CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206488Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 206488
Submission date: 6/26/2015
Drug: eteplirsen
Applicant: Sarepta Therapeutics
Indication: treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping

Reviewing Division: Division of Neurology Products

Discussion:
The pharm/tox reviewer and supervisor found the nonclinical information for eteplirsen adequate to support approval for the above indication.

No developmental and reproductive toxicity studies were conducted with eteplirsen because the patient population is almost entirely male. Potential effects on male fertility were assessed by histopathological evaluation of male reproductive organs in toxicity studies. No adverse effects were noted.

The pharm/tox reviewer and supervisor recommend that carcinogenicity studies be conducted as postmarketing requirements, which is reasonable.

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that the information is adequate to support approval from a pharm/tox perspective.

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/s/

PAUL C BROWN
07/05/2016

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: May 25, 2016

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 206-488 (eteplirsen; EXONDYS 51; Sarepta Therapeutics)

NDA 206-488 was submitted on June 26, 2015, to support marketing approval for eteplirsen in the treatment of patients with Duchenne muscular dystrophy "…who have a confirmed mutation of the dystrophin gene amenable to exon 51 skipping." On January 8, 2016, the sponsor provided additional clinical data, which resulted in a 3-month extension of the user fee goal date.

Clinical development was conducted under IND 77429. The following nonclinical data were submitted in the NDA:

- Pharmacology (cynomolgus monkey)
- Safety pharmacology (cynomolgus monkey)
- PK/ADME
- Toxicology
    - 12-week and 3-month studies (mdx and C57BL mice)
    - 26-week study (mdx mouse)
    - 12- and 39-week studies (cynomolgus monkey)
    - 10-week juvenile animal study (Sprague-Dawley rat)
- Genetic Toxicology
    - Ames assay
    - In vitro chromosomal aberration assay in CHO cells
    - In vivo micronucleus assay (mouse)

This battery of nonclinical studies is consistent with advice provided by the Division during clinical development (*cf. Memoranda of Meeting Minutes, dated July 20, 2011, April 12, 2013, and August 22, 2013; email communication, dated June 4, 2013; Advice/Information Request Letters, dated August 5, 2010 and January 21, 2011*). Nonclinical studies were reviewed by Dr. Wilcox under IND 77429 (*cf. Preliminary Pharmacology and Toxicology Safety Review, Barbara J. Wilcox, Ph.D., March 7, 2010;*

Pharmacology/Toxicology IND Review and Evaluation, Barbara J. Wilcox, Ph.D., March 11, 2013; Pharmacology/Toxicology Review Memorandum, Barbara J. Wilcox, June 4, 2013; Pharmacology/Toxicology Memorandum, Barbara J. Wilcox, November 18, 2013) and Dr. Hawver under the NDA (*cf. Pharmacology/Toxicology NDA Review and Evaluation, NDA 206488, David B. Hawver, Ph.D., January 22, 2016*). Based on a comprehensive review of the nonclinical studies, Dr. Hawver has concluded that the nonclinical data provided by the sponsor support approval of eteplirsen for the proposed indication.

A brief summary of the nonclinical data is provided below. A more detailed description and discussion of the data are provided in Dr. Hawver's review.

Pharmacology

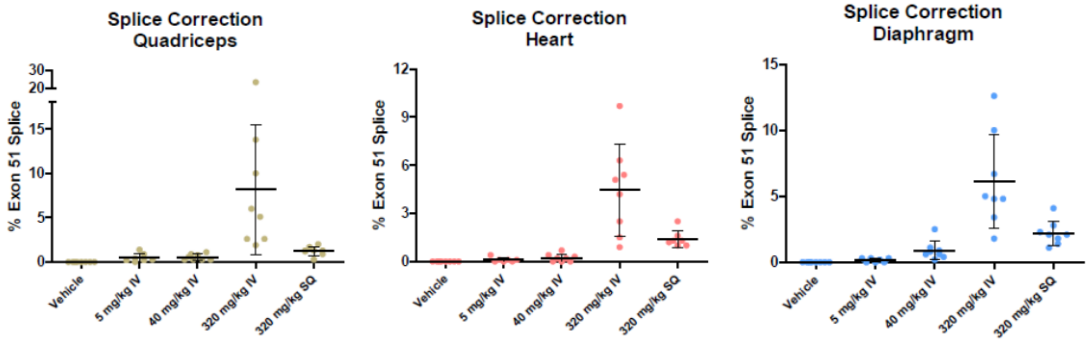
Eteplirsen is an antisense oligonucleotide, a phosphorodiamidate morpholino oligomer (PMO) designed to hybridize to dystrophin pre-mRNA, induce skipping of exon 51, and restore the mRNA reading frame to produce a truncated, but functional, dystrophin protein. The sponsor assessed exon skipping activity in one study, examining selected tissues from cynomolgus monkeys (main study: 3-4/sex/group; 28-day recovery: 2/sex/group) administered eteplirsen at doses of 0, 5, 40, or 320 mg/kg IV, or 320 mg/kg SC QW for 12 weeks. At the main-study necropsy, the following tissues were collected: bicep, tibialis anterior, quadriceps, heart, diaphragm, kidney, and liver; only quadriceps, heart, and diaphragm were analyzed. RNA was collected and RT-PCR was performed to detect exon skipping. Tissues from recovery animals were not analyzed for exon skipping.

The data (summarized in the following table and figure from the sponsor) indicate dose-related exon skipping.

Table 10 Average percentage of exon 51 splicing

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

SD standard deviation



Similar low levels of exon skipping were detected at 5 and 40 mg/kg IV in all three muscle types analyzed, but a greater than dose-proportional increase in exon skipping was evident in quadriceps and heart at the high dose of 320 mg/kg IV. In diaphragm, there was a slightly less than dose-proportional increase in exon skipping at 320 mg/kg IV. At 320 mg/kg, SC dosing resulted in substantially less exon skipping than IV, which may have informed the sponsor's selection of IV dosing for humans. In all three muscles, variability increased markedly at 320 mg/kg, particularly with IV dosing (ranges at 5, 40, and 320 mg/kg IV: 0.0-1.4, 0.3-1.1, and 1.9-23.3% for quadriceps; 0.0-0.4, 0.0-0.7, and 0.9-9.7% for heart; 0.0-0.3, 0.2-2.5, and 1.8-12.6% for diaphragm).

The Week 11 TK data ($AUC_{(0\text{-}t)}$ [µg*hr/mL]; mean ± SD) are provided in the following table:

ETEPLIRSEN DOSES (mg/kg)					
MALES			**FEMALES**		
5	**40**	**320**	**5**	**40**	**320**
15.4 ± 3.33	137 ± 50.0	1190 ± 537.0	13.6 ± 5.58	149 ± 24.7	855.0 ± 390.0

Plasma exposures at 5, 40, and 320 mg/kg in monkey are ~0.1, 1, and 8 times that in humans at the proposed clinical dose of 30 mg/kg QW.

The sponsor also cited published studies, both in the NDA and in the AC briefing document (dated April 25, 2016), which supported selection of eteplirsen as a clinical candidate (Arechavala-Gomeza V *et al. Hum Gene Ther* 18:798-810, 2007) and which demonstrate pharmacodynamic effects of various compounds and strategies (e.g., PTC124, gentamicin, U7 snRNA, rAAV6-microdystrophin, HDAdv [virus-mediated gene delivery of dystrophin cDNA], full-length dystrophin gene transfer, truncated microdystrophin), including PMOs and PPMOs (cell-penetrating peptide-tagged PMO), in in vitro assays and in vivo animal models of DMD.

Selected findings from published studies of systemic administration of PMO23 or modified PMOs cited by the sponsor and from a few additional recently published studies are summarized below. (The PMO administered in a particular study is stated to be AVI-4225 if identified by the sponsor as such.) In all studies discussed, any increase in dystrophin resulting from PMO administration was of an internally truncated, rather than full-length, dystrophin.

- Wu B *et al. Mole Therap* 19(3):576-583, 2011

This study in mdx and C57BL mice tested the effects of a mouse-specific PMO targeting exon 23 (AVI-4225) at doses of 15 and 60 mg/kg IV Q2W for 1 year and doses of 300 mg/kg and 1.5 g/kg IV Q2W for 6 months. At 15 mg/kg, dystrophin-positive fibers were increased up to 5% of wild type (WT) in skeletal muscle, but no increase was detected in cardiac muscle. The maximum amount of dystrophin protein was 2.6% of WT, based on Western blot analysis. No significant improvement in muscle pathology (% non-centranucleated fibers) or function (grip-force generation) was demonstrated. At 60

mg/kg, the level of exon skipping (by RT-PCR) ranged from "barely detectable" to 40% in skeletal muscle. Dystrophin-positive fibers were 10-50% but "with highly variable intensity" in skeletal muscle. Levels of dystrophin protein were up to 17.1% of WT but were reported to be highly variable among muscle groups in an individual animal and in the same muscle type among animals. Significant improvement in muscle pathology and function was observed. Further enhancement of exon skipping and muscle pathology and function was observed at the higher doses (300 mg/kg and 1.5 g/kg) after 6 months of dosing. At 300 mg/kg, dystrophin-positive fibers and levels of dystrophin protein were >50% and >15% of WT, respectively; at 1.5 g/kg, there was a further increase, to ~100% and 25-50% of WT, respectively.

- Malerba A *et al. Hum Gene Ther* 20:955-965, 2009

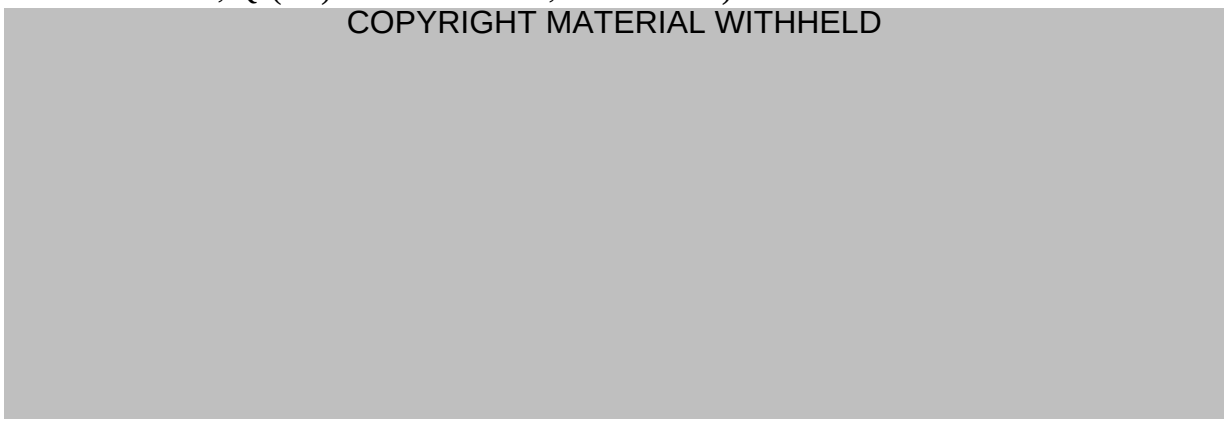
In this study, a PMO designed to skip exon 23 was administered IV to mdx mice at total doses of 20 or 200 mg/kg, given either as a single dose or weekly for 4 doses of 5 or 50 mg/kg. Following a single 200 mg/kg dose, the number of dystrophin-positive fibers in quadriceps was 8% and dystrophin expression in quadriceps and tibialis anterior was 3-5% of WT. When the same dose was administered as 50 mg/kg QW, the number of dystrophin-positive fibers in quadriceps was 57%; dystrophin expression was increased ≤15-17% in quadriceps and diaphragm and ≤10-13% in tibialis anterior compared to WT, although dystrophin expression was detected in only 1 of 3 quadriceps sampled. Recovery of muscle pathology, characterized as "partial," was not consistently observed among the different muscle groups with either dosing regimen. At a single 20 mg/kg dose or 5 mg/kg QW, increases in dystrophin expression (figures below taken from the publication) were observed in quadriceps but not tibialis anterior (although the number of dystrophin-positive fibers was 21% of WT in tibialis anterior), and there was no improvement in muscle pathology. No functional tests were conducted.

No effect on cardiac muscle was detected with either regimen at either dose.

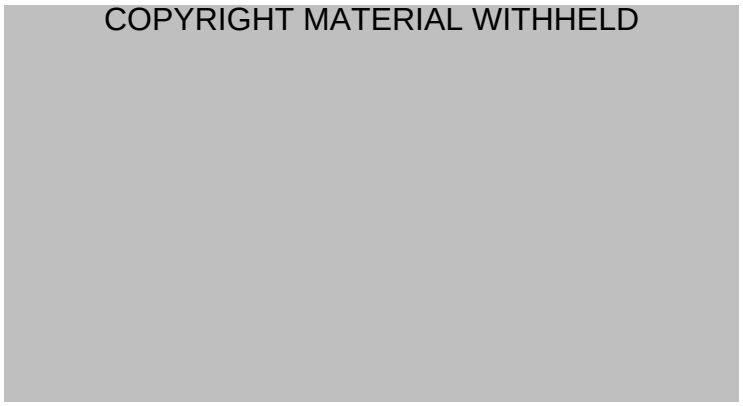
- Malerba A *et al. Mole Therap* 19(2):345-354, 2011

In a subsequent study in mdx mouse, a mouse-specific PMO (AVI-4225) was administered in cycles. One cycle consisted of 4 weekly IV injections followed by 6 weeks without dosing; this was repeated 2 (20 weeks) or 5 (50 weeks) times, at either 5 or 50 mg/kg. At 20 and 50 weeks, increases in dystrophin-positive fibers were observed at both doses (figure below taken from the publication; TA = tibialis anterior; Dia =

diaphragm; Gast = gastrocnemius; Tri = trices brachii; Qa = total quadriceps; Qa(VL) = vastus lateralis; Qa(RF) = rectus femoris; Sol = soleus).



At 20 weeks, the percent of dystrophin-positive fibers was 23-27% in tibialis anterior and ~30% in quadriceps. Further increases were detected in some, but not all, muscles at 50 weeks. Significant increases in dystrophin protein were observed at both doses. From the figure below (taken from the publication), it is evident that the increase in dystrophin protein was >10% at both doses and time points. Dystrophin expression increased notably with duration of dosing only in tibialis anterior.



It is unclear to what extent the different dosing regimen used is responsible for the greater skeletal muscle dystrophin expression in this study compared to Wu *et al.* (2011).

Improvement in muscle pathology (% centrally nucleated fibers) was observed at both doses and time points, although the sponsor noted that in muscles in which the % dystrophin-positive fibers were ≤15% of WT (rectus femoris and diaphragm), there was no effect on muscle pathology. Effects on muscle physiology and function are summarized in the following figure (taken from the publication):

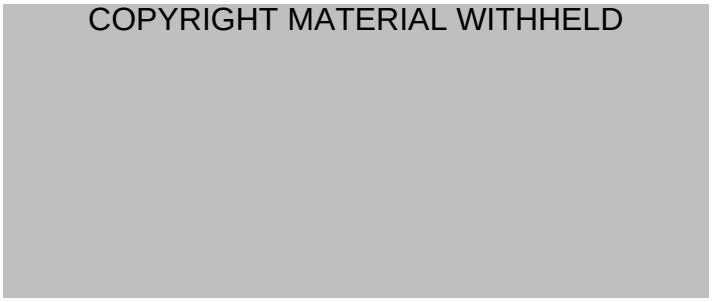
Improvement in muscle physiology and function was observed at both doses; however, at Week 50, improvement was observed only at the higher dose tested (50 mg/kg) but not on all parameters (e.g., % of maximum force was similar to untreated mdx mouse).

There was no increase in dystrophin expression in cardiac muscle.

- Alter J *et al. Nature Med* 12(2):175-177, 2006)

In this study, a PMO designed to skip exon 23 (AVI-4225) was administered IV (2 mg or ~60-80 mg/kg) in mdx mice, either as a single injection or 3 or 7 weekly injections. Following a single dose, dystrophin expression (Western blot) was up to 5% of WT in intercostal and quadriceps muscles. Expression was increased to >20% of WT in intercostal and quadriceps muscles (but only 5% in diaphragm) following 3 weekly doses (levels were not provided for tibialis anterior). Further increases were detected with 7 weekly doses, up to 50% of WT in gastrocnemius and quadriceps, and up to 10-20% in tibialis anterior, intercostal, abdominal, and triceps muscles. However, no expression was detected in the heart.

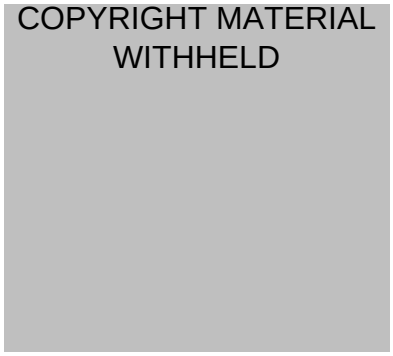
The Western blots for dystrophin expression in various muscles after a single (b) or 3 weekly (c) IV injections are provided in the figure below, taken from the publication:



Functional effects were assessed by measurement of isometric tetanic force in tibialis anterior after 3 weekly doses. Significant improvement was observed, although still lower than in WT. In tibialis anterior, improved maximum isometric tetanic force was observed, associated with dystrophin expression <20% of WT. The authors state that these data suggest "…that low levels of dystrophin [<20%] can have a crucial physiological impact in some muscles."

- Fletcher S *et al. J Gene Med* 8:207-216, 2006

Fletcher *et al.* (2006) administered a PMO (AVI-4225) to neonatal mdx mouse at a dose of 25 mg/kg IP, either as an acute dose or daily for 7 days, followed by 3 doses per week for 2 additional weeks. No dystrophin was detected in tibialis anterior or diaphragm following a single dose. After multiple doses, the authors report increases in dystrophin expression in these muscles (Western blot in figure below, from the publication). However, the poor quality of the Western blot makes it difficult to appreciate the responses. No functional assessment was conducted.



- Heemskerk *et al. J Gene Med* 11:257-266, 2009

In this study, short (20-mer) and long (25-mer) 2OMePS and a 25-mer PMO (AVI-4225) were administered at a dose of 14.52 µmol/kg IM or IV to mdx mouse every other day for 3 weeks. Western blots are provided for various skeletal muscles and heart (% of WT) in the following figure, taken from the publication.

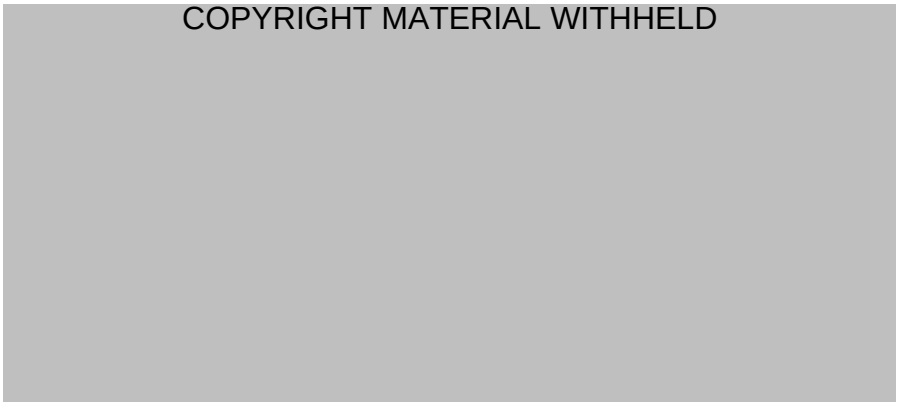


Figure 4. Western blot analysis of skeletal and heart muscle of *mdx* mice systemically treated with the long and short 2OMePS and PMO AONs targeting mouse exon 23. NCL-DYS1 was used to detect dystrophin. Dystrophin levels were quantified as percentages of wild type gastrocnemius dystrophin and are shown underneath the western blot. The results obtained were comparable for the different mice within each treatment group and therefore only a single sample per muscle is shown per group. No protein was detected in nontreated *mdx* mouse muscle (*Mdx*). After treatment, dystrophin was detectable in each skeletal muscle analysed. Dystrophin levels of PMO treated animals were higher than those treated with either the short or long 2OMePS AON for the skeletal muscles, which corresponds to the RT-PCR data. In heart, protein levels found are low for each AON, again in accordance with the low skipping levels found at the RNA level

For the PMO, dystrophin expression was 10-30% of WT, except in heart (0.6% of WT). Exon skipping was ~40% (RT-PCR) in skeletal muscles, but did not correlate with dystrophin expression, which varied among muscles. No functional measures were assessed.

- Yokota T *et al. Ann Neurol* 65:667-676, 2009

This study was conducted in a Beagle model of canine X-linked muscular dystrophy, which harbors a point mutation in exon 7; exons 6, 7, and 8 must be skipped in order to restore an open reading frame. Three dogs were administered an equimolar mixture of three PMOs IV at different doses and dosing regimens (total dose of 120 mg/kg QW for 5 weeks, total dose of 120 mg/kg Q2W for 5.5 months, or total dose of 200 mg/kg QW for 7 weeks. All animals were sacrificed 2 weeks after the last dose.

