6MWD cutoff at 320 and 350 meters instead of 330 meters, and leaving everything else the same.

Table 26 A comparison to Sponsor's Table 1 when baseline 6MWD is cut at 320 or at 350 – Study 044

Baseline 6MWD cut at 320	**Age ≤ 7 years**				**Age > 7 years**			
	Group 1 6MWD ≤ 320		**Group 2 6MWD > 320**		**Group 3 6MWD ≤ 320**		**Group 4 6MWD > 320**	
	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo
N	11	2	39	27	31	15	36	15
Mean	12.4	-139.5	-8.4	-15.3	-129.1	-129.0	-26.1	-38.3
SD	49.8	62.9	61.7	50.0	92.9	95.6	61.2	75.5
Median	**24.9**	**-139.5**	**1.0**	**-26.0**	**-121.0**	**-111.0**	**-22.4**	**-28.5**
Min	-108.7	-184.0	-215.0	-96.0	-303.0	-311.0	-194.0	-185.5
Max	66.0	-95.0	112.4	105.5	48.2	24.0	102.0	89.0
Diff in median	**164.4**		**27.0**		**-10.0**		**6.1**	
Baseline 6MWD cut at 350	**Group 1 6MWD ≤ 350**		**Group 2 6MWD > 350**		**Group 3 6MWD ≤ 350**		**Group 4 6MWD > 350**	
	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo	6 mg/kg	Placebo
N	19	7	31	22	37	20	30	10
Mean	6.1	-44.3	-10.0	-17.3	-115.3	-104.0	-22.6	-42.9
SD	49.2	73.6	64.9	54.0	96.8	100.2	54.8	77.2
Median	**22.0**	**-26.0**	**1.0**	**-27.1**	**-109.0**	**-103.2**	**-22.4**	**-22.1**
Min	-108.7	-184.0	-215.0	-96.0	-303.0	-311.0	-141.0	-185.0
Max	68.0	42.0	112.4	105.5	61.0	89.0	102.0	48.0
Diff in median	**48.0**		**28.1**		**-5.8**		**-0.3**	

Source: Review's analysis

As shown in the above table the benefit seen in the group with age > 7 and 6MWD > 330 m disappeared quickly when the cutoff point of baseline 6MWD changed from 330 to 320 or 350. What the sponsor believed to be a treatment effect could be simply the results of this variability.

Next, let us discuss the analysis model and results using the model with the additional variables of age with baseline 6MWD group and its interaction with the treatment group.

First, the significance of the interaction term of the subgroup (baseline 6MWD and age group) with treatment group (p=0.047) could be an indication of the contradicting directions pointed out above. The reduced p-value from 0.415 in the primary analysis to 0.118 in the sponsor's analysis in Table 2 could be a result of the dominant effect from group 1 (age ≤ 7 and 6MWD ≤ 330) due to its contribution as a group with substantially large treatment difference.

In the primary MMRM analysis, each patient contributed 4 assessments in the repeated measure analysis. However, in the analysis shown in sponsor's Table 2, the contribution from this smallest group of 17 patients created a dominant GROUP effect. If we exclude group 3 (age > 7 and 6MWD ≤ 330) in the analysis, as did the sponsor, an even larger dominant group effect could be the reason of the smaller p-value of 0.012. One could get even smaller p-value by cutting baseline 6MWD at 310 meters instead of 330 meters. In that case, the treatment difference in group 1 would be over 200 meters; not because drisapersen-treated patients did better, but because the only placebo-treated patient left in the group had a change from baseline of -184 meters.

In order to elaborate the above points, let us have more details of the two models presented in the sponsor's Table 2 by comparing the estimates of the coefficients from the models with and without group 3 (age > 7 and 6MWD $\leq$ 330). These estimates represent how much each effect contributed to the estimated treatment difference. The estimates of the coefficients of the group effect are relative to group 4 (age > 7 and 6MWD > 330).

Table 27 Coefficient estimate of the MMRM analysis with/without group 3 presented by sponsor in Table 2 – Study 044

Coefficient estimate relative to Group 4	Group	Estimate	Treatment difference	p-value of interaction group*treatment	p-value of treatment difference
MMRM with Group 3 (row 2 of Table 2)	1	-2.85	20.08	0.047	0.118
	2	35.59			
	3	8.20			
	Group 1*treatment	25.56			
	Group 2*treatment	-32.32			
	Group 3*treatment	-29.18			
MMRM excluding group 3 (row 3 of Table 2)	1	-15.41	32.93	0.013	0.012
	2	34.38			
	Group 1*treatment	32.18			
	Group 2*treatment	-31.56			

Source: Reviewer's analysis

In the above table, there was a substantial change in the effect of group 1 (from -2.85 to -15.41) and group 1 by treatment interaction (from 25.56 to 32.18) when group 3 was excluded. Other effects were little changed. The exclusion of group 3 increased rather than reduced the group by treatment interaction (from 0.047 to 0.013).

The following 2 tables present similar results when baseline 6MWD is cut at 320 meters or 350 meters.

Table 28 Coefficient estimate of the MMRM analysis with/without group 3 where baseline 6MWD is cut at 320 meters – Study 044

Coefficient estimate relative to Group 4	Group	Estimate	Treatment difference	p-value of interaction group*treatment	p-value of treatment difference
MMRM with Group 3 (row 2 of Table 2)	1	-5.11	21.20	0.246	0.126
	2	26.83			
	3	-1.34			
	Group 1*treatment	35.21			
	Group 2*treatment	-21.26			
	Group 3*treatment	-18.25			
MMRM excluding group 3 (row 3 of Table 2)	1	-17.59	30.96	0.074	0.0326
	2	27.59			
	Group 1*treatment	40.68			
	Group 2*treatment	-22.25			

Source: Reviewer's analysis

Table 29 Coefficient estimate of the MMRM analysis with/without group 3 where baseline 6MWD is cut at 350 meters – Study 044

Coefficient estimate relative to Group 4	Group	Estimate	Treatment difference	p-value of interaction group*treatment	p-value of treatment difference
MMRM with Group 3 (row 2 of sponsor's Table 2)	1	23.14	15.57	0.238	0.2153
	2	35.07			
	3	13.33			
	Group 1*treatment	-0.25			
	Group 2*treatment	-32.07			
	Group 3*treatment	-26.96			
MMRM excluding group 3 (row 3 of sponsor's Table 2)	1	11.19	23.42	0.126	0.0621
	2	33.18			
	Group 1*treatment	6.50			
	Group 2*treatment	-30.20			

Source: Reviewer's analysis

I agree that the phase-3 study had the patient population with more impaired patients compared to the phase-2 studies and there seemed to be some disadvantage for the drisapersen group due to randomization. These factors simply made the study difficult to show treatment effect if any, but did not prove that the treatment had an effect.

In addition, in the sponsor's analysis that added the subgroup of age combined with baseline 6MWD as an effect in the MMRM model, the baseline 6MWD was accounted twice. The model included baseline 6MWD, and age with baseline 6MWD combined effect. Such model is inappropriate. We could consider adding age group as an additional factor in the model as a post-hoc analysis. When age group of ≤ 7 years and > 7 years was added to the primary model, the treatment difference is 11.8 m with a nominal p-value of 0.347, similar to the results from the primary analysis.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

The 6 mg/kg weekly dosing drisapersen regimen was included in all three efficacy studies. The two small phase-2 studies showed significant or near significant treatment difference while the large phase-3 trial was negative on the primary endpoint.

The positive phase-2 trial 117, though small in size, appeared to have consistent results in sensitivity and post-hoc analyses. The phase-3 trial 044, in contrast, with much larger size showed equally consistent negative results.

An information amendment was submitted in which the sponsor made an argument that the 6 mg/kg drisapersen was effective after excluding the group of patients with older age and more severe disease stage (age > 7 and baseline 6MWD > 330) at entry of the study.

I found that the argument to be weak and not well supported by the data. When the cutoff point of baseline 6MWD in the sponsor's suggested group changed from 330 m to 320 m or 350 m, there was a large swing in the median change of 6MWD in group 1 and treatment difference in group 4 simply disappeared.

5.2 Conclusions and Recommendations

Mixed outcomes with positive results in phase-2 trial 117 and negative results in phase-3 trial 044 have hampered determination whether there was any the treatment.

If another study was to be conducted, a stratified randomization taking into account of difference in age and baseline 6MWD needs to be implemented.

VI. Consultative Review:

MRI Assessments and Measurements

DEPARTMENT OF HEALTH AND HUMAN SERVICES **M E M O R A N D U M**

Food and Drug Administration
10903 New Hampshire Ave
Silver Spring, MD 20993

Intercenter Consultative Review

ICC1500291 – NDA 206,031

FOR CONSULTED CENTER'S (CDER) USE ONLY

Date: September 11, 2015

To:
Veneeta Tandon, Ph.D.
Ron Farkas, M.D., Ph.D.
CDER/OND/ODEI/DNP

From:
Daniel Krainak, Ph.D.
Gary Levine, M.D.
CDRH/OIR/DRH

RPM: Fannie Choy

Subject: NDA 206,031
KYNDRISA (drisapersen)
BioMarin Pharmceuticals, Inc.

Table of Contents

I. Summary

Quantitative fat fraction by MR is likely to represent underlying tissue content; however, the changes observed in the study are presented from a small subset of patients (often with one or more missing data points) across a variety of vendors. We have greater uncertainty about quantitative T2 measures in the context of edema as T2 may be influenced by many physiologic factors (including inflammation/edema, local bleeding/hematocrit, fat, [fat effects T1 more than T2] and more). Most of the commonly seen pathologies (infection/inflammation, tumor [benign or malignant], etc.) lead to an increase in T2 values. Therefore, an altered T2 is sensitive but not specific unless correctly interpreted in the context of the underlying pathophysiology.

The data presented are unconvincing for several reasons: the small number of subjects with fat fraction data at baseline, 24 weeks and 48 weeks (five control, one at 3/mg/wk and five at 6 mg/kg/wk with data at all three time points), variability in the MR systems used, and lack of data concerning the actual quality control measurements from phantoms. In addition, we recommend careful investigation by statistical experts for this dataset, in particular about whether the sponsor appropriately addressed concerns about multiple comparisons (small number of subjects, multiple treatment groups, multiple muscles, and multiple MR metrics), the small sample size, and potential for sampling bias.

Small changes in quantitative fat fraction MR values were observed between groups that on the order of the expected variability and reproducibility (around 3%) of the technique. The data were presented in terms of the group means, not individual traces, which may be more appropriate given the small sample. Sample plots for individual muscles and patients for rectus femoris and vastus lateralis for individuals with data at all three time points are provided as examples, though other muscles may show slightly different trends.

Studies suggest a relationship between quantitative fat fraction measured by MR and patient function. The magnitude of the changes observed in the study population is on the order of the uncertainty in the measurement technique (approximately 3%). Based on existing literature it is unclear if changes in fat fraction provide of 2.7 – 5.2% in the placebo group (N = 5) compared to 0.9 – 3.8% in the 6 mg/kg/wk group (N = 6) over 48 weeks would be indicative of a functional difference between groups.

Specific questions from the lead reviewer are addressed at the end of this consulting memo.

II. Background

When we initially reviewed the information provided by the sponsor, we determined that there was insufficient information to assess the reliability and quality of the data. In addition, the limited dataset (small number of participants) and relationships of some of the MRI-based quantitative measures (specifically T2 measures) to the parameter(s) of interest (such as edema) were unclear. We interactively requested additional information related to the MRI dataset from DMD114876. The sponsor responded to our inquiry and provided additional supporting information including the image acquisition guidelines and related publications. In this review, we consider the original information and the response to the additional information request provided by the sponsor related to MR imaging in this study.

Name of product

KYNDRISA (drispersen)
BioMarin Pharmaceuticals, Inc,

Intended Use

Treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing.

III. Scope of this consultative review

The lead reviewer requested:

> The sponsor suggests that muscle MRI is supportive of efficacy, specifically based on "fat fraction" and "edema". Please provide advice on the reliability and meaningfulness of the effects observed. For example, for this type of MRI measurement, how much confidence is there that the "fat fraction" or "edema" measurements are really representing what they purport to represent – particularly in the setting of use of a drug that might alter physiology from that observed in natural history? Are these types of measures objective and reproducible? Is there a way to put the effect size in the context of potential clinical meaningfulness?

The scope of this review is limited to the use of MRI assessments and measurements in NDA206,031.

IV. Summary of the clinical trial

Of nine clinical studies cited, one included analyzable MRI data:

DMD114876 included a pilot MRI study of six different muscles of the right thigh at various time points (baseline, week 24 - 24 weeks after the start of treatment, week 48 – 24 weeks after last treatment). Normalized T2-weighted and fat fraction signal showed a shift in patients treated with drispersen compared to the placebo subjects:

- T2-weighted signal decreased (-0.07 to -0.23; N = 14) compared to controls (0.07 – 0.14; N = 10)
- Apparent fat fraction increased (2.7 – 5.2%; N = 5) in placebo group compared to 6 mg/kg/wk treatment (0.9 – 3.8%; N = 6).
- Effects persisted up to 24 weeks post-treatment

Study No.	Phase	Study objectives	Study design	Treatment*
Placebo-controlled studies				
DMD114876	II	Efficacy, tolerability, safety, PD, and PK	Double-blind, randomised, placebo-controlled Parallel-dose level groups	24 weeks SC: 6 mg/kg/wk 3 mg/kg/wk placebo 24 week off-treatment follow-up

Table 2: Muscle biopsy (*tibialis anterior*[1]) and biomarker assessments in drisapersen clinical studies

Study No.	Group	Number (N)	Biopsy pre-treatment	Biopsy post-treatment	Biopsy Analysis[2]	Muscle pathology[2]
Placebo-controlled studies						
DMD114876	6mg/kg/wk 3mg/kg/wk Placebo	18 17 16	Yes, all	all: 24wk 3rd biopsy at 12wk or 36wk[3]	DL, ES, DysP	sCK, MRI

From section 5.2.3 of the Mechanism-Based Assessment Of Drisapersen's Clinical Pharmacological

Effects

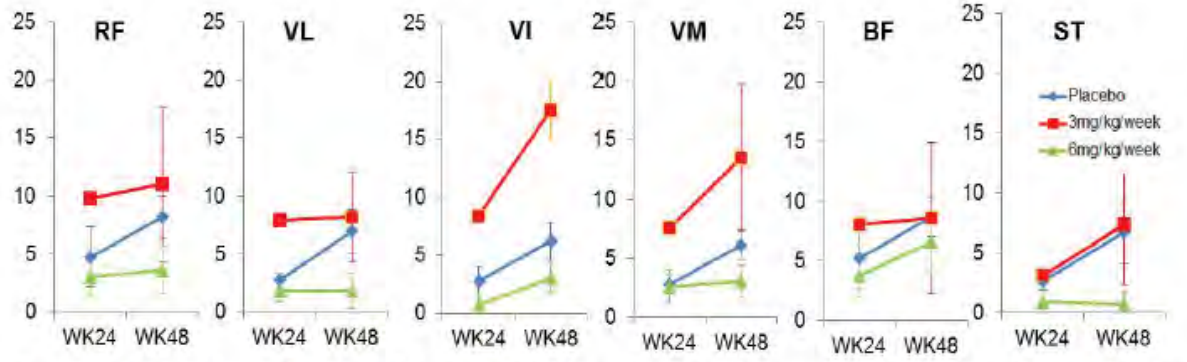

Figure 21: Reduced rate of fat infiltration fraction in subjects receiving 6 mg/kg drisapersen in DMD114876.

Plots showing the apparent fat fraction (y-axis, %) change from baseline (with SE) in each of the muscles for all subjects with evaluable data at each visit.; 6 muscle groups were examined. ; RF: rectus femoris; VL: vastus lateralis; VI: vastus intermedius; VM: vastus medialis; BF: bicep femoris; and ST: semitendinosus. The number of subjects in each of these treatments are as follows, placebo group n=5, 5, 3 mg/kg/week treatment group n=1, 2and 6 mg/kg/week group;n=6, 5 at the Week 24 and Week 48 visit, respectively. Note that the 3mg/kg was only based on n=1 (24wk) and n=2 (48wk) and is therefore not considered to be representative.

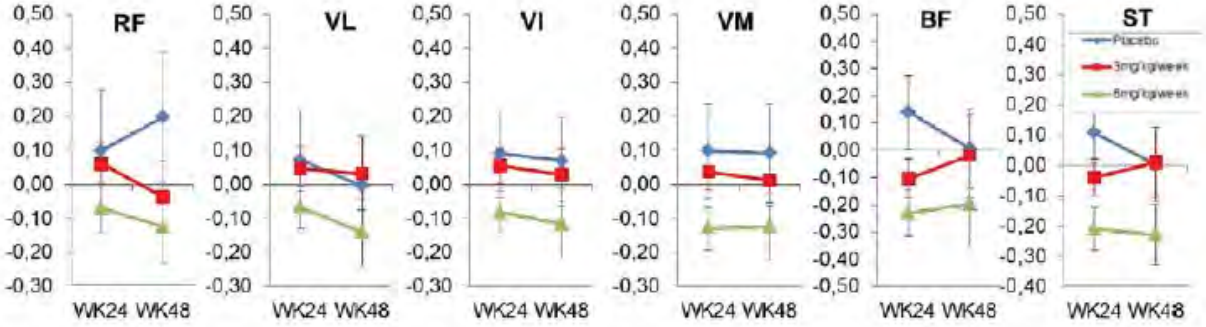

Figure 22: Reduced T2 weighted signal in subjects receiving drisapersen in DMD114876.

Plots (with SE) showing the normalised T2-weighted signal (y-axis,) change from baseline in each of the muscles for all subjects with evaluable data at each visit. All: all subjects; placebo group; 3 mg/kg/week treatment group; 6 mg/kg/week treatment group; RF: rectus femoris; VL: vastus lateralis; VI: vastus intermedius; VM: vastus medialis; BF: bicep femoris; and ST: semitendinosus. The number of subjects in each of these groups, is placebo: n=10, 9; 3 mg/kg/week: n=7, 7; 6 mg/kg/week: n=14, 11, at the Week 24 and Week 48 visit, respectively.

DMD114044 – MRI data were only acquired in a small number of subjects and data were not analyzed due to technology issues at acquisition.

Image Acquisition Guidelines, Protocols, and Quality Control in DMD114876

Consistent with literature recommendations, the sponsor asked all participants to avoid any excessive physical activity beyond their normal levels for at least one week prior to examination. The acquisition protocol required images to be acquired on the same system for the same subject. Quality control assessments were included as part of the study and there was a centralized data repository and analysis site that provided quality checks. However, if data were of insufficient quality (for example, subject motion, deviation from the study protocol, etc.), subjects were not rescanned.

Reviewer comment

The sponsor cites MRI data showing trends for reduction in edema (swelling) and adipose (fatty tissue) replacement signals based on T2 mapping and fat fraction methodology. However, the data presented are unconvincing for multiple reasons. Both quantitative fat fraction and T2-mapping techniques may be performed in multiple ways and the methodology used or variability in the methodology may impact the accuracy and uncertainty associated with the measurement. The sponsor notes that multiple MR system vendors were used including GE, Siemens, and Philips. There may be differences between vendors or models even when attempting to control differences between manufacturers.

Validation for the quantitative techniques (fat fraction or T2 mapping) used in the study was presented primarily as literature references wand supplemental articles were used to determine validation of these techniques. See sections below on the techniques and reproducibility.

While quantitative fat fraction is a logical extension of qualitative assessment of fatty infiltration as a measure of changing fat content in tissue, the relevancy and meaningfulness of changes in T2 values are less obvious. T2 may be changed by multiple factors and the relationship between the disease process and T2 change is not adequately presented. Much of the literature related to MR measurements in this patient population is recent, performed by a small number of groups using a variety of methods in small numbers of participants, and occasionally with mixed results. More justification for the relevancy of T2 mapping as related to the disease would be needed to understand the relationship of T2 to DMD and treatments.

If the data are further considered, we recommend careful investigation from a statistical expert as the limited number of individuals, multiple muscles, and multiple metrics for comparison were noted, but the method for controlling for multiple comparisons was unclear. Also, the same number of individuals was not scanned at all three time points (baseline, week 24, and week 48) and the missing data should be appropriately accounted for.

V. Description of MR techniques for evaluating fat fraction and edema

Fat Fraction

Fat fraction techniques (often referred to as DIXON-method techniques) are often used in the liver to produce qualitative and potentially quantitative fat fraction images that show the difference between water and triglyceride fat fraction. The Dixon technique typically uses gradient recalled echo (GRE) MR images from two or three echo times (TEs) known as the in-phase (IP) and out-of-phase (OP) or opposed phase. The IP image is the sum of water and fat, the OP is the difference between the water and fat. From these images, the fat/water proton ratio may be determined.

Based on the phantom and repeatability studies provided to support premarket notifications for these types of techniques, including (note that this list is not exhaustive):

K122035 – Resonant Health Services - Hepafat
K103411 – GE Medical Systems – IDEAL-IQ
K133526 – Philips Medical Systems - mDIXON-Quant

All of the 510(k)-cleared quantitative Dixon-based fat fraction methods have been restricted to the liver. Variability (scanner-to-scanner reproducibility) is in the range of 3-11% in phantoms or in vivo (95% limit of agreement). Interanalyst 95% confidence intervals were around 1.5% for volumetric liver measurements of fat fraction. Some sponsors provided evidence to support ± 3 - 3.5% reproducibility claims. Correlation to pathology was provided for a subset of the submissions. However, please note that the applications cleared (510[k]) to date have been in the liver whereas the current application uses the technique in leg muscle tissue. Also, note there may be significant image acquisition differences between vendors, for example, Philips mDIXON technique uses a six-echo pulse sequence instead of the traditional two-point method.

The data provided and previous articles suggest large muscle-to-muscle variability. Gaeta and others (2012) observed a range of fat fraction values in 20 boys with DMD from mean of 46.3% in the gluteus maximums to a mean of 2.7% in the gracilis. Morrow and others (2014) found significant differences between muscles in adults, with small absolute differences with mean fat fractions in the 0.6 – 2.6% range and limits of agreement for individual regions of interest ranging from -1.25 to 1.01% (thigh) and -1.55 to 0.95% (calf) across all muscles examined (rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, semimembranosus, semitendinosus, biceps femoris, adductor magnus, sartorius, gracilis, tibialis anterior, peroneus longus, lateral gastrocnemius, medial gastrocnemius, soleus and tibialis posterior muscles). Overall, the study population in Morrow and others (2014) had very low fat fraction values (highest value less than 16% with average values less than 3%) across all volunteers (n = 47) and muscles, which is not representative of the values typically found in DMD patients. Notably, single site studies such as Morrow and others (2014) may have smaller uncertainty.

