CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206488Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

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)

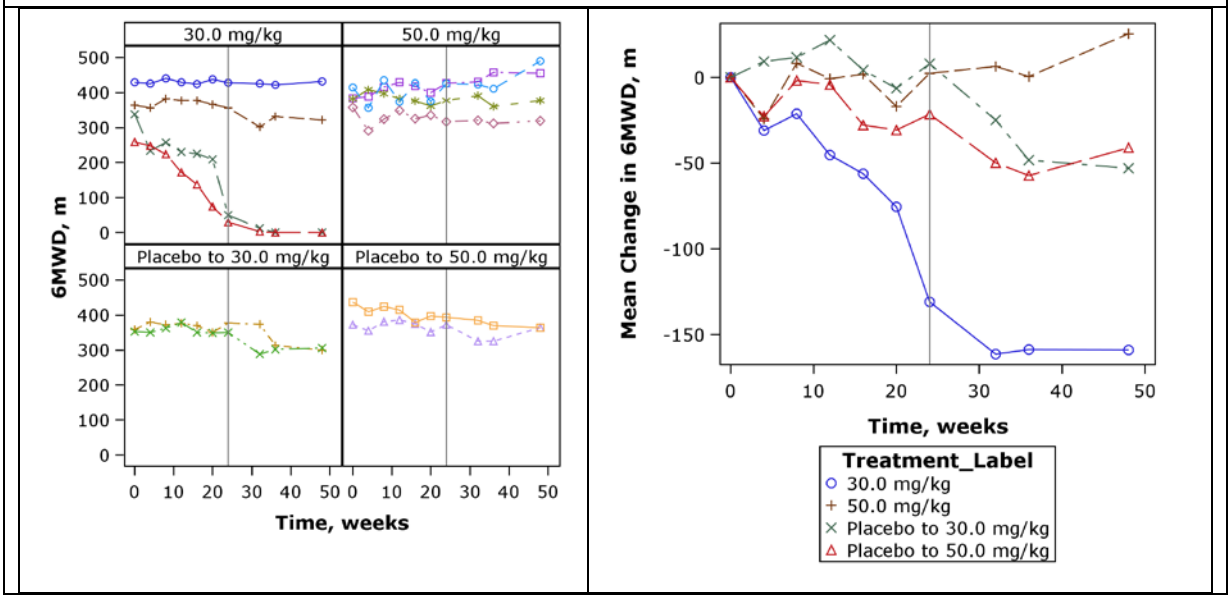
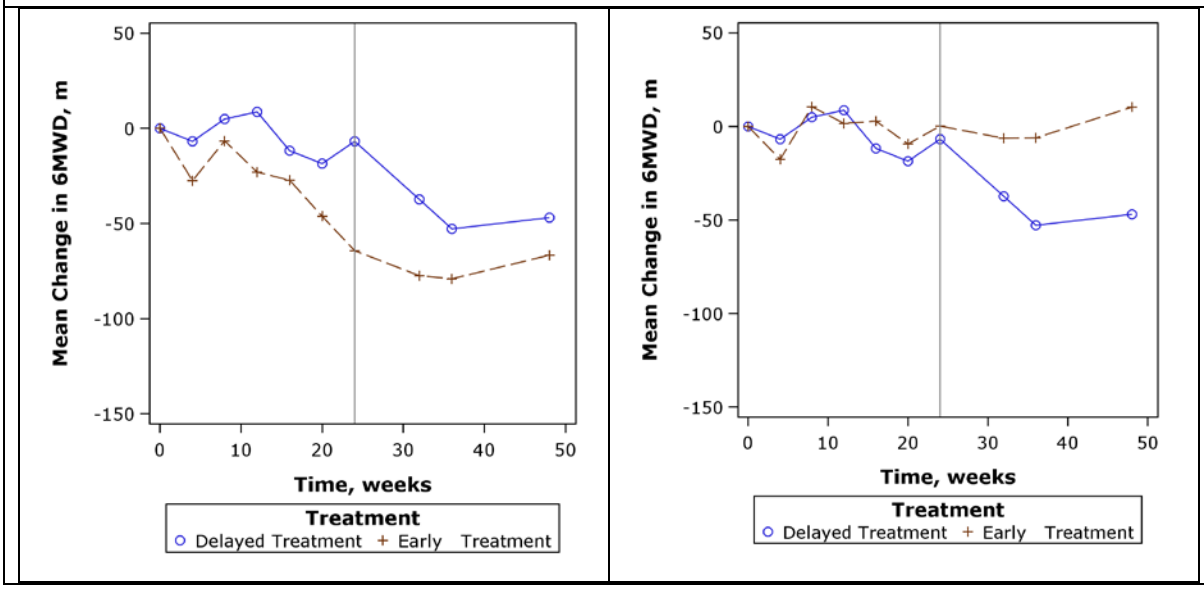


Figure 2. (Left) Mean Change in 6MWD vs Time by Early Treatment and Delayed Treatment Group in ITT population. (N=12) (Right) Mean Change in 6MWD vs Time by Early Treatment and Delayed Treatment Group in non-ITT population (N=10).