Western blot analysis indicated express of up to 50% of WT dystrophin levels, "but some muscles expressed only trace amounts…" The average dystrophin protein expression was greatest in the dog receiving the highest total dose (~26% of WT). In the heart, "Dystrophin expression was…detected…but less than in skeletal muscle and concentrated in small patches…" Muscle function was assessed using a 15 meter timed running test and a combined clinical grading score. All three dogs demonstrated functional improvement, with the older dog "with more advanced symptoms" showing the most improvement.

<u>Other published studies</u>

- Godfrey C *et al. Hum Mole Gene* 24(15):4225-4237, 2015

In a recent study conducted to address the issue of how much dystrophin is needed to protect muscles, a peptide-conjugated PMO (PPMO) designed to skip exon 23 was administered to mdx mice. Following an acute IV dose of 3, 6, 9, or 12.5 mg/kg PPMO,

doses of 9 and 12.5 mg/kg were associated with dystrophin expression in the tibialis anterior 5-15% of WT; none was detected at the lower acute doses. Both 9 and 12.5 mg/kg were associated with protection against eccentric contraction-induced muscle damage of the tibialis anterior, compared to WT. "Slight" improvement in muscle function was observed only at 12.5 mg/kg, which was associated with more homogenous dystrophin expression. The authors concluded that 15% of WT ("low level dystrophin restoration") was sufficient to protect muscle (eccentric contraction-induced muscle damage) but not sufficient to "substantially" improve muscle function (maximum isometric force). From the acute dose data, the authors concluded that lower but homogenous expression of dystrophin appeared more beneficial than higher, but patchy, expression. The effects of repeated dosing (Q2W) at 12.5 mg/kg of the PPMO on muscle pathology and function were tested in the tibialis anterior. Western blot analysis indicated homogenous dystrophin expression ~50% of WT, which positively correlated with maximal isometric force and reduced muscle pathology.

- Wu B *et al Am J Path* 181(2):392-400, 2012

A PMO23 conjugated with an arginine-rich peptide (PPMO AVI-5038) was administered to mdx mice at an acute dose of 30 mg/kg IV, at 1.5 or 6 mg/kg IV Q2W for 1 year, or 30 mg/kg IV QM for 1 year. Two days after an acute 30 mg/kg IV dose, efficiency of exon skipping was "nearly" 100% (RT-PCR). Dystrophin protein (WB) was ~20% of WT in the skeletal muscles examined. In cardiac muscle, exon skipping efficiency was ≤40%, but no dystrophin protein was detected. By 2 weeks post dose, dystrophin protein was ≥80% of WT. Reduced muscle pathology was evident in all skeletal muscles examined at 2.5 months and, except for diaphragm, up to 5 months post dose.

A dose of 1.5 mg/kg IV Q2W for 1 year resulted in <20% of dystrophin positive fibers and dystrophin protein ranging from "…barely detectable to <5% of WT level in any skeletal muscle," similar to the values previously observed after a single dose. No difference in muscle pathology or muscle function (grip force generation) from WT was observed.

At doses of 6 mg/kg IV Q2W and 30 mg/kg IV QM, there were increases in dystrophin-positive fibers (15-50 and ~100%, respectively) and dystrophin protein (20-50 and >50%, respectively); however, dystrophin protein "as low as 25%" was also detected in skeletal muscle. Muscle pathology was improved at both doses; however, at the lower dose, "…sporadic foci of degeneration and regeneration and monocyte infiltration remained in all muscles." In cardiac muscle, dystrophin expression was <5% of WT at the lower dose and "reached 15%" at the higher dose.

The authors cited a review by Moulton & Moulton (2010), who report that administration of PPMO AVI-5038 at a dose of 9 mg/kg IV QW to healthy cynomolgus monkey for 4 weeks "…induced an average of 40%, 25%, and 2% exon-skipped product in diaphragm quadriceps, and heart (respectively)…With the same dose schedule at a lower dose of 3 mg/kg, little exon-skipped product was detected, similar to the saline-treated monkeys."

The authors conclude that "These results indicate a nonlinear relationship between the dose and the efficiency of dystrophin induction with systemically delivered PPMO. In clinical application, therefore, a threshold dose of PPMO may be required to achieve significant levels of dystrophin production and consequent functional improvement for DMD patients."

PK/ADME

The PK/ADME of ^{14}C-eteplirsen (120 mg/kg IV) was assessed in an acute-dose study in male mdx mouse. Total radioactivity was quantitated in blood/plasma and selected tissues (brain, diaphragm, heart, kidney, biceps brachii, tibialis anterior, biceps femoris, and quadriceps) in samples collected for up to 144 hrs postdose. In addition, tissue distribution (1 mouse/time point) was assessed using quantitative whole-body autoradiography (QWBA) for up to 1344 hrs (56 days) postdose. Urine and feces were collected for up to 336 hrs postdose.

Plasma PK parameters were as follows: $t_{1/2}$ ~ 6 hrs, Cl = 348 mL/hr/kg, and V_d = 175 mL/kg. In the selected tissues examined, peak levels were detected at the first time point (0.25 hrs postdose) and were highest in kidney (263000 ng-eq/g) and lowest in brain (1770 ng-eq/g). Levels of radioactivity were fairly similar among muscles, with the highest peak levels in biceps femoris (43700 ng-eq/g) and lowest in biceps brachii (9550 ng-eq/g). Widespread tissue distribution was detected by QWBA, with highest peak levels in kidney (165000 ng-eq/g). At 56 days postdose, radioactivity was detected only in kidney and urine. The major route of elimination was via the urine (~60%).

Plasma protein bind was low in all species examined (<25%), consistent with a PMO backbone. The sponsor provided no data on dystrophin-positive fibers or dystrophin expression in tissues.

Toxicology

The pivotal toxicity studies were conducted in mdx or C57BL mice (12-week, 3-month, and 26-week), cynomolgus monkey (12- and 39-week), and juvenile Sprague-Dawley rat (10-week).

Mouse: In the 12-week study, eteplirsen was administered weekly at doses of 0, 12, 120, and 960 mg/kg IV or 960 mg/kg SC to mdx mice and at doses of 0 and 960 mg/kg IV to C57BL mice. In the 3-month and 26-week studies, the mouse surrogate, AVI-4225, was administered to mdx and C57BL mice according to the same study design. The subchronic studies were conducted in males and females and included a 28-day recovery period, whereas in the 26-week study, only males were tested and the recovery period was of 8 weeks' duration.

The primary target organ was kidney. Microscopic findings included basophilic tubules, tubular vacuolation, and degeneration/regeneration. Tubular degeneration/regeneration, the only kidney finding considered adverse, was observed in the 12-week study of

eteplirsen only in the C57BL mouse (960 mg/kg IV). In the 3-month study of AVI-4225, tubular degeneration/regeneration was not detected in mdx or C57BL mouse at doses up to 960 mg/kg IV or SC. In the 26-week study of AVI-4225, tubular degeneration, characterized as minimal, was observed at doses of 120 and 960 mg/kg IV in mdx mouse and in C57BL mouse (960 mg/kg IV) at the end of the dosing period, and at 960 mg/kg IV in mdx and C56BL mice at the end of recovery. Selected kidney (tubule) findings from the 26-week study are summarized in the following table:

FINDING	ETEPLIRSEN DOSE (mg/kg)					
	MDX				C57BL	
	0	12	120	960	0	960
MAIN STUDY						
cytoplasmic basophilia/microvacuolation						
minimal	0/16	0/16	1/16	9/16	0/16	0/16
slight	0/16	0/16	0/16	7/19	0/16	3/16
moderate	0/16	0/16	0/16	0/16	0/16	13/16
total	**0/16**	**0/16**	**1/16**	**16/16**	**0/16**	**16/16**
degeneration						
minimal	**0/16**	**0/16**	**1/16**	**12/16**	**0/16**	**7/16**
RECOVERY						
cytoplasmic basophilia/microvacuolation						
minimal	0/9	0/10	0/9	5/8	0/9	6/11
slight	0/9	0/10	0/9	3/8	0/9	3/11
moderate	0/9	0/10	0/9	0/8	0/9	0/11
total	**0/9**	**0/10**	**0/9**	**8/8**	**0/9**	**9/11**
degeneration						
minimal	**0/9**	**0/10**	**0/9**	**6/8**	**0/9**	**5/11**

A toxicity observed only in the 26-week study was dilatation of the lateral ventricles in brain (data summarized below) of mdx, but not C57BL, mouse.

SEVERITY	AVI-4225 DOSES (mg/kg)					
	MDX				C57BL	
	0	12	120	960	0	960
MAIN STUDY						
minimal	0/16	0/16	2/16	0/16	0/16	0/16
slight	0/16	0/16	1/16	3/16	0/16	0/16
moderate	0/16	0/16	0/16	1/16	0/16	0/16
total	**0/16**	**0/16**	**3/16**	**4/16**	**0/16**	**0/16**
RECOVERY						
slight	**0/9**	**0/10**	**0/9**	**1/8**	**0/9**	**0/11**

The NOAEL in the 26-week study was the LD, based on kidney tubular degeneration and dilatation of the lateral ventricles at the two higher doses. However, both findings were characterized as minimal or slight in all cases, except for dilatation of the lateral ventricles of moderate severity in one animal at the HD.

The sponsor conducted a more extensive histological evaluation of muscles in the 3-month and 26-week studies of AVI-4225 (summary tables below).

3-MONTH STUDY	DOSE (mg/kg)									
	MALES					FEMALES				
	0	12	120	960	960 SC	0	12	120	960	960 SC
MAIN STUDY										
larynx										
minimal	7/10	5/10	5/10	1/10	6/10	5/10	3/10	5/10	1/10	6/10
mild	2/10	2/10	0/10	1/10	0/10	5/10	0/10	0/10	0/10	0/10
moderate	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
total	**10/10**	**7/10**	**5/10**	**2/10**	**6/10**	**10/10**	**3/10**	**5/10**	**1/10**	**6/10**
biceps femoris										
minimal	2/10	5/10	4/10	5/10	5/10	0/10	4/10	4/10	5/10	3/10
mild	5/10	1/10	0/10	0/10	2/10	6/10	5/10	4/10	0/10	4/10
moderate	3/10	0/10	0/10	0/10	0/10	4/10	0/10	0/10	0/10	0/10
total	**10/10**	**6/10**	**4/10**	**5/10**	**7/10**	**10/10**	**9/10**	**8/10**	5/10	**7/10**
diaphragm										
minimal	0/10	0/10	1/10	8/10	3/10	0/10	2/10	2/10	4/8	5/10
mild	2/10	6/10	6/10	2/10	7/10	4/10	8/10	7/10	4/8	5/10
moderate	8/10	4/10	2/10	0/10	0/10	6/10	0/10	1/10	0/8	0/10
total	**10/10**	**10/10**	**9/10**	**10/10**	**10/10**	**10/10**	**10/10**	**10/10**	**8/8**	**10/10**
quadriceps										
minimal	2/10	5/10	6/10	6/10	5/10	1/10	1/10	1/10	6/10	7/10
mild	8/10	5/10	2/10	1/10	2/10	4/10	9/10	9/10	3/10	2/10
moderate	0/10	0/10	0/10	0/10	0/10	5/10	0/10	0/10	0/10	0/10
total	**10/10**	**10/10**	**8/10**	**7/10**	**7/10**	**10/10**	**10/10**	**10/10**	**9/10**	**9/10**
tongue										
minimal	8/10	2/10	3/10	4/10	3/10	6/10	7/10	6/10	3/10	1/10
mild	1/10	0/10	0/10	0/10	0/10	2/10	0/10	2/10	0/10	0/10
total	**9/10**	**2/10**	**3/10**	**4/10**	**3/10**	**8/10**	**7/10**	**8/10**	**3/10**	**1/10**
RECOVERY										
larynx										
minimal	3/5	1/5	1/5	1/5	2/5	3/5	2/5	3/5	--	1/5
mild	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5		0/5
total	**4/5**	**2/5**	**1/5**	**1/5**	**2/5**	**3/5**	**2/5**	**3/5**		**1/5**
biceps femoris										
minimal	1/5	4/5	4/5	2/5	2/5	2/5	2/5	2/5	3/5	4/5
mild	3/5	0/5	0/5	0/5	2/5	2/5	3/5	3/5	1/5	1/5
moderate	1/3	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5
total	**5/5**	**5/5**	**4/5**	**2/5**	**4/5**	**5/5**	**5/5**	**5/5**	**4/5**	**5/5**
diaphragm										
minimal	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5
mild	4/5	3/5	4/5	3/5	2/5	4/5	3/5	5/5	4/5	4/5
moderate	1/5	2/5	1/5	1/5	2/5	1/5	2/5	0/5	0/5	0/5
total	**5/5**	**5/5**	**5/5**	**5/5**	**4/5**	**5/5**	**5/5**	**5/5**	**5/5**	**5/5**
quadriceps										
minimal	1/5	2/5	1/5	3/5	4/5	0/5	0/5	1/5	3/5	4/5
mild	2/5	3/5	3/5	1/5	1/5	5/5	5/5	4/5	1/5	1/5
moderate	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
total	**5/5**	**5/5**	**4/5**	**4/5**	**5/5**	**5/5**	**5/5**	**5/5**	**4/5**	**5/5**
tongue										
minimal	3/5	0/5	2/5	0/5	2/5	3/5	3/5	5/5	1/5	4/5
mild	2/5	2/5	1/5	1/5	0/5	0/5	1/5	0/5	0/5	0/5
total	**5/5**	**2/5**	**3/5**	**1/5**	**2/5**	**3/5**	**4/5**	**5/5**	**1/5**	**4/5**

26-WEEK STUDY	DOSE (mg/kg)			
	0	12	120	960
MAIN STUDY				
larynx				
minimal	7/16	5/16	3/16	1/15
slight	3/16	0/16	0/16	0/15
total	**10/16**	**5/16**	**3/16**	**1/15**
biceps femoris				
minimal	2/16	12/16	9/16	3/16
slight	9/16	2/16	0/16	0/16
moderate	5/16	0/16	0/16	0/16
total	**16/16**	**14/16**	**9/16**	**3/16**
diaphragm				
minimal	0/16	1/16	5/16	6/16
slight	8/16	12/16	11/16	1/16
moderate	8/16	3/16	0/16	0/16
total	**16/16**	**16/16**	**16/16**	**7/16**
quadriceps				
minimal	2/16	10/16	12/16	6/16
slight	11/16	2/16	0/16	0/16
moderate	3/16	0/16	0/16	0/16
total	**16/16**	**12/16**	**12/16**	**6/16**
tongue				
minimal	**6/16**	**2/16**	**2/16**	**0/16**
RECOVERY				
larynx				
minimal	5/9	2/10	2/9	1/8
slight	0/9	0/10	0/9	0/8
total	**5/9**	**2/10**	**2/9**	**1/8**
biceps femoris				
minimal	2/9	7/10	6/9	3/8
slight	6/9	3/10	0/9	0/8
moderate	1/6	0/10	0/9	0/8
total	**9/9**	**10/10**	**6/9**	**3/8**
diaphragm				
minimal	1/9	0/10	1/9	5/8
slight	6/9	4/10	7/9	3/8
moderate	2/9	6/10	1/9	0/8
total	**9/9**	**10/10**	**9/9**	**8/8**
quadriceps				
minimal	2/9	9/10	6/9	5/8
slight	7/9	1/10	1/9	0/8
moderate	0/9	0/10	0/9	0/8
total	**9/9**	**10/10**	**7/9**	**5/8**
tongue				
minimal	**4/9**	**3/10**	**1/9**	**2/8**

The Day 176 TK data from the 26-week study are summarized in the following table:

AVI-4225 DOSES (mg/kg)							
C_0 (µg/mL)				$AUC_{(0-t)}$ (µg*hr/mL)			
MDX			C57BL	MDX			C57BL
12	**120**	**960**	**960**	**12**	**120**	**960**	**960**
16	340	92	190	18	284	2199	1720

Plasma exposure (AUC) at the NOAEL was substantially less than plasma eteplirsen exposures anticipated in humans at the proposed clinical dose of 30 mg/kg IV QW (127 µg*hr/mL). Plasma exposure at the MD (associated with minimal-to-slight dilatation of lateral ventricles) and HD was ~2 and 17 times, respectively, that in humans (127 µg*hr/mL) at the clinical dose of 30 mg/kg IV QW. These data demonstrate dose-related improvement in muscle pathology at plasma exposures (AUC) 0.1-17 times that in human at the proposed clinical dose.

Monkey: In the pivotal studies, eteplirsen was administered weekly at doses of 0, 5, 40, and 320 mg/kg IV; the 12-week study also included a group receiving 320 mg/kg SC. Recovery periods were included in both studies (28 days and 8 weeks in the 12- and 39-week studies, respectively). Males and females were tested in the 12-week study, but the 39-week study was conducted only in males.

As in mouse, kidney was the primary target organ. In the 12-week study, kidney findings were limited to tubular basophilia and vacuolation at 960 mg/kg IV and SC. In the 39-week study, tubular degeneration (characterized as minimal or slight) was observed only in recovery animals (2/3 at 320 mg/kg IV). Therefore, the NOAEL was the MD of 40 mg/kg. It should be noted, however, that the 39-week study was conducted in a very minimal number of animals (5/group for main study and 3/group for recovery) compared to that expected for a pivotal toxicity study (minimum of 8/group for main study and 4/group for recovery, if a single sex is evaluated).

The Week 39 TK data are summarized in the following table:

ETEPLIRSEN DOSES (mg/kg)					
C_0 (µg/mL)			$AUC_{(0-t)}$ (µg*hr/mL)		
5	**40**	**320**	**5**	**40**	**320**
84 ± 24	746 ± 139	4184 ± 1754	48 ± 19	434 ± 145	2552 ± 608

The plasma exposure (AUC) at the NOAEL is ~3 times that in humans at a dose of 30 mg/kg IV QW. The HD, associated with minimal-to-slight tubular degeneration, was associated with plasma exposure ~20 times that in human at 30 mg/kg IV QW

Reproductive and Developmental Toxicology

Because the patient population is primarily male, standard reproductive and developmental studies were not required. To assess male fertility, the sponsor conducted a histopathological evaluation of male reproductive organs in the pivotal toxicity studies,

including sperm analysis in the 26-week mdx mouse study of AVI-4225 and testicular examination in a stage-aware manner in the 39-week monkey study of eteplirsen. No adverse effects on the male reproductive system were observed.

A juvenile animal toxicology study was conducted in Sprague-Dawley rat. Eteplirsen was administered to juvenile male rats at doses of 0, 100, 300, and 900 mg/kg IV QW from PNDs 14 to 77. In addition to the standard parameters, TDAR, blood immunophenotyping, bone growth (densitometry, morphology), physical development (preputial separation), and neurobehavioral development (auditory startle habituation, locomotor activity, FOP, and Cincinnati water maze) were assessed, as well as a focused histopathological evaluation of male reproductive organs.

No drug-related effects were observed on TDAR, immunophenotyping, physical development, neurobehavioral development, or on the male reproductive system. Kidney and bone were the primary target organs. Kidney findings consisted of tubular dilatation, vacuolation, and basophilia at all doses, with dose-related incidence and severity. Tubular necrosis, characterized as minimal (20/32) or slight (1/32), was detected only at the HD and only at the end of the dosing period (i.e., not at the end of recovery).

The primary effects on bone were small (<10%), but dose-related decreases were observed in bone mineral content and density (by DXA analysis) in femur, with all doses affected, and in lumbar spine at the HD.

The PND 77 TK data are summarized in the following table (mean ± SE):

ETEPLIRSEN DOSES (mg/kg)					
C_{max} (µg/mL)			**$AUC_{(0-t)}$ (µg*hr/mL)**		
100	**300**	**900**	**100**	**300**	**900**
449±24.3	1293±72.3	4570±358	204±10.6	768±44.3	2937±218

Plasma exposures (AUC) at the LD, MD, and HD were ~2, 6, and 23 times that in humans at the clinical dose of 30 mg/kg IV QW.

Genetic Toxicology

Eteplirsen was negative in a standard battery of genetic toxicology (Ames, in vitro chromosomal aberration in CHO, in vivo mouse micronucleus) assays.

Carcinogenicity

Carcinogenicity studies have not been conducted for eteplirsen. As agreed to by the division, carcinogenicity studies in two species would be conducted post approval as PMRs.

Conclusions and Recommendations

The sponsor conducted an abbreviated battery of nonclinical studies to support clinical development and marketing approval of eteplirsen. All studies were adequate by design and conduct, except for the chronic toxicity study in monkey, which was conducted in a small number of animals and is minimally acceptable.

The pharmacological activity of eteplirsen was difficult to assess in animal models of DMD because of its species-specific sequence and in normal animals because of the lack of dystrophin gene mutations. The sponsor assessed exon skipping in only one study, a 12-week toxicity study in monkey, which demonstrated low levels (<1%) of exon 51 skipping at the low and mid IV doses, but a greater-than dose proportional increase at the highest IV dose tested (4.5-8.2%). The same high dose administered SC resulted in lower exon skipping (1.3-2.2%).