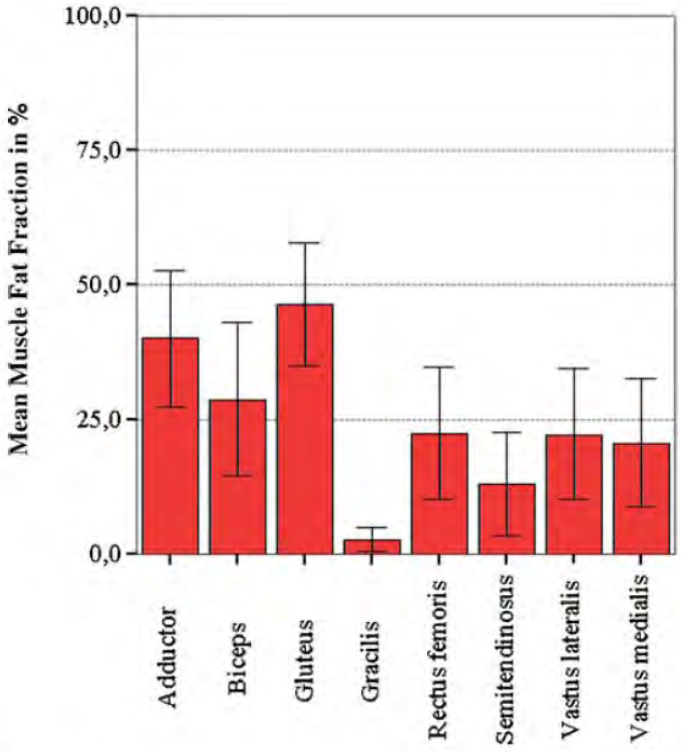

Fig. 1 Bar chart shows mean muscle fat fraction (MFF ± SD) of eight muscles in 20 patients. The gluteus maximus muscle has the highest mean MFF and the gracilis muscle has the lowest

Figure from Gaeta and others 2012

As an example from the study DMD114876, for subject 28 at randomization

Rectus femoris right	27.66
Vastus lateralis right	21.15
Vastus intermedius right	15.74
Vastus medialis right	14.41
Biceps femoris right	38.53
Semitendinosus right	18.06

Spatial heterogeneity in the fat fraction may also be observed within and between dystrophic muscles and even in muscles with similar fat fraction values.

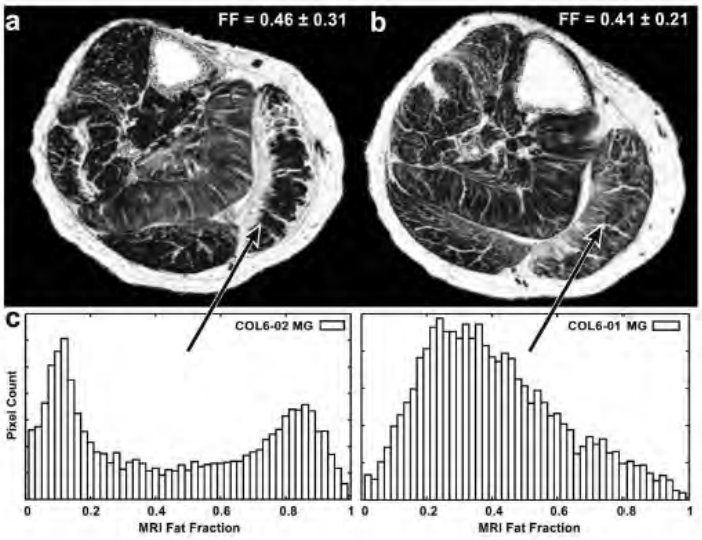

Figure 7.
Examples of histograms from the medial gastrocnemius of two different COL6 subjects with similar fat fraction as measured by MRI. Subject (a) showed fatty infiltration inward from the external fascia, which was associated with a bimodal fat fraction distribution (c), whereas subject (b) showed a unimodal distribution characteristic of a more diffuse pattern of fat infiltration.

Figure from Triplett and others (2014) High spatial heterogeneity within muscles was observed within dystrophic muscles.

The reproducibility of fat fraction measures:

Based on the evidence provided for liver, quantitative fat fraction measures based on MRI, when carefully performed are objective measurements and have reproducibility measures which may be approximately 3-5% when carefully conducted. The sponsor provided several references related to the reproducibility of quantitative fat fraction in muscle.

Ponrartana and others (2014) looked at repeatability of quantitative fat fraction in seven healthy children at a single site, single MR system (Philips) using Philips mDIXON protocol in a “coffee-break” experiment (that is scan – reposition the patient – scan within a short time-frame, same session). Mean muscle fat fraction was less than 13% for all individuals and all muscles (gluteus maximus, rectus femoris, vastus medialis, lateralis, and intermedius, semimembranosus, semitendinosus, biceps femoris, combined adductors, anterior and posterior tibialis, peroneus longus, gastrocnemius, and soleus). Triplett and others (2014) observed day-to-day variability in fat fraction of CV = 12% in control muscle (n = 6) and CV = 5.3% in DMD (n = 26) in children based on a two-day repeatability study. Philips 3T system was used for image acquisition in this study. Forbes and others (2013) compared reproducibility of three centers (two with 3T Siemens systems and one with 3T Philips system) using two-compartment coaxial phantoms, but fat fraction reproducibility was not examined using this phantom. Two healthy adults (ages 35 and 42) were examined at all three centers to look at within-subject variation in the soleus and the mean within-subject CV was 7.2 ± 1.3% across centers using an MRS technique to determine lipid fraction. Bonati and others (2015) recently described using quantitative MRI in DMD to achieve reproducibility for quantitative fat fraction of 0.9% in a single scanner, single site study. Morrow and others (2014) examined muscles in 47 adults using MR techniques with 15 adults undergoing repeat examinations two weeks apart and found limits of agreement -1.55 to 1.01% in adults with low fat fraction values.

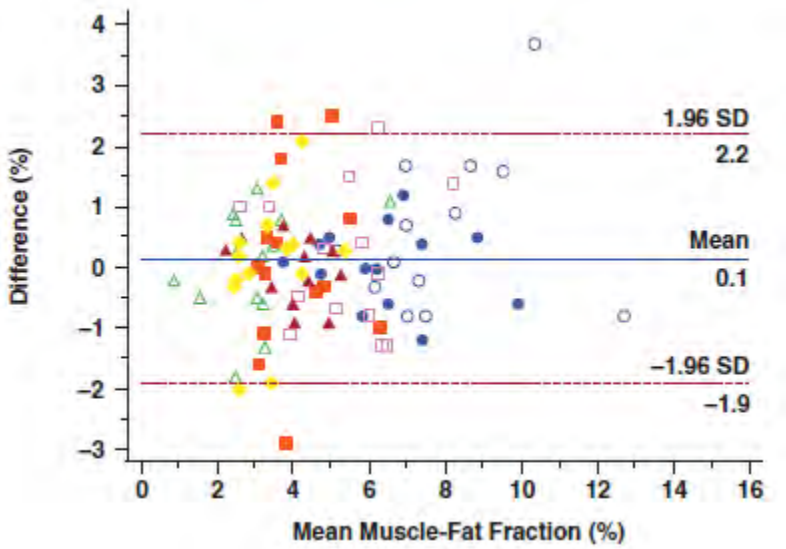

Figure from Ponrartana and other 2014 showing the Bland-Altman plot for a coffee-break test-retest experiment of seven healthy children. Each symbol represents different subject, and each data point represents mean value from each individual muscle measured. The largest difference between two tests was 3.7%.

Thus, reproducibility results across studies may vary and well-controlled studies may achieve better reproducibility.

Histopathology validation of fat fraction

Gaeta and others (2011) compared muscle fat fraction measured by the two-point Dixon technique with muscle biopsy as the reference standard in 27 patients with neuromuscular disorders ages 7 – 67. Six individuals had DMD. Results showed mean differences -0.3 ± 1.3% for mean fat fraction values of around 20% (range 3 – 46%) with limits of agreement ranging from -2.8% to 2.2%.

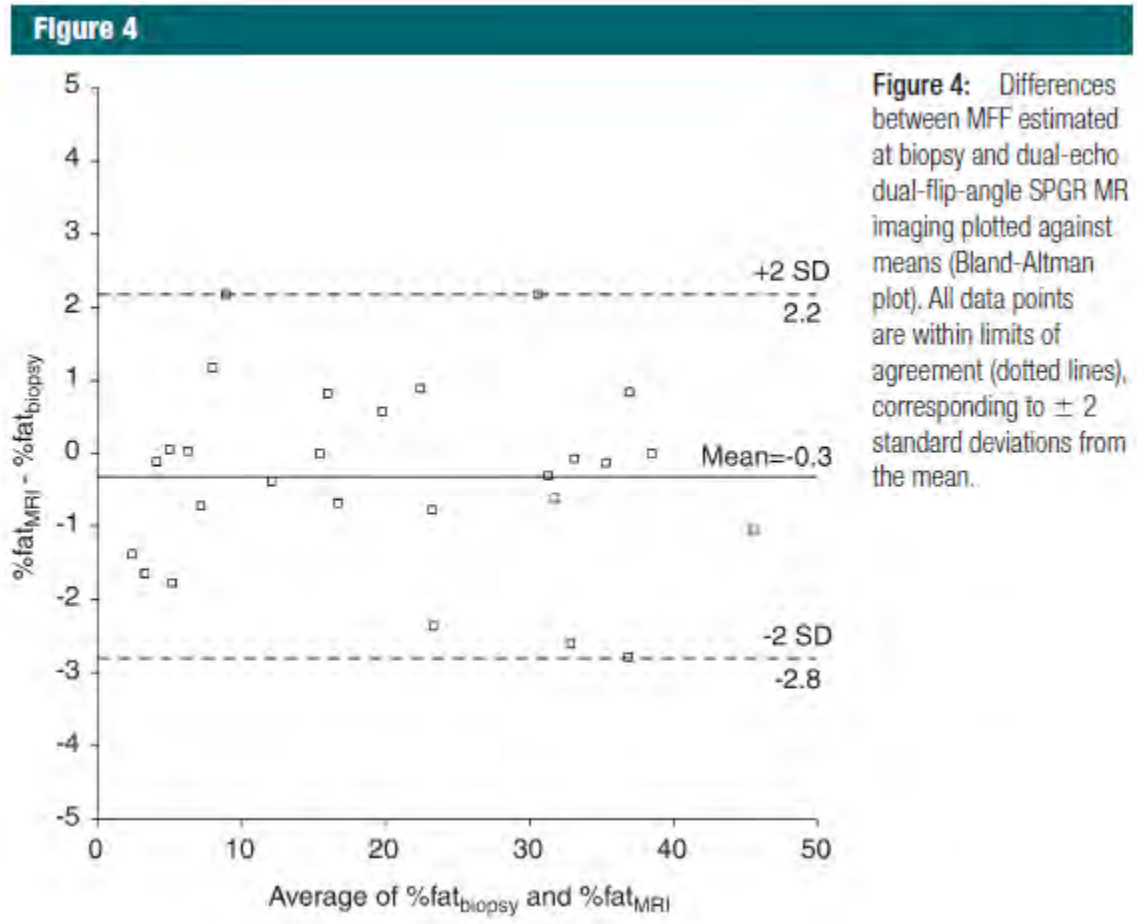

Figure 4: Differences between MFF estimated at biopsy and dual-echo dual-flip-angle SPGR MR imaging plotted against means (Bland-Altman plot). All data points are within limits of agreement (dotted lines), corresponding to ± 2 standard deviations from the mean.

Figure from Gaeta and others (2011) comparing two-point Dixon fat fraction measurements with biopsy.

T2 relaxation & edema

T2 is a magnetic resonance property described as the transverse or spin-spin relaxation and specific pulse sequences may be used to evaluate this property in tissue. Often data are acquired at multiple echo times and modeled as a single exponential function to determine T2 for the voxel or region of interest. T2 measurement by MRI can be accurate and reproducible, but the reliability of the data depends on the technique used.

Measurements of T2 relaxation times are also influenced by fat content as shown in this table from Arpan and others (2013) comparing non-fatsat and fatsat T2-relaxation time. Note the larger differences between techniques in DMD compared to controls. While fat content is not the sole contributor to T2 signal changes, fat content does impact T2 relaxation times. The authors also note that T2 measure obtained from non-fatsat images showed better correlations with functional measures in comparison with those obtained from fatsat. These authors also observed increased heterogeneity in T2 measurements of DMD compared to controls.

Table 2. T_2 relaxation times (ms) for non-fat-saturated (non-fatsat) and fat-saturated (fatsat) images of controls and subjects with Duchenne muscular dystrophy (DMD)

Muscle	Non-fatsat		Fatsat	
	Control	DMD	Control	DMD
SOL	40.3 ± 1.1	50.6 ± 5.5[a]	38.2 ± 0.8	45.3 ± 3.8[a]
MG	39.2 ± 1.9	50.8 ± 7.9[a]	37.1 ± 0.5	45.2 ± 4.3[a]
PER	39.7 ± 1.5	51.2 ± 8.4[a]	37.3 ± 0.6	44.2 ± 3.9[a]
TA	38.7 ± 1.3	45.7 ± 5.0[a]	37.3 ± 1.2	41.5 ± 1.6[a]

MG, medial gastrocnemius; PER, peroneal; SOL, soleus; TA, tibialis anterior.
Values are expressed as mean ± standard deviation (SD).
[a]Significantly different between groups ($p \leq 0.001$)

The relationship between edema and T2 values is unclear. Typically, a fluid sensitive technique (such as STIR or FLAIR) would be used to assess localized increases in signal intensity (for example, bright areas) that would be indicative of inflammation or edema. Quantitative T2 measures may be influenced by physiologic factors other than edema and fat such as iron content, hematocrit, inflammation, fibrosis, or tissue structure alteration. T2 values may also be influenced by steroid use. Therefore, the interpretability of changes in T2 relaxometry values is unclear.

The reproducibility of T2 measures:

Forbes and others (2013) found day-to-day variability T2 w/o fat saturation in medial gastrocnemius, peroneus longus and peroneus brevis, soleus, tibialis anterior, long head of the biceps femoris, gracilis, semetendinosus, and vastulis lateralis (two day study using the same MR system) mean coefficient of variability (CV) ranged 1.3 – 5.9% in ~10 age-matched controls and 1.7 – 5.6% in 25 – 30 boys with DMD where CV = std(repeated measure)/mean(repeated measure).

VI. Example data for fat fraction in two muscles from DMD114876

Given the large patient-to-patient variability and how the sponsor provided the results, two muscles were selected from the sponsor's dataset to examine the trends across time (rectus femoris and vastus lateralis). Statistical comment would be appropriate as there are concerns about the small number of individuals with data points at 0, 24, and 48 weeks as well as that six muscles were examined. Data included in this subset of five placebo, one 3 mg/kg/wk and five 6 mg/kg/wk were from Siemens and GE scanners across a variety of models and at both 1.5T and 3T. There was very limited data demonstrating reproducibility over time in the same patient and even less demonstrating that measurements across MR system manufacturer, field strength, model, and software version provided similar results.

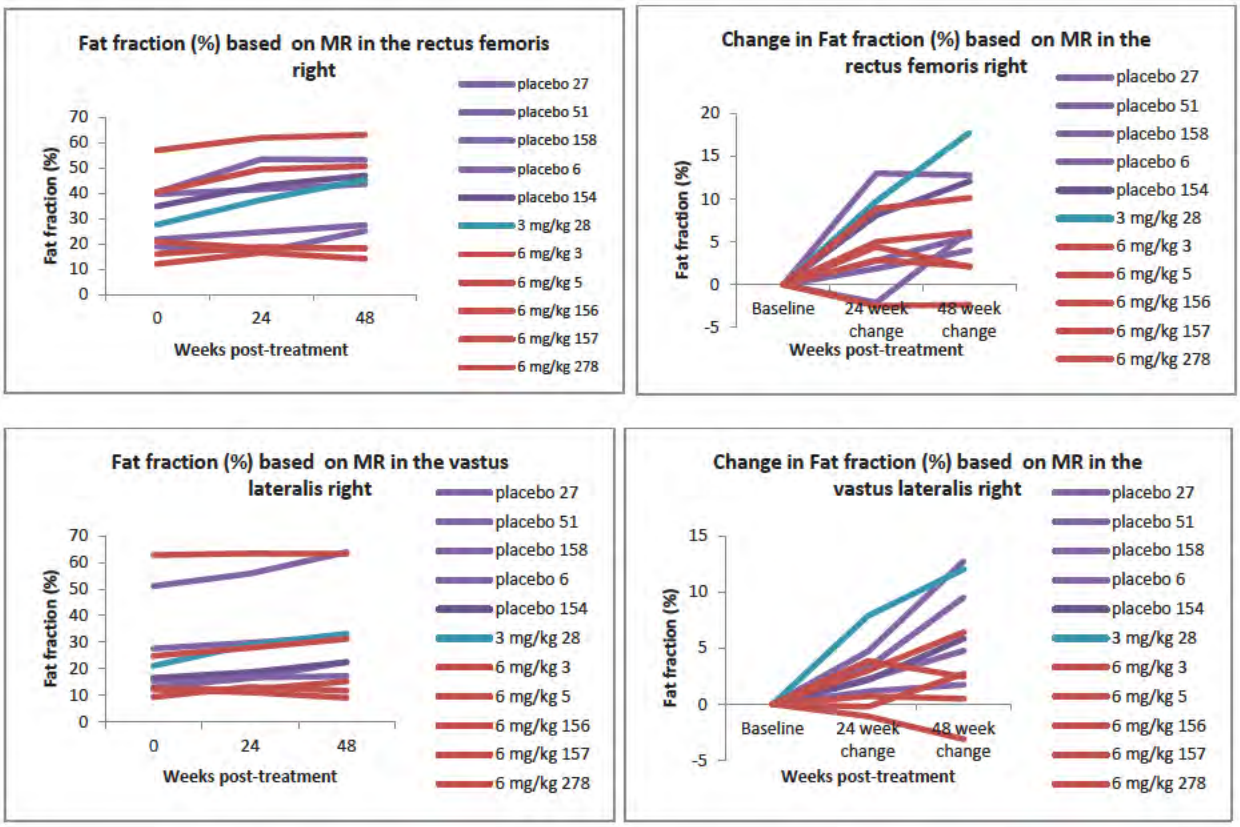

Figure: Two example muscles (rectus femoris [upper row] and vastus lateralis [lower row]) showing individual's data across all three time points as raw values (left column) and change (right column).

The limited data show substantial overlap between treatment groups, large variability by muscle and a wide range of fat fraction observed at baseline.

VII. Response to specific questions from the consultant

Please provide advice on the reliability and meaningfulness of the effects observed. *Response was separated include the potential reliability and meaningfulness.*

1. For example, for this type of MRI measurement, how much confidence is there that the "fat fraction" or "edema" measurements are really representing what they purport to represent – particularly in the setting of use of a drug that might alter physiology from that observed in natural history? Are these types of measures objective and reproducible?

Fat fraction may be evaluated through visual qualitative assessment or quantitative fat fraction techniques. Visual assessment was performed in the DMD1411876 study using the Mercuri scale. Finanger and others (2012) in a review of MR techniques in DMD note:

> "Mercuri et al developed a fourpoint grading system to categorize disease severity, based on visual inspection of fatty tissue infiltration (Mercuri et al., 2002; Mercuri et al., 2005). This strategy was recently used to screen DMD subjects in a clinical trial involving injection with antisense oligonucleotides (van Deutekom et al., 2007). However, there is considerable interest in utilizing quantitative imaging to monitor disease progression and efficacy of treatment strategies, which is the focus of the remainder of this article."

Visual inspection techniques such as the Mercuri scale may have intra- and inter-reader variability and are typically less sensitive to small changes than quantitative techniques (especially those such as quantitative fat fraction that have relatively reliable performance). Therefore, we (like Finanger and others [2012]) have focused on the quantitative fat fraction in our review.

See above for an explanation of the Dixon technique to measure fat fraction. Reproducibility could be considered to be roughly about 3-11% (with well-controlled image acquisition and analysis) depending on the exact technique used. The use of multiple vendors and field strengths without head-to-head reproducibility data for the systems precludes the ability to estimate the uncertainty exactly. Also, validation and reproducibility data referenced in literature was often for different muscles than those in the study and in patients with varying functional characteristics (such as ambulatory and non-ambulatory), and therefore, it is challenging to precisely estimate the uncertainty for the current study. However, the image acquisition protocol in DMD114876 ensured that subjects were scanned on the same scanner with the same software version at all time-points, which should reduce within-subject variance. Data were centrally analyzed and the use of quantitative fat fractions techniques increases the confidence in the limited data provided.

Changes in fat fraction based on quantitative Dixon-methods are likely to reflect true changes in the fat content in muscle; however, the description of the technique in the current study is insufficient to determine if the methodology was adequately controlled and validated to detect changes in magnitude <5%. Quantitative fat fraction methods may be accurate and reproducible when carefully controlled (uncertainty from the method alone may be less than 3% based on evidence provided for commercial products intended for use in the liver as well as other references and possibly less than 1% if carefully controlled). Quantitative fat fraction by MR can be considered a logical progression and substantial improvement from subjective assessment of fatty infiltration by MR.

The meaningfulness of the effects observed for changes in quantitative fat fraction would have to be considered in the correlation to changes in the fat fraction with any other clinical outcomes investigated to date. See response to question 2 below.