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.

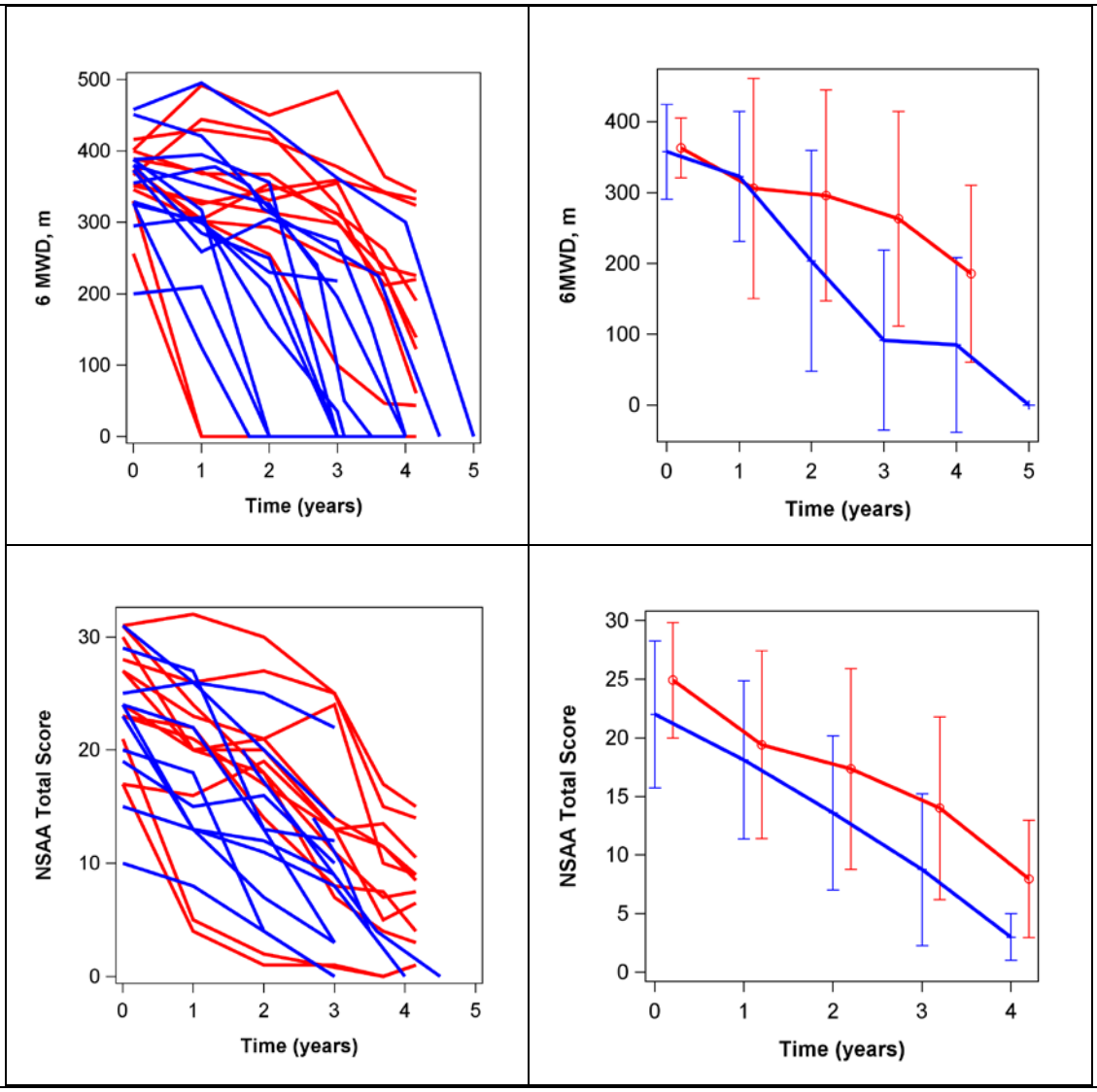
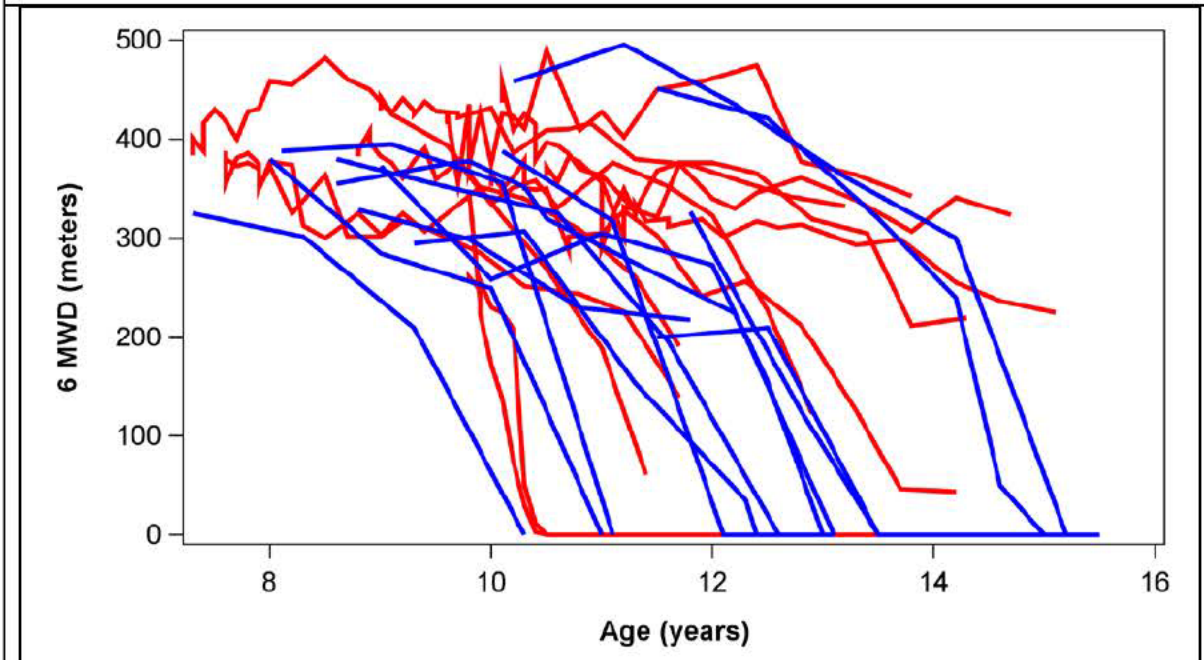