Effects on exon skipping or dystrophin protein expression were not assessed in the mdx mouse; however, the histopathology data in the mdx mouse (with the mouse surrogate, AVI-4225) demonstrated improvement in muscle pathology after 3 and 6 months of dosing at all doses, but notably greater improvement with increased dose. There are published studies of AVI-4225 in which dystrophin expression was measured at similar doses (e.g., 15 mg/kg in Wu *et al.* [2011]); however, there are too many differences in the methodologies used to be able to estimate dystrophin expression in the sponsor's 26-week study based on the published data.

The sponsor also cited a number of publications in animal models of DMD (mdx mouse), some of which were stated to have been conducted with the mouse surrogate (AVI-4225).

In general, major issues that have been identified for AONs in the treatment of DMD include (1) difficulty in delivering sufficient amounts of AONs to tissues, (2) the high variability in dystrophin expression both between muscles and within the same muscle following treatment, (3) expression is undetectable or low in cardiac muscle compared to skeletal muscle, and (4) the difficulty in identifying a minimum amount of dystrophin expression anticipated to have a functional effect (*cf.* Aoki Y *et al. BioMed Res Int* Article ID 402369: 8 pg, 2013; Chamberlain JS *Basic Appl Myol* 7(3&4):251-255, 1997; Fairclough RJ *et al. Nature Rev Genetics* 14:373-378, 2013; Godfrey C *et al. Hum Mole* Gene 24(15):4225-4237, 2015; Lu Q-l *et al Mole Therap-Nucleic Acids* 3, e152, 2015; Moulton HM, Moulton JD *Biochem Biophys Acta* 1798: 2296-2303, 2010; Wu B *et al. Am J Pathol* 181(2):392-400, 2012; Verhaart IEC *et al Nucl Acid Therap* 23(3):228-237, 2013; Yang L *et al. PLoS One* 8(4):e61584, 2013; White PJ *et al. Exp Rev Mole Med* 11(e10), March 2009; Wilton SD *et al Trend Mole Med* 21(7):417-426, 2015).

Difficulties in delivery of AONs to the target organ have been reported in numerous publications and have led to investigation of modified AONs, such as peptide-conjugated PMOs and PMO:leash duplexes in an attempt to increase delivery to skeletal and cardiac muscles (*cf.* Gao X *et al Mole Therap* 12(7):1333-1341, 2014; Gebski BL *et al. Human*

Mole Genet 12(15):1801-1811, 2003; Goyenvalle A *et al. Mole* Therap 19(1):198-205, 2010; Moulton & Moulton (2010); Wu B *et al. PNAS* 105:14814-14819, 2008; Wu B *et al. Am J Pathol* 181(2):392-400, 2012; Wu B *et al Gene Ther* 21(9):785-793, 2014; Yin H *et al. Hum Mole Gene* 17(24):3909-3918, 2008).

However, published literature also identify the limitations of animal studies of AONs, including:

- For mdx mouse, the pharmacological activity of the human product cannot be tested because of differences in the dystrophin gene mutation, requiring use of a mouse-specific PMO, exon 23 skipping AON and, therefore, resulting in a different truncated dystrophin. The mdx mouse also has a milder phenotype, with a normal lifespan, though to be the result of a compensatory increase in utrophin in this species.
- For normal monkey, which has a normal dystrophin gene, exon skipping would be expected to reduce dystrophin expression and the lack of muscle damage may result in underestimation of tissue distribution (and pharmacological activity). Damaged muscle with "leaky" fibers is thought to allow greater entry of AON to the site.

In addition, published studies are not routinely conducted in a rigorous manner (e.g., they are typically not conducted blind), they involve relatively small numbers of animals, and do not provide data needed for an independent evaluation. However, the value of such data is strengthened by consensus among studies, particularly if conducted by different (independent) labs.

With these caveats in mind, the published studies do suggest that systemically administered unmodified PMO AONs can increase dystrophin expression sufficient to improve muscle pathology or function, depending on the dose and duration of dosing. The data also suggest that a certain threshold level of dystrophin is necessary to exert beneficial effects on muscle pathology or function, although what that threshold is has not been established. The sponsor's studies demonstrate a greater than dose-proportional increase in exon 51 skipping with eteplirsen in monkey and a dose-related improvement in muscle pathology with a mouse-specific surrogate (AVI-4225) in the mdx mouse.

Regarding toxicity, the primary toxicities observed with eteplirsen were renal tubule degeneration/necrosis in all species tested (mdx mouse, juvenile rat, cynomolgus monkey), dilatation of lateral ventricles in mdx mouse, and decreases in bone mineral content and density in juvenile rat.

Safety margins based on plasma (AVI-4225 or eteplirsen) exposures at the NOAELs in adult animals are low (<1 in mdx mouse, 3.4 in monkey); however, plasma exposures at the highest doses tested, which were associated with minimal-to-slight toxicity (except for moderate dilatation of lateral ventricles), were 17 and 20 times the anticipated human exposure. In juvenile rat, a NOAEL was identified for kidney toxicity (tubular

degeneration/necrosis) but not bone changes. Plasma exposure at the NOAEL for kidney toxicity was 6 times that anticipated in humans.

The kidney toxicity was minimal and is monitorable and bone growth is monitorable in children. Dilatation of lateral ventricles is not monitorable and may be relevant to DMD patients, but was not thought to be of sufficient concern to halt clinical development, in part because of the delayed onset and presumed slow progression of the effect. Presuming that the toxicities of concern can be monitored in humans, the nonclinical data would support doses of 30 mg/kg and higher in humans.

Recommendations

Overall, the nonclinical studies of eteplirsen and the mouse surrogate (AVI-4225) are adequate to support approval of the NDA, with appropriate labeling and PMRs for carcinogenicity studies in two species.

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/s/

LOIS M FREED
05/25/2016

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206488
Supporting document:	SD1
Applicant's letter date:	June 26, 2015
CDER stamp date:	June 26, 2015
Product:	EXONDYS 51 (eteplirsen injection)
Indication:	Duchenne Muscular Dystrophy (DMD)
Applicant:	Sarepta Therapeutics, Inc.
Review Division:	Neurology Products
Reviewer:	David B. Hawver, Ph.D.
Supervisor:	Lois M. Freed, Ph.D.
Division Director:	Billy Dunn, M.D.
Project Manager:	Fannie Choy, R.Ph.

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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Eteplirsen is a phosphorodiamidate morpholino oligomer (PMO) consisting of a sequence of 30 nucleobases. Eteplirsen is designed to hybridize with a specific target sequence within exon 51 of human dystrophin pre-mRNA and result in the skipping of exon 51, restoration of an open reading frame in the mature dystrophin mRNA, and production of an internally truncated, but still functional, dystrophin protein in patients with Duchenne muscular dystrophy (DMD) with mutations amenable to exon 51 skipping (~13% of DMD patients).

1.2 Brief Discussion of Nonclinical Findings

Pharmacological studies have demonstrated that administration of eteplirsen can induce exon 51 skipping in dystrophin mRNA in human muscle cell cultures, muscle explant cultures, and in transgenic hDMD mice, cynomolgus monkeys, and DMD patients after direct IM injections. Eteplirsen-induced dystrophin protein expression was shown by Western immunoblot in cell cultures derived from a DMD patient and in extensor digitorum brevis (EDB) muscles of DMD patients after direct IM injection, and by immunohistochemistry (IHC) in the latter. A series of published studies presented evidence for exon skipping and induction of dystrophin protein expression in mouse and dog DMD models using species-specific exon skipping PMOs. These studies often correlated these changes with reductions in muscle pathology and/or improvements in muscle function or resistance to injury. However, the published studies typically used small numbers of animals per group and lacked the level of detail needed to support the reliability of the conclusions in a regulatory setting. The most robust finding among the studies provided and referenced in this submission was the wide variability in the extent of PMO-induced dystrophin expression within a single muscle and among different muscles, suggesting that caution is warranted in generalizing from the results of biopsies taken from only one or a few sites, muscle types, or patients.

Pivotal toxicology studies of eteplirsen were conducted in male monkeys (39-week study) and juvenile male rats (10-week study). A 26-week study was conducted with a mouse-specific surrogate (AVI-4225) in male transgenic *mdx* mice. In all three species, the primary target organ was the kidney, as evidenced by dose-dependent renal tubular cytoplasmic basophilia and/or vacuolation and, at the high dose, tubular degeneration/necrosis.

In the *mdx* mouse study, dilatation of the lateral ventricles of the brain was observed at the mid and high doses. The mechanism of this effect and its relevance to humans is unknown. Juvenile rats showed slight reductions in bone length, width, area, mineral content, and mineral density at the high dose, with dose-dependent trends noted in some parameters at lower doses. Mean eteplirsen plasma exposures (AUC) at the NOAELs for monkey and juvenile rat were 20-fold and 6-fold, respectively, greater than that in patients dosed once weekly with 30 mg/kg IV eteplirsen.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data submitted adequately support the approval of eteplirsen for the treatment of DMD in patients with mutations amenable to exon 51 skipping therapies.

1.3.2 Additional Non Clinical Recommendations

Carcinogenicity studies in two species should be conducted as a post-marketing requirement.

1.3.3 Labeling

The sponsor's proposed labeling for the nonclinical sections is acceptable with the following exceptions noted below:

8.2 Lactation

Risk Summary

There (b) (4) are no human or animal data to assess the effects (b) (4) of treatment with EXONDYS 51 during lactation on ~~milk production,~~ the presence of eteplirsen in milk, ~~or the effects of EXONDYS 51 on the~~health of a breast-fed (b) (4), or milk production.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for EXONDYS 51 and any potential adverse effects on the breastfed (b) (4) from EXONDYS 51 or from the underlying maternal condition.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been conducted with eteplirsen.

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Number
1173755-55-9

Generic Name
Eteplirsen Injection

Code Name
AVI-4658

Proprietary Name
EXONDYS 51

Chemical Name

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

Molecular Formula

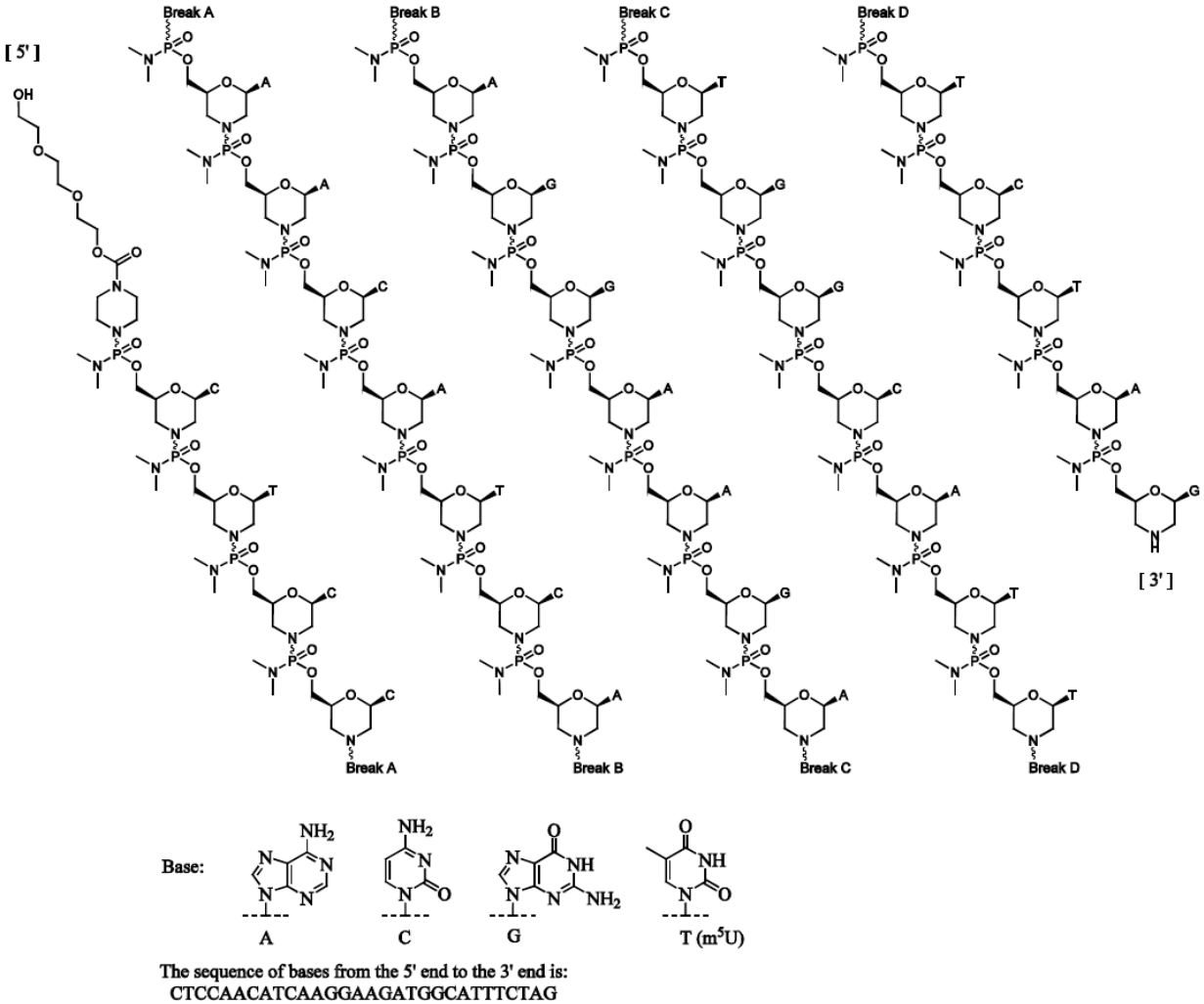
$C_{364}H_{569}N_{177}O_{122}P_{30}$

Molecular Weight

10305.7

Biochemical Description and Structure

Eteplirsen is a charge-neutral phosphorodiamidate morpholine oligomer (PMO) consisting of a sequence of 30 of the following four nucleobases: 5-methyluracil (m^5U; thymine; T), adenine (A), cytosine (C), and guanine (G).



The sequence of bases from the 5' end to the 3' end is:
CTCCAACATCAAGGAAGATGGCATTTCTAG

(page 5 of sponsor's Nonclinical Overview)

(b) (4)

2.2 Relevant INDs, NDAs, BLAs, and DMFs

IND 77429 Eteplirsen Injection for DMD, received August 9, 2007

2.3 Drug Formulation

Eteplirsen is supplied as a 50 mg/mL sterile, isotonic, phosphate-buffered (pH 7.5) solution without preservatives in 2-mL (100 mg) and 10-mL (500 mg) (b) (4) vials. Dilution to 100-150 mL with normal saline is necessary prior to administration at 30 mg/kg once weekly via IV infusion over 35 to 60 minutes.

Table 1: Formulation for Eteplirsen Drug Product, 50 mg/mL

Component	Reference to Standards	Quantity (mg/mL)	Function
Eteplirsen	In-house specification	50	Active ingredient
Sodium chloride	USP	8.0	(b) (4)
Potassium chloride	USP	0.2	
Potassium phosphate monobasic	NF	0.2	
Sodium phosphate dibasic, anhydrous	USP	1.14	
Sodium hydroxide	NF	q.s.[1]	pH adjustment
Hydrochloric acid	NF	q.s.[1]	pH adjustment
Water for Injection	USP	q.s.[2]	(b) (4)

[1] q.s. = quantity sufficient for pH adjustment
[2] q.s. = quantity sufficient to achieve final volume

(page 2 of sponsor's Description and Composition of the Drug Product)

2.4 Comments on Novel Excipients

There are no novel excipients in the drug product.

2.5 Comments on Impurities/Degradants of Concern

Based on the lowest percentages of impurities/degradants present in the batches of drug substance used in the pivotal toxicity studies (the 39-week study in monkey and the 10-week study in juvenile rat), the proposed specification limits for two classes of process impurities exceed levels that have been qualified: (b) (4)

(b) (4)

(b) (4)

After internal discussion with FDA experts, it was concluded that the sponsor's proposed specification limits for (b) (4) and (b) (4) impurities are not likely to present a safety concern, and, therefore, are acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

Eteplirsen is proposed for the chronic (lifetime) treatment of patients with DMD who have a confirmed mutation of the *DMD* gene amenable to exon 51 skipping, at the dose of 30 mg/kg once weekly via IV infusion over 35 to 60 minutes.

2.7 Regulatory Background

Original IND 77429 (Eteplirsen for DMD) was received on August 9, 2007, and placed on Full Clinical Hold on September 4, 2007, based on the lack of adequate CMC and nonclinical information (no nonclinical studies of eteplirsen were submitted).

The investigation of eteplirsen for DMD was granted Fast Track designation on November 27, 2007.

An Incomplete Response to Hold letter was sent to the sponsor on May 22, 2008, based on the lack of batch analysis data for the drug product and the lack of study reports for the safety pharmacology and genotoxicity studies needed to support the proposed clinical trial.

The Full Clinical Hold was removed on June 25, 2010, based on review of the information provided in the sponsor's Complete Response to Clinical Hold dated May 21, 2010.

In the Preliminary Comments for the End of Phase 2 meeting sent on March 12, 2013, the sponsor was informed that 1) the proposal to assess carcinogenicity in only one species would need to be justified in a request for waiver of the standard requirement for carcinogenicity assays in two species; 2) nonclinical studies for drugs intended for treatment of DMD may be conducted in male animals only, but, if a single sex is used, the number of animals per group will need to be doubled; and 3) carcinogenicity study(ies) may be submitted post approval, considering the seriousness of the indication, provided that the available nonclinical and clinical data support such a strategy. There was no further discussion of these issues during the meeting.

In the Preliminary Comments for the Pre-NDA meeting sent on September 17, 2014, the sponsor was informed that 1) confirmation should be provided that microscopic evaluations of male reproductive organs in the juvenile rat and 39-week monkey studies were conducted in a stage-aware manner; 2) justification for the planned assessment of

carcinogenicity in only one species should be provided in the NDA, with supporting data; and 3) assessment of carcinogenicity for eteplirsen may be conducted post-approval. There was no further discussion of these issues during the meeting.

NDA 206488 was submitted as an original NDA, for rolling review, with the initial submission received on May 20, 2015, providing the complete nonclinical and CMC content in Modules 2, 3, and 4, as well as a request for a deferral for a carcinogenicity assessment of eteplirsen. The final submission of this rolling NDA, including the complete clinical content in Modules 2 and 5, as well as an application for Priority Review Designation, was received on June 26, 2015.