More evidence would have to be provided to suggest T2 measurements and T2 signal intensity measures as representative of edema. T2 measurements and changes in T2 may reflect muscle damage, edema, fibrosis, inflammation, fatty inflammation (Arpan et al. 2013; Willcocks et al. 2014), as well as other factors (for example, iron, hematocrit, or exercise). Willcocks and others (2014) provide limited supporting evidence that changes in T2 signal are correlated with functional measures based on a small study (16 boys with DMD; 15 controls) in muscles (soleus, peroneal, tibialis anterior) different than those used in the current study (rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, bicep femoris, semitendinosus). However, Willcocks and others (2014) contrasts previous results from Kim and others (2010) who presented mixed results about changes in T2 values in the gluteus muscles in 11 boys with DMD over time with steroid treatment.

2. Is there a way to put the effect size in the context of potential clinical meaningfulness?

Based on a small literature search, most of the evidence for correlating MR findings to clinical outcomes is relatively recent and conducted in small studies. There is some limited supporting literature in the form of small studies for fat fraction measurements by MR correlating with functional outcomes in DMD (for examples, see Bonati and others 2015; Gaeta and others 2012; Wren and others 2007) but the results vary in MR technique, muscles analyzed, and clinical outcome measures.

Fischmann and others (2013) examined the relationship between motor function measurement (MFM) and fat fraction (based on the 2-pt DIXON technique – Siemens 3T) and found a correlation between fat fraction and a few muscle groups (left and right quadriceps, left and right hamstrings, and left and right adductors).The study (Fischmann and other 2013) included both ambulatory and non-ambulatory patients and examined loss of ambulation and motor function compared with fat fraction measured by MRI. They propose that a change of fat content of 2% should be detectable after six months and that fat fraction

could be used to predict loss in ambulation; however a longitudinal study would be necessary to validate this or any similar model.

Gaeta and others (2012) reported on preliminary experience with fat-fraction and correlation with clinical assessments. Twenty (20) ambulatory boys with DMD were scanned with a 1.5T Philips MR system using a 2-point DIXON method. Analyzed muscles included: gluteus maximus, adductor magnus, rectus femoris, vastus lateralis, vastus medialis, biceps femoris, semitendinosus, and gracilis. In the discussion, they note that "an increase of 20% in MFF (muscle fat fraction) is associated with a high risk of functional reduction" after finding correlations between fat fraction and functional measures (Medical Research Council score [MRCS], timed Gower score, and time to run 10 meters).

Thus, based on existing literature, it is unclear if changes in fat fraction provide of 2.7 – 5.2% in the placebo group (N = 5) compared to 0.9 – 3.8% in the 6 mg/kg/wk group (N = 6) over 48 weeks would be indicative of a functional difference between groups.

VIII. Previous additional information request

We requested additional information interactively (sent to the sponsor August 6, 2015 and response received on August 17, 2015).

You have provided MRI data related to DMD114876. We are concerned about the data quality of the MR-based information as the acquisition and analysis methods may impact the results.

a. Please provide the complete Image Acquisition Guidelines (IAG) documentation. Please be certain to include a detailed description of the acquisition including descriptions of the pulse sequence, critical parameters (such as TE, TR, voxel size, etc.), and note any differences in acquisition between MR system manufacturers or models used in the study. Please describe your procedure for image acquisition quality control and justify why any differences in image acquisition parameters between patients or visits would not impact the results. Please highlight any protocol deviations.
b. Please describe the image analysis procedures including any analysis quality control activities. Please clarify the outputs from your analysis (for example, quantitative fat fraction, T2 relaxation time, T2 signal intensity, etc.).
c. Please provide the quantitative values for each participant by muscle for each visit. Please note any missing data.
d. Please provide an assessment of repeatability, reproducibility and uncertainty for the quantitative techniques (fat fraction and T2 relaxation time) used in the study.

IX. References

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X. Acknowledgement of supervisory concurrence

Digital Signature Concurrence Table	
Reviewer Sign-Off Daniel Krainak	
Medical Officer Sign-Off Gary Levine, M.D.	
Division Sign-Off	

VII. Clinical Pharmacology Review:

Clinical Pharmacology, Pharmacometrics and Genomics

CLINICAL PHARMACOLOGY REVIEW

NDA Number:	206031
Applicant Name:	BioMarin Pharmaceutical Inc.
Submission Dates:	April 27, 2015; Aug 24, 2015; Aug 28, 2015; Aug 31, 2015;
Brand Name:	Unknown
Generic Name	Drisapersen
Dosage Form:	Single-use clear glass vials containing 200 mg/mL of drisapersen sodium (0.5 mL and 0.8 mL deliverable volume) dissolved in aqueous phosphate buffer
Dosage Strengths:	200 mg/mL
Proposed Indication:	For the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing
OCP Division (s):	DCP 1, DPM, Genomics and Targeted Therapy
Primary Reviewers:	Atul Bhattaram, Bei Yu, Bart Rogers
Team Leaders:	Kevin Krudys, Angela Men, Christian Grimstein

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1 EXECUTIVE SUMMARY

Biomarin is seeking approval for drisapersen for the treatment of Duchenne muscular dystrophy (DMD) in patients with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing. Drisapersen is an exon skipping oligonucleotide designed to restore the mRNA reading frame and subsequently produce an internally-deleted dystrophin protein. The proposed treatment regimen is to initiate patients with a drisapersen dose of 6 mg/kg twice weekly for the first 3 weeks of treatment. Subsequently, drisapersen is to be administered 6 mg/kg once weekly. It is recommended that drisapersen be administered subcutaneously. Injection sites should be rotated.

The efficacy and safety of drisapersen was evaluated in 2 placebo-controlled clinical studies that had a duration of 48 weeks and 1 placebo-controlled clinical study that had a duration of 24 weeks. In addition, findings from a long term open-label study (up to 168 weeks) were compared to a natural history cohort and were submitted as supportive evidence of effectiveness. The sponsor submitted results of 7 clinical studies to characterize the PK of drisapersen in DMD patients.

The primary purpose of this review from the perspective of the Office of Clinical Pharmacology was to evaluate the sponsor's comparison of findings from the open-label 3 year clinical study (DMD114673) with the sponsor's natural history data.

The findings from the Office of Clinical Pharmacology are as follows:

- Potential issues with the matching analyses comparing the effect of drisapersen on 6 minute walk distance (6MWD) in study DMD114673 with natural history controls were identified. In this study, 12 patients were treated with 6 mg/kg drisapersen for 168 weeks. Drisapersen treated patients were matched with natural history controls on the basis of age and 6MWD. The analysis, according to sponsor, showed improvement in 6MWD in some patients when compared to their matched controls. However, the matching analysis was conducted using one natural history database and did not take into consideration other important prognostic factors such as genetic mutations and rise time. Due to these issues, it is difficult to make any definitive conclusions regarding efficacy of long term treatment with drisapersen.
- Drisapersen was able to lower serum creatine kinase (CK) across all clinical studies. No clear association between changes in serum CK levels and 6 minute walk distance was observed. Changes in serum CK likely reflect pharmacodynamic activity of drisapersen.
- Drisapersen plasma concentrations, after several weeks of treatment, were similar in all clinical efficacy trials (DMD114117, DMD11876 and DMD114044) irrespective of the observed effect on 6MWD.
- The impact of drisapersen binding antibodies and antidystrophin antibodies on efficacy and safety was inconclusive.

- No significant QT prolongation was observed in a clinical efficacy study (DMD 114876).

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the submission (NDA 206031). The review concludes that:

- The long term study DMD114673 does not provide supportive evidence of effectiveness for drisapersen at this time.
- The findings regarding changes in serum CK following drisapersen treatment likely represent a pharmacodynamic effect of drisapersen, however they are not correlated with clinical benefit.
- If found to be safe and effective, drisapersen should be indicated for all mutations amenable to exon-51 skipping.
- There is inadequate information on the effects of drisapersen in patients younger than 5 years of age and not concomitantly treated with corticosteroids. The sponsor should conduct a controlled clinical trial to evaluate drisapersen in this age group.
- The impact of anti-drisapersen antibodies on clinical efficacy and safety should be evaluated in a long term study (> 48 weeks), e.g., the immunogenicity could be assessed in an ongoing study DMD114673.

1.2 Phase 4 Commitments

None

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

The current submission consisted of 7 clinical studies to characterize single and multiple dose-PK of drisapersen in DMD patients between doses of 0.5 mg/kg/week and 9 mg/kg/week (for 9 mg/kg dose level, only single dose PK was evaluated).

Following multiple doses of 3-6 mg/kg/week subcutaneously, the median Tmax is between 2 and 4 hours; the inter-subject variability (% CV) was low to moderate (22-46% for Cmax and 25-47% for AUC0-t). There is a trend of dose proportionality between 3 mg/kg/week and 6 mg/kg/week.

Plasma trough levels and muscle tissue levels increased over time with once weekly dosing and approached steady state after 24 weeks. Drug accumulation in plasma and muscle was observed following multiple doses of drisapersen at 6 mg/kg/week.

Drisapersen is highly bound to human plasma protein in vitro (≥ 98.2%). Unchanged (parent) drisapersen was the major circulating drug-related component detected in the plasma. Drisapersen is not expected to be a substrate of CYP450, and it's not an inhibitor or inducer of major CYP450 isozymes at the therapeutic dose in vitro. Drisapersen and its shortened metabolites are excreted primarily in urine.

Findings from three clinical studies (DMD114117, DMD114876, DMD114044) were used to provide information on benefit/risk ratio of drisapersen. In addition, findings from an open label, longterm study (168 weeks) were compared to subjects from a natural history cohort to provide supportive evidence of effectiveness. Information on biomarkers such as dystrophin and creatine kinase were collected in various studies.

Effect of drisapersen on primary clinical endpoint

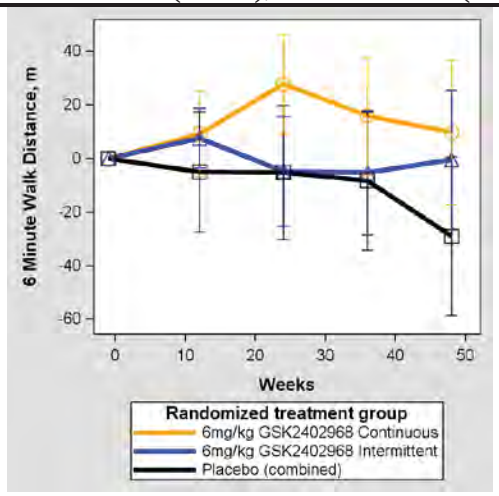
DMD usually first manifests in boys 3 to 7 years of age when they are noted to develop proximal muscle weakness. By 10 to 14 years of age, most boys with DMD have transitioned to full-time wheelchair use [Craig M McDonald et al, Muscle & Nerve, 2010]. Drisapersen was evaluated for its effect on 6 minute walk distance (6MWD) as the primary endpoint. A decline of approximately 30 meters from an average performance on the 6MWD in DMD to a threshold 6MWD of 325 meters or 55%-predicted would place a patient with at risk for more precipitous decline in ambulatory function over the subsequent year [Craig M McDonald et al, Muscle & Nerve, 2013].

Sponsor conducted studies that evaluated dose-response and impact of continuous versus intermittent dosing regimens and enrolled patients with a wide range of baseline prognostic factors known to influence DMD disease progression. Briefly, study DMD114876 evaluated efficacy and safety of 3 and 6 mg/kg doses of drisapersen every week. Study DMD114117 evaluated efficacy and safety of a 6 mg/kg dose of drisaperen administered as a continuous regimen (every week) or as an intermittent regimen (6 mg/kg drisapersen twice weekly on the 1st, 3rd and 5th weeks, once weekly on the 2nd,

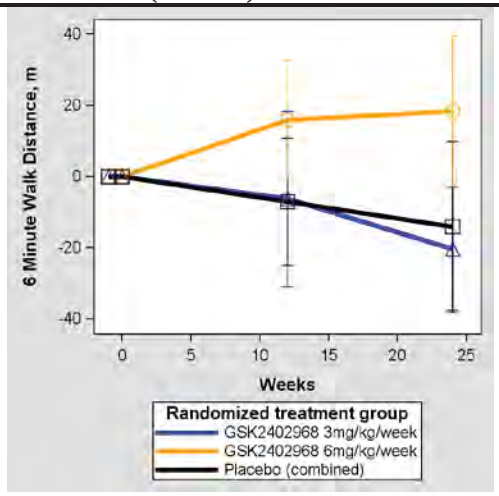
4th and 6th weeks, and no active drug on the 7th to 10th weeks of each 10 week cycle). All subjects in study DMD114117 were initially administered doses twice weekly for the first three weeks (loading dose). The intermittent regimen cycle started after completion of the loading dose regimen. Study DMD114044 studied the effect of 6 mg/kg dose of drisapersen administered weekly. No loading doses were administered in study DMD114044.

Figure 1 shows the mean change in 6MWD across studies in ITT population. Patients in DMD114876 study treated with drisapersen 6 mg/kg relative to placebo showed a change of +27 m at 24 weeks (p=0.0609). Patients in DMD114117 study treated with drisapersen 6 mg/kg relative to placebo showed a change of +35 m at 25 (p=0.0104) and 49 (p=0.0501) weeks relative to placebo. Patients in DMD114044 study treated with drisapersen relative to placebo showed a change of +10 m (p=0.415) at the end of 48 weeks. For further details about clinical significance of the changes in 6MWD and other secondary endpoints, please refer to the review by Dr Veneeta Tandon (Medical Officer, DNP).

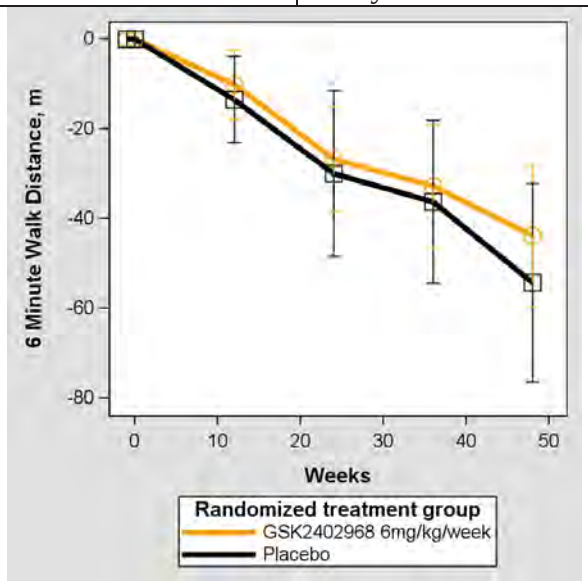
Figure 1. Mean Change from Baseline (95% CI) in 6MWD (m) Population in Studies DMD114117 (N=53), DMD114876 (N=51), DMD114044 (N=162).



Study DMD114117



Study DMD114876



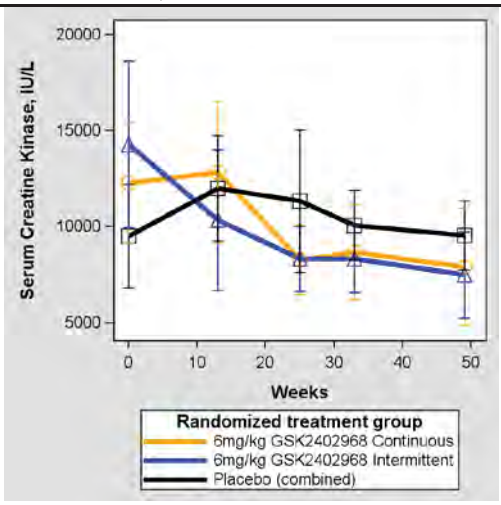
Study DMD114044

Findings from long term study DMD 114673 (N=12) were compared with a natural history control. Patients in the natural history control were on a stable dose of corticosteroids. While matching analyses suggested that some patients have better walking ability than matched controls it should be noted that the matching analyses included mutations not amenable to exon 51 skipping therapy. It is not clear how to evaluate impact of other factors such as supportive care in matching analyses. While improvements in 6MWD in some DMD114673 study patients could be due to drisapersen, definitive treatment benefit upon long term administration of drisapersen cannot be adequately quantified with the available data.

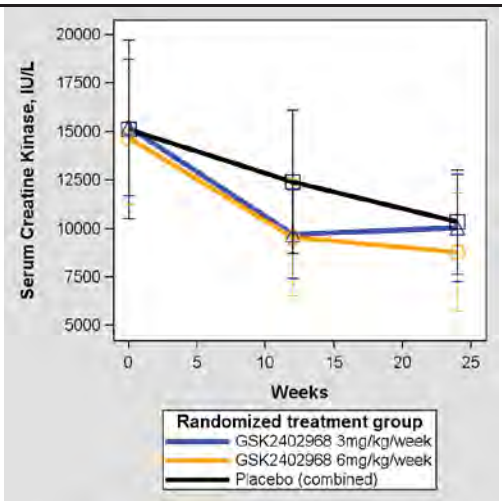
Effect of drisapersen on biomarker (creatine kinase)

Serum creatine kinase is a marker of muscle damage in DMD. Patients with DMD have high levels of creatine kinase (Figure 2). Normal values from healthy subjects are in the range of 60 to 174 IU/L. Drisapersen decreased creatine kinase levels by 30-40% across studies. While serum CK decreases were observed in DMD114044 study, there were no significant findings on the primary endpoint of 6MWD. For further details refer to the review by Dr Veneeta Tandon (Medical Officer, DNP)

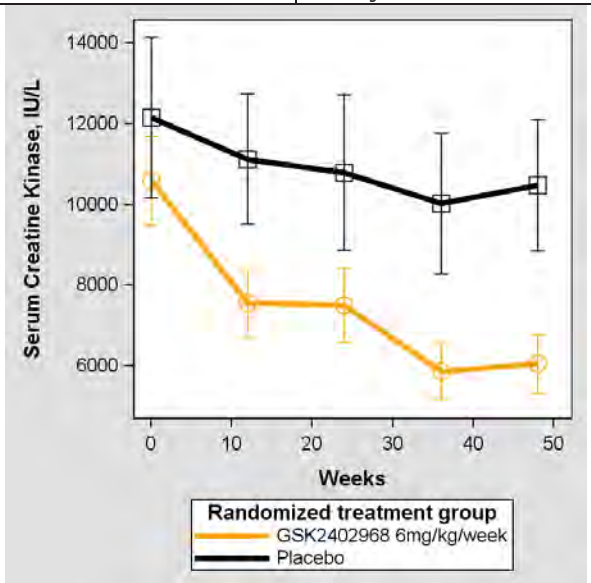
Figure 2. Mean Change in Creatine kinase (95% CI) in Studies DMD114117, DMD114876, DMD114044.



Study DMD114117



Study DMD114876



Study DMD114044

Safety Findings

The adverse events (AEs) of interest are injection site reactions, thrombocytopenia and glomerular nephritis. Table 1 shows overview of on-treatment adverse events in Study DMD114044.

Table 1. Overview of On-Treatment Adverse Events (Safety Population)

	Number (%) of Subjects		
	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)	Total (N=186)
Any AE of special interest	37 (61)	114 (91)	151 (81)
Injection site reaction	10 (16)	97 (78)	107 (58)
Renal effects	20 (33)	80 (64)	100 (54)
Inflammation	16 (26)	33 (26)	49 (26)
Coagulation	9 (15)	9 (7)	18 (10)
Hepatic effects	0	7 (6)	7 (4)
Thrombocyte counts	0	0	0

Source : Source : Table 36 on Page 103 in dmd114044csrbody.pdf

The labeling proposes strategies to handle these safety findings. Please refer to the review by Dr Evelyn Mentari, Clinical Safety Reviewer, DNP, for further details.

Immunogenicity Findings

The total incidence of anti-drug antibodies (ADA) formation was 29.4% in 109 patients during 48-week drisapersen treatment. Trough concentrations of drisapersen were increased by 130% in patients who were ADA positive compared to those who were ADA negative on Week 47. Given the multiple confounding factors associated with the disease, the impact of ADA on 6MWD is inconclusive.

It seems that ADA unlikely has an impact on AEs/SAEs and relevant lab parameters based on the available data.

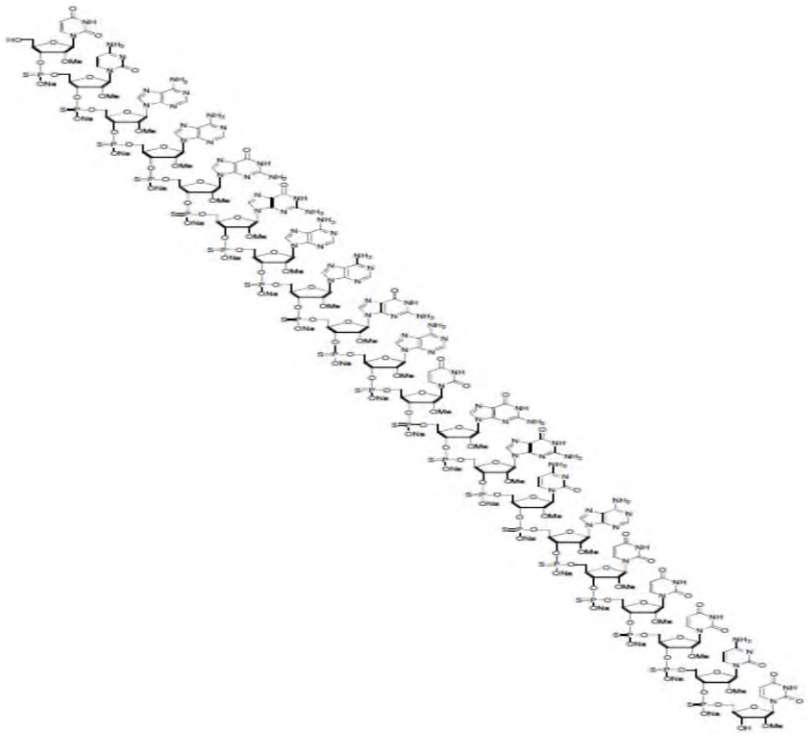
2 QUESTION BASED REVIEW

2.1 General Attributes of the Drug

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

Drisapersen (GSK2402968 and PRO051) is a 20mer chemically-modified antisense oligonucleotide with molecular mass of 7395.27 (averaged isotopic distribution).

Drisapersen sodium solution, 200 mg/mL is a sterile, clear, colorless to yellow solution, essentially free from particulates, containing 200 mg/mL of drisapersen sodium (the sodium salt of a 20-mer 2'-O-methyl-phosphorothioate oligoribonucleotide). The structure of drisapersen sodium is presented below:



2.1.2 What are the proposed mechanism of action and therapeutic indications?

Drisapersen is an exon skipping oligonucleotide inducer of dystrophin synthesis indicated for the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing.