Figure 4. (Top) Changes in 6MWD in 12 Eteplirsen Treated Patients (—) and 13 Historical Controls (—) With Age (Bottom) Changes in NSAA Total Score in 12 Eteplirsen Treated Patients and 12 Historical Controls With Age.



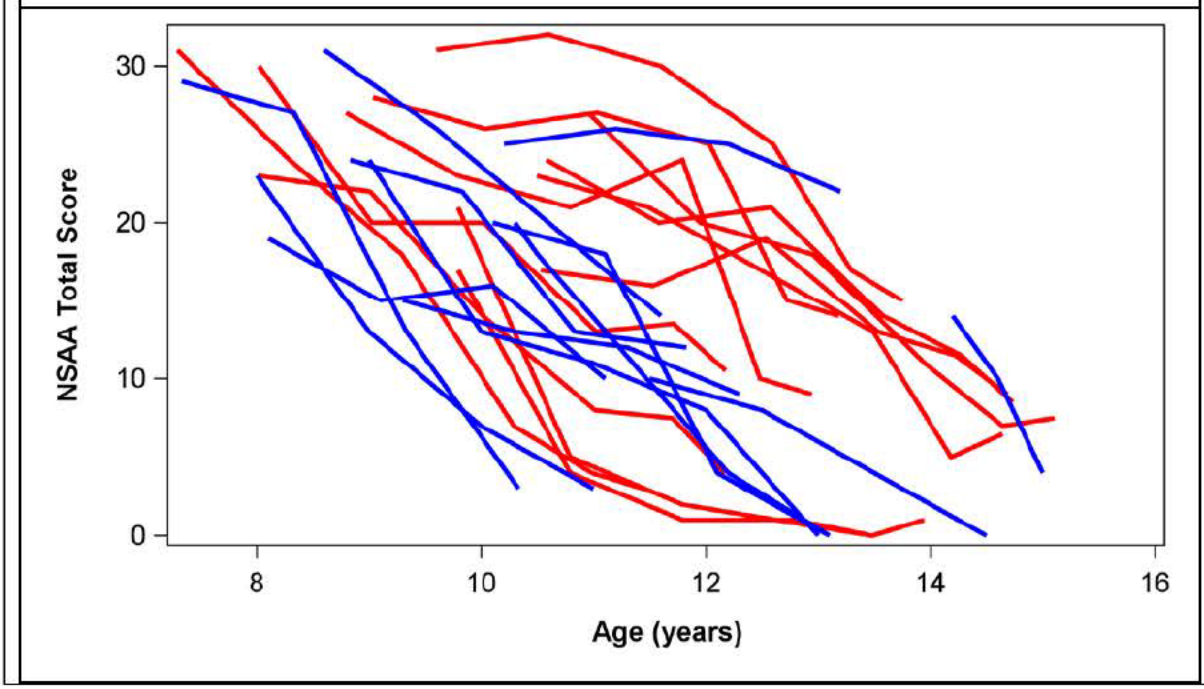
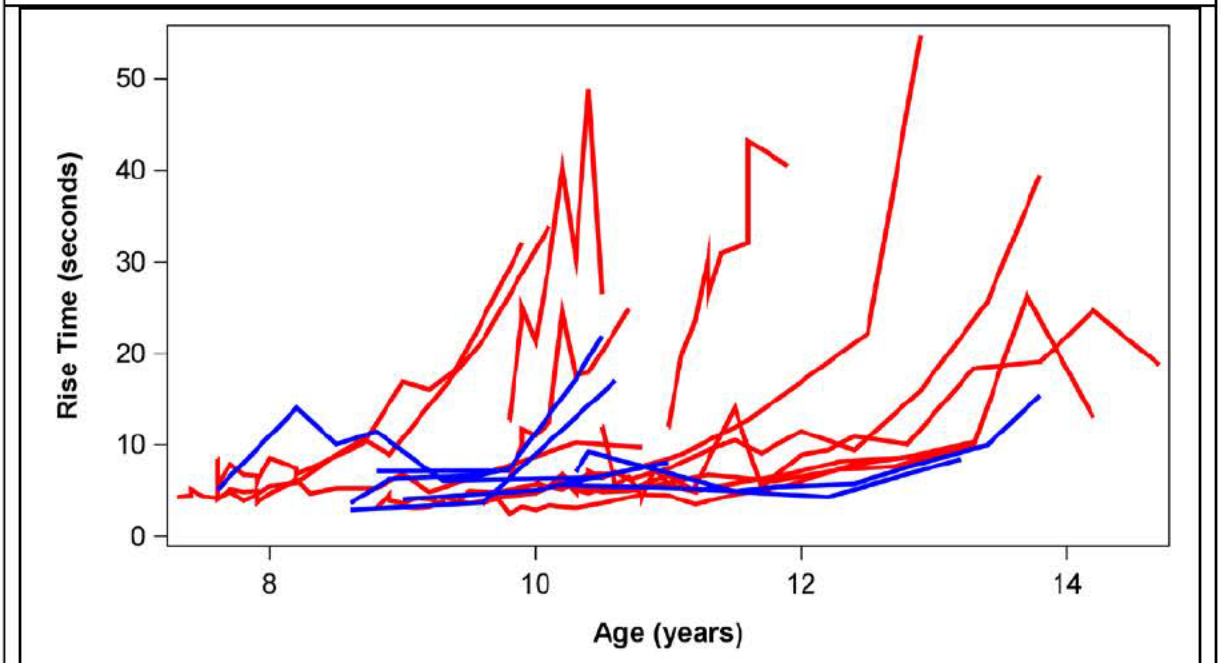
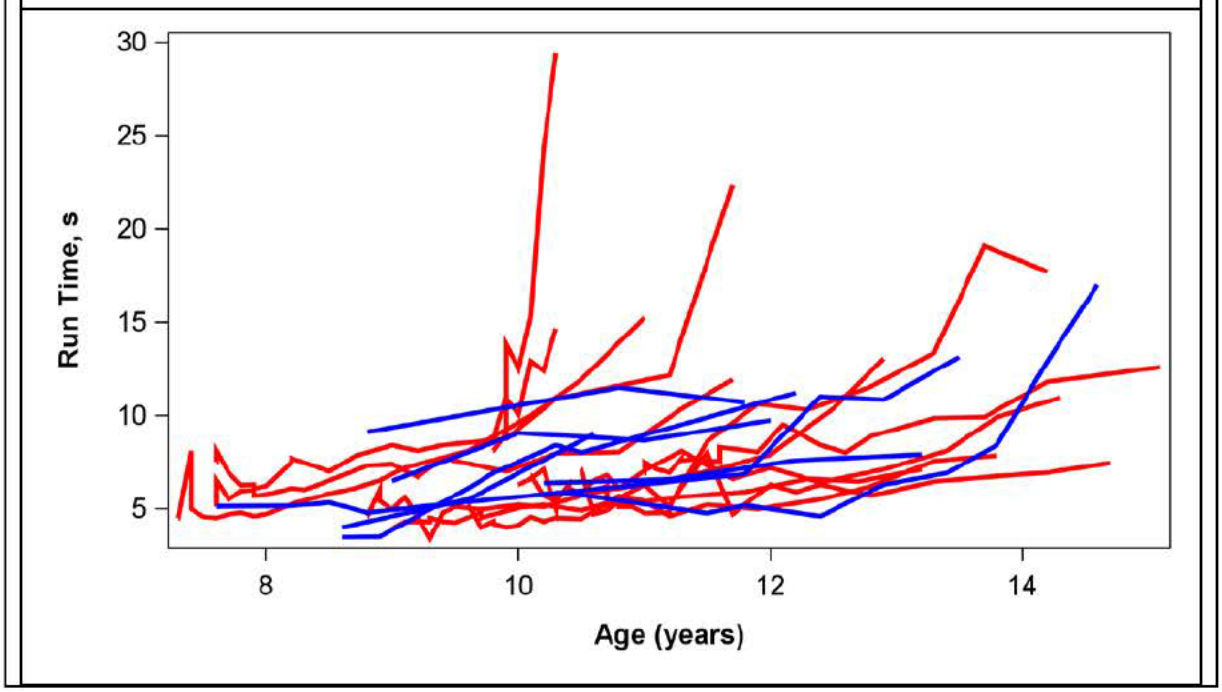