3 Studies Submitted

3.1 Studies Reviewed

Exon splicing activity of eteplirsen in cynomolgus monkey tissue samples from (b) (4) study 1152-014 (Sponsor Study 4658-SSA-005)

Eteplirsen -- Effects on cardiovascular, respiratory, neurological, renal and liver functions following subcutaneous intravenous infusion in the conscious cynomolgus monkey monitored by telemetry (Sponsor Study AVI-4658-GLP-0704)

Absorption, distribution, metabolism, and excretion of ^{14}C-eteplirsen following intravenous injection to mice (Sponsor Study 4658-PKD-006)

In vitro plasma protein binding of ^{14}C-eteplirsen in mouse, rat, monkey, human (Sponsor Study 4658-PKD-001)

Metabolism of ^{14}C-eteplirsen by mouse, rat, monkey, and human hepatic microsomes in the absence and presence of NADPH (Sponsor Study 4658-PKD-002)

Evaluation of CYP induction following exposure of primary cultures of human hepatocytes to eteplirsen (Sponsor Study 4658-PKD-003)

Inhibitory potential of eteplirsen towards human hepatic microsomal CYP isoenzymes (Sponsor Study 4658-PKD-004)

Summary Assessment of ^{14}C-eteplirsen as a Substrate or Inhibitor of Human Drug Transporters (Sponsor Study 4658-PKD-005)

Eteplirsen: A Pilot Single Dose Intravenous Injection Toxicity Study in the Juvenile and Adult Rat (Sponsor Study 4658-TOX-002)

Eteplirsen: A 10-Week Intravenous Injection Toxicity Study in the Juvenile Rat (Sponsor Study 4658-TOX-003)

Eteplirsen: 12-week intravenous and subcutaneous toxicity study in mice (Sponsor Study AVI-4658-GLP-0902)

AVI-4225: 3-month intravenous and subcutaneous toxicity study in mice (Sponsor Study 4225-GLP-0903)

26-Week Toxicity and Toxicokinetic Study of AVI-4225 in Mice with an 8-Week Recovery (Sponsor Study 4225-TOX-001)

Eteplirsen: 12-week intravenous and subcutaneous toxicity study in cynomolgus Monkeys (Sponsor Study 4658-GLP-0901)

39-Week Intravenous Injection Toxicity and Toxicokinetic Study of Eteplirsen in Sexually Mature Male Cynomolgus Monkeys with an 8-Week Recovery (Sponsor Study 4658-TOX-0011)

Bacterial Reverse Mutation Assay (Sponsor Study 4658-GLP-0701)

In Vitro Mammalian Chromosome Aberration Test (Sponsor Study 4658-GLP-0703)

Mouse Bone Marrow Erythrocyte Micronucleus Test Following Intravenous Administration of Eteplirsen (Sponsor Study 4658-GLP-0702)

3.2 Studies Not Reviewed

AVI-4225 -- Effects on cardiovascular, respiratory, neurological, renal and liver functions following intravenous infusion in the conscious cynomolgus monkey monitored by telemetry (Sponsor Study AVI-4225-GLP-0808; not reviewed because eteplirsen was not assessed; the mouse-specific homologue, AVI-4225 was used)

AVI-6002: Evaluation on cloned hERG channels expressed in human embryonic kidney (HEK293) cells (Sponsor Study AVI-6002-VIT-001; not reviewed because eteplirsen was not assessed; AVI-6002 is a PMO*plus* compound under development for a different indication)

Effects of AVI-6002 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers (Sponsor Study AVI-6002-VIT-002; not reviewed because eteplirsen was not assessed)

AVI-6003: Evaluation on cloned hERG channels expressed in human embryonic kidney (HEK293) cells (Sponsor Study AVI-6003-VIT-001; not reviewed because eteplirsen was not assessed; AVI-6003 is a PMO*plus* compound under development for a different indication)

Effects of AVI-6003 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers (Sponsor Study AVI-6003-VIT-002; not reviewed because eteplirsen was not assessed)

3.3 Previous Reviews Referenced

Nonclinical review of IND 77429 by Barbara Wilcox, March 10, 2013

4 Pharmacology

4.1 Primary Pharmacology

Exon splicing activity of eteplirsen in cynomolgus monkey tissue samples from (b) (4) study 1152-014 (Sponsor Study 4658-ssa-005; AVI Biopharma Study AVI-4658-GLP-0901)

Samples of quadriceps muscle, heart, and diaphragm tissues, collected on Day 79 from cynomolgus monkeys (4/sex/group, except 3 LDM) after 12 weekly doses of eteplirsen at 0, 5, 40, or 320 mg/kg IV, or 320 mg/kg SC, were analyzed for exon 51 skipping of the dystrophin gene using nested polymerase chain reaction (PCR) performed on total RNA preparations. Primary and secondary PCR protocols were performed on RNA prepared on 3 separate days, and results were averaged to yield each individual animal data point on the scattergrams. Results are shown in the sponsor's summary table below:

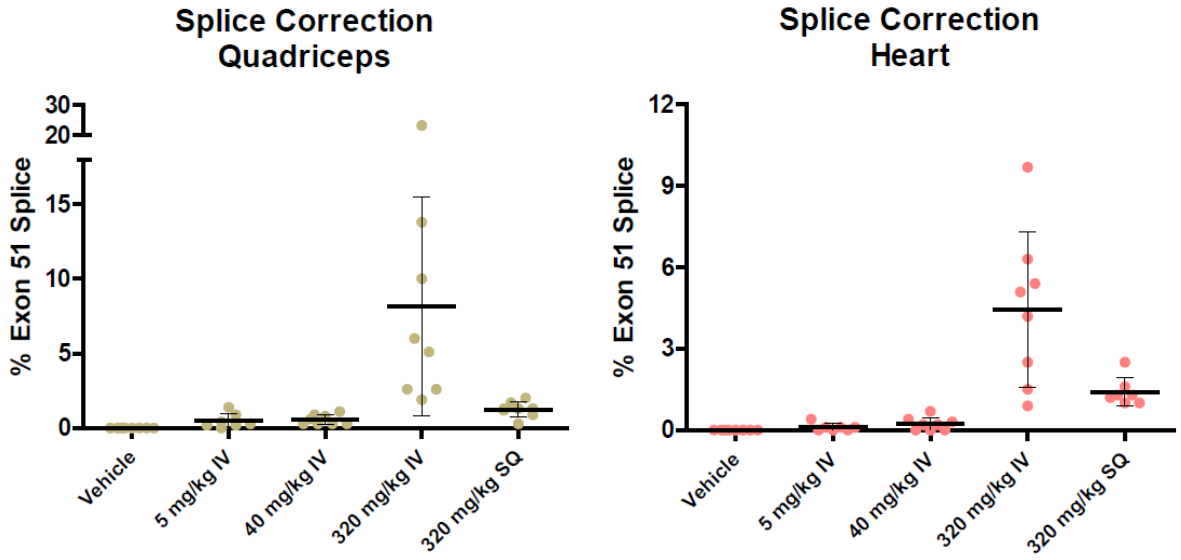
Table 10 Average percentage of exon 51 splicing

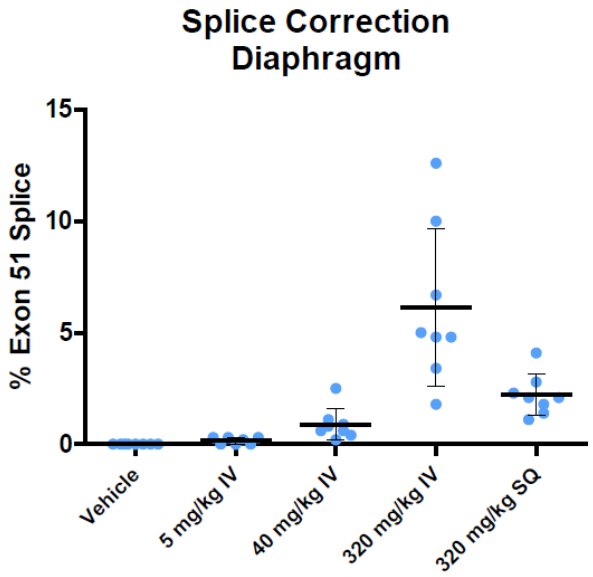
Tissue	*Average % Exon 51 Splicing ± 1 SD*				
	0 mg/kg IV	5 mg/kg IV	40 mg/kg IV	320 mg/kg IV	320 mg/kg SC
Quadriceps muscle	0.0 ± 0.0	0.5 ± 0.5	0.6 ± 0.3	8.2 ± 7.4	1.3 ± 0.5
Heart	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.2	4.5 ± 2.9	1.4 ± 0.5
Diaphragm	0.0 ± 0.0	0.2 ± 0.2	0.9 ± 0.7	6.1 ± 3.5	2.2 ± 0.9

SD standard deviation

(page 8 of Study Report)

All three target muscles showed the anticipated pharmacodynamic response, increased skipping of exon 51 of the dystrophin gene, after treatment with IV or SC eteplirsen. The greatest effects were achieved with 320 mg/kg IV; however, as shown in the figures below, the responses to 320 mg/kg IV also showed greater variability among individuals.





(page 9 of Study Report)

"Bicep and tibialis anterior muscle, kidney, and liver samples collected on Day 79 for exon skipping were not analyzed. Tissues collected on Day 106 for exon skipping were also not analyzed." *(page 3 of Study Report)*

Brief Summary of the Pharmacodynamic Results of Clinical Study AVI-4658-33, Restoring Dystrophin Expression in Duchenne Muscular Dystrophy: A Phase I/II Clinical Trial Using AVI-4658 (eteplirsen)

Patients with DMD amenable to exon 51 skipping therapy aged 10-17 years were injected with a single dose of 0.09 mg (N=2; Group 1) or 0.9 mg (N=5; Group 2) eteplirsen into a 1-cm^2 area of the extensor digitorum brevis (EDB) muscle of one foot and placebo (saline) into the same area of the other foot. Doses were divided into 9 IM injections of 100 µL each (first 5 enrolled) or 4 IM injections of 225 µL each (last 2 enrolled) for each foot. Biopsies were taken from the EDB muscle of each foot 28 days after dosing, except for one sample from a Group 1 patient collected on Day 21 and one sample from a Group 2 patient collected on Day 19. EDB muscle samples were analyzed for exon 51 skipping (by PCR), dystrophin expression (by Western blot), percentage of dystrophin-positive muscle fibers (by IHC), and intensity of alpha-sarcoglycan and beta-dystroglycan (by IHC).

According to the study report, the initial PCR assay demonstrated exon 51 skipping in the EDB muscle biopsies from 5/5 patients injected with 0.9 mg eteplirsen, but not in muscles injected with 0.09 mg eteplirsen or placebo. Subsequent assessments using increased PCR amplification detected low level exon 51 skipping in 2/2 EDB muscles injected with 0.09 mg eteplirsen.

Expression of dystrophin protein was confirmed by Western blot in 5/5 biopsies from EDB muscles injected with 0.9 mg eteplirsen, but not in one from an EDB muscle

injected with 0.09 mg eteplirsen (the other was not tested) or in one from a placebo-injected EDB muscle (the other 6 were not tested).

The percentage of dystrophin-positive muscle fibers was reported to be 44.0%, 48.0%, 63.0%, 65.0%, and 78.6% (mean 59.7%) in EDB muscles injected with 0.9 mg eteplirsen, compared to 0% injected with 0.09 mg eteplirsen or placebo.

No consistent differences in the intensity of expression of dystrophin-associated proteoglycans (alpha-sarcoglycan and beta-dystroglycan) were observed between eteplirsen- and placebo-injected EDB muscles; all were reduced compared to age-matched healthy controls. "The lack of increase of the levels of dystrophin associated glycoproteins on the sarcolemma of the treated site might be related to the relative short interval between the AVI-4568 injection and the muscle biopsy; the fact that the levels of restored dystrophin were lower compared to wild type muscle, or both" *(page 3 of Section 13.1.6 of Study Report)*.

Brief Summary of Key Data from Published Articles Cited by the Sponsor

Arechavala-Gomeza et al. (2007) showed that eteplirsen was able to induce exon 51 skipping in human fetal muscle cultures (40-50%), muscle cells cultured from a DMD patient with an exon 48-50 deletion, muscle explants from a DMD patient, and in gastrocnemius muscles of hDMD mice after direct IM injection (22.1%). Induction of detectable levels of dystrophin protein was observed after transfection of muscle cells cultured from a DMD patient with a deletion of exon 50. Because neither the number of cultures or animals used in these studies nor individual culture/animal data were not provided, the reliability of these results is questionable.

Several published studies have investigated the ability of AVI-4225 to induce exon 23 skipping in the *mdx* mouse model of DMD, in which a nonsense point mutation in exon 23 of the dystrophin gene results in the absence of full-length dystrophin, leading to progressive muscle degeneration. Alter et al. (2006) showed that injection of *mdx* mice (N=4-6/group) with 2 mg IV AVI-4225 once weekly for 1, 3, or 7 weeks led to duration-dependent increases in exon 23 skipping (by PCR) and dystrophin expression in several skeletal muscles (especially quadriceps and gastrocnemii, in which >70% of fibers were positive for dystrophin by IHC and levels in Western blots were ~50% of normal levels after 7 injections), but no expression was observed in heart. Variations in the extent of expression were observed between different muscles and between different areas of the same muscle. Functional improvement in AVI-4225-treated *mdx* mice was suggested by observations of increased normalized maximum isometric tetanic force (compared to control mice, measured 2 weeks after the third injection, despite dystrophin expression in <20% of fibers in this muscle by IHC), colocalization of dystrophin with α-sarcoglycan, β-dystroglycan, and neuronal nitric oxide synthase at the sarcolemmal membrane, and reduced levels of creatine kinase (CK) activity in serum (compared to *mdx* controls, 2 weeks after the 7th injection).

Wu et al. (2011) demonstrated that injection of *mdx* mice (N=10/group) with 60 mg/kg IV AVI-4225 biweekly for 50 weeks induced expression of dystrophin in skeletal muscle

(10-50% of fibers by IHC, up to ~17% of normal levels by Western blot) and heart (<5% of fibers by IHC, up to ~2% of normal levels by Western blot). The intensity of staining of dystrophin positive fibers was “highly variable” within and between different muscles Controls showed <1.5% revertant fibers in all muscles tested. Functional improvement was suggested by improvements in grip-force generation and cardiovascular parameters (stroke volume, cardiac output, end-diastolic volume, end-systolic pressure, maximum and minimum upstroke velocity, and ejection fraction, measured under dobutamine stress) compared to saline controls. Similar experiments at higher doses for 6 months resulted in greater dystrophin expression: >50% of skeletal muscle fibers by IHC, >15% of normal levels by Western blot at 300 mg/kg, but <4% of cardiac muscle fibers; almost 100% of skeletal muscle fibers and >40% of cardiac muscle fibers by IHC (though most stained weakly) at 1500 mg/kg AVI-4225 biweekly, associated with improvement in grip force to near normal levels but no apparent toxicity. The authors note that “it is most probable that a truncated dystrophin lacking only exon 23 produced in the *mdx* mice muscles will retain better function than most truncated dystrophins expected from DMD patients after exon skipping” *(page 581).*

Malerba et al. (2011) showed that injection of *mdx* mice (N=4-8/group) with 5 or 50 mg/kg IV AVI-4225 for 20 or 50 weeks in 10-week cycles (each cycle: once weekly for 4 weeks followed by 6 weeks off drug) led to duration-dependent increases in the percentage of dystrophin positive fibers and (after 20 weeks only) dose-dependent increases in the intensity of dystrophin expression within the positive fibers in skeletal muscles and diaphragm (no dystrophin expression was observed in cardiac muscle fibers). Functional improvements were suggested by the following observations after 50 weeks in the high dose group: colocalization of dystrophin with neuronal nitric oxide synthase in 80-90% of dystrophin positive fibers, increased specific force in the tibialis anterior muscle, improved forelimb grip strength, and normalized physical activity and locomotor behavior; however, eccentric contraction-induced damage of the tibialis anterior muscle was similar to controls.

Sharp et al. (2011) reported that direct IM injection of 10-60 µg AVI-4225 into the tibialis anterior muscles of aged female *mdx* mice (N=4-7/group) induced dose-dependent dystrophin expression in 18-65% of muscle fibers by IHC correlated with improved resistance to injury induced by 10 repeated eccentric contractions 4-6 weeks after the injection; saline-injected contralateral control muscles showed dystrophin in 1-2% of fibers. These results suggest that induction of dystrophin in ≥ 20% of skeletal muscle fibers is needed to achieve a measurable level of protection against contraction-induced injury. The level of dystrophin protein measured by Western blot was also correlated with improved resistance to injury, but less strongly than the percentage of dystrophin-positive muscle fibers measured by IHC. No correlation was observed between dystrophin expression and specific muscle force.

The sponsor conducted a 26-week study in male *mdx* mice (N=16/group) demonstrating dose-dependent reductions in the incidence and severity of myofiber degeneration in biceps femoris, quadriceps, diaphragm, larynx, and tongue after once weekly dosing at

12, 120, and 960 mg/kg IV AVI-4225 (see Study 4225-TOX-001 in the Toxicology section below).

Exon skipping as a therapeutic strategy has also been studied in the CXMD beagle model of DMD, in which a mutation in intron 6 of the *dystrophin* gene leads to exclusion of exon 7 and a loss of the open reading frame in the mRNA transcript. The open reading frame can be restored by administration of two exon skipping PMOs, one targeting exon 6 and one targeting exon 8, resulting in expression of an internally truncated functional dystrophin protein. Yokota et al. (2009) showed that treatment with a combination of 3 dog-specific exon 6- and exon 8-skipping PMOs (Ex6A, Ex6B, and Ex8A, each at 40 mg/kg IV once weekly for 5 weeks in one dog; 40 mg/kg IV biweekly for 22 weeks in another dog; and 66 mg/kg IV once weekly for 7 weeks in a third dog) resulted in increased expression of dystrophin in muscle fibers by IHC and immunoblot (though the extent and intensity of staining varied widely within and between different muscles—e.g., lower in cardiac muscle), stabilization of muscle pathology, and maintenance of ambulation compared to untreated age-matched litter mates.

The sponsor concluded that these studies in *mdx* mice and CXMD dogs demonstrated that low levels of dystrophin expression in skeletal muscle and heart induced by exon skipping PMOs were sufficient to support improved function compared to vehicle controls. However, these conclusions are weakly supported by the published studies referenced, which were conducted using only 3-10 *mdx* mice and CXMD dog per group, and did not include sufficient level of detail to allow independent assessment of the results. Repeated IV administration of species specific exon skipping PMOs to *mdx* mice and CXMD dogs consistently induced expression of dystrophin-related immunoreactivity in muscle fibers that varied widely within a single muscle and between different muscles, but was lower in heart compared to most skeletal muscles.

References

Arechavala-Gomeza V, et al. Comparative analysis of antisense oligonucleotide sequences for targeted skipping of exon 51 during dystrophin pre-mRNA splicing in human muscle. *Hum Gene Ther* 2007;18:798-810.

Alter J, et al. Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. *Nature Med* 2006;12(2):175-177.

Malerba A, et al. Chronic systemic therapy with low-dose morpholino oligomers ameliorates the pathology and normalizes locomotor behavior in *mdx* mice. *Mol Ther* 2011;19(2):345-354.

Sharp PS, et al. Physiological characterization of muscle strength with variable levels of dystrophin restoration in *mdx* mice following local antisense therapy. *Mol Ther* 2011;19(1):165-71.

Wu B, et al. One-year treatment of morpholino antisense oligomer improves skeletal and cardiac muscle functions in dystrophic *mdx* mice. *Mol Ther* 2011;19(3):576-583.

Yokota T, et al. Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. *Ann Neurol* 2009;65(6):667-76.

4.2 Secondary Pharmacology

No secondary pharmacology studies were conducted.

4.3 Safety Pharmacology

Effects on cardiovascular, respiratory, neurological, renal, and liver functions
(Sponsor Study 4658-GLP-0704; (b) (4) Study AA42301; eteplirsen: October 26, 2007; GLP, except for formulation analysis; QA; AVI-4658 Lot # 37GD-XX03 [15 mg/mL], -XX03 [60 mg/mL], -XX04 [120 mg/mL])

Six telemeterized male cynomolgus monkeys (2-3 years old, 2.7-2.9 kg) were treated with 0 (PBS), 40, 160, and 320 mg/kg eteplirsen SC injections at 2.67 mL/kg into the dorsal cervical/interscapular region on Days 0, 7, 14, and 21 in a Latin Square design. The dose volume administered to each animal was divided approximately equally into three separate injection sites, alternating weekly with three additional sites. On Days 38 and 45, the same animals were treated with 0 (PBS) or 320 mg/kg IV eteplirsen using a catheter (implanted into the posterior vena cava via a femoral vein) attached to a remote syringe pump (2.67 mL/kg, 1 mL/min).

The following parameters were evaluated: mortality/morbidity, clinical signs, body weight, body temperature, blood pressure (systolic, diastolic, mean arterial), heart rate, ECG, EMG, inspiratory time, expiratory time, respiration rate, AUC of rectified diaphragmatic EMG burst (AUC_{EMG}, correlated with tidal volume), AUC_{EMG} * respiratory rate (index of minute volume), level of consciousness, motor function, eye movements, coagulation (APTT, PTT), clinical chemistry (BUN, ALP, ALT, AST, albumin, bilirubin, serum osmolality, Na, K, Cl, creatinine, glucose, urea, total protein, alpha amylase, lipase, sedimentation rate), urinalysis (volume, osmolality, specific gravity, appearance, pH, sediment, Na, K, Cl, creatinine), hematology (hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, packed cell volume, RBC count, mean corpuscular volume, reticulocyte count, platelet count, total WBC count, differential WBC count).

No toxicologically important effects were observed on any of the parameters assessed. The study was adequately performed.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption, distribution, metabolism, and excretion of ^{14}C- eteplirsen following intravenous injection to mice (Sponsor Study 4658-pkd-006; (b) (4) Study 8284272)

Adult male DMD*mdx* mice (C57BL/10ScSn-DMD*mdx*) were given a single bolus IV injection of 120 mg/kg ^{14}C-eteplirsen (300 µCi/kg, 48 mg/mL) in Dulbecco's PBS without Mg^{++} or Ca^{++}. Terminal blood samples were collected from 3 mice per time point at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, and 144 hours postdose for analysis of radioactivity concentrations in blood and plasma. Radioactivity was also measured in the following tissues collected from 3 mice per group at 0.25, 1, 4, 8, 24, 48, 96, 120, and 144 hours postdose: brain, diaphragm, heart, kidneys, biceps, brachii, tibialis anterior, biceps femoris, and quadriceps. Drug-related radioactivity was determined in urine and feces collected over 336 hours postdose. Tissue distribution was analyzed by quantitative whole-body autoradiography (QWBA) in 1 animal per time point at 4, 24, 72, 120, 336, and 1344 hours postdose.