2.1.3 Should drisapersen be indicated for patients amenable to exon-51 skipping who were not studied in the clinical development program?

Yes. Despite studying nine different DMD mutations amenable to exon-51 skipping, not all amenable mutations were enrolled in the clinical development program. Proposed product labeling states that drisapersen is to be indicated for all DMD mutations that are amenable to treatment with exon 51 skipping. In theory, drisapersen can restore the mRNA reading frame to produce an internally-deleted dystrophin for a number of DMD deletion mutations not studied in the clinical development program.

Patients with other ultra-rare DMD deletion mutations that are amenable to exon-51 skipping do exist (e.g. 13-50, 52-63). For some amenable mutations only 1-2 patients exist in the DMD Leiden database (www.dmd.nl). Given the strict inclusion criteria for the drisapersen clinical trials, these patients may have been ineligible to participate. Hence, given the lack of available subjects for study, coupled with inherent heterogeneity in disease, along with the unknowns regarding the functionality of the internally-deleted dystrophin; determining efficacy in patients with ultra-rare DMD mutations amenable to exon-51 skipping is difficult. Last, there are no reasons to believe that the safety of drisapersen is in any way different in these ultra-rare populations of patients. Thus, if drisapersen is ultimately found to be safe and effective to warrant approval, then drisapersen should be indicated for all exon-51 amenable mutations.

2.1.4 What are the proposed dosages and routes of administration?

The proposed dosage and route of administration is the following:

- Loading dose: Initiate with 6 mg/kg twice weekly for the first 3 weeks of treatment
- Maintenance dose: 6 mg/kg once weekly
- Administer drisapersen subcutaneously. Rotate injection sites

Patients should receive concomitant glucocorticosteroid therapy during drisapersen treatment. The dosing regimen was evaluated in Study DMD 114117 (A phase II, double blind, exploratory, parallel-group, placebo-controlled clinical study to assess two dosing regimens of GSK2402968 for efficacy, safety, tolerability and pharmacokinetics in ambulant subjects with Duchenne muscular dystrophy).

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and the clinical studies used to support dosing or claims?

The dosing and claims are based on changes in 6 minute walk distance (primary endpoint) in double blind, randomized, placebo controlled studies. Studies DMD114117, DMD114678 are 48 weeks in duration with the primary endpoint at 24 weeks. The Phase III clinical trial design is similar to early clinical studies with the primary endpoint at 48 weeks. There were differences in entry criteria between early clinical studies and Phase

III study. Early clinical studies enrolled patients who could rise from the floor within 15 seconds. In Phase III study, this enrollment criteria was not implemented. Patients with rise time greater than 15 seconds have worse prognosis.

2.2.2 What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology studies?

The response endpoint in clinical trials is 6 minute walk distance. This endpoint is not measured in clinical pharmacology studies.

2.2.3 Are the active moieties in plasma appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

Yes.

2.2.4 Exposure-Response

2.2.4.1 What are the characteristics of the exposure-response relationships for efficacy?

A link between drisapersen plasma or muscle biopsy concentrations with changes in 6MWD cannot be definitively quantified at this time. The drisapersen treated group (6 mg/kg) relative to placebo in study DMD114117 and DMD114876 showed improvement in 6MWD; these studies (combined) had 36 patients receiving drisapersen. In study DMD114044, 122 patients were treated with 6 mg/kg drisapersen. In this study, drisapersen did not show any benefit relative to placebo.

The reviewer conducted analyses to understand differences in findings across studies. Drisapersen plasma concentrations collected pre-dose were compared across studies (Table 2). Table 2 shows that at Week 24 drisapersen pre-dose plasma concentrations were similar across the studies. Hence, differences in efficacy between studies are due to factors other than drisapersen concentrations.

Table 2. Drisapersen Plasma Concentration at Pre-dose in Studies DMD114044, DMD114876, DMD114117.

Visit	n	Trough concentration (ng/mL) Median (SD)
Week 8	47	12.7 (747)
Week 12	47	19.1 (12.9)
Week 24	49	40.9 (37.3)
Week 36[a]	38	55.2 (1059)
Week 48	106	61.4 (576)

Study DMD114044

Source: Table 60 on Page 132 in dmd114044csrbody.pdf. Shown are median (SD)

Parameter	Week 0		Week 12		Week 23	
	n		n		n	
C168 (ng/mL)[1]	5	5.1 (118)	7	21.1 (76.4)	7	35.8 (92)

Study DMD114876

Source: Table 64 on Page 149 in dmd114876csrbodyefficacy.pdf. Shown are geometric mean (%CV)

Visit	6 mg/kg Drisapersen Continuous	
	n	Concentration (ng/mL) [a]
Week 1 Day 1	18	0.00
Week 1 Day 4	18	5.86 ± 10.3
Week 24	16	27.1 ± 19.1
Week 29	18	37.9 ± 27.8
Week 34	17	37.4 ± 40.7
Week 37	17	47.9 ± 84.2

Study DMD114117

Source: Table 58 on Page 128 in dmd114117csrbody.pdf. Shown are median (SD)

While not definitive, the failure of DMD114044 could be due to enrollment of patients with advanced stage of the disease. To identify reasons the reviewer characterized disease progression and looked at influence of baseline prognostic factors such as age, 6MWD, rise time, corticosteroid dosing regimen on the rate of change in 6MWD. The analysis showed that age, 6MWD and rise time influence the rate of change in 6MWD. These findings are similar to those reported in literature for other mutations. It is the opinion of the reviewer that more research is needed to identify other potential prognostic factors such as physiotherapy and concomitant medications for cardiac related issues on the rate of change in 6MWD and ultimately their inclusion in exposure response analyses.

2.2.4.2 What are the characteristics of the exposure-response relationships for safety?

The adverse events (AEs) of interest are injection site reactions, thrombocytopenia and glomerular nephritis. Table 3 shows the adverse events of special interest by time to first occurrence in study DMD114876. The onset of injection site reactions in treatment group is less than a week in some patients. Analysis linking drisaperen concentrations with safety was not conducted.

Table 3. AEs of Special Interest by Time to First Occurrence (Safety Population) in Study DMD114876

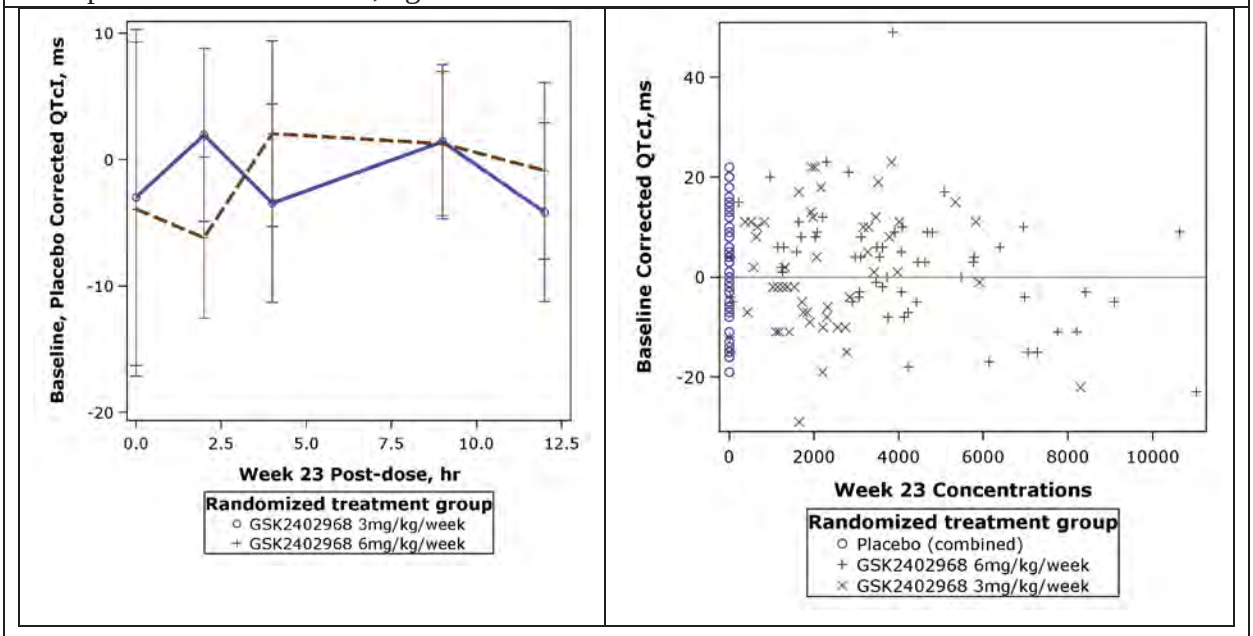
Treatment Group/ Special Interest Category	Number (%) of Subjects Time Since Start of Study Medication											
	<1 Week	<2 Weeks	<3 Weeks	<4 Weeks	<8 Weeks	<12 Weeks	<16 Weeks	<20 Weeks	<24 Weeks	<36 Weeks	<48 Weeks	≥48 Weeks
Placebo (N=16)												
Any AE of Special Interest	0	0	0	0	0	3 (19)	6 (38))	8 (50)	9 (56)	9 (56)	9 (56)	0
Injection site reaction	0	0	0	0	0	2 (13)	5 (31)	5 (31)	5 (31)	6 (38)	6 (38)	0
Renal effects	0	0	0	0	0	1 (6)	1 (6)	4 (25)	5 (31)	5 (31)	5 (31)	0
Inflammation	0	0	0	0	0	0	0	0	1 (6)	1 (6)	1 (6)	0
Coagulation	0	0	0	0	0	0	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	0
Hepatic effects	0	0	0	0	0	0	0	1 (6)	1 (6)	1 (6)	1 (6)	0
Thrombocyte counts	0	0	0	0	0	0	0	0	0	0	0	0
Drisapersen 3 mg/kg (N=17)												
Any AE of Special Interest	4 (24)	8 (47)	9 (53)	9 (53)	10 (59)	10 (59)	11 (65)	11 (65)	11 (65)	11 (65)	11 (65)	0
Injection site reaction	3 (18)	8 (47)	9 (53)	9 (53)	10 (59)	10 (59)	11 (65)	11 (65)	11 (65)	11 (65)	11 (65)	0
Renal effects	1 (6)	1 (6)	1 (6)	1 (6)	2 (12)	2 (12)	2 (12)	2 (12)	2 (12)	2 (12)	2 (12)	0
Inflammation	0	0	0	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	0
Coagulation	0	0	0	0	0	0	0	0	0	0	0	0
Hepatic effects	0	0	0	0	0	0	0	0	0	0	0	0
Thrombocyte counts	0	0	0	0	0	0	0	0	0	0	0	0
Drisapersen 6 mg/kg (N=18)												
Any AE of Special Interest	6 (33)	9 (50)	9 (50)	10 (56)	13 (72)	15 (83)	16 (89)	16 (89)	16 (89)	16 (89)	16 (89)	0
Injection site reaction	6 (33)	8 (44)	8 (44)	9 (50)	11 (61)	13 (72)	13 (72)	13 (72)	13 (72)	13 (72)	13 (72)	0
Renal effects	0	0	0	0	2 (11)	4 (22)	4 (22)	4 (22)	5 (28)	5 (28)	5 (28)	0
Inflammation	1 (6)	2 (11)	2 (11)	3 (17)	3 (17)	3 (17)	3 (17)	3 (17)	5 (28)	5 (28)	5 (28)	0
Coagulation abnormalities	0	0	0	0	0	0	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	0
Hepatic effects	0	0	0	0	0	0	1 (6)	1 (6)	1 (6)	1 (6)	1 (6)	0
Thrombocyte counts	0	0	0	0	0	0	0	0	0	0	0	0

Source : Table 38 on Page 109 in dmd114876csrbodyefficacy.pdf

2.2.4.3 Does this drug prolong QT/QTc Interval?

No formal TQT study was conducted although the sponsor collected information on QT in various studies. No large changes in mean QT interval were detected in an efficacy study (DMD114876). In this clinical study, patients were randomized to placebo, 3 mg/kg and 6 mg/kg drisapersen administered weekly. Each group consisted of 16-18 patients. The largest upper bound of the 2-sided 90% confidence interval (CI) for the mean change from baseline (placebo corrected) was less than 10 ms after administration of drisapersen on Week 23 (Figure 3). Evidence of dose response on QT prolongation was not observed. Baseline corrected QTcI did not show any relationship with drisapersen concentrations (Figure 3).

Figure 3: (Left) Mean and 90% CI ΔΔQTcI Time Course for Drisapersen Treatment Groups. (Right) Relationship Between Baseline Subtracted QTcI,msec and Drisapersen Concentrations, ng/mL



2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known E-R relationship?

Preclinical and clinical findings, according to sponsor, led 6 mg/kg to be considered the maximum tolerated dose.

PK modeling suggested that with a loading dose regimen of twice weekly dosing for 3 weeks, on average approximately steady state drisapersen concentrations are achieved 6 weeks earlier compared to weekly administration. Hence, all subjects in the successful study DMD114117 received a twice-weekly loading dose of 6 mg/kg (or placebo) for the first 3 weeks to achieve plasma levels of drisapersen that are anticipated to provide a therapeutic response, thereby potentially providing a faster clinical benefit. However, patients in the Phase III study DMD114044 did not receive loading doses.

The dose selection for DMD114044 was based on open-label clinical study with drisapersen in subjects with DMD. PRO051-02 (DMD114673 [acute phase]), showed exon 51 skipping with doses of 2 to 6 mg/kg given weekly for 5 weeks. No differences were observed with regard to muscle function and muscle strength between dose groups, or change over time within any dose group. However, it was likely that the duration of dosing was too short to demonstrate any functional changes. Subjects then received drisapersen 6 mg/kg/week for at least 48 weeks in the open-label extension to PRO051-02 (DMD114673 [extension phase]), and it was generally well tolerated. The efficacy data obtained at the 24-week timepoint in the open-label extension for study PRO051-02 (DMD114673 [extension phase]) suggested that the 6 mg/kg/week dose provided a clinically meaningful benefit in the majority of subjects, with a mean change in the 6MWD test of 36.8 m (range -58 m to +115 m) and was therefore supportive of the choice of dose in for study DMD114044.

Dose selection for DMD 114876 was based on PK/PD modeling. Based on PK/PD modeling, it was predicted that at steady-state, the 6 mg/kg/week dose would induce dystrophin expression greater than 30% of control. The 3 mg/kg/week dose was chosen as modeling predicted 3 mg/kg/week of drisapersen would produce dystrophin expression in the range of 18-22%.

There is lack of reliable data on dystrophin expression in DMD114044 to confirm model based predictions.

2.2.4.5 Immunogenicity

2.2.4.5.1 What is the incidence (rate) of the formation of the anti-product antibodies (APA), including the rate of pre-existing antibodies, the rate of APA formation during and after the treatment, time profiles and adequacy of the sampling schedule?

Sparse PK plasma samples in clinical study DMD114044 were analyzed for anti-drug antibody (ADA). A summary of ADA detected in all evaluable plasma samples of patients in the study is presented in the table below:

Study Week	Number of positive subjects and titer information	Placebo Subjects	Treated Subjects
0	Positive /total (total of samples analysed)	1/17 (47)	0/0 (0)
	Median titer (range)	200 (200)	NA
	Rate of ADA formation (%)	5.9	NA
8	Positive /total (total of samples analysed)	0/23 (23)	1/47 (47)
	Median titer (range)	NA	50 (50)
	Rate of ADA formation (%)	NA	2.1
12	Positive /total (total of samples analysed)	0/25 (25)	0/46 (46)
	Median titer (range)	NA	NA
	Rate of ADA formation	NA	NA

24	Positive /total (total of samples analysed) Median titer (range) Rate of ADA formation (%)	0/13 (13) NA NA	8/51 (51) 300 (100-3200) 15.7
36	Positive /total (total of samples analysed) Median titer (range) Rate of ADA formation (%)	0/17 (17) NA NA	10/41 (41) 1000 (50-6400) 24.4
47/48	Positive /total (total of samples analysed) Median titer (range) Rate of ADA formation (%)	0/50 (50) NA NA	30/107 (107) 800 (100-6400) 28.0
Total positive subjects (%)		1 /50 (2)	32/109 (29.4)

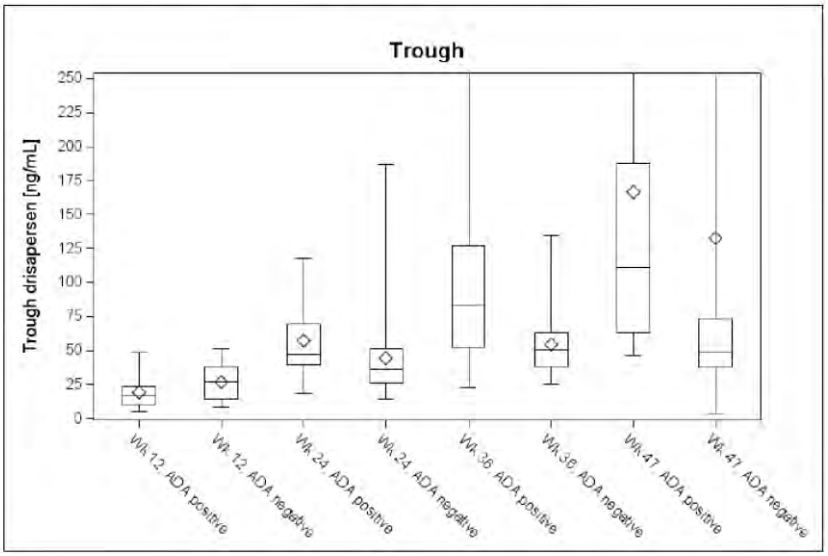
ADA formation is mainly detected after 12 weeks of drug treatment, at Weeks 24, 36, and 47/48. The incidence of ADA formation is 15.7% on Week 24, 24.4% on Week 36, and 28% on Week 47/48. The total incidence of ADA formation is 29.4% in 109 patients during the drug treatment.

One patient in placebo group showed ADA formation at Week 0.

2.2.4.5.2 Does the immunogenicity affect the PK of the therapeutic protein?

Yes. Trough concentrations of drisapersen are increased in ADA positive patients compared to those of ADA negative, which is more pronounced on Weeks 36 and 47. For instance, median trough concentrations of drisapersen are increased by 130% in ADA positive patients to those of ADA negative patients at Week 47.

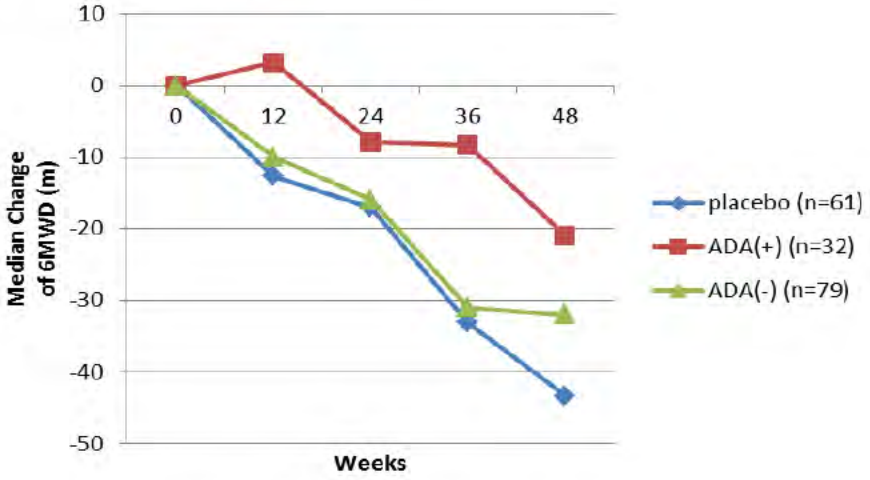
Figure 2.1: Boxplot of Drisapersen Trough Concentrations by Visit



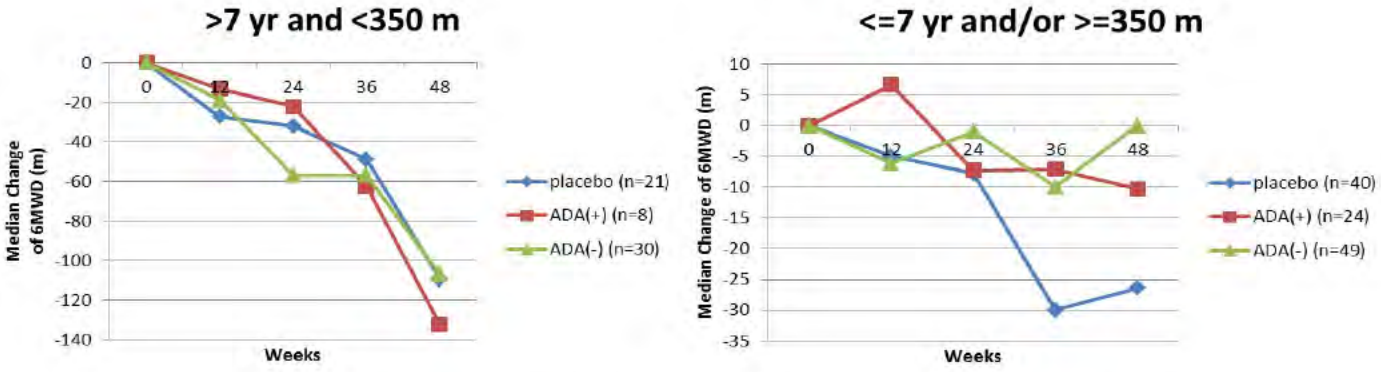
2.2.4.5.1 What is the impact of anti-product antibodies on clinical efficacy?

Due to multiple confounding factors associated with the disease, the impact of ADA on 6MWD is inconclusive based on the available data.

ADA positive patients showed a consistent less pronounced decline in mean and median change from original baseline in 6MWD at all visits compared to ADA negative subjects (Figure showed below).



However, unbalanced baseline age and the associated baseline 6MWD can contribute to the difference on 6MWD between ADA positive and negative patients (figure shown below).