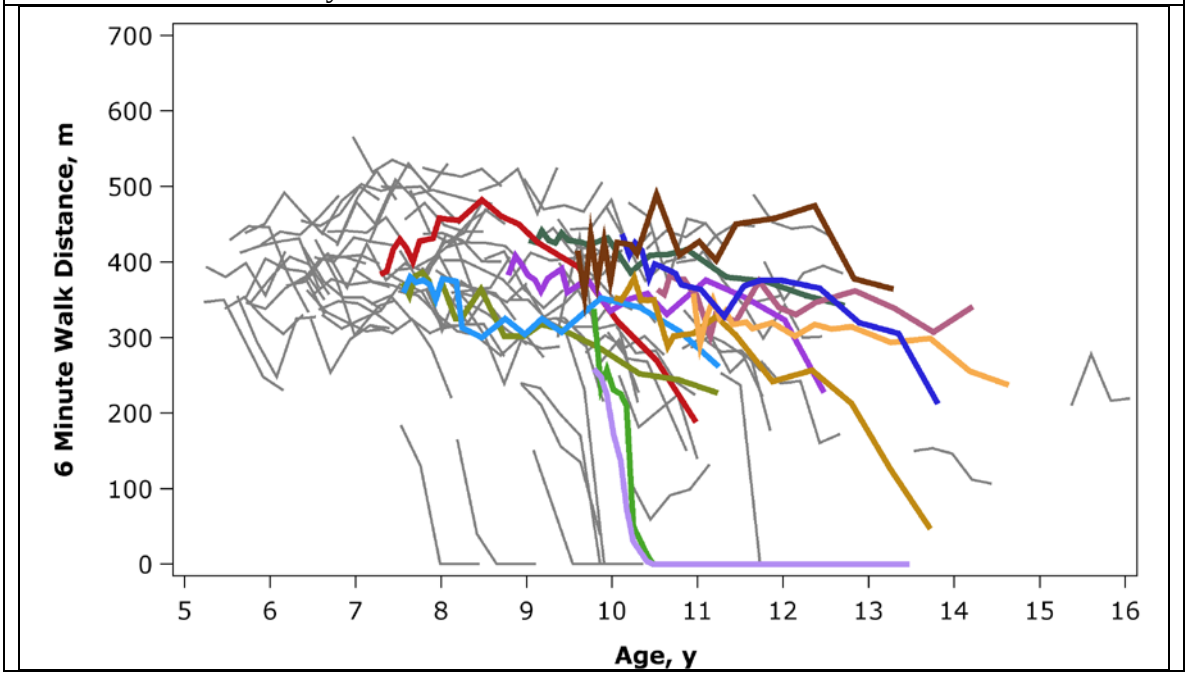
Figure 5. (Top) Changes in Rise Time in 12 Eteplirsen Treated Patients (—) and 8 Historical Controls (—) With Age (Bottom) Changes in 10 meter Run Time in 12 Eteplirsen Treated Patients and 7 Historical Controls With Age.