No signs of overt toxicity were observed. Pharmacokinetic parameters for total radioactivity in blood and plasma are shown in the sponsor's table below:

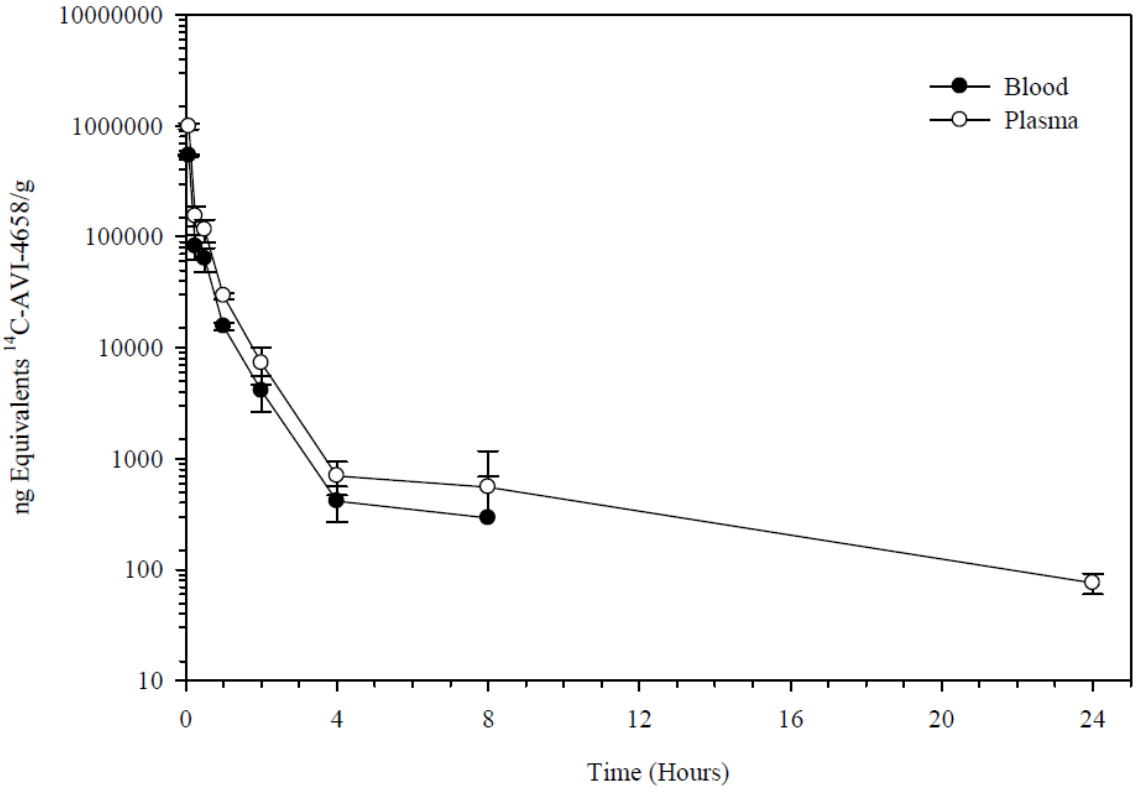
Matrix	C_0 (ng eq/g)	$t_{½}$ (hours)	$AUC_{(0-t)}$ (ng eq·hour/g)	$AUC_{(0-\infty)}$ (ng eq·hour/g)	CL (mL/hour/kg)	Vd (mL/kg)
Blood	1380000	NA	185000	NA	NA	NA
Plasma	2490000	6.03	344000	345000	348	175

Abbreviations: C_0 = Extrapolated concentration at time zero (t_0); $t_{1/2}$ = Elimination half-life; $AUC_{(0-t)}$ = area under the curve from 0 to last quantifiable time point; $AUC_{(0-\infty)}$ = Area under the curve from 0 to infinity; CL = Total body clearance; Vd = Volume of distribution.
eq Equivalents ^{14}C-AVI-4658.
NA Not applicable.

(page 6 of Study Report)

As noted in the sponsor's figure below, radioactivity was only detectable in blood through 8 hours postdose, and in plasma through 24 hours postdose.

Figure 1
Mean concentrations of radioactivity in blood and plasma at specified times after a single intravenous administration of ^{14}C-AVI-4658 to male C57BL/10ScSn-DMDmdx mice (Group 1, 120 mg/kg)



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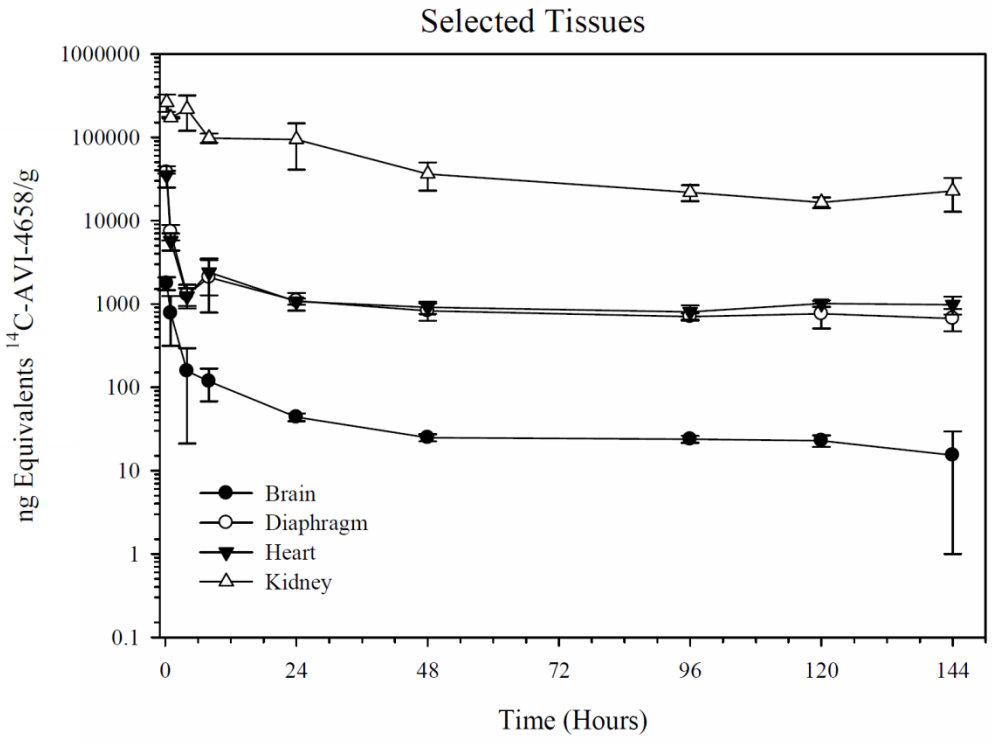
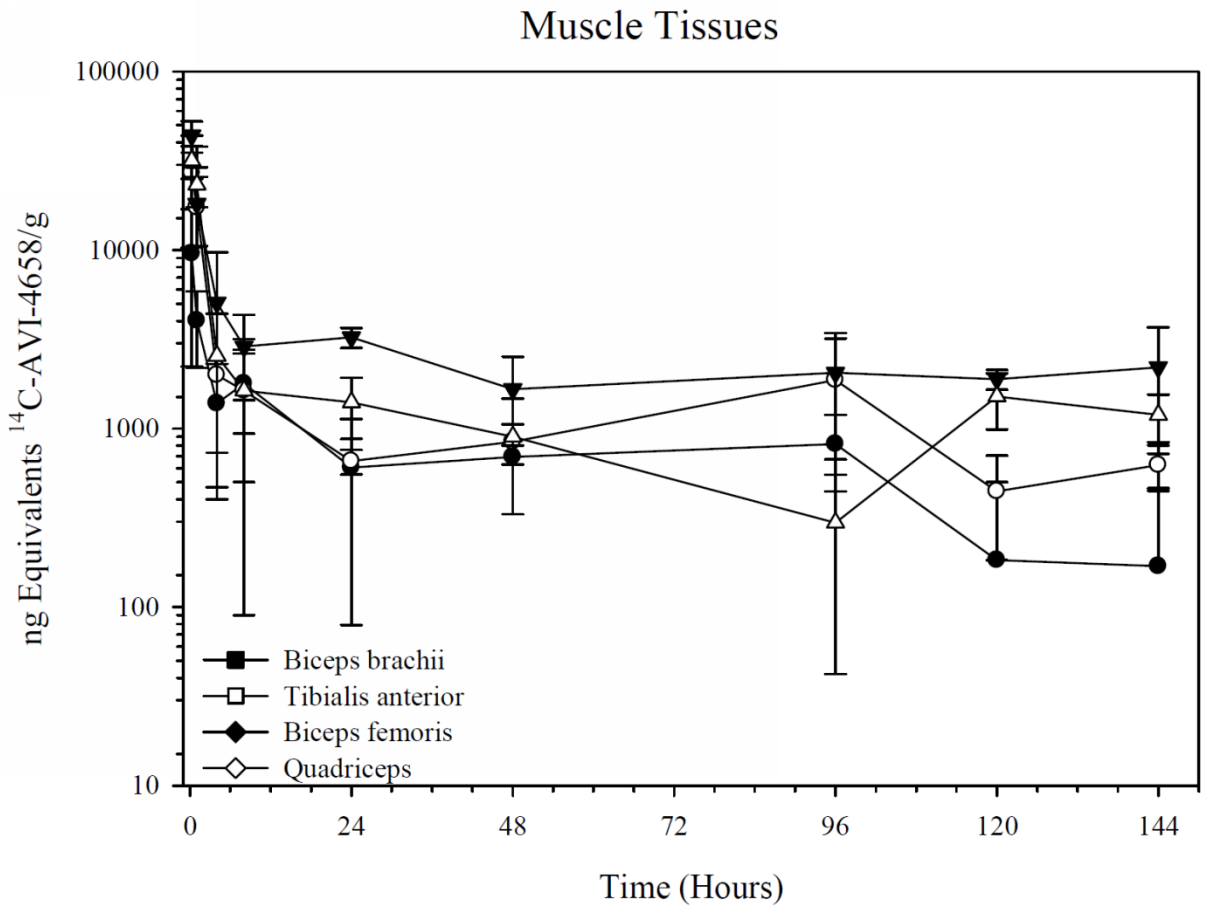
The sponsor's table and figures below show the mean concentrations of radioactivity in the various tissues collected, at each time point:

	Mean Concentration (ng Equivalents ^{14}C-AVI-4658/g)								
	Sacrifice Time (Hours)								
Sample	0.25	1	4	8	24	48	96	120	144
Brain	1770	775	157	118	43.8	24.8	23.8	22.9	15.3
Diaphragm	38000	7320	1290	2080	1090	826	703	760	668
Heart	35000	5690	1240	2390	1070	910	803	1010	979
Kidney	263000	172000	217000	97700	94000	36200	21800	16500	22600
Muscle Forelimb:									
Biceps brachii	9550	4020	1380	1780	604	692	818	183	169
Muscle Hindlimb:									
Tibialis anterior	27000	17300	1990	1630	655	841	1870	444	624
Biceps femoris	43700	18100	5060	2880	3240	1660	2050	1890	2200
Quadriceps	31600	23200	2550	1630	1400	899	296	1510	1190

(page 7 of Study Report)

Figure 2
Concentrations of radioactivity in tissues at specified times after a single intravenous administration of ^{14}C-AVI-4658 to male C57BL/10ScSn-DMDmdx mice (Group 1, 120 mg/kg)





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The highest concentration of drug-related radioactivity was observed in kidney and the lowest in brain. In the diaphragm, heart, and hindlimb muscles, levels were approximately 13% of those in kidney at the T_{max} of 0.25 hours postdose. Six days after dosing, radioactivity concentrations in kidney were 9% of peak levels; those in hindlimb skeletal muscle were 2-5% of peak levels.

The percentage of total radioactivity administered present in urine and feces is shown in the sponsor's table below:

	Intravenous Administration					
	% Mean Total Recovery	% Dose in Urine	% Dose in Feces	% Dose in Carcass	% Dose in Cage Rinse	% Dose in Other[a]
Group 2 (0-336 hours postdose)	83.2 ± 2.02%	32.3 ± 17.9%	22.1 ± 11.6%	1.11 ± 0.0372%	17.4 ± 1.89%	10.2%

a Cage wash and cage wipe.

(page 7 of Study Report)

Most of the radioactivity measured in the cage rinse, wipe, and wash is likely to represent drug-related material in urine that had stuck to the glass caging. Therefore, the primary route of excretion was renal, accounting for approximately 60% of the administered dose; fecal excretion accounted for ~22% of the administered dose. Excretion of radioactivity was nearly complete by 6 days postdose; only ~1% of the administered dose remained in the carcass.

QWBA showed rapid and widespread distribution of radioactivity into tissues, with peak levels at 4-24 hours postdose in most tissues (4 hours postdose was the earliest time point assessed). The highest peak concentrations of drug-related radioactivity (ng-equivalents of ^{14}C-eteplirsen/g) were observed in urine (6420000), kidney cortex, kidney (165000), kidney medulla (83100), large intestine contents (15800), cecum contents (7980), adrenal gland (6310), lung (6040), small intestine contents (5470), bulbo-urethral gland (5170), liver (3550), large intestine (3520), pigmented skin (2880), lymph nodes (2850), epididymis (2700), cecum (2640), bone marrow (2600), salivary glands (2460), and spleen (2330). Radioactivity in skeletal muscle peaked at 24 hours postdose (1570), and remained detectable at 14 days postdose (636). Levels in myocardium peaked at 4 hours postdose (1160) and were also detectable at 14 days postdose (499). Levels in the diaphragm peaked at 4 hours postdose (1170), and remained detectable at 5 days postdose (936). Low peak concentrations were found in blood (512) and bile (453), and levels were below the level of quantification in the following tissues: arterial wall, bone, brain, eye lens, eye, nasal turbinates, seminal vesicle, and spinal cord. Radioactivity was no longer detectable in tissues by 56 days postdose, with the following exceptions: kidney cortex (1790), kidneys (1630), kidney medulla (1460), and urine (1440).

In vitro plasma protein binding of ^{14}C- eteplirsen in mouse, rat, monkey, human (Sponsor Study 4658-pkd-001; (b) (4) Study 8284271)
The extent of plasma protein binding of ^{14}C-eteplirsen (8, 24, 80, 240, and 800 µg/mL) was evaluated by ultrafiltration of pooled samples from at least 3 male CD-1 mice, SD rats, and cynomolgus monkeys, and from 3 adult human males using standard techniques. Plasma protein binding was low in all species tested: 17.9-25.4% (mouse), 1.8-18.5% (rat), 0.2-8.4% (monkey), and 6.1-16.5% (human).

Metabolism of ^{14}C- eteplirsen in mouse, rat, monkey, and human hepatic microsomes (Sponsor Study 4658-pkd-002; (b) (4) Study 8284270)
The extent of metabolism of ^{14}C-eteplirsen (8 and 80 µM) by hepatic microsomes (1 mg/mL) from mouse, rat, monkey, and human was assessed in the presence of NADPH (1 mM) for 0, 30, 45, 60, and 120 minutes at 37 °C in vitro, compared to controls incubated in the absence of NADPH for 0 and 120 minutes. ^{14}C-eteplirsen was not significantly metabolized by liver microsomes in any species under the conditions tested.

Evaluation of CYP induction following exposure of primary cultures of human hepatocytes to eteplirsen (Sponsor Study 4658-pkd-003; (b) (4) Study 8278651)
The induction potential of eteplirsen (up to 6.65625 mg/mL) was assessed in primary cultures of human hepatocytes using standard procedures. mRNA levels of CYP450 isozymes 1A2, 2B6, and 3A4 were measured using RT-PCR, and enzyme activity assays were performed using standard isozyme-specific substrates. No induction of CYP isozymes 2B6 or 3A4 was observed. CYP1A2 was significantly induced in hepatocytes from 2 of 3 human donors by mRNA analysis and from all 3 donors by enzyme activity assay. The level of induction of CYP1A2 by eteplirsen (2.5- to 6-fold for mRNA, 4- to 7.5-fold for enzyme activity, compared to solvent control) was much lower than for the positive control, omeprazole (46- to 90-fold for mRNA, 22- to 58-fold for enzyme activity).

Inhibitory potential of eteplirsen towards human hepatic microsomal cytochrome P450 isoenzymes (Sponsor Study 4658-pkd-004; (b) (4) Study 8278650)
The inhibitory potential of eteplirsen (up to 6.66 or 10.65 mg/mL) on CYP450 isozyme activity was assessed using in vitro preparations of human liver microsomes incubated with standard CYP-selective substrates. No inhibition exceeding 50% was observed for CYPs 2B6, 2C8, 2D6, or 3A4/5. CYPs 1A2, 2C9, and 2C19 were competitively inhibited by eteplirsen: IC_{50} = 6.52, 2.75, and 1.16 mg/mL, respectively; K_i = 7.58, 0.676, and 0.553 mg/mL, respectively. No metabolism-dependent inhibition of CYP isozymes was observed in similar studies after preincubation with NADPH for 30 minutes.

Evaluation of eteplirsen as a substrate and inhibitor of a panel of human drug transporters (Sponsor Study 4658-pkd-005; (b) (4) Study 8284329)
Chinese Hamster ovary (CHO) cells stably transfected with human transporters (organic anion transporter 1 [OAT1]; OAT3; organic cation transporter 1 [OCT1]; OCT2; organic anion transporting polypeptide 1B1 [OATP1B1]; or OATP1B3) were used to evaluate uptake of ^{14}C-eteplirsen (8 and 80 µg/mL) and the potential of eteplirsen (80 and 800 µg/mL) to inhibit uptake of known substrates. Eteplirsen was not a substrate of any of these transporters, but showed weak inhibition of OCT1 and OATP1B1 under the conditions tested (activity was reduced 14-26% and 18-32%, respectively, compared to solvent control).

Caco-2 cell monolayers were used to assess the potential for eteplirsen to act as a substrate or inhibitor of P-glycoprotein (P-gp) and breast cancer resistant protein (BCRP). Eteplirsen was not a substrate or inhibitor of P-gp or BCRP under the conditions tested.

Inside out membranes prepared from insect cells (Sf9) infected with recombinant baculovirus encoding cDNA for human multidrug resistance protein 2 (MRP2) or human bile salt export pump (BSEP) were used to assess the potential for eteplirsen to act as a substrate or inhibitor of MRP2 or BSEP. Eteplirsen was not a substrate or inhibitor of MRP2 or BSEP under the conditions tested.

5.2 Toxicokinetics

Interspecies Comparison of Eteplirsen Pharmacokinetic Parameters

Study	NOAEL Dose (mg/kg Q1W)	T_{max} (hrs) (Mean ± SD)	$t_{½}$ (hrs) (Mean ± SD)	C_{max} (µg/mL) (Mean ± SD)	$AUC_{(0\text{-}24\ hr)}^{\#}$ (µg*hr/mL) (Mean ± SD)
Human Study 202 Week 152	30	1.12 ± 0.08	3.543 ± 0.643	85.067 ± 15.913	127.457 ± 25.798
Monkey Study 001 Week 39	320	0.281 ± 0.088	4.24 ± 0.17	1843.750 ± 508.638	2551.895 ± 608.503
Juvenile Rat Study 003 Day 77 pp	300	0.0833	NA	1293 ± 72.3 (SE)	768 ± 44.3 (SE)

[#]$AUC_{0\text{-}36\ hr}$ for the monkey study; however, $AUC_{24\text{-}36\ hr}$ was only ~1% of $AUC_{(0\text{-}36\ hr)}$; NA-Not Available

6 General Toxicology

6.1 Single-Dose Toxicity

Eteplirsen: A Pilot Single Dose Intravenous Injection Toxicity Study in the Juvenile and Adult Rat
(Sponsor Study 4658-tox-002; [redacted] (b) (4) Study 902364; eteplirsen on September 22, 2011; AVI-4658 Lot # DEV-518, LY01, purity 93%; non-GLP; non-QA)

Juvenile rats (Crl:CD[SD]; 6M/group; 34.5-48.0 g at dosing) were injected via a tail vein on Postnatal Day (PND) 14 with vehicle (Dulbecco's Phosphate Buffered Saline w/o Mg^{++} or Ca^{++}), 600 mg/kg (LD), or 960 mg/kg (HD) eteplirsen and euthanized under isoflurane anesthesia by exsanguination from the abdominal aorta approximately 24 hours after treatment. Parameters evaluated included mortality, clinical signs, body weight, hematology (3/group, except 4 HD), clinical chemistry (3/group, except 2 HD), necropsy, and histopathology (limited to kidneys).

Drug-related effects were limited to increased BUN (LD 13%; HD 22%, compared to Con), which was correlated with increased incidence of minimal tubular vacuolation and basophilia (1 Con; 5 LD; 6 HD) and minimal hemorrhage in renal papilla (1 HD).

Adult rats (Crl:CD[SD]; 6M/group; 423-475 g at dosing) were injected via a tail vein on PND 77 with vehicle (Con; Dulbecco's Phosphate Buffered Saline w/o Mg^{++} or Ca^{++}) or 960 mg/kg (HD) eteplirsen and euthanized under isoflurane anesthesia by exsanguination from the abdominal aorta approximately 24 hours after treatment. Parameters evaluated included mortality, clinical signs, body weight, hematology (3/group), clinical chemistry (3/group), urinalysis (24-hour collection after dosing), necropsy, and histopathology (limited to kidneys).