2.2.4.5.2 What is the impact of anti-product antibodies on clinical safety?

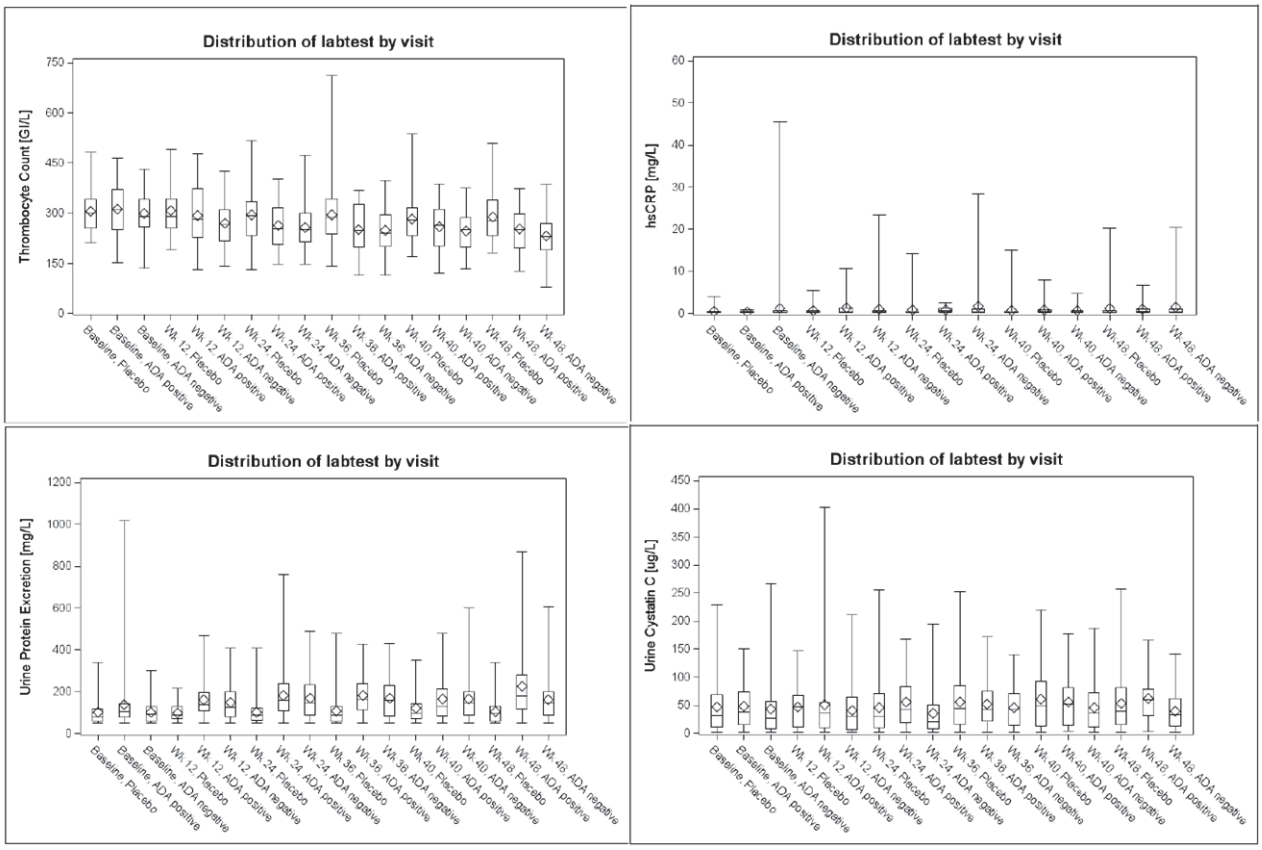
It seems that ADA unlikely has an impact on AEs/SAEs and relevant lab parameters based on the available data.

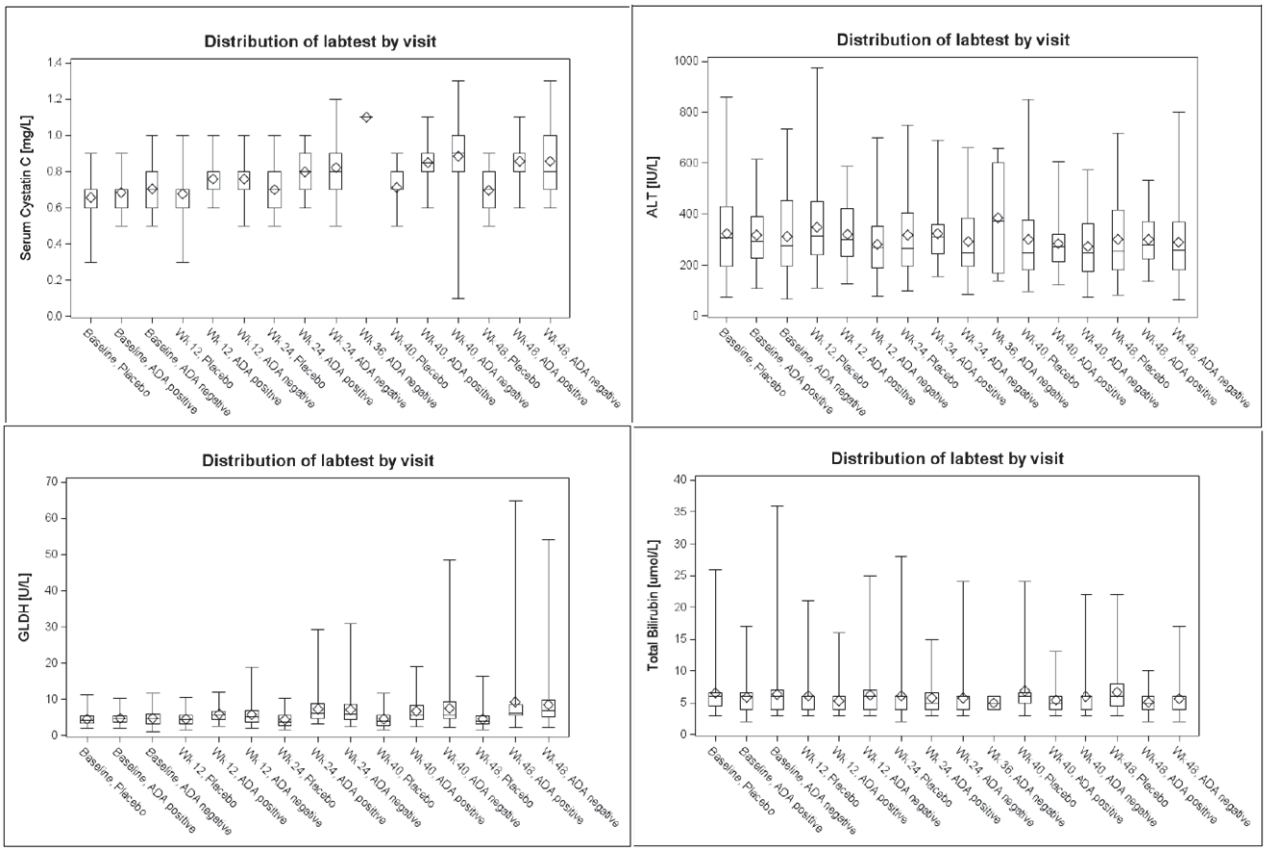
The first ADA positive sample for all except one ADA positive subject was at Week 24 or later. Therefore, only (S)AEs starting from Week 24 onwards were included in the analysis. None of the SAEs included in the analysis were drug related. Three SAEs (Subject 527, Glomerulonephritis, Subject 1270 Intracranial venous sinus thrombosis and

spinal pain) excluded from the analysis were drug related. All three SAEs occurred after Week 24 in subjects with inconclusive ADA status. Subjects on active treatment who have only negative ADA samples, but did not have an end of study sample were considered inconclusive regarding ADA status and were excluded from the analysis.

No increase in the percentage AEs, AESIs (injection site reaction, renal toxicity, inflammation, hepatic toxicity, coagulation, thrombocytes) was observed in ADA positive subjects, compared to ADA negative subjects. There is an over 1 fold increase of SAE in ADA positive patients compared to negative patients: One SAE (myocardial ischaemia), 3% of patients in ADA positive group; two SAEs (lumbar vertebral fracture and tibia fracture), 1% of patients in ADA negative group.

All laboratory parameters were generally in the same range with similar mean/median between ADA positive and ADA negative patients for all visits (figures showed below).





2.2.4.5.3 What is the incidence (rate) of the formation of the anti-dystrophin antibodies?

There is no reliable data on the incidence of the formation of anti-dystrophin antibodies.

Since dystrophin protein is theoretically lacking in DMD patients and a truncated-form of the protein produced by drisapersen treatment could elicit an immune response, serum samples had been collected from patients in clinical studies except for PRO051-01 to test the presence of anti-dystrophin antibodies using western blot analysis. A summary of results of the detection of anti-dystrophin antibodies in the clinical studies is presented below:

Study	Total number subjects evaluated	Anti-dystrophin antibody in placebo treated subjects		Anti-dystrophin antibody in drisapersen treated subjects	
		Subjects evaluated (n)	Positive subjects in any sample (n (%))	Subjects evaluated (n)	Positive subjects in any sample (n (%))
DMD114673 (acute phase)	12	na	na	12	0
DMD114673 (extension phase)	12	na	na	12	0
DMD114117	53	18	0	35	0
DMD114118	20	5	0	15	0
DMD114876	51	16	0	35	0
DMD114044	186	61	2 (3%)	125	1 (1%)
DMD114349	233	na	na	233	9 treated / 5 placebo(5%)
Total	**567**	**100**	**2 (2%)**	**467**	**12 (3%)**

Note: Note: All studies described in this section were performed using the sodium salt of drisapersen. Drisapersen sodium may be referred to as drisapersen, PRO051, GSK2402968, or h51AON23 in the study reports provided.
Key: n: number of subjects, na: not applicable, since the DMD114673 and the DMD114349 studies were open-label studies

The data showed that the rate of the formation of anti-dystrophin antibodies is low (3%) in drisapersen treated patients, which is comparable with that of placebo patients (2%).

A limitation of the assay is that the western blotting was performed using full-length dystrophin protein. In the unlikely event that an induced anti-dystrophin antibody would be only specific to the epitope unique to the truncated form of the protein resulting from the exon-skipping treatment, this antibody may not be detected with this assay. In addition, no confirmatory assay could be developed as no purified dystrophin protein is available so any positive samples could not be confirmed. Thus, the incidence of the formation of anti-dystrophin antibodies is inconclusive.

2.2.5 What are the PK characteristics of the drug?

2.2.5.1 What are the single and multiple dose PK parameters?

The current submission consisted of 7 clinical studies to characterize single and multiple dose-PK of drisapersen for DMD patients between doses of 0.5 mg/kg/week and 9 mg/kg/week (for 9 mg/kg dose level, only single dose PK was evaluated).

A summary of main PK parameters (Cmax and AUC0-24h) and tissue levels of drisapersen in the clinical studies is shown below:

Study Ref No	Treatments (route, dose, regimen)	Parameter				Mean tissue concentration [c]		Study summary location
		Cmax (µg/mL) [a]		$AUC_{0\text{-}24h}$ (µg.hr/mL) [b]		(µg/g)	wk in study	
		Day 1	End of study	Day 1	End of study			
DMD114673 (acute phase)	SC, 0.5 mg/kg/wk for 5 weeks SC, 2 mg/kg/wk for 5 weeks SC, 4 mg/kg/wk for 5 weeks SC, 6 mg/kg/wk for 5 weeks	1.69 3.62 5.27 9.13	1.02 4.11 6.80 11.0	7.5 26.9 40.8 76.7	5.9 25.9 44.2 103	- - - 6.9	- - - 5	section 2.2.1.2.1
DMD114673[d] (extension phase)	SC, 6 mg/kg/wk SC, start of re-dosing	- 8.2	8.7 -	- 105	103 -	14.4 20.3	24 69	section 2.2.3.1.1
DMD114118	SC, 3 mg/kg single dose SC, 6 mg/kg single dose SC, 9 mg/kg single dose	4.99 8.14 8.94	- - -	44.6 87.8 97.8	- - -	- - -	- - -	section 2.2.2.1.1
DMD114117[e]	Continuous: SC, 6 mg/kg/wk or Intermittent: alternating 6 mg/kg biweekly and 6 mg/kg/wk for 6 weeks followed by 4 weeks off-dose period	- -	4.85 4.81	- -	45.5 52.5	11.0 9.8	24 24	section 2.2.2.2.1
DMD114876[f]	SC, 3 mg/kg for 24 weeks SC, 6 mg/kg for 24 weeks	3.05 5.73	2.84 6.24	23.0 46.7	30.2 57.3	2.7 10.8	24 24	section 2.2.2.3.1
DMD114044	SC, 6 mg/kg/wk for 48 weeks	-	-	-	-	4.1 5.2 9.3 16.6 14.0	8 12 24 36 48	section 2.2.2.4.1
DMD114349[g]	SC, 6 mg/kg/week up to 104 weeks divided into sub-groups of placebo in feeder study active treatment in feeder study	 5.68 5.81	 6.57 6.22	 55.2 58.4	 80.3 74.1			section 2.2.3.2.1

Note: All studies described in this section were performed using the sodium salt of drisapersen. Drisapersen sodium may be referred to as drisapersen, PRO051, GSK2402968, or h51AON23 in the study reports provided. PK parameters are reported in ng in the reports, however stated in µg in this summary document

Key:

a Geometric mean (CV%) ; b Median (range); c mean tissue concentrations provided in µg/g tissue; wk is the week in which the biopsy was obtained during the study; d up to 177 weeks; tissue concentrations reported are from visit 37 (week 24) and visit 81 (week 69) respectively, re-dosing commenced approximately one year after week 177; e no profile obtained at first dose, end of study is 29 week; subjects started with SC 6 mg/kg twice weekly for 3 weeks and thereafter continued with either the continuous or intermittent regimen; tissue concentrations determined in tibialis anterior muscle reported here (quadriceps excluded from mean); f tissue concentrations at week 24 reported in table; g Cmax and AUC reported are from subjects who had PK profiles on both occasions, end of study is week 48 profile, subjects were dosed up to 104 weeks

- = not determined/available; SC.=subcutaneous, wk = week

Following single dose administration, the inter-subject variability (% CV) at lower dose levels at 0.5 – 2 mg/kg was 66-75% for Cmax and 14-30% for AUC0-t. This large inter-subjects variability for Cmax was decreased to 20-50% following 5 weekly doses.

Overall, at dose levels of 3-9 mg/kg the inter-subject variability (% CV) was 7-48% for Cmax and 12-48% for AUC0-t following single dose administration. Following multiple doses of 3-6 mg/kg/week, the inter-subject variability (% CV) was low to moderate (22-46% for Cmax and 25-47% for AUC0-t).

Following multiple doses at 0.5 -2 mg/kg/week for 5 weeks, no obvious drug accumulation was observed. However following multiple doses at 6 mg/kg/week, AUC0-168 increased about 2-fold over 48 weeks of weekly dosing of drisapersen. Drug accumulation in muscle was also observed following multiple doses of drisapersen. In one study (DMD114044), mean concentrations of drisapersen in muscle tissue homogenates increased with increasing time up to about 36 weeks of dosing at 6 mg/kg/week.

Plasma trough levels and muscle levels increased over time with once weekly dosing and approach steady state after 24 weeks. Muscle tissue concentrations of drisapersen can be detected at 12 weeks after cessation of dosing at 3-6 mg/kg/week.

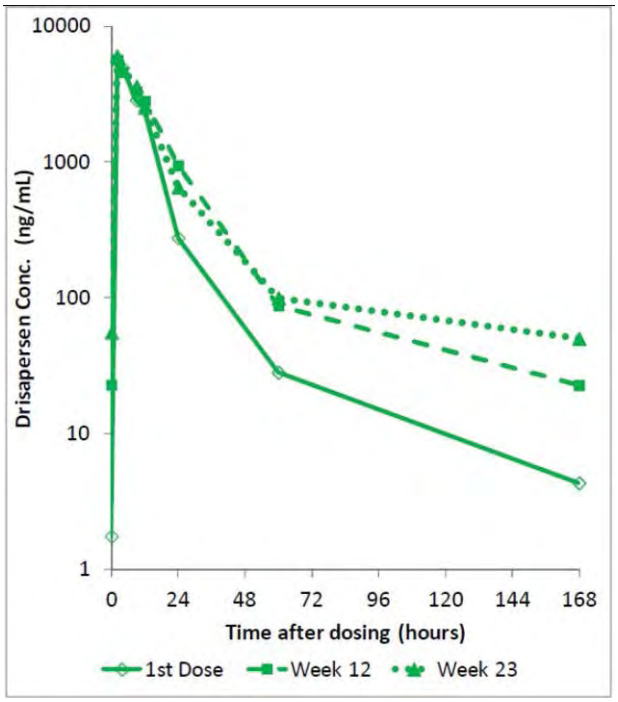
Extraction efficiency to determine drisapersen levels in muscle homogenates is unknown and the impact on accuracy has not been determined. Therefore the actual concentrations measured in tissue should be considered estimates.

2.2.5.2 How does the PK of the drug and its major metabolites in healthy adults compare to that in patients?

Drisapersen PK was evaluated in DMD patients.

2.2.5.3 What are the characteristics of drug absorption, distribution, metabolism and elimination)?

Following SC administration of drisapersen at 6 mg/kg/week, the median Tmax is between 2 and 4 hours. Mean drisapersen plasma concentration-time profiles following SC administration of 6 mg/kg/week after single and multiple doses are shown below:



Values of AUC0-24 of drisapersen are comparable following a 4-h IV infusion and a SC administration at 6 mg/kg.

Drisapersen is highly bound to human plasma protein in vitro (≥ 98.2%) at concentrations of 0.7 – 700 ug/mL.

Unchanged (parent) drisapersen was the major circulating drug-related component detected in the plasma. Minor metabolites resulting from the sequential loss of nucleotides from the 3' end were detected. Drisapersen is not expected to be a substrate of CYP450, and it's not an inhibitor or inducer of major CYP450 isozymes at the therapeutic dose in vitro.

Drisapersen and its shortened metabolites are excreted primarily in urine.

2.2.5.4 Based on PK parameters, what is the degree of linearity in the dose-concentration relationship?

Following single dose of drisapersen, over the dose range of 0.5 mg/kg to 6 mg/kg, Cmax and AUC parameters increased dose proportionally; however, less than dose proportionality has been shown between 6 mg/kg and 9 mg/kg.

Following multiple doses of drisapersen, there is a trend of dose proportionality between 3 mg/kg/week and 6 mg/kg/week.

2.2.5.5 How do the PK parameters change with time following chronic dosing?

Drispersen accumulation following weekly doses of 6 mg/kg has been observed in plasma and muscle tissues. Plasma trough levels and muscle levels increase over time with once weekly dosing and approach steady state after 24 weeks. Refer to Section 2.2.5.1.

2.2.5.6 What is the inter- and intra-subject variability of PK parameters in volunteers and patients?

Inter-individual variability on clearance is 20.3% and central volume of distribution is 20.3% and 26.6% respectively. The inter-occasion variability on absorption rate constant is 52.7%.

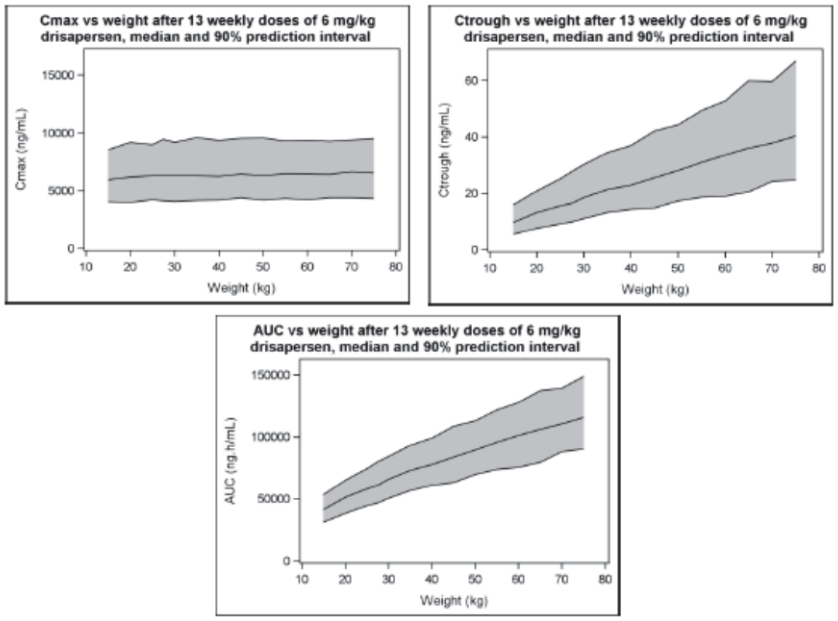
2.3 Intrinsic Factors

2.3.1 What intrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Factors influencing exposure

Body weight has an effect on exposure. Patients with higher body weight have higher AUC compared to lighter patients (Figure 4). There is no influence of body weight on Cmax when drisapersen is dosed on a mg/kg basis.

Figure 4. Predicted Week 13 drisapersen PK parameter estimates based on the final model (median and 90% prediction interval) versus body weight, for 6 mg/kg dosing. Top left: peak concentrations; top right: trough concentrations (before week 13 intake); bottom: AUC0-168h.



Source : Figure 14 on Page 59 in kns14082clinicalstudyreportincludingappendices.pdf

Factors influencing response

Baseline prognostic factors such as 6 minute walk distance, rise time, and age have an impact on DMD disease progression. Rise time is reported to be an early predictor of milestone events like loss of ambulation.

The Phase III trial DMD114044 conducted in 186 (N=124 in drisapersen group; N=62 in placebo group) patients did not show any treatment related benefit on 6MWD. The study enrolled patients with a wide range of rise time. DMD114117 was conducted in patients with rise time ≤ 7 seconds and showed treatment related benefit on 6MWD. DMD114876 study was conducted in patients with rise time <15 seconds. It is possible that differences in patient population characteristics could have contributed to differences between studies. It is not clear if the loading doses administered in DMD114117 study contributed towards a positive finding. However, drisapersen concentrations at 24 weeks were similar between the failed Phase III study and positive early clinical study (Table 2).

2.3.2 Based upon what is known about E-R relationships and their variability, what dosage regimen adjustments are recommended for each group?

2.3.2.1 Elderly

There are no labeling statements regarding dose adjustment in elderly. The patient population that would be treated with drisapersen would be younger.