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?

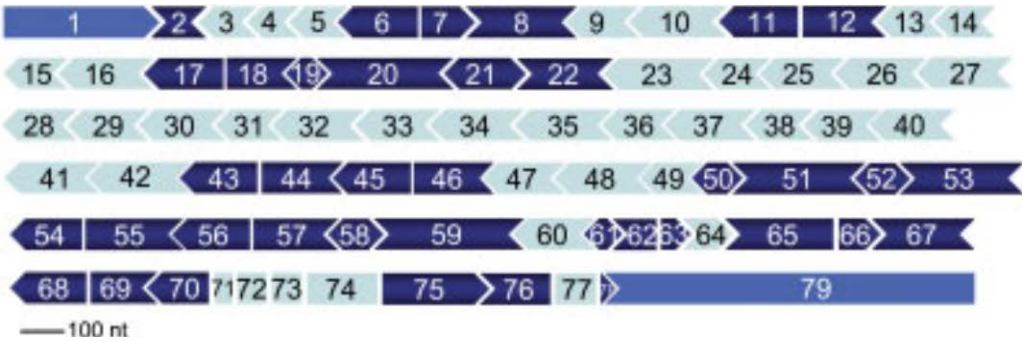
Eteplirsen is an exon skipping phosphorodiamidate morpholino oligomer (PMO) which is intended to restore the mRNA reading frame to induce a truncated dystrophin protein production. The proposed indication is for the treatment of Duchenne Muscular Dystrophy (DMD) in patients who have a confirmed mutation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

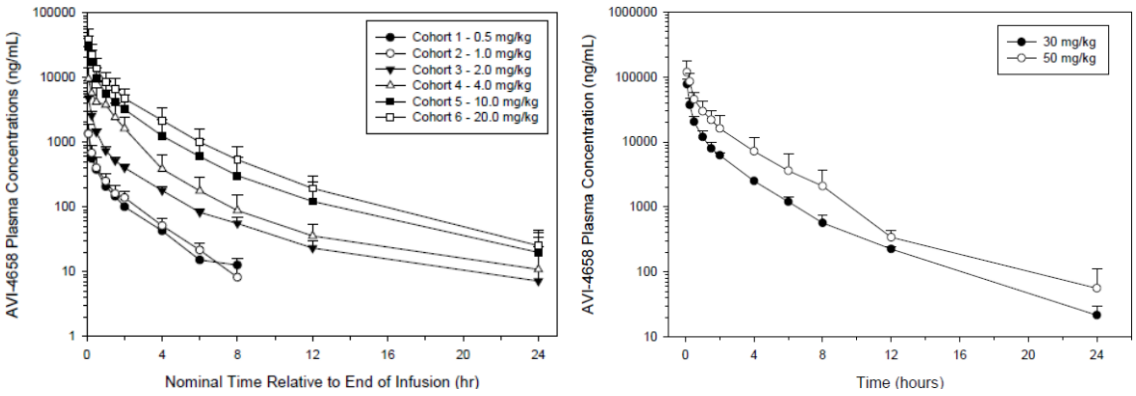


Table 1. Eteplirsen plasma PK parameters across 0.5~20 mg/kg dose range at Weeks 1, 6, and 12 (Study AVI-4658-28)

	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>

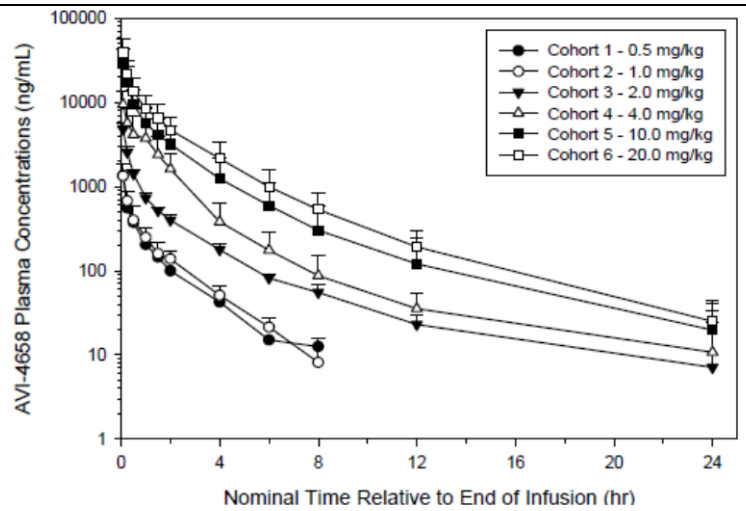


Table 1. Mean PK parameters of AVI-4658 averaged across Weeks 1, 6, and 12

	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.