Drug-related effects reported in HDM adult rats included increased BUN (74%, compared to Con), urinary protein (2.8x), and urine protein/creatinine ratio (2.9x), which were correlated with the following lesions in kidneys: tubular vacuolation and basophilia (4 moderate; 2 marked); tubular necrosis (4 minimal; 1 slight); tubular dilatation (3 minimal; 2 slight); basophilic cast (3 minimal); and hemorrhage in renal medulla and papilla (3 minimal); and hyaline cast (1 minimal). One HDM also had enlarged kidneys. No lesions were observed in adult Con.

6.2 Repeat-Dose Toxicity

Eteplirsen: 12-week intravenous and subcutaneous toxicity study in mice

(Sponsor Study AVI-4658-GLP-0902; (b) (4) Study 1152-015; eteplirsen on April 21, 2009; AVI-4658 Lot # 42GD-LY01, purity 96%; Lot # 43GD-LY01, purity 95%; Lot # 38GD-LY01, purity 96%; GLP; QA)
See nonclinical review of IND 77429 by Barbara Wilcox dated March 10, 2013, for a more detailed review of this study.

Mdx mice (C57BL/10ScSn-DMD^{mdx}/J; 15/sex/group) were injected IV with vehicle (Con; Dulbecco's Phosphate Buffered Saline w/o Mg^{++} or Ca^{++}), 12 mg/kg (LD), 120 mg/kg (MD), or 960 mg/kg (HD) eteplirsen, or 960 mg/kg SC eteplirsen (HD SC) once weekly for 12 weeks at 9.6 mL/kg (6 mL/kg for LD & MD). Non-*mdx* mice (C57BL/6NCrl; 15/sex/group) were injected IV with vehicle (Con) or 960 mg/kg (HD) eteplirsen once weekly for 12 weeks at 9.6 mL/kg. Five animals/sex/group were maintained without dosing for a 28-day recovery period following the final dose. Parameters evaluated included mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, necropsy, organ weights, and histopathology.

Mortality was limited to 1/15 HDM SC found dead of uncertain cause on Day 93, and 1/15 Con M *mdx* mice that died following an accident on Day 57. No drug-related effects on clinical signs, body weight, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, or macroscopic findings were reported. Reversible increases were observed in relative kidney weights in HD non-*mdx* mice (M 2.6%; F 12.2%) compared to Con. Partially reversible minimal to mild renal tubular cytoplasmic basophilia and tubular vacuolation was reported in MD, HD, HD SC *mdx* mice, and HD non-*mdx* mice. Fully reversible minimal renal tubular degeneration/regeneration was noted in HD non-*mdx* mice. Lymphoid necrosis in spleen (minimal) and thymus (minimal to moderate) was increased in incidence and severity at the HD compared to controls, and was partially reversible. Minimal to moderate infiltration of macrophages containing foamy basophilic cytoplasm and basophilic granules observed in the skin and injection site of HD SC *mdx* mice was not reversible.

AVI-4225: 3-month intravenous and subcutaneous toxicity study in mice

(Sponsor Study AVI-4225-GLP-0903; (b) (4) Study 1152-021; eteplirsen on July 15, 2009; AVI-4225 Lot # DEV-441, LY01, purity 93%; GLP; QA)
See nonclinical review of IND 77429 by Barbara Wilcox, dated March 10, 2013, for a more detailed review of this study.

Mdx mice (C57BL/10ScSn-DMD^{mdx}/J; 15/sex/group) were injected IV with vehicle (Con; Dulbecco's Phosphate Buffered Saline w/o Mg^{++} or Ca^{++}), 12 mg/kg (LD), 120 mg/kg (MD), or 960 mg/kg (HD) AVI-4225, or 960 mg/kg SC AVI-4225 (HD SC) once weekly for 12 weeks at 9.6 mL/kg (HD) or 6 mL/kg (LD & MD). Non-*mdx* mice (C57BL/6NCrl; 15/sex/group) were injected IV with vehicle (Con) or 960 mg/kg (HD) AVI-4225 once weekly for 12 weeks at 9.6 mL/kg. Five animals/sex/group were maintained without dosing for a 28-day recovery period following the final dose. AVI-4225 is the murine

surrogate of eteplirsen. Parameters evaluated included mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, necropsy, organ weights, and histopathology.

No drug-related effects were observed on mortality, clinical signs, body weight, food consumption, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, or macroscopic findings. Increases were observed in relative kidney weights in HD non-*mdx* mice (M 9%; F 8%) compared to Con; these increases were maintained in HD recovery non-*mdx* mice (M 7%; F 15%). Minimal renal tubular cytoplasmic basophilia, vacuolation, and basophilic granules were reported in HD *mdx* mice, HD SC *mdx* mice, and HD non-*mdx* mice; these effects were partially reversible. Minimal to mild basophilic granules in the urinary bladder in MD, HD, and HD SC mdx and HD non-*mdx* mice were also partially reversible. Minimal to moderate infiltration of macrophages containing basophilic granules were observed in the injection site of HD SC *mdx* mice, and similar changes of minimal severity were noted in HD *mdx* mice and HD non-*mdx* mice; these changes were fully reversible. Dose-dependent reductions were observed in the incidence and severity of myofiber degeneration in muscles (larynx, biceps femoris, diaphragm, quadriceps, and tongue), consistent with the anticipated pharmacodynamic action of AVI-4225 in *mdx* mice; effects were greater with IV compared to SC dosing.

Eteplirsen: 12-week intravenous and subcutaneous toxicity study in cynomolgus monkeys

(Sponsor Study AVI-4658-GLP-0901; (b) (4) Study 1152-014; eteplirsen on May 7, 2009; AVI-4658 Lot # 42GD-LY01, purity 96%; Lot # 43GD-LY01, purity 95%; Lot # 38GD-LY01, purity 96%; Lot # 58GD-LY01, purity 99%;GLP; QA)
See nonclinical review of IND 77429 by Barbara Wilcox dated March 10, 2013, for a more detailed review of this study.

Cynomolgus monkeys (~3 years old; 2-3 kg; 6/sex/group) were injected IV with vehicle (Con; Dulbecco's Phosphate Buffered Saline w/o Mg^{++} or Ca^{++}), 5 mg/kg (LD), 40 mg/kg (MD), or 320 mg/kg (HD) eteplirsen, or 320 mg/kg SC eteplirsen (HD SC) once weekly for 12 weeks at 3.2 mL/kg (Con, HD, HD SC), 2.5 mL/kg (LD), or 2 mL/kg (MD). Two animals/sex/group were maintained without dosing for a 28-day recovery period following the final dose. Parameters evaluated included mortality, clinical signs, body weight, food consumption, ophthalmology, ECG, hematology, coagulation, clinical chemistry, urinalysis, necropsy, organ weights, histopathology, and toxicokinetics.

No drug-related effects were observed on mortality, clinical signs, body weight, food consumption, ophthalmology, ECG, hematology, coagulation, clinical chemistry, urinalysis, organ weights, or macroscopic findings.

Mortality was limited to 1 LDM sacrificed moribund Day 18 following watery feces during Weeks 1-2, and distended abdomen, decreased activity, few/absent feces, and prostration in Week 3; no cause of death was determined. Drug-related effects in survivors were primarily observed in kidney, and included minimal to moderate tubular basophilia, minimal to mild vacuolation (HD, HD SC), and minimal basophilic granules

(LDM, MDM). Recovery animals showed minimal tubular basophilia and vacuolation. Minimal to moderate basophilic granules in macrophages at the injection site were observed mainly in HD SC animals and were partially reversible.

A 10-Week Intravenous Injection Toxicity Study in the Juvenile Rat

Study number:	4658-tox-003
Study report location:	edr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 1, 2011 (eteplirsen)
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	AVI-4658: Lot # DEV-518, LY01, purity 92% Lot # 2502074, purity 95%

Key Study Findings

- Dose-related microscopic changes in kidney (including minimal to marked tubular vacuolation/basophilia and dilatation, and minimal to slight tubular necrosis, among others) were correlated at 900 mg/kg with increased relative kidney weight, gross findings in kidney (enlargement, mottled, irregular surface, pale), and changes in urinalysis (decreased creatinine clearance, cystatin C/creatinine ratio, pH, and chloride; increased protein/creatinine ratio and incidence of blood in urine) and clinical chemistry (increased urea nitrogen and creatinine).
- Minimal to moderate accumulation of vacuolated and basophilic macrophages was reported in lung at 900 mg/kg.
- The renal and lung changes were partially reversed after a 4-week recovery period.
- Dose-related reductions were observed in bone length, width, area, mineral content, and mineral density, achieving statistical significance at the HD and showing dose-related trends in some parameters at the MD and/or LD.
- The NOAEL for the treatment of juvenile rats once weekly for 10 weeks with IV eteplirsen was the MD of 300 mg/kg, based on renal tubular necrosis and clinical pathology changes consistent with impaired renal function observed at the HD of 900 mg/kg.

Methods

Doses:	0, 100, 300, 900 mg/kg eteplirsen
Frequency of dosing:	once weekly (Q1W) PNDs 14-77; 10 injections total
Route of administration:	IV injection via the tail vein
Dose volume:	10 mL/kg
Formulation/Vehicle:	Dulbecco's PBS w/o Mg^{++} or Ca^{++}
Species/Strain:	Sprague-Dawley rat; Crl:CD(SD)
Number/Sex/Group:	see sponsor's tables below; males only
Age:	PND 14 at dosing initiation
Weight:	24.2-44.0 g at dosing initiation
Satellite groups:	see sponsor's tables below
Unique study design:	see sponsor's tables below
Deviation from study protocol:	Minor deviations were reported; these did not affect the overall interpretation or validity of the study

Experimental Design

Group No.	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Males
1/ Vehicle Control[a]	0	10	0	140
2/ AVI-4658	100	10	10	144
3/ AVI-4658	300	10	30	160
4/ AVI-4658	900	10	90	160

[a] Dulbecco's phosphate buffered saline (1x) without magnesium or calcium

Phase I – Toxicology Subsets

Group No.	No. of Males		
	Subset A	Subset B	Subset C
	Main Study	Recovery	Behavior/Reproduction
1/ Vehicle Control	32	32	32
2/ AVI-4658	32	32	32
3/ AVI-4658	32	32	32
4/ AVI-4658	32	32	32

Phase II – Laboratory Investigation Subsets

Group No.	No. of Males				
	Subset D	Subset E	Subset F	Subset G	Subset H
	Toxicokinetic		Clinical Pathology	Immunology	
	Day 14/15 pp	Day 77/78 pp	Day 15 pp	Treatment Period	Recovery Period
1/ Vehicle Control	8	8	12	8	8
2/ AVI-4658	28	8	12	-	-
3/ AVI-4658	28	8	12	8	8
4/ AVI-4658	28	8	12	8	8

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Observations and Results

Mortality

All animals were evaluated twice daily for mortality and morbidity by cageside observation.

One of 32 main study MD animals was found dead after dosing on PND 21, of uncertain cause; no clinical signs or body weight changes indicative of morbidity were reported.

Clinical Signs

Detailed observations were conducted once daily pre-weaning and once weekly post-weaning for all animals, and at scheduled termination for main study, recovery, and behavior/reproduction animals.

No drug-related clinical signs were reported.

Body Weights

All animals were weighed on PNDs 4, 7, 10, 14, 17, and 21; twice weekly thereafter; and at scheduled termination for main study, recovery, and behavior/reproduction animals.

Mean body weight was reduced 4-5% in HD animals compared to controls from PND 38 to 77. Mean body weight gain (PND 21 to 77) was reduced 5% in HD animals compared to controls.

Food Consumption

Food consumption was quantitatively measured in main study, recovery, and behavior/reproduction animals twice weekly from PND 21 to 28 and once weekly thereafter, on days of body weight measurement.

No drug-related effects on food consumption were reported.

Physical Development

Behavior/reproduction animals were assessed for preputial separation once daily from PND35 until separation was observed.

No drug-related effects on the day of preputial separation were reported.

Auditory startle

Auditory startle habituation was assessed in recovery animals on PND 56 (± 2) and PND 100 (± 2). After a 4-minute acclimation period, responses were measured in 50 identical trials at 120 dBA (± 4) with an 8-second inter-trial interval.

No drug-related effects were reported.

Motor activity

Locomotor activity was assessed in behavior/reproduction animals for 6 ten-minute intervals on PND 73 (± 4) and PND 93 (± 1).

No drug-related effects were reported.

Cincinnati water maze

Learning and memory was assessed using the Cincinnati water maze in recovery animals between PND 68 and 76, and in behavior/reproduction animals between PND 96 and 104.

No drug-related effects were reported.

Functional Observation Battery

Behavior/reproduction animals were assessed on PND 73 (± 4) and PND 93 (±1) in a standard Functional Observation Battery, consisting of Qualitative Observational Assessments (observations in home cage, removal from home cage, observations in arena, handling observations, observations on the surface, and observations on top of box) and Quantitative Observations (grip strength, hindlimb splay, and rectal body temperature).

No drug-related effects were reported.

Bone measurement

As shown in the tables below, femur length was reduced in MD and HD animals compared to controls (p≤0.5 for HD), and femur width was reduced in recovery animals at all doses compared to controls.

Text Table 4
Summary of Bone measurements – Percent Difference – Left Femur – End of Treatment Period

				Group	Vehicle Control	AVI-4658 100 mg/kg		AVI-4658 300 mg/kg		AVI-4658 900 mg/kg	
			Units		Value	Value	% vs Gr.1	Value	% vs Gr.1	Value	% vs Gr.1
Femur	Male	Length	mm	Mean	38.25	38.33	0.2	37.90	-0.9	**37.74**	-1.3
				SD	0.98	0.72		0.79		0.66	
		Width	mm	Mean	4.61	**4.79**	3.9	4.65	0.9	4.60	-0.3
				SD	0.31	0.30		0.32		0.27	

Values in bold are significantly different from Vehicle Control values

Text Table 5
Summary of Bone measurements – Percent Difference – Left Femur – Recovery Period

				Group	Vehicle Control	AVI-4658 100 mg/kg		AVI-4658 300 mg/kg		AVI-4658 900 mg/kg	
			Units		Value	Value	% vs Gr.1	Value	% vs Gr.1	Value	% vs Gr.1
Femur	Male	Length	mm	Mean	40.26	40.50	0.6	40.24	0.0	39.65	-1.5
				SD	0.85	0.91		1.44		1.28	
		Width	mm	Mean	4.89	4.85	-0.8	4.85	-0.7	4.77	-2.4
				SD	0.33	0.28		0.28		0.26	

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Bone densitometry

Recovery animals were assessed for bone mineral density (BMD), bone mineral content (BMC), and bone area using Dual Energy X-Ray Abosorptiometry at the end of the treatment and recovery periods (same 12 animals if possible). Global, proximal, mid, and distal sites of the right femur and L1-L4 lumbar vertebrae were scanned.

As shown in the summary tables below, BMC and BMD of the femur were dose-dependently reduced in all dose groups compared to controls; bone area was reduced in MD and HD animals. The reductions in bone area and BMC in the femur achieved statistical significance at the HD (p≤0.5). Bone area, BMC, and BMD of the L1-L4 lumbar vertebrae were reduced at the HD compared to controls. The reductions in bone area and BMC in the femur achieved statistical significance at the HD.

Text Table 6
Summary of DXA parameters – Percent Difference – Right Femur – End of Treatment Period

				Group	Vehicle Control	AVI-4658 100 mg/kg		AVI-4658 300 mg/kg		AVI-4658 900 mg/kg	
			Units		Value	Value	% vs Gr.1	Value	% vs Gr.1	Value	% vs Gr.1
Right Femur	Global Male	Area	cm²	Mean	1.687	1.697	0.5	1.669	-1.1	**1.598**	-5.3
				SD	0.065	0.053		0.083		**0.086**	
		BMC	g	Mean	0.421	0.415	-1.3	0.403	-4.3	**0.384**	-8.7
				SD	0.024	0.017		0.022		**0.042**	
		BMD	g/cm²	Mean	0.249	0.245	-1.8	0.241	-3.2	0.240	-3.7
				SD	0.007	0.006		0.006		0.015	
	Proximal Male	Area	cm²	Mean	0.528	0.542	2.8	0.520	-1.5	0.503	-4.8
				SD	0.019	0.028		0.034		0.027	
		BMC	g	Mean	0.154	0.155	0.3	0.146	-5.2	**0.141**	-8.5
				SD	0.009	0.010		0.010		**0.015**	
		BMD	g/cm²	Mean	0.292	0.285	-2.4	0.282	-3.7	0.280	-4.0
				SD	0.011	0.008		0.009		0.016	
	Mid Male	Area	cm²	Mean	0.387	0.378	-2.4	0.380	-1.8	0.375	-3.2
				SD	0.025	0.015		0.016		0.013	
		BMC	g	Mean	0.085	0.084	-1.3	0.083	-2.7	0.079	-7.3
				SD	0.008	0.005		0.006		0.009	
		BMD	g/cm²	Mean	0.219	0.222	1.3	0.217	-0.8	0.210	-4.3
				SD	0.009	0.008		0.010		0.017	
	Distal Male	Area	cm²	Mean	0.511	0.511	0.0	0.498	-2.6	0.491	-3.9
				SD	0.023	0.020		0.022		0.033	
		BMC	g	Mean	0.136	0.132	-2.8	0.128	-5.7	0.126	-6.9
				SD	0.007	0.008		0.007		0.017	
		BMD	g/cm²	Mean	0.265	0.258	-2.9	0.257	-3.3	0.256	-3.6
				SD	0.012	0.009		0.009		0.020	

Values in bold are significantly different from Vehicle Control values

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Text Table 7
Summary of DXA parameters – Percent Difference – Lumbar Spine – End of Treatment Period

			Group	Vehicle Control	AVI-4658 100 mg/kg		AVI-4658 300 mg/kg		AVI-4658 900 mg/kg	
		Units		Value	Value	% vs Gr.1	Value	% vs Gr.1	Value	% vs Gr.1
Lumbar Vertebrae Male	Area	cm²	Mean	2.044	2.084	1.9	2.091	2.3	2.000	-2.2
			SD	0.068	0.084		0.082		0.134	
	BMC	g	Mean	0.425	0.437	2.7	0.437	2.6	0.408	-4.0
			SD	0.032	0.026		0.032		0.046	
	BMD	g/cm²	Mean	0.208	0.210	0.8	0.209	0.4	0.204	-2.0
			SD	0.010	0.006		0.009		0.010	

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Ophthalmoscopy

n/a

ECG

n/a

Hematology

Blood samples were collected from the abdominal aorta from the second 6 animals per group for clinical pathology, main study, and recovery animals on PNDs 15, 78, and 105, respectively, or upon unscheduled death. The following parameters were evaluated: red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, mean platelet volume, white blood cell count (WBC), differential blood cell count, reticulocyte count, red cell distribution width (RCDW). Coagulation parameters were collected for main study and recovery animals and analyzed for APTT and PTT.

No drug-related effects were reported on PND 15. The following slight changes were reported at the HD compared to controls on PND 78: decreased RBC, HGB, HCT (5-6%); increased RCDW (7%), WBC (18%), neutrophil count (40%), monocyte count (57%). HD recovery animals showed very slight decreases in RBC and HGB (3-4%).

Clinical Chemistry

Blood samples were collected from the abdominal aorta from the first 6 animals per group for clinical pathology, main study, and recovery animals on PNDs 15, 78, and 105, respectively, or upon unscheduled death. The following parameters were measured: BUN, TPROT, ALB, GLOB, A/G ratio, ALT, ALP, AST, Ca^{++}, Cl^{-}, CHOL, CREAT, GLU, PHOS, K^{+}, Na^{+}, TBILI, and TRIG.

The following changes at the HD compared to control on PND78 were correlated with microscopic lesions in renal tubules: increased BUN (138%), CREAT (37%), PHOS (5%). Additional changes at the HD included: increased CHOL (13%), K^{+} (8%), Cl^{-} (2%); decreased TPROT (3%) and ALB (4%). HD recovery animals (PND105) showed only minimal increases in BUN (12%) and Cl^{-} (1%), consistent with partial reversibility of the renal lesions.

Urinalysis

Urine was collected over 24 hours from main study animals (PND78) and recovery animals (PND105) for analysis of the following parameters: color, appearance, specific gravity, total volume, pH, glucose, bilirubin, ketones, blood, urobilinogen, creatinine, creatinine clearance, calcium, chloride, protein, total protein/creatinine ratio, potassium, sodium, cystatin C, cystatin C/creatinine ratio.

The following changes at the HD compared to control on PND 78 were correlated with microscopic lesions in renal tubules: decreased creatinine clearance (28%), cystatin C/creatinine ratio (20%), pH (5.5-7.5 vs. 7.0-7.5 in control), chloride (50%); increased protein/creatinine ratio (74%), incidence of blood in urine. HD recovery animals (PND 105) showed only decreased cystatin C/creatinine ratio (18%), consistent with partial recovery of the renal tubular lesions.

Gross Pathology

Full necropsy was conducted on main study and recovery animals on the day of unscheduled death or sacrifice, PND 78 (terminal sacrifice), or PND 105 (recovery sacrifice), unless autolysis precluded evaluation. Partial necropsy (limited to the reproductive system) was conducted on behavior/reproduction animals on PND 105 (or upon unscheduled death). Animals were terminated by exsanguination after isoflurane anesthesia (main study, recovery, behavior/reproduction, TK PND 14/15, clinical pathology) or carbon dioxide asphyxiation (TK PND 77/78, immunology, and immunology recovery).