2.3.2.2 Pediatric Patients

No dose adjustments are recommended. The dose/dosing regimen, as studied in DMD 114117, is the recommended dose in pediatric patients.

2.3.2.3 Race

Not enough information to determine the race impact on PK and the clinical responses of drisapersen. No dose adjustments are recommended.

2.3.2.4 Renal Impairment

Although renal related adverse events are reported, no specific dose adjustments are being proposed. For renal related adverse events, the label recommends:

Glomerular Renal Effects: Monitor urine protein. Suspend [TRADENAME] when urine protein is > 1 gram per 24 hours. Discontinue [TRADENAME] if patient develops glomerulonephritis

2.3.2.5 Hepatic Impairment

No clinical studies have been conducted to evaluate the PK of drisapersen in hepatic impaired patients. Hepatic metabolic function is less relevant to drisapersen as the main route of metabolism is through exonucleases and non-hepatic specific cytochrome enzymes.

Thus, no dose adjustments are recommended.

2.3.3 What pregnancy and lactation use information is there in the label?

Pregnancy
Risk Summary
Drisapersen has not been studied in female patients. Reproduction studies in female animals have not been conducted. It is not known whether drisapersen can cause fetal harm when administered to pregnant women or if drisapersen affects the female

reproductive capacity. The pharmacologic mechanism of action of drisapersen is not expected to result in adverse developmental outcomes.

Lactation
Risk Summary
Drisapersen has not been studied in female patients. It is not known whether drisapersen is excreted in human milk. The effect of drisapersen on human milk production or on the breast fed child has not been studied.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Analysis looking at the influence of extrinsic factors such as physiotherapy, concomitant medications other than corticosteroids on response was not conducted. It should be noted that patients in placebo and treatment groups were on stable corticosteroid doses.

2.4.2 What are the drug-drug interactions?

2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?

No.

Drisapersen is not expected to be a substrate of CYP450 enzymes, as the main route of its metabolism is through exonucleases and non-hepatic specific cytochrome enzymes.

Two in vitro studies have been conducted to evaluate the effect of drisapersen to inhibit or induce the major CYP enzymes in human hepatocytes. The studies show that drisapersen is unlikely to inhibit CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5, and is unlikely to induce CYPs 1A2, 2B6 and 3A4/5 at the proposed therapeutic dose.

2.4.2.2 Is the drug an inhibitor and/or an inducer of PGP transport processes?

Drisapersen is unlikely to be an inhibitor/or inducer of membrane transporters.

No studies have been conducted to evaluate drisapersen's potential interaction (as a substrate, inhibitor, or inducer) with uptake and efflux membrane transporters.

AONs (including drisapersen) appear to be taken up into cells via endocytosis and not by uptake membrane transporters. In addition, ASOs have long tissue half-lives, up to several weeks. Clearance from tissues is very slow and typically involves metabolism by exo- and endonucleases (though this latter appears less important for drisapersen). This suggests no involvement of membrane transporters.

2.4.2.3 Does the label specify co-administration of another drug?

Yes. Patients will receive concomitant corticosteroid therapy.

2.4.2.4 What other co-medications are likely to be administered to the target population?

Corticosteroids, beta blockers, ACE inhibitors, medications to manage pain and other co-morbidities.

2.4.2.5 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

No

2.4.2.6 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions?

No

2.4.2.7 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

No

2.4.3 What issues related to dose, dosing regimens, or administration are unresolved and represent significant omissions?

No.

2.5 General Biopharmaceutics

2.5.1 What is the relative bioavailability of the proposed to-be-marketed formulation to the immediate release formulation?

The commercial formulation has been used in all clinical studies including the pivotal study (except for studies of PRO051-01 and PRO051-02). Thus, no formal comparative bioavailability or bioequivalence studies were conducted.

2.6 Comparing DMD114673 study with natural history cohort

DMD114673 is an open label long term study that evaluated efficacy of 6mg/kg drisapersen. Sponsor concluded that drisapersen improved walking ability in some patients when compared to matched controls from a natural history study. Do these findings provide supporting evidence of efficacy?

Reviewer's Comments: While improvements in 6MWD in some DMD114673 study patients could be due to drisapersen, definitive treatment benefit upon long term administration of drisapersen cannot be adequately quantified with the available data.

Sponsor's Analyses

Data

The natural history cohort data was obtained through an observational single center study recording functional time tests, pulmonary function, age, weight, height and medication use collected as part of routine follow-up clinics from genetically confirmed and corticosteroid treated DMD subjects attending the Leuven Neuromuscular Reference Centre (NMRC) for clinical care and management.

In the DMD114673, all subjects were genetically confirmed with DMD and as having a mutation suitable for exon 51 skipping therapy and were all receiving continuous corticosteroid treatment. Only one drisapersen subject (Subject 207) had a short period of intermittent corticosteroid treatment during the observation period. Of the 12 subjects enrolled, five subjects were in functional decline and seven subjects were stable in their functional abilities as assessed by their treating physicians. Subjects were originally dosed for five weeks in groups of three at 0.5, 2.0, 4.0, and 6.0 mg/kg/week of drisapersen, with a subsequent 13 week follow-up period. Following this dose escalation phase, and after a break of 6 – 15 months, subjects entered an extension study where they were treated with drisapersen at 6.0 mg/kg/week for 72 weeks. A break in dosing was implemented for eight weeks, and dosing recommenced at Week 81 on an intermittent regimen (8 weeks of 6mg/kg/week, 4 weeks no dosing = 12 week cycle) until Week 188. In addition to the 6 minute walk distance (6MWD), the rise from floor (RFF) times and the boy's pulmonary function were reviewed.

Data Analysis

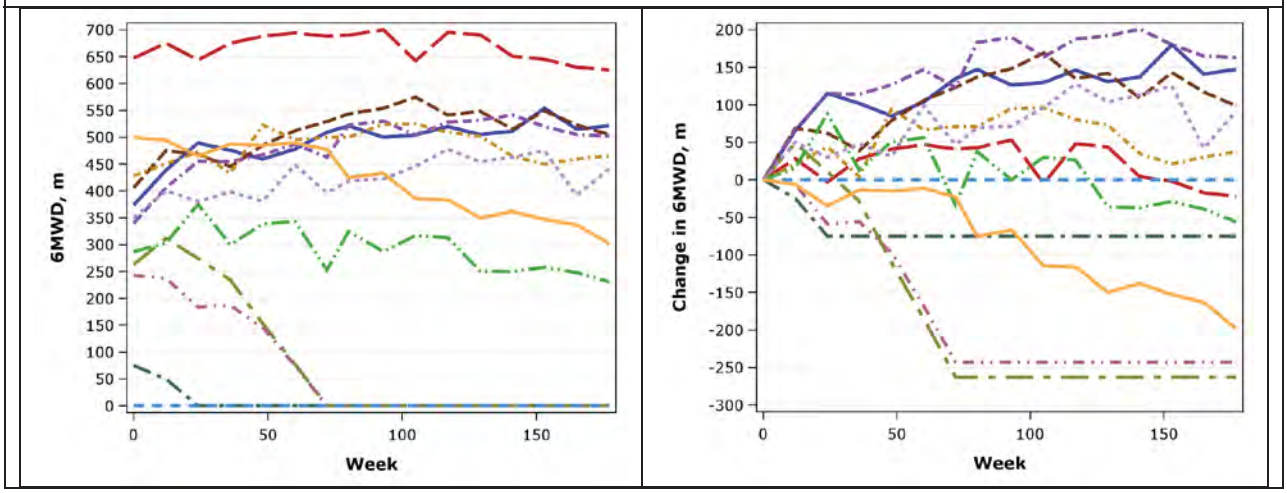
Two analyses were performed by matching according to DMD114673 baseline 6MWD and age, and baseline RFF and age. The matching criteria were pre-defined prior to any matching being performed and was based on recommendation from a N. Goemans and the McDonald publication showing the influence of age, baseline 6MWD, and other

timed function tests as reliable predictors of outcome (Pane, Mazzone et al. ; McDonald, Henricson et al. 2013; McDonald, Henricson et al. 2013).
Matching was performed by first identifying subjects within the natural history database that matched any of the 12 subjects from the DMD114673 study based on baseline age (within six months) and 6MWD (within 30 meters) or RFF (within 0.5 seconds). The first data-matched time point for the natural history cohort was used as the control baseline. The results from the 6MWD at this baseline time point were plotted together with the results for the applicable matched DMD114673 subject over time (with time being represented as age of the boys). All matches were plotted from the first matching record with the applicable DMD114673 subject at baseline onwards.

Results

Figure 5 shows the 6MWD data in 12 DMD114673 study subjects up to 168 weeks.

Figure 5. (Left) 6MWD (Right) Change from Baseline 6MWD in DMD 114673 Subjects. Data collected till 168 weeks is shown here.



A total of 75 natural history subjects matches with the 12 DMD114673 subjects were available and included in the analysis. Table 4 describes the number of natural history matches made based on age and 6MWD per DMD114673 subject and those excluded due to having no more than two assessments available. This table also presents the 6MWD range at baseline and the age range of the matched subjects over the assessment period to put into context the functionality expected from that particular group of DMD subjects. All matched natural history subjects are included in the per subject plots. Only matched natural history subjects with more than two assessments from the point of matching are including in the individual subject narratives.

Table 4. Number of natural history cohort matches per DMD114673 study subject

DMD114673 Subject	NH Matches Identified	NH Matches Included[a]	Subject Age Range (Years) during Observation Period		Baseline 6MWD Range (Metres)	
			DMD114673	Natural History	DMD114673	Natural History
101	5	2	10.9 – 14.3	10.5 - 14.1	374	356-399
102	10	7	8.0 – 11.4	7.6 – 12.4	406	377-435
103	1	0	11.8 – 15.2	11.7	75	60
104	0	0	10.3	N/A	647	N/A
105	10	4	9.2 – 12.6	8.8 – 13.9	340	315-366
106	3	1	9.6 – 13.0	9.4 – 13.0	263	275-281
107	6	4	11.4 – 14.8	11.1 – 15.6	243	221-248
201	13	0[b]	14.3 – 17.7	13.8 - 17.5	0	0
202	4	2	7.5 – 10.9	7.1 – 11.7	429	400-425
205	4	3	12.0 – 15.4	12.0 – 14.6	287	259-310
206	7	5	5.9 – 9.3	5.5 – 8.8	350	322-379
207	1	1	9.9 – 13.3	10.1 – 14.1	500	475

a. Subjects excluded if they did not have more than 2 assessments available - consideration was taken in terms of timeframe between those 2 assessments.
b. All subjects were non-ambulant and therefore full matching was not performed based on 6MWD

Source: Table 1 on Page 6 in dmd114673-nhclinicalstudyreportincludingappendices.pdf

Six of the drisapersen subjects ("101", "102", "105", "202", "206" and "207") were defined as having stable function at baseline (those with a 6MWD of at least 300 metres).

Figure 6, Figure 7, Figure 8 and Figure 9 show the change in 6MWD with age in individual subjects from the DMD114673 study. Also shown are changes in 6MWD for the matches from natural history.

Figure 6. Longitudinal Changes in 6MWD by Individual DMD 114673 Subjects (black solid line) Along With Natural History Matches Based on Age and Baseline 6MWD (solid color lines).

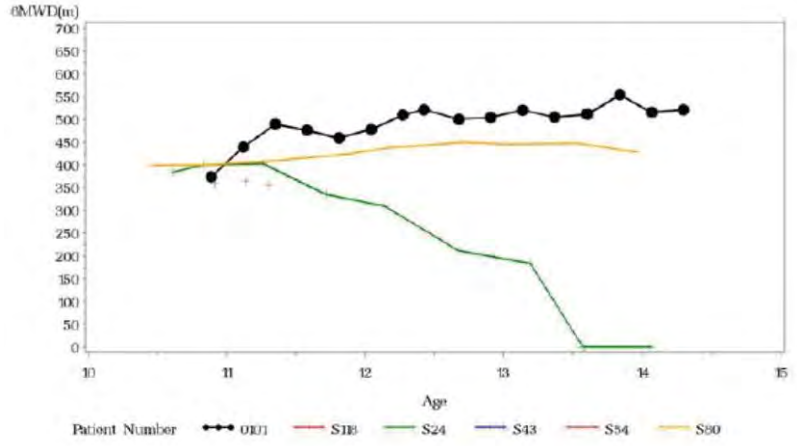
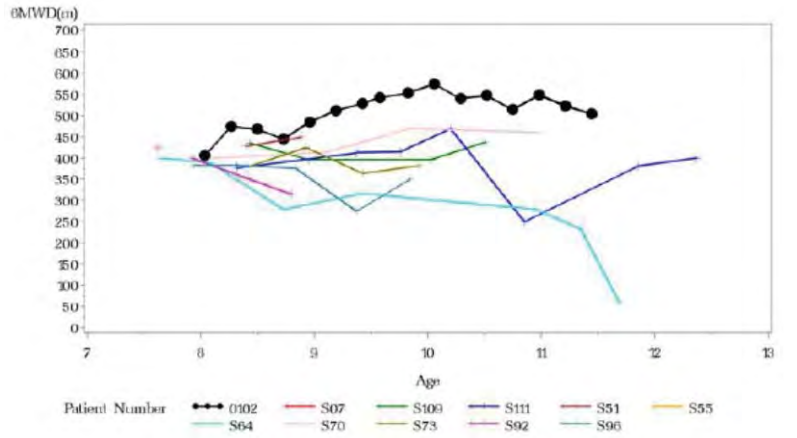
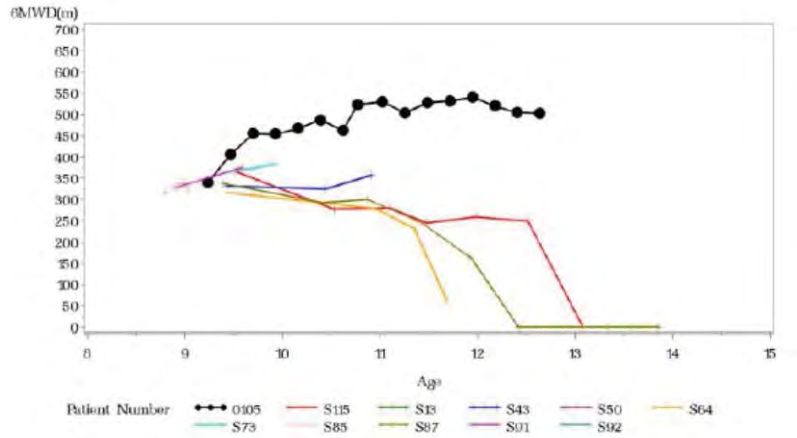
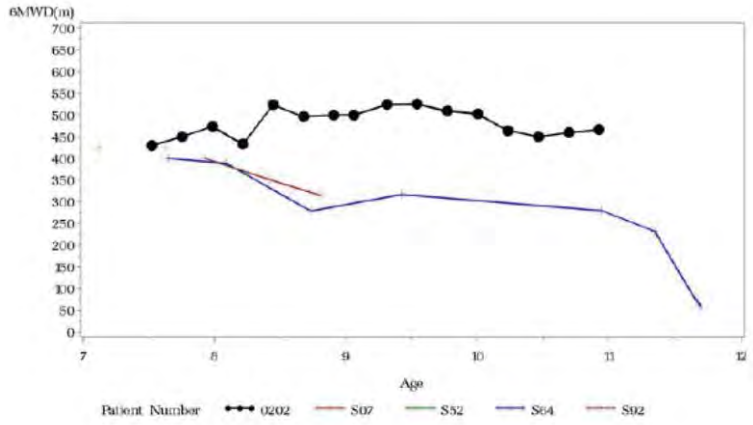
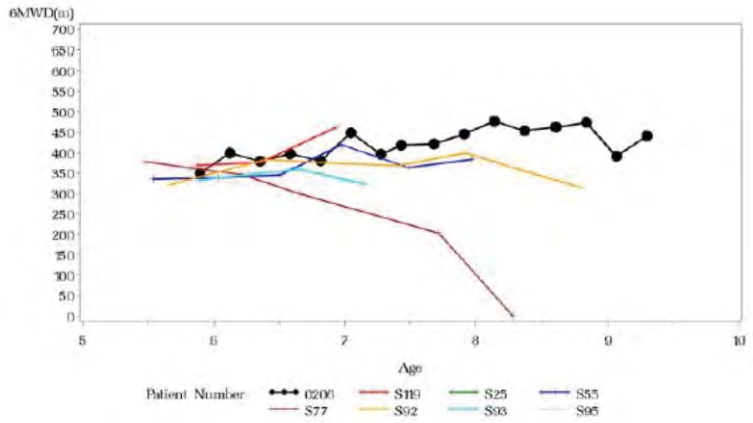
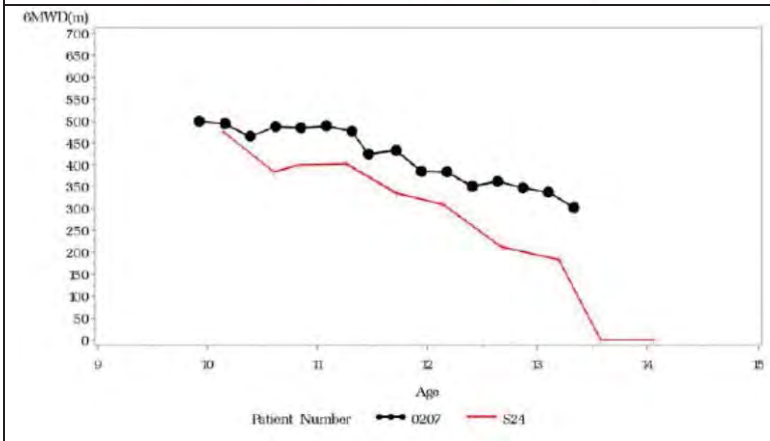
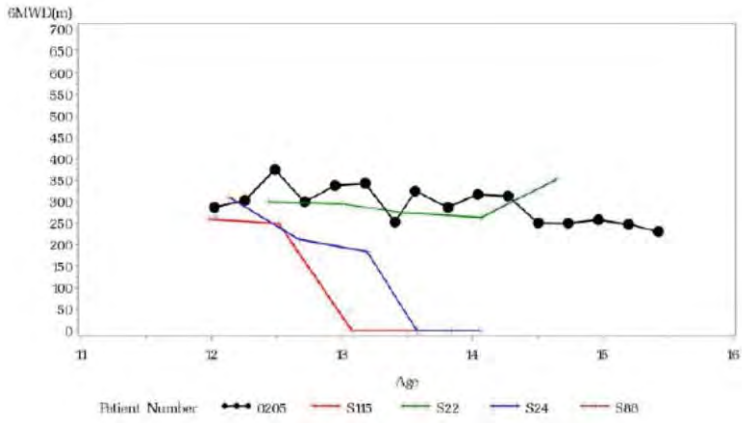
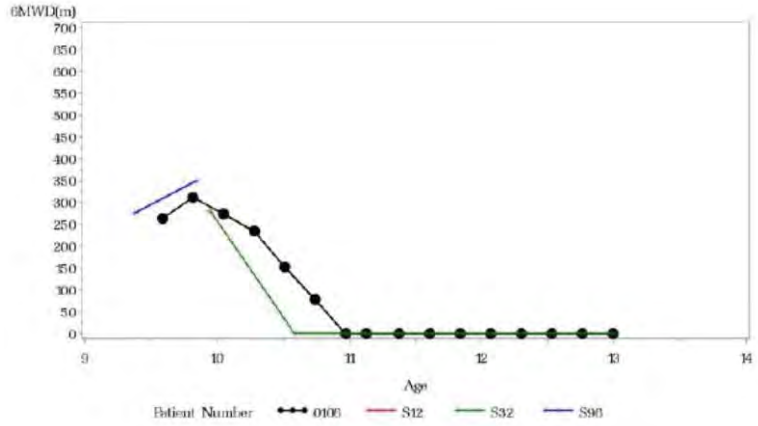
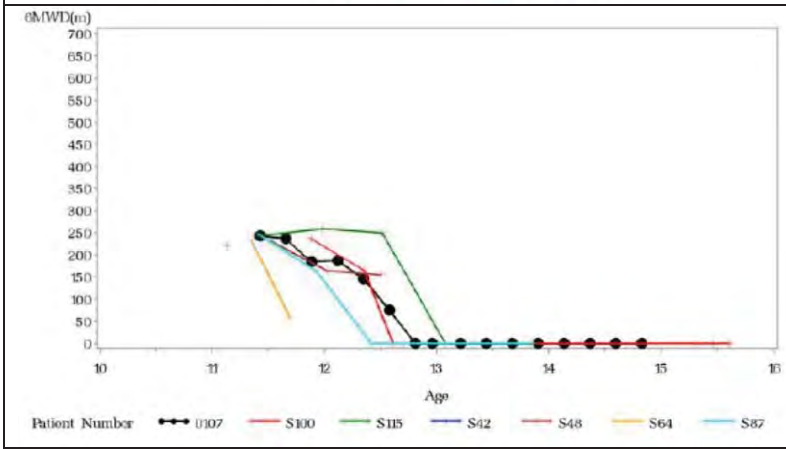
	Patient 0101 vs Natural History Matches • At 14 y, NH patient S80 walked 428 m (NH) while drisapersen treated patient walked 515 m. • Distance walked in 6 minutes improved by 87 m
	Patient 0102 vs Natural History Matches • At 11 y, NH patient S70 walked 461 m (NH) while drisapersen treated patient walked 549 m. • Distance walked in 6 minutes improved by 88 m.
	Patient 0105 vs Natural History Matches • At 11 y, NH patient S43 walked 358 m (NH) while drisapersen treated patient walked 530 m. • Distance walked in 6 minutes improved by 172 m.

Figure 7. Longitudinal Changes in 6MWD by Individual DMD 114673 Subjects (black solid line) Along With Natural History Matches Based on Age and Baseline 6MWD (solid color lines).

	Patient 0202 vs Natural History Matches • At 11 y, NH patient S64 walked 280 m (NH) while drisapersen treated patient walked 466 m. • Distance walked in 6 minutes improved by 186 m.
	Patient 0206 vs Natural History Matches • At 7 y, NH patient S119 walked 463m m (NH) while drisapersen treated patient walked 441 m at 9.3y. • Similar walking trajectory.
	Patient 0207 vs Natural History Matches • Decline in ambulation. Similar to NH match.