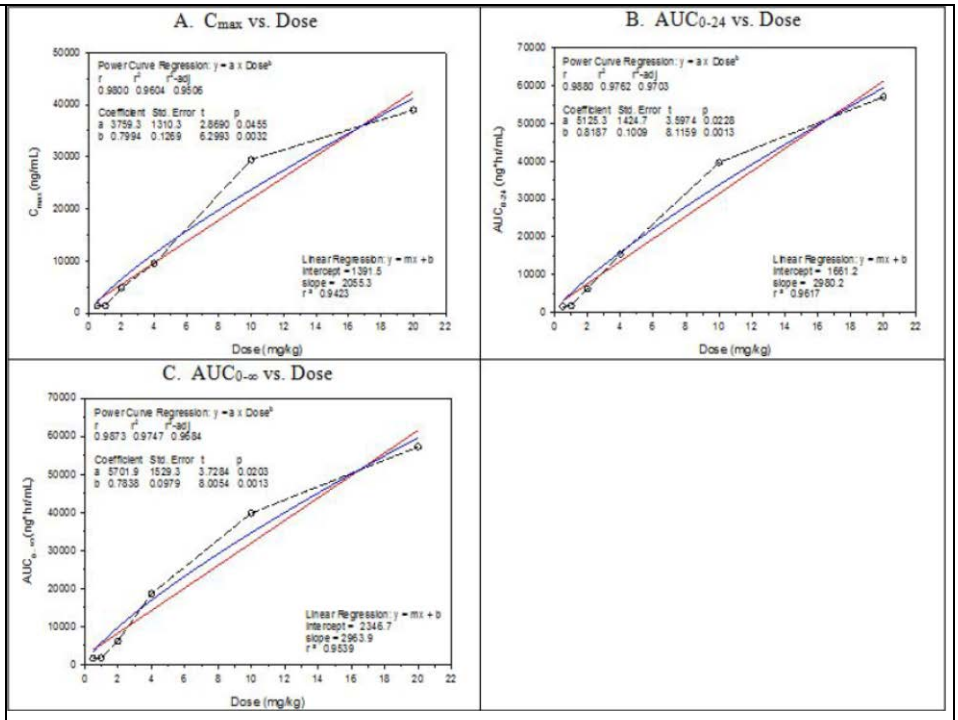


Table 2. Mean AVI-4658 renal clearance parameters across Weeks 1, 6, 12

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)

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</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

	• These assays are considered validated and acceptable. **Table.** Assay performance for AVI-4658

Analyte		AVI-4658 (plasma)	AVI-4658 (urine)
Method:		HPLC-FL	HPLC-UV
Standard Curve:	Range:	10-1000 ng/mL	25-2500 µg/mL
	Precision:	6.4-9.8%	*
	Accuracy:	97.0-107%	96.6-102.4%
LLOQ:		10 ng/mL	25 µg/mL
ULOQ:		1000 ng/mL	2500 µg/mL
LQC:		30 ng/mL	75 µg/mL
	Precision:	31.6%ǂ	*
	Accuracy:	101%	102%
MQC:		150 ng/mL	1000 µg/mL
	Precision:	18.2%	
	Accuracy:	108%	103%
HQC:		750 ng/mL	2000 µg/mL
	Precision:	4.9%	*
	Accuracy:	96.5%	103%