The following drug-related macroscopic changes were reported in kidney at the HD: enlargement (30/32), mottled appearance (9), and irregular surface (6), pale discoloration (5). These changes correlated with microscopic findings of renal tubular basophilia and vacuolation. HD recovery animals showed enlarged kidney (6/32).

Organ Weights

Weights were recorded for main study and recovery animals for the following organs: adrenal glands, brain, heart, kidneys, liver, lung, pituitary gland, prostate gland, spleen, testes, thymus, thyroid/parathyroid. Organs weighed from behavior/reproduction animals included epididymides, seminal vesicles, prostate gland, and testes.

Relative kidney weight was increased 80% at the HD compared to control, correlated with renal tubular basophilia and vacuolation; and remained 29% greater in HD recovery animals, consistent with partial recovery of the renal lesions.

Histopathology

The following tissues from all main study and recovery animals were examined microscopically: gross lesions, injection site, kidney, and lung. Bone marrow smears were prepared but not analyzed.

The following tissues were evaluated only in control, HD, and unscheduled death main study animals: adrenal gland, aorta, bone and bone marrow (sternum, femur), brain, cecum, colon, duodenum, epididymis, esophagus, eye, harderian gland, gut-associated lymphoid tissue, heart, ileum, jejunum, lacrimal gland, liver, lymph node (mandibular, mesenteric), mammary gland, muscle (thigh), pancreas, pituitary gland, prostate gland, salivary gland (submandibular), seminal vesicle, skin, spinal cord (cervical, thoracic,

lumbar), spleen, stomach, testis, thymus, thyroid/parathyroid gland, tongue, trachea, urinary bladder.

The battery of tissues examined was adequate.

No Peer Review was conducted; a signed pathology report was provided.

The primary target organs were the kidney, lung, and injection site. As shown in the sponsor's summary tables below, the most prominent changes in kidney were tubular vacuolation and basophilia and tubular dilatation, which were moderate to marked in most HD animals but of minimal severity in LD and MD animals. These lesions "consisted of flattened or enlarged tubular epithelial cells with granular basophilic and vacuolated cytoplasm primarily located in the proximal convoluted tubules of the cortical region but also extended in the medullary and papillary regions of both kidneys" *(page 14 of Final Pathology Report)*. All renal changes listed in the tables below were minimal in LD and/or MD animals but showed dose-dependent increases in incidence. Minimal to slight tubular necrosis and increased incidence of minimal interstitial inflammation were only observed in HD animals.

In lung, minimal to moderate accumulation of vacuolated and basophilic macrophages was reported at the HD, "characterized by the presence of a small number of macrophages with cytoplasmic vacuoles and basophilia in the alveolar lumen of the lungs" *(page 14 of the Final Pathology Report)*.

Minimal to slight inflammation and minimal to moderate hemorrhage were reported at the injection site in all groups, including control, but the incidence and/or severity was increased at the HD.

Summary of Microscopic Findings in the Kidneys–Scheduled Euthanasia–End of Treatment, Subset A (Day 78 pp)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**100**	**300**	**900**
No. Animals Examined	**32**	**32**	**32**	**32**
Kidney (No. Examined)	**32**	**32**	**32**	**32**
Vacuolation and basophilia: tubular	0	24	30	32
Minimal	0	24	30	0
Slight	0	0	0	4
Moderate	0	0	0	19
Marked	0	0	0	9
Dilatation: tubular	0	2	14	32
Minimal	0	2	14	2
Slight	0	0	0	12
Moderate	0	0	0	11
Marked	0	0	0	7
Cast: basophilic	0	6	9	29
Minimal	0	6	9	26
Slight	0	0	0	3
Cast: hyaline	0	0	2	9
Minimal	0	0	2	9
Necrosis: tubular	0	0	0	21
Minimal	0	0	0	20
Slight	0	0	0	1
Basophilic material: intravascular	0	0	1	30
Minimal	0	0	1	12
Slight	0	0	0	14
Moderate	0	0	0	4
Mineralization: tubular	0	0	6	15
Minimal	0	0	6	14
Slight	0	0	0	1
Inflammation: interstitial	4	4	3	10
Minimal	4	4	3	10
Hemorrhage	0	0	0	1
Minimal	0	0	0	1

(page 13 of Final Pathology Report)

Summary of Microscopic Findings in the Lungs – Scheduled Euthanasia – End of Treatment, Subset A (Day 78 pp)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**100**	**300**	**900**
No. Animals Examined	**32**	**32**	**32**	**32**
Lungs (No. Examined)	**32**	**32**	**32**	**32**
Macrophage accumulation: vacuolated and basophilic	0	0	0	27
Minimal	0	0	0	23
Slight	0	0	0	3
Moderate	0	0	0	1

(page 14 of Final Pathology Report)

Summary of Injection Site Microscopic Findings – Scheduled Euthanasia – End of Treatment, Subset A (Day 78 pp)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**100**	**300**	**900**
No. Animals Examined	**32**	**32**	**32**	**32**
Injection Site (No. Examined)	**32**	**32**	**32**	**32**
Inflammation	8	5	4	16
Minimal	7	4	3	13
Slight	1	1	1	3
Hemorrhage	9	4	2	17
Minimal	5	4	1	5
Slight	2	0	1	12
Moderate	2	0	0	0

(page 15 of Final Pathology Report)

As shown in the sponsor's table below, the incidence of the kidney and lung findings observed in recovery animals was similar to that observed at the end of the dosing period; however, the severity was reduced, indicating partial recovery. No drug-related changes at the injection site were reported in recovery animals.

Summary of Microscopic Findings in the Kidneys and Lungs – Scheduled Euthanasia – Recovery, Subset B (Day 105 pp)

	Males			
Group	**1**	**2**	**3**	**4**
Dose (mg/kg)	**0**	**100**	**300**	**900**
No. Animals Examined	**32**	**32**	**32**	**32**
Kidney (No. Examined)	**32**	**32**	**32**	**32**
Vacuolation and basophilia: tubular	0	22	31	32
Minimal	0	22	31	18
Slight	0	0	0	13
Moderate	0	0	0	1
Dilatation: tubular	0	4	17	32
Minimal	0	4	17	26
Slight	0	0	0	6
Cast: basophilic	0	1	6	31
Minimal	0	1	6	31
Basophilic material: intravascular	0	0	0	2
Minimal	0	0	0	2
Mineralization: tubular	0	0	4	20
Minimal	0	0	4	20
Inflammation: interstitial	7	7	7	20
Minimal	7	7	7	20
Lungs (No. Examined)	**32**	**32**	**32**	**32**
Macrophage accumulation: vacuolated and basophilic	0	0	0	28
Minimal	0	0	0	27
Slight	0	0	0	1

(page 15 of Final Pathology Report)

The NOAEL was the MD of 300 mg/kg, based on the renal tubular necrosis observed at the HD of 900 mg/kg.

T-Cell Dependent Antibody Response (TDAR)

Blood was collected by jugular venipuncture from control, MD, and HD immunology animals on PND 57 and prior to KLH injections on PNDs 43 and 50; and from control, MD, and HD immunology recovery animals on PND 100 and prior to KLH injections on PNDs 86 and 93. KLH was injected via the tail vein as a solution of 150 µg in 0.05 mL (3 mg/mL) sterile water for injection. Samples were analyzed for serum levels of anti-KLH IgM and IgG using a semi-quantitative ELISA method.

No drug-related effects were reported.

Immunophenotyping

Blood was collected by jugular venipuncture from immunology animals on PND 57 and immunology recovery animals on PND 100 for analysis of white blood cell count (total, absolute, and differential), and immunophenotyping for the following subsets of lymphocytes: CD3+ (Total T cells), CD3+/CD4+ (Helper T cells), CD3+/CD8a+ (Cytotoxic T cells), CD3-/CD45RA+ (B cells), and CD3-/CD161a+ (Natural-killer cells).

A reversible 43% decrease in the absolute number of natural killer cells was reported at the HD compared to control on PND 57.

Sperm Assessments

Behavior/reproduction animals were assessed for reproductive potential. Sperm from the left vas deferens was assessed for motility. Sperm from the left cauda epididymis was analyzed for concentration and morphology.

No drug-related effects were observed on sperm motility, concentration, or morphology.

Dosing Solution Analysis

Samples were collected from the vehicle control and each dosing formulation on Days -14 and 30, and were analyzed in duplicate for concentration of eteplirsen.

All samples were within 5% of target concentrations. No significant peaks were detected in vehicle control samples.

Toxicokinetics

Blood was collected from TK PND 14/15 animals via the abdominal aorta after isoflurane anesthesia at 5 minutes after dosing on PND 14 for 8 controls, and for LD, MD, and HD groups (4/group/timepoint) at 5, 15, and 30 minutes, and 1, 3, 8, and 24 hours after dosing. Blood was collected from TK PND 77/78 animals via jugular venipuncture at 5 minutes after dosing on PND 77 for 8 controls, and for LD, MD, and HD groups (4/group/timepoint) at 0 (predose), 5, 15, and 30 minutes, and 1, 3, 8, and 24 hours after dosing.

As shown in the sponsor's summary tables below, eteplirsen plasma exposures generally increased dose-proportionately; accumulation in plasma with repeated dosing was not observed.

Table 2 **Toxicokinetic Parameters of AVI-4658 in Male Sprague-Dawley Rat Plasma Following Intravenous Injection of AVI-4658**

Day 14 Post Partum - (F1 Generation Pups - Phase II)

Group	Dose Level	Tmax	Cmax		AUC(0-t)		AUC(0-inf)	T1/2			
No.	(mg/kg)	(h)	(µg/mL) *	SE	(µg•h/mL) *	SE	(µg•h/mL) *	(h)	Cmax/D	AUC(0-t)/D	AUC(0-inf)/D
2	100	0.108	584	69.3	378	10.6	378	1.93	5.84	3.78	3.78
3	300	0.0833	1825	142	994	26.8	994	2.01	6.08	3.31	3.31
4	900	0.0833	5243	303	3328	133	3330	2.04	5.83	3.70	3.70

Day 77 Post Partum - (F1 Generation Adults - Phase II)

Group	Dose Level	Tmax	Cmax		AUC(0-t)		AUC(0-inf)	T1/2			
No.	(mg/kg)	(h)	(µg/mL) *	SE	(µg•h/mL) *	SE	(µg•h/mL) *	(h)	Cmax/D	AUC(0-t)/D	AUC(0-inf)/D
2	100	0.0833	449	24.3	204	10.6	RNR	RNR	4.49	2.04	–
3	300	0.0833	1293	72.3	768	44.3	RNR	RNR	4.31	2.56	–
4	900	0.0833	4570	358	2937	218	2952	3.68	5.08	3.26	3.28

RNR Result not reported because the AUC(0-inf) was extrapolated by more than 20% or Rsq was <0.800.

– Not calculated.

* The data were converted from ng/mL to ug/mL.

(page 23 of Final Toxicokinetic Report; t = 24 hrs)

26-Week Toxicity and Toxicokinetic Study of AVI-4225 in Mice with an 8-Week Recovery

Study number:	4225-tox-001; (b) (4) Study 8245175
Study report location:	edr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 21, 2011 (dosing initiation)
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	AVI-4225 Lot # DEV-502, LY01, purity 93%, Lot # DEV-514, LY01, purity 92%

Key Study Findings

- Drug-related macroscopic changes were observed in the brain of 4 *mdx* mice: large ventricles (1/16 MD; 1/4 HD unscheduled death/sacrifice); depressed area (1/16 HD); and soft (1/16 HD).
- The macroscopic changes in brain were correlated with microscopic findings of minimal to moderate dilatation of the lateral ventricles in brain (3/16 MD: 2 minimal, 1 mild; 4/16 HD: 3 slight, 1 moderate; 1/8 HD recovery: minimal; and 1/4 HD unscheduled death/sacrifice: moderate).
- Drug-related changes were observed in kidney of all HD *mdx* mice: cytoplasmic basophilia ± microvacuolation (9/16 minimal, 7 slight), and minimal degeneration of tubules (7/16); these changes were still evident in 8-week recovery animals.
- NOAEL = MD of 120 mg/kg/wk based on adverse moderate dilatation of lateral ventricles at HD of 960 mg/kg/wk IV AVI-4225.
- Dose-related reductions in myofiber degeneration in muscle tissues (biceps femoris, quadriceps, diaphragm, larynx, and tongue) in *mdx* mice were consistent with the anticipated pharmacodynamic activity of AVI-4225 on dystrophic muscle.

Methods

Doses:	0, 12, 120, 960 mg/kg AVI-4225
Frequency of dosing:	once weekly (Q1W) for 26 weeks
Route of administration:	IV bolus injection via tail vein
Dose volume:	10 mL/kg
Formulation/Vehicle:	Dulbecco's PBS w/o Mg^{++} or Ca^{++}
Species/Strain:	male *mdx* mice (C57BL/10ScSn-DMD*mdx*/J) male non-*mdx* control mice (C57BL/6NCrl)
Number/Sex/Group:	see sponsor's table below
Age at dosing initiation:	8-12 weeks (*mdx*); 8 weeks (non-*mdx*)
Weight at dosing initiation:	19.7-33.5 g (*mdx*); 19.7-25.1 g (non-*mdx*)
Satellite groups:	8-week recovery; hormone levels; toxicokinetics (see table below)
Unique study design:	male fertility (see table below, footnote "e")
Deviation from study protocol:	Minor deviations were reported; these did not affect the overall interpretation or validity of the study

Group[a]	Subgroup[b]	No. of Animals Male	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)
C57BL/10ScSn-DMDmdx/J mice (mdx)				
1 (Control)[c]	1 (Toxicity)[d,e]	34	0	0
	2 (Hormone)[f]	16	0	0
	3 (Toxicokinetic)	6	0	0
2 (Low)	1 (Toxicity)[d,e]	28	12	1.2
	2 (Hormone)	10	12	1.2
	3 (Toxicokinetic)	48	12	1.2
3 (Mid)	1 (Toxicity)[d,e]	28	120	12
	2 (Hormone)	10	120	12
	3 (Toxicokinetic)	48	120	12
4 (High)	1 (Toxicity)[d,e]	28	960	96
	2 (Hormone)	10	960	96
	3 (Toxicokinetic)	48	960	96
C57BL/6NCrl mice (non-mdx control strain)				
5 (Control)[c]	4 (Toxicity)[d,e]	34	0	0
	5 (Hormone)[f]	16	0	0
	6 (Toxicokinetic)	6	0	0
6 (High)	4 (Toxicity)[d,e]	28	960	96
	5 (Hormone)	10	960	96
	6 (Toxicokinetic)	48	960	96

a Animals received a dose volume of 10 mL/kg.

b Hormone level and toxicokinetic animals (Subgroups 2, 3, 5, and 6) were included solely for the purpose of blood sample collection.

c Groups 1 and 5 received vehicle control article [Dulbecco's Phosphate Buffered Saline (1x) without magnesium or calcium] only.

d The last 12 animals/group (based on survival) from the toxicity subgroups (Subgroups 1 and 4) were designated as recovery animals and were dosed with test or vehicle control article once weekly for 26 weeks, after which dosing was discontinued, and animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for 8 weeks after dosing.

e For male fertility assessment, the first six control toxicity animals from each strain were used during the predose phase, the first six toxicity animals/group from each strain were used at the terminal sacrifice, and the first four toxicity animals/group from each strain were used at the recovery sacrifice.

f Blood was collected from the first six control hormone animals from each strain at a single interval during the predose phase for hormone sampling.

(page 17 of Study Report; "Toxicity" Subgroups 1 and 4 will be referred to below as "main study" groups)

Observations and Results

Mortality

All animals were observed twice daily for mortality.

Death or moribund sacrifice occurred at the following overall incidence (main study, hormone, and TK groups combined): 4/56 control *mdx*, 5/86 LD *mdx*, 3/86 MD *mdx*, 7/86 HD *mdx*, 5/56 control non-*mdx*, and 3/86 HD non-*mdx* mice. The incidences of early death or sacrifice were not dose-related in the combined groups or in any of the subgroups.

One main study HD *mdx* mouse (A60103; found dead Day 22) had moderate dilation of the ventricles correlated with large brain and large ventricles; the cause of death was uncertain. Six other mice from various main study groups were found dead of uncertain cause on Days 44-158 of the dosing phase or Day 35 of the recovery phase.

Eight main study animals were sacrificed moribund with the following conditions, which are unlikely to be drug-related: dermatitis (1/28 control *mdx*, 1/28 MD *mdx*, 2/28 HD *mdx*, and 1/28 control non-*mdx* mice); swollen, inflamed penis (1/28 control non-*mdx* mice); swollen abdomen correlated with inflammation of the pancreas (1/28 control non-*mdx* mice); and general debilitation correlated with vascular inflammation (1/28 control *mdx* mice).

Eleven TK and Hormone mice in various dose groups were found dead or sacrificed moribund based on morbidity of uncertain cause.

Clinical Signs

All animals were evaluated by cageside observation once daily (except on days of detailed observations).

Detailed observations were conducted once prior to dosing initiation in all animals, and in main study animals prior to dosing on Day 1, once daily at ~10 minutes postdose during the dosing period, on the day of termination, and once weekly during the recovery period.

No drug-related clinical signs were reported. Sore tail and scabs on tail were observed across all dose groups and were likely caused by the injection procedure. Hypoactivity and/or hunched posture was observed in a few animals in control and AVI-4225 groups throughout the study but was not considered adverse because it occurred only once per animal. Swollen abdomen, thorax, and/or perineal area of uncertain cause were observed in several control and AVI-4225 non-*mdx* mice. Convulsions were observed in one mouse in the MD Hormone group on Day 123, 3 days following the final injection; per protocol, no postmortem evaluation was conducted. Based on the low incidence and lack of dose response, the convulsions are not likely to be drug-related.

Body Weights

Body weights were recorded for all animals prior to dosing initiation, weekly during Weeks 1-13, and once every 4 weeks thereafter through the end of the dosing and recovery periods.

No drug-related effects on body weight were reported.

Food Consumption

Food consumption was measured quantitatively in main study animals during Weeks 1-13, and once every 4 weeks thereafter through the end of the dosing and recovery periods.

No drug-related effects on food consumption were reported.

Ophthalmoscopy

n/a

ECG

n/a

Hematology

Blood samples were collected from nonfasted main study animals via the vena cava after sodium pentobarbital anesthesia on the day of scheduled sacrifice (first 8/group) or recovery sacrifice (first 6/group) for analysis of the following parameters: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, white blood cell count, differential blood cell count, reticulocyte count, blood cell morphology.

Drug-related effects on hematology were limited to a reversible 23% reduction in platelet count in HD *mdx* mice compared to controls.

Clinical Chemistry

Blood samples were collected from nonfasted main study animals via the vena cava after sodium pentobarbital anesthesia on the day of scheduled sacrifice (second 8/group) or recovery sacrifice (second 6/group) for analysis of the following parameters: urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST), calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, creatine kinase (CK), triglycerides.

As shown in the summary table below, AST, ALT, and CK were dose-dependently decreased in *mdx* mice compared to controls, consistent with the microscopic findings of dose-dependent reduction in myofiber degeneration in multiple muscles. No other important changes were observed in *mdx* or non-*mdx* mice.

	DOSE (mg/kg)							
	0		12		120		960	
PARAMETER	Day 177	Recov	Day 177	Recov	Day 177	Recov	Day 177	Recov
AST	883	843	624	756	257* (↓71%)	790	104* (↓88%)	296* (↓65%)
ALT	180	153	101* (↓44%)	96	66* (↓63%)	114	35* (↓81%)	49* (↓68%)
CK	5265	7096	3432	11402	1324* (↓75%)	5701	910* (↓83%)	2573* (↓64%)

*P≤0.05

Urinalysis

n/a

Gross Pathology

Necropsy was conducted on all main study animals on the day of unscheduled death or sacrifice, Day 177 (terminal sacrifice), or at least 8 weeks after the 26th dose (recovery sacrifice), unless autolysis precluded evaluation. Animals were terminated by exsanguination under sodium pentobarbital anesthesia.

Drug-related macroscopic changes were limited to observations in the brain of four *mdx* animals: large ventricles (1/16 MD), depressed area (1/16 HD), and soft (1/16 HD) in groups sacrificed at the end of the dosing period; and large brain, large ventricles (1/4 HD unscheduled death/sacrifice animals—this animal was found dead on Day 22 of uncertain cause. These macroscopic changes were correlated with microscopic findings of dilatation of the lateral ventricles in the brain.

Organ Weights

Weights were recorded for the following organs: adrenal glands, brain, epididymides, heart, kidneys, liver with gallbladder (drained), lung, pituitary gland, salivary glands (mandibular), seminal vesicles, spleen, testes, thymus, thyroid/parathyroid.

No important effects on organ weights were observed.