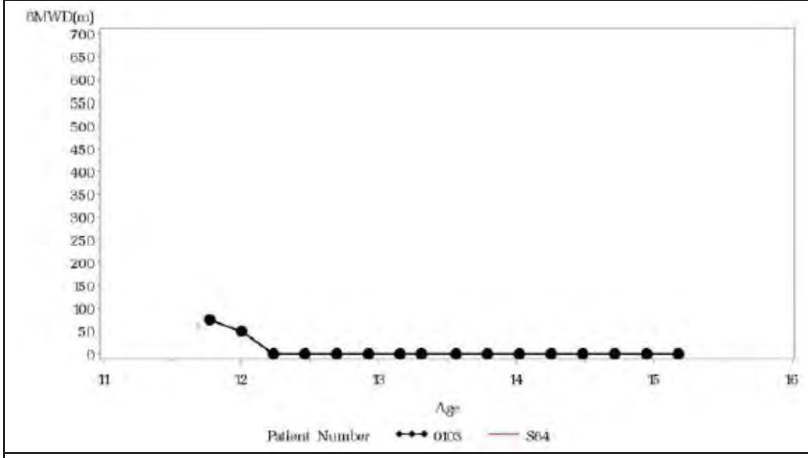
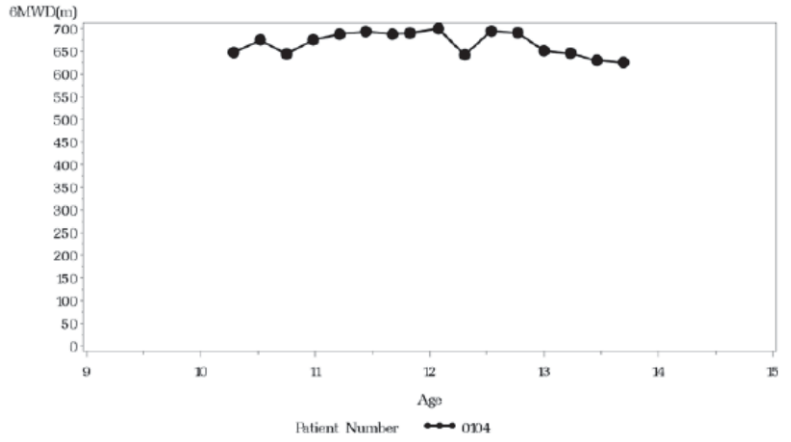
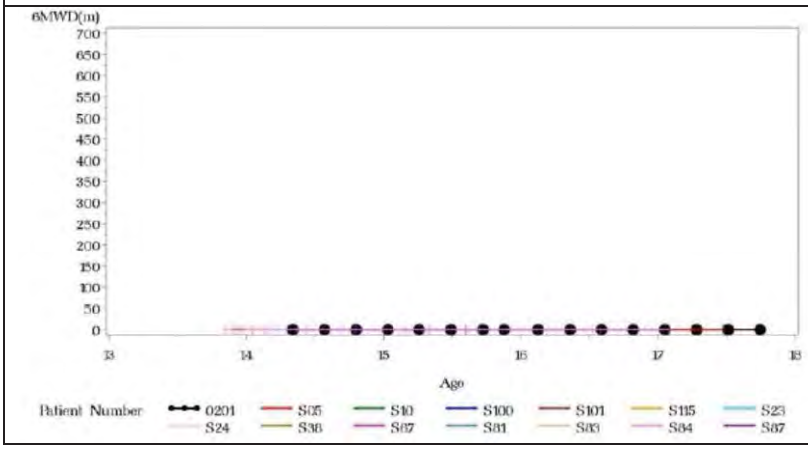
NA- Not Available

Figure 8. Longitudinal Changes in 6MWD by Individual DMD 114673 Subjects (black solid line) Along With Natural History Matches Based on Age and Baseline 6MWD (solid color lines).

	Patient 0205 vs Natural History Matches • Decline in ambulation. Similar to NH match.
	Patient 0106 vs Natural History Matches • Decline in ambulation. Similar to NH match.
	Patient 0107 vs Natural History Matches • Decline in ambulation. Similar to NH match.

NA- Not Available

Figure 9. Longitudinal Changes in 6MWD by Individual DMD 114673 Subjects (black solid line) Along With Natural History Matches Based on Age and Baseline 6MWD (solid color lines).

	Patient 0103 vs Natural History Matches • Decline in ambulation. No NH match.
	Patient 0104 vs Natural History Matches • Stable patient with baseline 6MWD of 650 m. No NH match.
	Patient 0201 vs Natural History Matches • Non ambulant patient at baseline. No NH match.

Sponsor's Conclusions

A matched control study has shown a clear difference in 6MWD when comparing subjects with stable baseline function treated with drisapersen over 3.4 years when compared with a natural history cohort (Table 5).

Table 5. Conclusions Based on Matching Analysis

Patient Number	Conclusions Based on Matching Analysis	Improvement in 6MWD compared to natural history
101	428 m (NH), 515 m (Drisapersen) at 14 y.	87 m
102	461 m (NH), 549 m (Drisapersen) at 11y.	88 m
103	No matches	
104	No matches. Baseline 6MWD > 600m.	
105	358 m (NH), 530 m (Drisapersen) at 11y.	172 m
106	Loss of ambulation. Similar match in NH.	
107	Loss of ambulation. Similar match in NH.	
201	Non ambulant.	
202	280 m (NH), 466 m(Drisapersen) at 11 y.	186 m
205	Decline in ambulation. Similar to NH match.	
206	463m (NH, 7y), 441 m(Drisapersen) at 9.3y. Similar trajectories.	
207	Decline in 6MWD. Similar to NH match.	

No drisapersen subjects with stable baseline function lost ambulation compared with 25% of natural history subjects. A difference was less evident in those with a declined baseline function, with the exception of one drisapersen subject who was still walking 231 meters unlike his DMD peers who had lost ambulation at the age of 15.4 years.

Reviewer's Analysis

The reviewer was able to conduct analyses using sponsor's code and data. However, there are two issues that need to be considered:

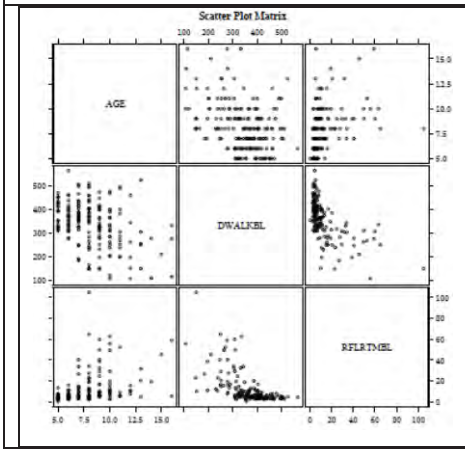
- Acceptability of matching distance measure (6MWD±30m, Age±0.5y).
- Matching analysis using data from patients with various mutations.

Acceptability of matching distance measure (6MWD±30m, Age±0.5y)

Data from the placebo group in DMD114044 study was used to understand the impact of baseline prognostic factors such as age, 6MWD, rise time and use of corticosteroids on DMD disease progression (reflected in 6MWD).

Figure 10 shows the scatter plot and correlation between age, 6MWD and rise time at baseline visit in Study 114044. Figure 10 shows that age, baseline 6MWD and rise time are correlated. For example, patients who have trouble rising from floor have lower walking ability and are older in age. These correlations indicate that one or two out of three prognostic factors are adequate for matching analysis.

Figure 10. (Left) Scatterplot matrix (Right) Correlation Coefficients Between Age (AGE), Baseline 6MWD (DWALKBL) and Rise Time (RFLRTMBL) in Study 114044.

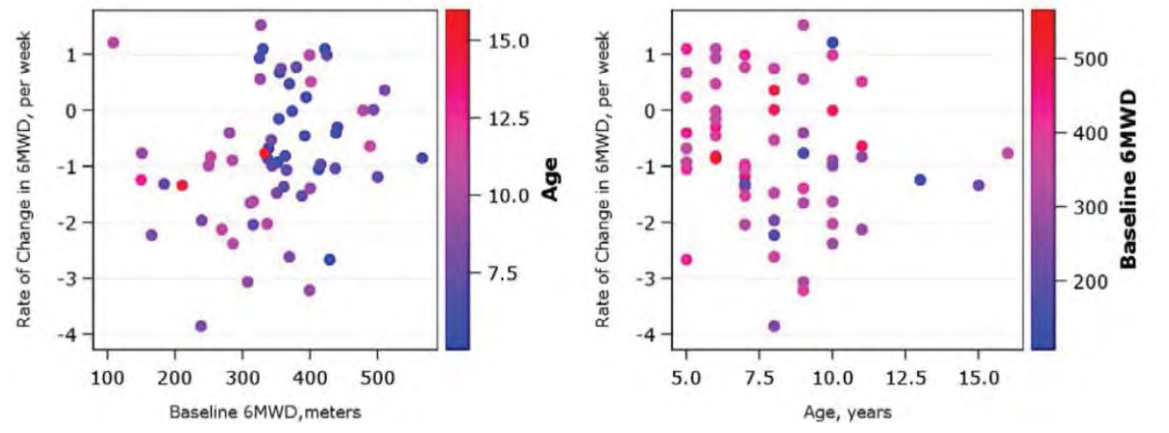


Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	AGE	DWALKBL	RFLRTMBL
AGE Age in years	1.00000 186	-0.38479 <.0001 186	0.37034 <.0001 161
DWALKBL Distance walked baseline (meters)	-0.38479 <.0001 186	1.00000 186	-0.58651 <.0001 161
RFLRTMBL Baseline rise from floor time (seconds)	0.37034 <.0001 161	-0.58651 <.0001 161	1.00000 161

The reviewer estimated rate of change in 6MWD in each DMD114044 study patient using non linear mixed effects analysis. Figure 11 shows the rate of change in 6MWD (per week) by baseline 6MWD and age. Figure 11 suggests that for example, patients with baseline 6MWD of 280 m would have a different trajectory compared to patients with baseline of 350 m. A margin of 30 m would ensure that patients would be reasonably matched as wider margins would group patients with significantly different prognosis. . In addition to 6MWD, it is important that age is also reasonably matched between DMD114673 subjects and natural history. Figure 11 suggests that for example, patients with baseline age of 6 y are less likely to have worse prognosis compared to patients with baseline age of 10 y. Literature also suggests that patients below age of 7 years will have a different progression compared to patients above age of 7 years(Pane, Mazzone et al.). Overall, the sponsor's choice of matching distance measure (6MWD±30m, Age±0.5y) is acceptable (Table 4).

Figure 11. (Left) Relationship Between Rate of Change in 6MWD and Baseline 6MWD (Right) Relationship Between Rate of Change in 6MWD and Baseline Age

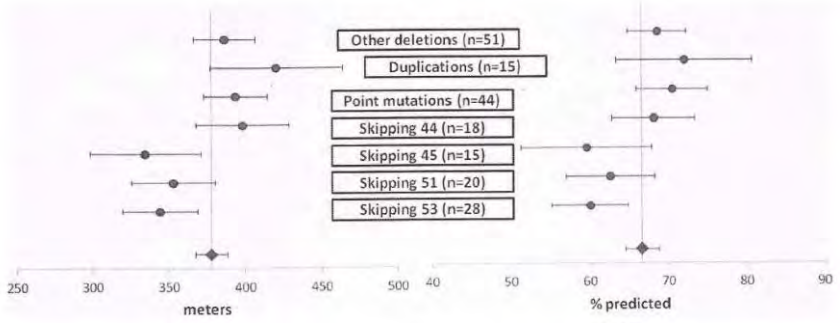


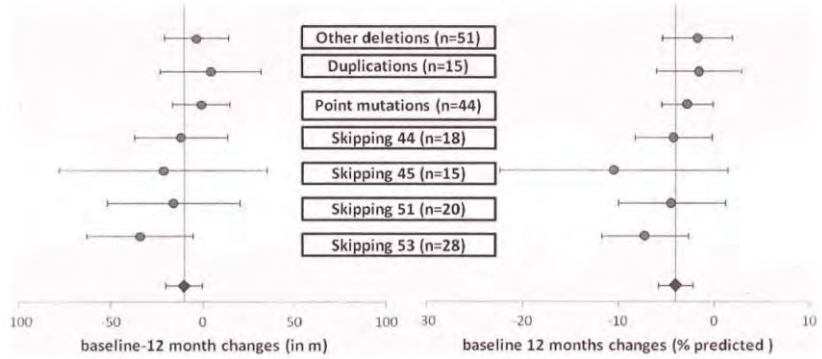
Matching analysis findings using different types of mutations

A recent publication discusses 12 month changes in patients among different types of mutations (deletions, duplications, point mutations) and among subgroups of deletions eligible to skip individual exons. The 6MWT was performed in 191 ambulant DMD boys at baseline and 12 months later (Pane, Mazzone et al.). Figure 12 shows the mean 6MWD data at baseline and change from baseline at the end of 12 months in different types of mutations. The authors concluded that

- Although boys with duplications had better results than those with the other types of mutations, the difference was not significant.
- Similarly, boys eligible for skipping of the exon 44 had better baseline results and less drastic changes than those eligible for skipping exon 45 or 53, but the difference was not significant.
- Even if there are some differences among subgroups, the mean 12 month changes in each subgroup were all within a narrow range from the mean of the whole DMD cohort

Figure 12. (Top) Mean raw scores (left panel) and % predicted (right panel) of 6MWD in individual subgroups. (Bottom) Mean 12 month changes (left panel) and % predicted (right panel) of 6MWD in individual subgroups.





Source: Pane, M., E. S. Mazzone, et al. "6 Minute walk test in Duchenne MD patients with different mutations: 12 month changes." PLoS One **9**(1): e83400.

Reviewer's Comments

- Figure 12 shows that patients with skipping 51 mutations have worse prognosis compared with point mutations or duplications. These differences need to be considered in the matching analysis. Including patients who have better prognosis than patients with skipping 51 mutations will lower the chance of detecting drug benefit.
- While improvements in 6MWD in some DMD114673 study patients could be due to drisapersen, definitive treatment benefit upon long term administration of drisapersen cannot be adequately quantified with the available data. For better interpretation of the data, the sponsor is recommended to

    - Increase the size of natural history data pool, especially from patients with skipping 51 mutations, for matching analysis. While matching analysis using a single natural history database might show drisapersen improves 6MWD, it is possible that matches exist in other databases.

    - Obtain information on presence of LTBP4 (latent transforming growth factor β binding protein 4) and SPP1 (secreted phosphoprotein 1, or

osteopontin) polymorphisms in natural history subjects as well as drisapersen subjects. LTBP4 haplotype is reported to modify age at loss of ambulation(Bello, Kesari et al.).

References

Bello, L., A. Kesari, et al. "Genetic modifiers of ambulation in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study." Ann Neurol **77**(4): 684-96.

McDonald, C. M., E. K. Henricson, et al. (2013). "The 6-minute walk test and other clinical endpoints in duchenne muscular dystrophy: reliability, concurrent validity, and minimal clinically important differences from a multicenter study." Muscle Nerve **48**(3): 357-68.

McDonald, C. M., E. K. Henricson, et al. (2013). "The 6-minute walk test and other endpoints in Duchenne muscular dystrophy: longitudinal natural history observations over 48 weeks from a multicenter study." Muscle Nerve **48**(3): 343-56.

Pane, M., E. S. Mazzone, et al. "Long term natural history data in ambulant boys with Duchenne muscular dystrophy: 36-month changes." PLoS One **9**(10): e108205.

Pane, M., E. S. Mazzone, et al. "6 Minute walk test in Duchenne MD patients with different mutations: 12 month changes." PLoS One **9**(1): e83400.

3 GENOMICS AND TARGETED THERAPY REVIEW

OFFICE OF CLINICAL PHARMACOLOGY
GENOMICS and TARGETED THERAPY GROUP REVIEW

NDA/BLA Number	206031
Submission Date	04/27/2015
Applicant Name	BioMarin Pharmaceuticals
Generic Name	Drisapersen
Proposed Indication	Treatment of Duchenne Muscular Dystrophy
Primary Reviewer	Hobart Rogers Pharm.D, Ph.D.
Secondary Reviewer	Christian Grimstein Ph.D.

EXECUTIVE SUMMARY

Drisapersen is a synthetic antisense oligonucleotide (AON) that is targeted against exon 51 of the dystrophin gene. Drisapersen is being developed for the treatment of Duchenne Muscular Dystrophy (DMD) in individuals who possess deletion mutations amenable to the skipping of exon-51 to restore the reading frame and produce an internally-deleted dystrophin protein. Individuals with these internally-deleted dystrophins have on average a much milder form of disease known as Becker Muscular Dystrophy (BMD). The sponsor is seeking an approval of drisapersen for all mutations amenable to skipping exon-51 in the *DMD* gene, however not all mutations were studied in the clinical trials. The purpose of this review is to evaluate whether drisapersen should be approved for all mutations amenable to exon-51 skipping by drisapersen. The review concluded that given the lack of available subjects for study, coupled with inherent heterogeneity in disease, along with the unknowns regarding the functionality of the internally-deleted dystrophin; determining efficacy in patients with ultra-rare DMD mutations amenable to exon-51 skipping is difficult. Furthermore, there are no reasons to believe that the safety of drisapersen is in any way different in these ultra-rare populations of patients. Hence, it is reasonable to conclude that the restoration of the reading frame by drisapersen should be beneficial for all DMD mutations amenable to exon-51 skipping. The findings of this review indicate that drisapersen, if found to be safe and effective in the studied population, should be indicated for all mutations amenable to exon-51 skipping.

1 Background

Duchenne Muscular Dystrophy (DMD) is characterized by an absence of the protein dystrophin. Dystrophin is a rod shaped cytoplasmic protein that connects the cytoskeleton of a muscle fiber the surrounding extracellular matrix through the cell membrane. Functionally, dystrophin acts to stabilize the sarcolemma membrane against the stress imposed by muscle contraction. The lack of dystrophin in DMD results in a severe disease observed in the first years of life with patients typically losing ambulation around the age of 12 years and the need for mechanical ventilation around 18 years of age. Another related genetic disease is Becker Muscular Dystrophy (BMD), where an internally-deleted dystrophin is produced. BMD results in a much milder phenotype with many patients remaining ambulant throughout life or even asymptomatic. The stark contrast between DMD and BMD phenotype is the presence of dystrophin. In DMD the reading-

frame of the mRNA is disrupted and little to no dystrophin is produced, whereas in BMD, the reading frame is intact and an internally-deleted, but somewhat functional dystrophin protein is produced.

The gene for dystrophin is one of the largest in the human genome consisting of 79 exons. DMD is an X-linked disorder; mutations occur in about 1 in every 3500 male births. There are a large variety of mutations, with one out of three mutations occurring *de novo*. Over 4500 pathogenic mutations are known to cause DMD. Large deletions are present in about 60% of patients, large duplications in about 10% and point mutations (confined mostly to coding exons) in about 30% of patients (PMID: 219693337). Of the deletion mutations, approximately 66% of patients carry a deletion of one or more exons, of which 70% cluster between exon 45 and 55 (PMID: 19156838).

Drisapersen is a synthetic chemically modified 2'-*O*-methyl-phosphorothioate (2OMePS) RNA antisense oligonucleotide composed of 20 nucleotides in a sequence specific for exon 51 of the dystrophin pre-mRNA. Drisapersen binds to exon 51 of the dystrophin pre-mRNA causing exon skipping during processing and restoring the reading frame to produce a truncated internally-deleted dystrophin. In theory, this exon 51 skipping would restore the reading frame of the mRNA to allow an internally-deleted dystrophin protein to be expressed. The resultant protein, while not complete, is expected to convert DMD patients to the less severe BMD phenotype.

2 Submission Contents Related to Genomics

The sponsor submitted the following labeling language for drisapersen:

Indications and Usage:
Drisapersen is an exon skipping oligonucleotide inducer of dystrophin synthesis indicated for the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing.

The sponsor's submitted data included the underlying DMD mutation for all patients. The sponsor's to-be labeled population compared to the studied population will be the focus of this review. The sponsor's proposed labeling states that the drug will be indicated for subjects with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing.

Of the DMD mutations amenable to treatment with drisapersen, the sponsor has studied nine (17-50, 38-50, 43-50, 45-50, 47-50, 48-50, 49-50, 50, and 52) different DMD deletion mutations in their clinical trials (Table 1).

Table 1. DMD Mutations Present in Placebo-controlled Studies*

	Phase II studies						Phase III study	
	DMD114117			DMD114879			DMD114044	
	Placebo (combined) (N=18)	Drisapersen 6 mg/kg/wk (N=18)	Drisapersen 6 mg/kg intermittent (N=17)	Placebo (combined) (N=16)	Drisapersen 3 mg/kg/wk (N=17)	Drisapersen 6 mg/kg/wk (N=18)	Placebo (N=61)	Drisapersen 6 mg/kg/wk (N=125)
Exon mutation, n (%)								
DMD 43-50 deletion	0	0	0	0	0	1 (6)	0	0
DMD 45-50 deletion	7 (39)	6 (33)	5 (29)	9 (56)	4 (24)	4 (22)	16 (26)	40 (32)
DMD 47-50 deletion	0	0	0	1 (6)	0	0	1 (2)	0
DMD 48-50 deletion	3 (17)	6 (33)	6 (35)	4 (25)	5 (29)	3 (17)	7 (11)	26 (21)
DMD 49-50 deletion	1 (6)	4 (22)	3 (18)	1 (6)	5 (29)	6 (33)	20 (33)	31 (25)
DMD 50 deletion	4 (22)	1 (6)	3 (18)	1 (6)	2 (12)	1 (6)	5 (8)	11 (9)
DMD 52 deletion	3 (17)	1 (6)	0	0	1 (6)	3 (17)	10 (16)	16 (13)
Other	0	0	0	0	0	0	2 (3)	1 (<1)

Source: Derived from Table 22 page 87 module 2.7.3
*One subject with each mutation 17-50, and 38-50 were enrolled as part of the "other" group

3 Key Questions and Summary of Findings

3.1 Are the studied populations in the sponsor's clinical trials representative of the to-be labeled population?

*No. The sponsor has studied nine different DMD mutations amenable to exon-51 skipping therapy. Drisapersen is to be indicated for all mutations amenable to skipping exon 51. Additional DMD mutations (e.g. 19-50, 52-63) are known to exist, however they are ultra-rare (1-2 subjects in database) in nature. A search of the Leiden DMD database (www.dmd.nl) using the known exon splicing (**Figure 1**), identified subjects composing of seven additional DMD mutations (i.e., 3-50, 13-50, 19-50, 29-50, 40-50, 52-58, 52-63) that may be amenable to exon-51 skipping based on the mechanism of action of drisapersen. Amenable mutations are those in which skipping of exon-51 would, in theory, restore the reading frame. For instance, in Figure 1, a subject with a deletion of exons 44-50 would not be amenable to exon-51 skipping as exons 43 and 52 cannot be spliced together, whereas, a deletion of exons 43-50 can be successfully spliced by exon-51 skipping.*

Figure 1. Depiction of the 79 Exons of the Dystrophin Gene and Splicing

Source: PMID 19156838
Note: In-frame exons are in light blue, out-of-frame in dark blue. Deletions are considered in-frame when the exons flanking the deletion "fit."