ǂ Precision >25%, outside of acceptable range
* Not reported

Population/ Demographics	• 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

PK Results	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

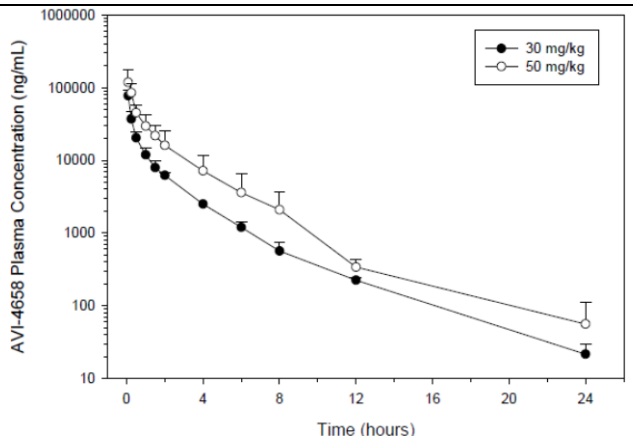


Table 1. Summary of plasma PK for AVI-4658 in ng/mL at Visit 13 (Week 12)

Treatment Group	Subject	T_{max} hr	C_{max} ng/mL	$AUC_{0\text{-last}}$ hr*ng/mL	$AUC_{0\text{-}\infty}$ hr*ng/mL	$AUC_{0\text{-}24}$ hr*ng/mL	AUC% ext %	CL_{PL} mL/hr/kg	V_{ss} mL/kg	Half-Life ($t_{1/2}$) hr	R^2 adjusted	MRT_{∞} 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> <u>Note</u>: • 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	*<u>Pharmacokinetics</u>:* • 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

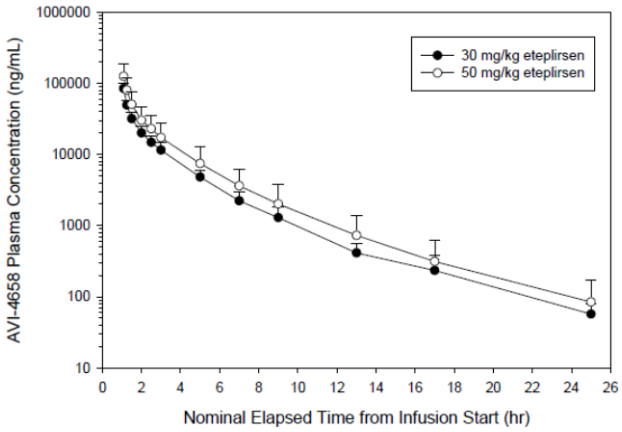
<table>
<tr><td></td><td>DMD patients who have successfully completed the 28-week study (4658-us-201).
• To explore the link between biomarkers for DMD at the clinical status</td></tr>
<tr><td>Formulation</td><td>• Eteplirsen (in phosphate buffered solution) of 100 mg/mL was supplied in single-use vials.
• 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 See table below • 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)

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

	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

		Percentage of Radioactivity						
	^{14}C-AVI-4658	Unbound			Bound		Recovered	
Species	(µg/mL)	Replicate	Mean	SD[a]	Replicate	Mean	Mean	SD
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			

Results	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.
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). Metabolism-dependent inhibition: • 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	Direct (reversible) inhibition: • 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

Metabolism-dependent inhibition:

	• 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 [redacted] (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. AVI-4658 as a substrate an inhibitor of P-gp or BCRP: • 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. AVI-4658 as an inhibitor of P-gp or BCRP: • 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. Assessment of AVI-4658 as a substrate or an inhibitor of MRP2: • 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%. Assessment of AVI-4658 as a substrate or an inhibitor of BSEP: • 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]

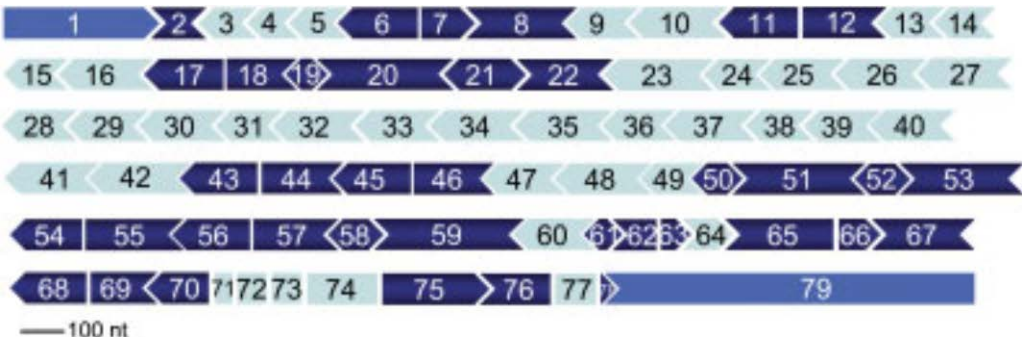
Source: Modified from page 49 Summary of Clinical Safety

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)

4.4 Cover Sheet and OCP Filing/Review Form

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