3.2 ***Should drisapersen be indicated for patients amenable to exon-51 skipping who were not studied in the clinical development program?***

Yes. Despite not all DMD mutations amenable to exon-51 skipping being enrolled in the clinical development program, if drisapersen is ultimately found to be safe and effective to warrant approval, then drisapersen should be indicated for all exon-51 amenable mutations.

Reviewer comment: In theory, restoring the reading frame by skipping exon-51 may result in a milder form of the disease (i.e. transition from DMD phenotype towards a BMD phenotype); therefore it has the potential to be efficacious for patients with all amenable mutations. However, given the ultra-rare occurrence of some exon-51 amenable mutations (e.g. 43-50 deletions) it is exceedingly difficult to find adequate numbers of patients for clinical studies. Moreover, given the strict inclusion criteria for the drisapersen clinical trials, these patients may have been ineligible to participate (e.g. non-ambulatory). Furthermore, given the inherent variability in disease, studying these ultra-rare mutation subsets may be challenging for determining efficacy or lack thereof.

Many unknowns remain in how the internally-deleted dystrophin can impact disease, both in quantity and quality. Successful exon-51 skipping in the case of each DMD deletion mutation would create a different internally-deleted dystrophin protein. For some mutations amenable to exon-51 skipping we have BMD subjects with the same internally-deleted "in-frame" mutations to infer some degree of functionality of that protein (PMID: 25633150, 22102647). Though generally less severe, even within BMD patients there can be a large heterogeneity in disease phenotype (PMIDs: 25633150, 2404853). While in-frame deletions in the proximal regions of the protein (exons 20-40) tend to be milder than those in the distal part (exons 40-55), it is still difficult to predict exactly what the functionality of the skipped dystrophin protein may be (PMIDs: 19156838,16770791,17041910). For example, a case report of a patient missing exons 17-48 only resulted in mild BMD, with the patient being ambulant at 61 years of age (PMID: 2404210). Thus, it is clear that the amount of exons present isn't directly correlated with functionality. Hence, while we can infer some functionality of an exon-51 skipped product, many unknowns remain on how it can affect clinical phenotype.

Given the lack of available subjects for study, coupled with inherent heterogeneity in disease, along with the unknowns regarding the functionality of the internally-deleted dystrophin, determining efficacy in single patients with a specific exon-51 skipping amenable mutation is difficult. Moreover, there are no reasons to believe that the safety of drisapersen is in any way different in these ultra-rare populations of patients. Thus, if drisapersen is approved, any DMD deletions amenable to exon-51 skipping (i.e., theoretical restoration of the reading frame) should be eligible to receive drisapersen.

3.3 **Is there a difference in the functionality of the exon-skipped truncated dystrophin produced by treatment with drisapersen?**

Potentially, however given the significant intra- and inter-subject variation in disease phenotype; it is likely that large numbers of DMD patients with different mutations would need to be studied in order to determine efficacy. Given the small numbers of subjects in the sponsor's submission with specific DMD deletions, numerical comparisons can only be made for a few of the exon-51 skipping amenable groups.

3.3.1 Sponsor's analysis

The sponsor states that *post hoc* analysis of 48-week ambulant, placebo-controlled studies suggested a that the effect of drisapersen was larger in subjects with a single DMD deletion (treatment difference at Week 48: 22.8 metres for DMD 50 deletion, 30.0 metres for DMD 52 deletion) than for subjects with multiple DMD deletions (treatment difference at Week 48: 9.7 metres for DMD 45-50 deletion, –11.7 metres for DMD 48-50 deletion, 12.2 metres for DMD 49-50 deletion. The sponsor argues that given the small numbers in each group, that these findings should be interpreted with caution.
The sponsor also evaluated the change from baseline 6MWD in the phase 2 placebo-controlled studies. The tendency for single DMD deletions to have improved treatment differences was not observed in this subset.

Furthermore, the sponsor demonstrated an increase in exon skip mRNA for each mutation that had at least five subjects. RT-PCR transcripts of mRNA increased regardless of the studied DMD mutation (**Table 2**).

Table 2. Increase in Exon Skip 51 mRNA at Week 48 in Different Mutation Groups After Drisapersen Compared to Placebo in Study DMD114044

Deletion	Placebo			Drisapersen (6 mg/kg/wk)		
	Mean ± SE (SD)	Median	n	Mean ± SE (SD)	Median	n
Exon 52	0.52 ± 0.13 (0.42)	0.4	10	2.40 ± 1.05 (4.08)	0.5	15
Exon 50	1.62 ± 0.62 (1.38)	1.0	5	2.65 ± 0.68 (1.92)	2.0	8
Exon 49-50	1.83 ± 0.46 (1.96)	1.2	18	3.69 ± 0.96 (5.35)	2.4	31
Exon 48-50	0.70 ± 0.30 (0.79)	0.5	7	1.51 ± 0.28 (1.39)	1.2	25
Exon 45-50	1.90 ± 0.53 (2.05)	1.6	15	3.26 ± 1.17 (7.23)	1.6	38
All deletions	1.45 ± 0.22 (1.68)	0.9	56	2.83 ± 0.48 (5.21)	1.6	118

Abbreviations: n= number, SD = standard deviation
Result of skip mRNA product in a.u. by nested PCR and lab-on-chip analysis. Assay not tested for linearity or accuracy. Rare deletions with only one treated subjects (47-50, 38-50, 17-50), and hence no comparative treatments, not shown.

Source: Summary of clin pharm page 37 module 2.7.2

3.3.2 Reviewer's analysis

The sponsor enrolled nine different DMD deletion mutations that were amenable to exon-51 skipping. The goal of drisapersen treatment is to restore the reading frame and

produce a truncated dystrophin protein similar to patients with BMD. While, each DMD mutation amenable to exon-51 skip will produce a different internally-deleted dystrophin, in theory, it is unlikely that an amenable mutation would not respond to treatment with drisapersen. Given the heterogeneity in disease phenotype DMD mutations, it is difficult to ascertain whether differences in DMD mutation affected efficacy. The sponsor's post-hoc analysis indicated that no significant differences in the 6MWD by DMD mutation type were observed. While there may be some differences in functionality of the exon-51 skipped transcripts; restoring the reading frame to produce dystrophin even if it may be different between DMD mutations is warranted.

4 Summary and Conclusions

Drisapersen is being sought for the indication of the treatment of DMD in all mutations amenable to exon-51 skipping. There were nine different DMD mutations represented in the sponsor's clinical trials; however two mutations had only one representative subject (17-50, 38-50). Although drisapersen was not studied in all DMD mutations amenable to exon-51 skipping, it is reasonable to extrapolate efficacy to ultra-rare populations (i.e., mutations with only one or two known subjects), given the inherent variability in disease, and our understanding of the mechanism of action in restoring the reading frame. Last, there are no reasons to believe that the safety of drisapersen is in any way different in these ultra-rare populations of patients. Hence, given the challenges of studying these ultra-rare populations of disease, coupled with the lack of any unique safety concerns, it is reasonable to approve drisapersen for all DMD mutations amenable to exon-51 skipping, if found to be safe and effective in the studied population.

5 Recommendations

It is the finding of this review that drisapersen, if found to be safe and effective to warrant approval, is likely to benefit all mutations amenable to exon-51 skipping and should be labeled accordingly.

Post-marketing studies

None.

5.1 Labeling recommendations

No additional labeling recommendations.
Drisapersen is an exon skipping oligonucleotide inducer of dystrophin synthesis indicated for the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing.

Philips, Howard

From: Farkas, Ronald
Sent: Wednesday, November 11, 2015 2:59 PM
To: Dunn, Billy; Bastings, Eric; Unger, Ellis; Temple, Robert
Cc: Rao, Ashutosh; Tandon, Veneeta; Bhattaram, Atul; Mentari, Evelyn; Breder, Christopher D
Subject: FW: CK doesn't change in frameshift DMD subjects
Attachments: Malik gentamycin readthrough.pdf

All,

This papers reports that CK was decreased in nonsense mutation DMD patients on gentamycin but not in frameshift patients. The fact that the paper was published by Mendell gives some pause, but CK is a pretty straightforward test and the difference in groups is so large that I think it is believable. This data may put the nail in the coffin of any alternative explanation other than increased dystrophin for the decrease in CK in the drisapersen studies. To me it's always been conceptually very appealing to think that the decrease in CK from drisapersen really does support that enough dystrophin might have been made to be beneficial to muscle physiology. This doesn't change the fact that the clinical evidence for drisapersen is still weak, but I don't think the clinical data ever disproved that the drug could have a small beneficial effect.

I acknowledge that my head is still ringing a bit from the meeting with Woodcock, but I don't think that's the cause of my current state of mind.

I think it would be good to tell Woodcock and the others at the CD briefing that we found this paper and are taking it seriously.

Tell me what you think about any or all of the above.

Thanks

Ron

From: Farkas, Ronald
Sent: Wednesday, November 11, 2015 1:41 PM
To: Tandon, Veneeta; Bhattaram, Atul; Mentari, Evelyn
Subject: CK doesn't change in frameshift DMD subjects

All,
The data below appears to be a very compelling piece of evidence that Ck ***is*** changing because of increased dystrophin. It was my mistake to not be aware of this earlier – but at least we found it now. Tell me what you think
Thanks
Ron

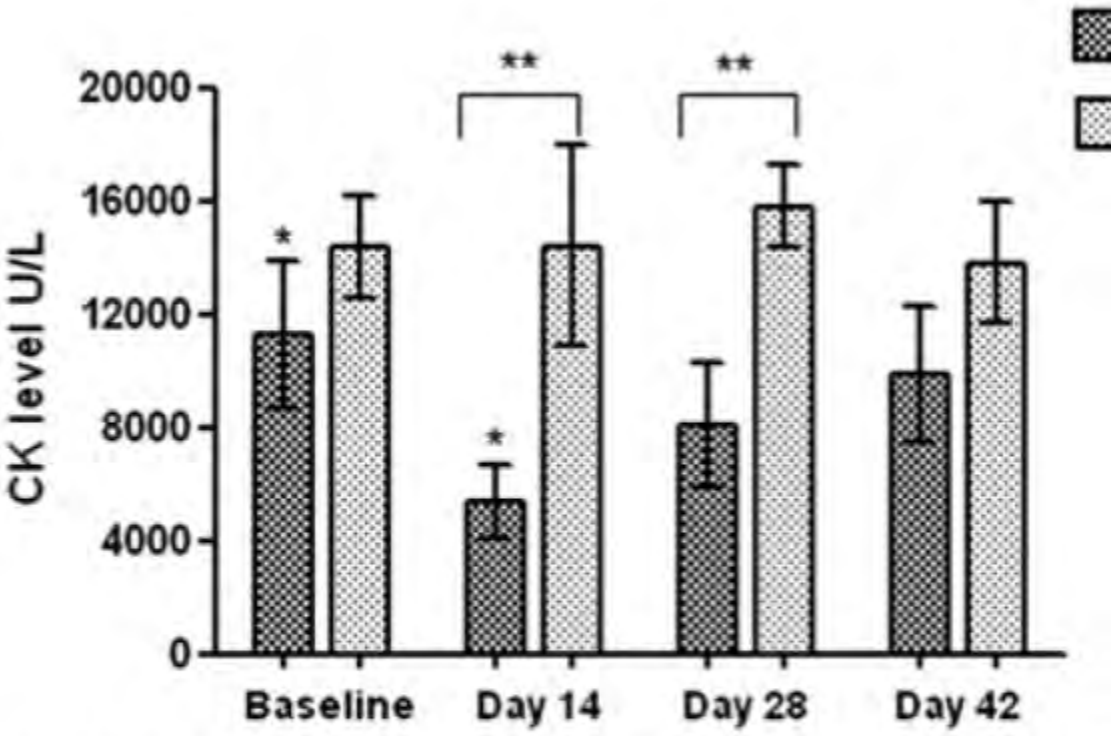


FIGURE 1: Serum creatine kinase (CK) levels in nonsense (Cohort 1) and framesh gentamicin daily for 14 days. For Cohort 1, n = 12 at all time points. For Cohort 2, 7 at day 28; n = 6 at day 42. Within Cohort 1, a statistically significant change com day 14 (*$p = 0.007$). Compared to frameshift controls, statistically significant changes and day 28 ($p = 0.03$).

Gentamicin-Induced Readthrough of Stop Codons in Duchenne Muscular Dystrophy

Vinod Malik, PhD,[1] Louise R. Rodino-Klapac, PhD,[1] Laurence Viollet, PhD,[1] Cheryl Wall, RN,[3] Wendy King, PT,[3] Roula Al-Dahhak, MD,[1] Sarah Lewis,[1] Christopher J. Shilling, MS,[1] Janaiah Kota, PhD,[1] Carmen Serrano-Munuera, MD,[3] John Hayes, PhD,[2] John D. Mahan, MD,[2] Katherine J. Campbell,[4] Brenda Banwell, MD,[5] Majed Dasouki, MD,[6,7] Victoria Watts,[6,7] Kumaraswamy Sivakumar, MD,[8] Ricardo Bien-Willner,[8] Kevin M. Flanigan, MD,[1,2,3,9] Zarife Sahenk, MD, PhD,[1,2,3] Richard J. Barohn, MD,[6,7] Christopher M. Walker, PhD,[2,3,4] and Jerry R. Mendell, MD[1,2,3]

Objective: The objective of this study was to establish the feasibility of long-term gentamicin dosing to achieve stop codon readthrough and produce full-length dystrophin. Mutation suppression of stop codons, successfully achieved in the *mdx* mouse using gentamicin, represents an important evolving treatment strategy in Duchenne muscular dystrophy (DMD).

Methods: Two DMD cohorts received 14-day gentamicin (7.5mg/kg/day): Cohort 1 (n = 10) stop codon patients and Cohort 2 (n = 8) frameshift controls. Two additional stop codon DMD cohorts were gentamicin treated (7.5mg/kg) for 6 months: Cohort 3 (n = 12) dosed weekly and Cohort 4 (n = 4) dosed twice weekly. Pre- and post-treatment biopsies were assessed for dystrophin levels, as were clinical outcomes.

Results: In the 14-day study, serum creatine kinase (CK) dropped by 50%, which was not seen in frameshift DMD controls. After 6 months of gentamicin, dystrophin levels significantly increased ($p = 0.027$); the highest levels reached 13 to 15% of normal (1 in Cohort 3, and 2 in Cohort 4), accompanied by reduced serum CK favoring drug-induced readthrough of stop codons. This was supported by stabilization of strength and a slight increase in forced vital capacity. Pretreatment stable transcripts predicted an increase of dystrophin after gentamicin. Readthrough efficiency was not affected by the stop codon or its surrounding fourth nucleotide. In 1 subject, antigen-specific interferon-γ enzyme-linked immunospot assay detected an immunogenic dystrophin epitope.

Interpretation: The results support efforts to achieve drug-induced mutation suppression of stop codons. The immunogenic epitope resulting from readthrough emphasizes the importance of monitoring T-cell immunity during clinical studies that suppress stop codons. Similar principles apply to other molecular strategies, including exon skipping and gene therapy.

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From the [1]Center for Gene Therapy, Research Institute at Nationwide Children's Hospital, [2]Department of Pediatrics, [3]Department of Neurology, and [4]Center for Vaccines and Immunity, Research Institute at Nationwide Children's Hospital, Ohio State University, Columbus, OH; [5]Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; [6]Department of Neurology and [7]Clinical Translational Research Center, University of Kansas, Kansas City, KS; [8]Neuromuscular Research Center, Scottsdale, AZ; and [9]Department of Neurology and Human Genetics, University of Utah, Salt Lake City, UT.

Additional Supporting Information can be found in the online version of this article.

Philips, Howard

From: Bautista, Philip
Sent: Thursday, November 12, 2015 4:35 PM
To: Dunn, Billy
Cc: Unger, Ellis; Temple, Robert; Bastings, Eric; Ngo, Diem-Kieu (CDR,USPHS); Morin, Steve; Klein, Richard M
Subject: RE: AC members for drisapersen

Hi Billy,

As you know, the FR Notice for the Nov. 24 PCNS meeting has published, listing the 2-hour OPH. The OPH registration deadline has passed and we have confirmed 31 public speakers at 3.5 minutes each. Taking transition times between speakers into consideration, we will need the entire 2 hours for the OPH. For these reasons, we are unable to shorten the OPH duration at this late stage. If DNP would like to increase the committee discussion time, we can adjust the meeting start or end times (start at 7:30am or end at 6:00pm).

Regarding the Patient Representatives, this is to confirm that two Patient Representatives (Cheryl Gunvalson and Chris Cassidy) will be participating in the November 24 PCNS meeting. Each will serve as a temporary voting member (2 patient votes, not 3 as you indicated). As the patient perspective is different than the caregiver perspective for DMD, OHCA recommended that two Patient Representatives participate in the Nov 24 PCNS meeting in order to ensure that both the patient and the caregiver perspectives are represented. We met with Jill Warner and the Advisory Committee Oversight Management Staff (ACOMS) to discuss OHCA's request, and they agreed that two patient perspectives are needed for the November 24th and January 22nd PCNS meetings on NDAs for DMD. We understand that this should have been communicated to DNP more clearly.

I've cc'd Steve Morin and Richard Klein in this email, in case they have anything to add regarding the need for two Patient Representatives.

Regards,

Phil

Phil Bautista, PharmD
Designated Federal Officer
Division of Advisory Committee and Consultant Management
Office of Executive Programs, CDER
(301) 796-9006

From: Dunn, Billy
Sent: Thursday, November 12, 2015 1:17 PM
To: Bautista, Philip
Cc: Unger, Ellis; Temple, Robert; Bastings, Eric
Subject: RE: AC members for drisapersen

Phil,

The Office and Division leadership look forward to your reply. Please send to all.

Billy

From: Dunn, Billy
Sent: Tuesday, November 10, 2015 3:44 PM
To: Bautista, Philip
Cc: Unger, Ellis; Temple, Robert; Bastings, Eric
Subject: RE: AC members for drisapersen

Phil,

In addition, the Office and Division leadership believe the public comment period needs to be reduced in duration. It is simply too long, and we need the time for committee discussion.

Billy

From: Unger, Ellis
Sent: Tuesday, November 10, 2015 2:03 PM
To: Dunn, Billy; Bautista, Philip
Cc: Temple, Robert; Bastings, Eric
Subject: Re: AC members for drisapersen

Agree completely.

Ellis Unger

Sent from my Blackberry

From: Dunn, Billy
Sent: Tuesday, November 10, 2015 01:19 PM
To: Bautista, Philip
Cc: Unger, Ellis; Temple, Robert; Bastings, Eric
Subject: AC members for drisapersen

Phil,

This email is from both the Division and the Office. Both ODE1 and DNP do not agree with the inclusion of 2 additional patient representatives on the committee as voting members. This will result in a total of 3 votes from one representative area. The Division and Office leadership have discussed this in detail and do not support this assignment of votes. Further, it should be noted that neither the Division nor the Office was involved in the plan to include these additional patient representatives and this issue took us by surprise. These additional patient representatives should not be included as voting members.

Billy

Philips, Howard

From: Unger, Ellis
Sent: Friday, November 13, 2015 3:25 PM
To: Mentari, Evelyn; Freed, Lois M; Farkas, Ronald
Cc: Yasuda, Sally; Choy, Fannie (Yuet); Temple, Robert
Subject: RE: drisapersen clinical safety slides

Evelyn,

They are coming along well.

I tried to suggest some edits using tracked changes but I was not successful. I started to write down suggested edits, but that was also difficult.

So…I went ahead and made some edits myself. And I am sorry, but the only way to find the changes is by examination.

See what your think. I have a couple of questions about the renal toxicity (whether it was reversible and whether it affected creatinine, see slide 9).

I'm also sending to Dr. Temple (he's the signatory on this).

Ellis

From: Mentari, Evelyn
Sent: Friday, November 13, 2015 2:33 PM
To: Unger, Ellis; Freed, Lois M; Farkas, Ronald
Cc: Yasuda, Sally; Choy, Fannie (Yuet)
Subject: drisapersen clinical safety slides

Hello,

I have attached my slides, updated according to the comments from yesterday. I have listed the changes below. I also changed "(b) (5)" to "patients" throughout the slides.
Lois, could you please edit Slide 17 as needed?

Thank you,
Evelyn

<< File: Drisapersen AC Clinical Safety 11-12-2015.ppt >>

Slide 3 -- Added placebo-controlled study information on thrombocytopenia
Slide 4 – Updated first bullet wording
Slides 5-6 -- Updated Titles. Enlarged Labels. Added arrows for drug discontinuation.
Slide 7 – "(b) (5)" changed to "would." Parentheses removed from "but not eliminate"

Slide 8 – Hyphen added to 24-hour urine
Slide 9 – Added information on thrombotic events with nephrotic syndrome. Reworded patient descriptions.
Slide 10 -- "(b) (5)" changed to "would."
Slide 15 – Added details to SAEs and changed the units for ISR duration to months and years
Slide 16 – Changed slide title to Injection Site Reactions: Mitigation.
Slide 17 – Updated title.
Slide 18 – Updated title.
Slide 19 – Removed the patient registry bullet.