Histopathology

The following tissues from all main study animals were examined microscopically:
testes, kidneys, muscle (biceps femoris, quadriceps and diaphragm), tongue, larynx, brain and IV injection site.

The following tissues were examined microscopically only in control and HD animals:
adrenal glands, aorta, bone and bone marrow (sternum, femur), cecum, colon, duodenum, epididymides, esophagus, eyes, gallbladder, heart, ileum, jejunum, lacrimal gland, liver, lung with large bronchi, lymph nodes (mesenteric), optic nerve, pancreas, pituitary gland, prostate gland, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicle, skin/subcutis, spinal cord (cervical, thoracic, and lumbar)

spleen, stomach, thymus, thyroid/parathyroid gland, trachea, urinary bladder, gross lesions (if any).

The battery of tissues examined was adequate.

All brain slides from all groups were peer reviewed.

Dilatation of the lateral ventricles in the brain was observed in 3/16 MD (2 minimal, 1 slight), 4/16 HD (3 slight, 1 moderate), 1/8 HD recovery (minimal), and 1/4 HD unscheduled death/sacrifice (moderate) *mdx* mice. The sponsor notes that hereditary hydrocephalus has been reported to occur at up to 4.1% in C57BL mice *(Mori A, 1968, Hereditary hydrocephalus in C57BL mouse, Brain and Nerve 20: 695-700)*. Based on the absence of similar observations in controls and C57BL non-*mdx* mice, the sponsor speculates that these findings may be attributable to "a strain-specific, test article-related exacerbation of a spontaneously occurring hereditary hydrocephalus" *(page 36 of Study Report)*. However, the mechanism of such an effect, and its relevance to humans, remains uncertain. The sponsor considered the changes in the HD group to be adverse based on their moderate severity.

As illustrated in the summary tables below, drug-related changes were also observed in kidneys, IV injection site, muscles (biceps femoris, quadriceps, and diaphragm), larynx, and tongue in *mdx* mice; and in the kidneys and IV injection site in non-*mdx* mice.

Incidence and Severity of Select Microscopic Findings in mdx (C57BL/10ScSn-DMDmdx/J) Mice - Terminal and Recovery Sacrifices

	AVI-4225							
	C57BL/10ScSn-DMDmdx/J mice							
Sacrifice	Terminal				Recovery			
Dose Level (mg/kg/dose)	0	12	120	960	0	12	120	960
Kidney								
Number Examined	16	16	16	16	9	10	9	8
Cytoplasmic Basophilia/Microvacuolation, Tubules								
Minimal	0	0	1	9	0	0	0	5
Slight	0	0	0	7	0	0	0	3
Degeneration, Tubule								
Minimal	0	0	1	12	0	0	0	6
Muscle, Biceps Femoris								
Number Examined	16	16	16	16	9	10	9	8
Myofiber, Degeneration								
Minimal	2	12	9	3	2	7	6	3
Slight	9	2	0	0	6	3	0	0
Moderate	5	0	0	0	1	0	0	0
Muscle, Quadriceps								
Number Examined	16	16	16	16	9	10	9	8
Myofiber, Degeneration								
Minimal	2	10	12	6	2	9	6	5
Slight	11	2	0	0	7	1	1	0
Moderate	3	0	0	0	0	0	0	0
Muscle, Diaphragm								
Number Examined	16	16	16	16	9	10	9	8
Myofiber, Degeneration								
Minimal	0	1	5	6	1	0	1	5
Slight	8	12	11	1	6	4	7	3
Moderate	8	3	0	0	2	6	1	0
Larynx								
Number Examined	16	16	16	15	9	10	9	8
Myofiber, Degeneration								
Minimal	7	5	3	1	5	2	2	1
Slight	3	0	0	0	0	0	0	0
Tongue								
Number Examined	16	16	16	16	9	10	9	8
Myofiber, Degeneration								
Minimal	6	2	2	0	4	3	1	2
Intravenous Injection Site								
Number Examined	16	15	16	16	9	10	9	8
Infiltrate, Macrophages								
Minimal	0	0	0	13	0	0	0	6
Fibrosis, Perivascular								
Minimal	2	2	2	6	0	1	2	0

Incidence and Severity of Select Microscopic Findings in non-mdx (C57BL/6NCrl)Mice - Terminal and Recovery Sacrifices

	AVI-4225			
Strain	C57BL/6NCrl mice			
Sacrifice	Terminal		Recovery	
Dose Level (mg/kg/dose)	0	960	0	960
Kidney				
Number Examined	16	16	9	11
Cytoplasmic Basophilia/Microvacuolation, Tubules				
Minimal	0	0	0	6
Slight	0	3	0	3
Moderate	0	13	0	0
Degeneration, Tubule				
Minimal	0	7	0	5
Intravenous Injection Site				
Number Examined	15	16	8	11
Infiltrate, Macrophages				
Minimal	0	13	0	3
Fibrosis, Perivascular				
Minimal	0	4	0	4
Slight	0	0	0	1

(pages 39 and 40 of Study Report)

Kidney findings of cytoplasmic basophilia with or without microvacuolation and tubular degeneration in MD and HD *mdx* mice and in HD non-*mdx* mice were "characterized by the presence of variably basophilic-staining cortical tubules with microvacuolated to finely granular cytoplasm" likely representing uptake of AVI-4225. Minimal tubular degeneration was described as scattered and multifocal,

> "with deeply basophilic-staining, swollen and vacuolated epithelial cells that often filled the lumen of mildly dilated tubular segments, or by mildly ectatic tubules lined by attenuated and vacuolated basophilic-staining epithelium and containing amphophilic fluid and occasionally low numbers of sloughed degenerate cells" *(page 37 of Study Report).*

These changes in kidney were maintained after 8 weeks of recovery, though there was a reduction in incidence and severity of cytoplasmic basophilia/microvacuolation in the non-*mdx* mice.

Drug-related increases in minimal macrophage infiltration and minimal perivascular fibrosis were observed at the injection site at the HD in *mdx* and non-*mdx* mice.

Dose-related reductions in myofiber degeneration were observed in biceps femoris, quadriceps, diaphragm, larynx, and tongue in *mdx* mice, consistent with the anticipated pharmacodynamic activity of AVI-4225 on dystrophic muscle. These changes persisted throughout the 8-week recovery period. Non-*mdx* mice did not show myofiber degeneration, as expected.

Hormone Levels

Blood samples were collected from nonfasted satellite animals via decapitation prior to dosing initiation, during Week 26 of dosing, or during Week 8 of recovery. Serum was analyzed for levels of follicle-stimulating hormone (FSH) by radioimmunoassay. Planned analysis of serum luteinizing hormone and testosterone levels were not conducted because sample volumes were insufficient for many animals.

No effects were observed on serum FSH levels in *mdx* or non-*mdx* mice.

Sperm Assessments

The following main study animals were used for analysis of sperm parameters: the first 6 control animals (sacrificed predose); the first 6 animals/group at terminal sacrifice; and the first 4 animals/group at the recovery sacrifice. Right and left vasa deferentia were analyzed for sperm motility. Epididymides were analyzed for total sperm count and sperm morphology.

No drug-related effects were observed on sperm motility, count, density, or morphology.

Dosing Solution Analysis

Two sets of duplicates samples from the top, middle, and bottom of LD and HD solutions from Day 1 were analyzed for homogeneity. Two sets of duplicates from control, LD, MD, and HD solutions from Weeks 1, 8, 13, and 26 were analyzed for concentration.

All homogeneity and concentration samples were within 10% of target concentrations. No significant levels of drug were found in control samples.

Toxicokinetics

Blood samples were collected via cardiac puncture after carbon dioxide inhalation on Days 1 and 176 at 0.0833, 0.25, 0.5, 1, 2, 4, 8, and 24 hrs postdose from satellite TK animals (3 mice per time point, with a few exceptions for which only one or two animals were available).

As shown in the sponsor's summary table below, AVI-4225 plasma exposures generally increased greater than dose-proportionately; accumulation in plasma with repeated dosing was not observed. Consistently high mean V_d values suggest that AVI-4225 distributed into tissues. Mean AVI-4225 plasma exposures at the end of the dosing period were similar in *mdx* and non-*mdx* mice given 960 mg/kg, but lower in the latter on Day 1.

Toxicokinetic Parameters for AVI-4225 in Male Mouse Plasma

Interval	Dose Group[a]	Dose Level (mg/kg/dose)	C_0 (ng/mL)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} [(ng•hr/mL)/(mg/kg/dose)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	V_d (mL/kg)	CL (mL/hr/kg)	MRT (hr)	Accumulation Ratio AUC_{0-t}
Day 1	2	12	72463	44767	3731	0.0833	16709	1392	16728	1.02	1054	717	0.407	NA
	3	120	852920	488667	4072	0.0833	226079	1884	NC	NC	NC	NC	NC	NA
	4	960	6496028	5046667	5257	0.0833	3431680	3575	3432395	3.09	1248	280	0.806	NA
	6	960	7252412	3673333	3826	0.0833	1493572	1556	NC	NC	NC	NC	NC	NA
Day 176	2	12	15560	15077	1256	0.0833	18367	1531	NA	1.01	877	601	1.01	1.10
	3	120	340142	258033	2150	0.0833	283738	2364	NA	2.31	1411	423	1.59	1.26
	4	960	92188	449667	468	1.00	2198932	2291	NA	1.87	1179	436	4.32	0.641
	6	960	190000	606500	632	0.250	1720313	1792	NA	2.51	2015	557	4.26	1.15

NA Not applicable.
NC Not calculated.
a Groups 2 through 4 consisted of dystrophic mdx mice (C57BL/10ScSn-DMDmdx/J mice), while Group 6 consisted of C57BL/6NCrl (non-mdx) mice

(page 9 of Final Toxicokinetic Report; t = 24 hrs)

39-Week Intravenous Injection Toxicity and Toxicokinetic Study of Eteplirsen in Sexually Mature Male Cynomolgus Monkeys with an 8-Week Recovery

Study number:	4658-tox-001; (b) (4) Study 8243147
Study report location:	edr
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 22, 2011 (dosing initiation)
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	AVI-4658: Lot # DEV-506, LY01, purity 95% Lot # DEV-503, LY01, purity 94% Lot # DEV-518, LY01, purity 92%

Key Study Findings

- Dose-related minimal to moderate multifocal basophilic cytoplasm was observed in renal tubules (LD: 4/5, minimal; MD: 1 minimal, 4 slight; HD: 5 moderate). These changes were reversible in LD and MD, but not in HD recovery animals.
- Two of 3 HD recovery animals showed the following additional changes in the kidneys: minimal to slight intratubular basophilic granular material, tubular dilatation and degeneration, and/or mononuclear inflammation.
- The NOAEL for once weekly dosing in monkey was the HD of 320 mg/kg.

Methods

Doses:	0, 5, 40, 320 mg/kg ETEPLIRSEN
Rationale:	320 mg/kg was the maximum feasible dose based on solubility
Frequency of dosing:	once weekly (Q1W) for 39 weeks
Route of administration:	IV injection over at least one minute via the saphenous vein
Dose volume:	3.2 mL/kg
Formulation/Vehicle:	Dulbecco's PBS w/o Mg^{++} or Ca^{++}
Species/Strain:	Cynomolgus monkey; Chinese origin
Number/Sex/Group:	5 M/group main study; 3 M/group 8-wk recovery
Age:	5-6 years old at dosing initiation
Weight:	5.3-7.2 kg at dosing initiation
Satellite groups:	n/a
Unique study design:	Testicular and semen volume, sperm parameters; hormone levels; urinary cystatin C & KIM-1; complement activation analysis
Deviation from study protocol:	Minor deviations were reported; these did not affect the overall interpretation or validity of the study

Group[a]	No. of Animals[b] Male	Dose Level (mg/kg)	Dose Concentration (mg/mL)
1 (Control)[c]	8	0	0
2 (Low)	8	5	1.56
3 (Mid)	8	40	12.5
4 (High)	8	320	100.0

a Animals received a dose volume of 3.2 mL/kg.
b Three animals/group were designated as recovery animals and were dosed with test or vehicle control article for 39 weeks, after which dosing was discontinued, and animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for 8 weeks after dosing.
c Group 1 received vehicle control article [Dulbecco's Phosphate Buffered Saline (1x PBS) without magnesium or calcium] only.

(page 13 of Study Report)

Observations and Results

Mortality

All animals were observed twice daily for mortality.

All animals survived to scheduled termination.

Clinical Signs

All animals were evaluated by cageside observation once daily (except on days of detailed observations). Detailed observations were conducted 5 times predose, prior to dosing on Day 1, once weekly thereafter within 10 minutes of dosing, and on days of scheduled sacrifice.

No drug-related clinical signs were reported.

Body Weights

Body weights were recorded 5 times predose, prior to dosing on Day 1, and weekly thereafter.

No drug-related effects on body weight were reported.

Food Consumption

Food consumption was recorded qualitatively once daily during predose, dosing, and recovery phases.

No drug-related effects on food consumption were observed.

Ophthalmoscopy

Ophthalmic examinations using an indirect ophthalmoscope and slit lamp were performed under ketamine anesthesia, after dilation with a mydriatic agent, once predose and once during Week 38.

No drug-related ophthalmology findings were reported.

ECG

Eight-lead ECGs were recorded under ketamine anesthesia once predose, during Week 38 prior to dosing and 1-2 hours postdose, and once within the final 8 days of recovery.

No drug-related effects on ECG parameters were reported.

Hematology

Blood samples were collected from all animals after overnight fast via a femoral vein twice predose; during Weeks 13, 26, and 39 at ~24 hours postdose; and on the day of sacrifice in recovery animals. The following parameters were analyzed: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin, concentration, platelet count, white blood cell count, differential blood cell count, reticulocyte count, blood cell morphology, prothrombin time, and activated prothrombin time.

White blood cell, neutrophil, and monocyte counts were decreased in MD (32%, 38%, and 22%, respectively) and HD (41%, 50%, and 38%, respectively) in Week 39 compared to controls. These changes are not considered adverse based on their mild severity and reversibility.

Clinical Chemistry

Blood samples were collected from all animals after overnight fast via a femoral vein twice predose; during Weeks 13, 26, and 39 at ~24 hours postdose; and on the day of sacrifice in recovery animals. The following parameters were analyzed: urea nitrogen, total protein, albumin, globulin, albumin/globulin ratio, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, gamma glutamyl transferase, calcium, chloride, cholesterol, creatinine, glucose, inorganic phosphorus, potassium, sodium, total bilirubin, sorbitol dehydrogenase, creatine kinase, triglycerides.

No drug-related effects on clinical chemistry parameters were reported.

Urinalysis

Urine samples were collected over 24 hours from animals fasted overnight (≥ 10 hours) once predose; from 0 to 24 hours after dosing on Days 1, 85, 176, and 260; and for 24 hours prior to dosing on Days 8, 92, 183, 267, and prior to sacrifice in recovery animals. Parameters analyzed included creatinine, cystatin C/creatinine ratio, KIM-1/creatinine ratio, quantitative total protein, quantitative total protein/creatinine ratio, clarity, color,

volume, specific gravity, pH, protein, glucose, ketones, bilirubin, urobilinogen, blood, and microscopic examination of sediment.

No drug-related effects on urinalysis parameters were reported.

Gross Pathology

Necropsy was conducted after overnight fast on 5 animals/group ~48 hours after the final (39th) weekly dose; and on 3/animals/group after 8 weeks (57 days) of recovery. Animals were terminated by exsanguination under sodium pentobarbital anesthesia.

No drug-related macroscopic effects were reported.

Organ Weights

Weights were recorded for the following organs: adrenal glands, brain, epididymides, heart, kidneys, liver with gallbladder (drained), lung, pituitary gland, prostate, salivary glands (mandibular), seminal vesicles, spleen, testes, thymus, thyroid/parathyroid.

No drug-related effects on organ weights were reported.

Histopathology

The following tissues from all animals were examined microscopically: adrenal glands, aorta, bone and bone marrow (sternum, femur), brain, cecum, colon, duodenum, epididymides, esophagus, eyes, gallbladder, heart, ileum, IV injection site, jejunum, kidneys, liver, lung with large bronchi, lymph nodes (mandibular, mesenteric), muscle (biceps femoris), optic nerve, pancreas, pituitary gland, prostate gland, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicle, skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, urinary bladder, gross lesions (if any).

The battery of tissues examined was adequate.

No Peer Review was conducted; a signed pathology report was provided.

Drug-related microscopic effects were limited to the kidneys. As shown in the sponsor's summary tables below, dose-related increases were observed in the incidence and severity of multifocal basophilic cytoplasm in renal tubules. These changes were reversible in LD and MD, but not in HD animals. Two of 3 HD recovery animals showed the following additional minimal to slight changes in the kidneys: intratubular basophilic granular material, tubular dilatation and degeneration, and mononuclear inflammation.

Accumulation of basophilic material in renal tubules is often observed in animals injected with oligonucleotide compounds, and likely represents accumulation of the test article in the cytoplasm. Such changes are generally not considered adverse unless they are associated with other microscopic changes (e.g., degeneration or necrosis) or impaired renal function.

Incidence and Severity of Test Article-Related Microscopic Findings - Terminal Sacrifice

	AVI-4658			
Sex	Males			
Dose Level (mg/kg)	0	5	40	320
Kidney				
Number Examined	5	5	5	5
Basophilic cytoplasm, tubules				
Not Present	5	1	0	0
Minimal	0	4	1	0
Slight	0	0	4	0
Moderate	0	0	0	5
Average Severity	0.0	0.8	1.8	3.0

Incidence and Severity of Test Article-Related Microscopic Findings - Recovery Sacrifice

	AVI-4658			
Sex	Males			
Dose Level (mg/kg)	0	5	40	320
Kidney				
Number Examined	3	3	3	3
Basophilic cytoplasm, tubules				
Not Present	3	3	3	0
Slight	0	0	0	2
Moderate	0	0	0	1
Average Severity	0.0	0.0	0.0	2.3
Basophilic granular material, intratubular				
Not Present	3	3	3	1
Minimal	0	0	0	2
Average Severity	0.0	0.0	0.0	0.7
Dilatation, tubule(s)				
Not Present	3	3	3	1
Minimal	0	0	0	1
Slight	0	0	0	1
Average Severity	0.0	0.0	0.0	1.0
Degeneration, tubule(s)				
Not Present	3	3	3	1
Minimal	0	0	0	1
Slight	0	0	0	1
Average Severity	0.0	0.0	0.0	1.0
Inflammation, mononuclear				
Not Present	3	3	3	1
Minimal	0	0	0	1
Slight	0	0	0	1
Average Severity	0.0	0.0	0.0	1.0

(pages 3-4 of Anatomic Pathology Report)

Testicular Measurements

Testicles were measured by a trained technician twice during the predose phase; during Weeks 13, 26, and 38 of dosing; and within 7 days of recovery sacrifice.

No drug-related effects on testicular volume were reported.

Semen Analysis

Semen was collected by a trained technician twice during the predose phase; during Weeks 13, 26, and 38 of dosing; and prior to recovery sacrifice. Parameters evaluated included semen volume; and sperm number, concentration, percent mobility, and morphology.

No drug-related effects on semen volume or sperm parameters were reported.

Hormone Levels

Blood samples were collected via the femoral vein twice predose; during Weeks 13, 26, and 38; on the day of scheduled termination; and within 7 days of recovery sacrifice. Serum was evaluated for levels of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) by radioimmunoassay (FSH & LH) or solid-phase chemiluminescent immunoassay (testosterone). Samples from Week 38 were discarded without analysis.

No drug-related effects on serum testosterone, FSH, or LH were reported.

Complement Activation Analysis

Blood samples were collected from nonfasted animals via a femoral vein at 10 minutes, 1 hour, and 6-8 hours postdose on Days 1 and 8 of dosing; during Weeks 13 and 39; and once prior to recovery sacrifice. Day 1 samples were analyzed for plasma levels of C3a-des-Arg, C5a-des-Arg, and Bb by enzyme immunoassay. Day 8, Week 13, and Week 39 samples were analyzed for serum levels of the complement alternative pathway component, C5b-C9, by enzyme immunoassay.

The concentration of the Bb fragment of complement increased on Day 1 in HD (12% at 1 hour postdose; 67.7% at 6-8 hours postdose) compared to controls, indicating partial activation of the alternative complement pathway. However, C3a-des-Arg concentrations, which typically increase following activation of complement pathways, were decreased on Day 1 in HD animals (18.8% at 1 hour postdose; 29.1% at 6-8 hours postdose), and the C5a-des-Arg fragment was below detectable levels at all time points in HD animals. These data suggest that the drug-induced activation of the complement pathway indicated by the increase in the Bb fragment was too weak to activate the terminal pathway of complement. No consistent effects on complement fragments were observed on Day 8, Week 13, Week 39, or prior to sacrifice of recovery animals.

Dosing Solution Analysis

Two sets of duplicates samples from the top, middle, and bottom of LD and HD solutions from Day 1 were analyzed for homogeneity. Two sets of duplicates from control, LD, MD, and HD solutions from Weeks 1, 4, 8, 13, 26, and 39 were analyzed for concentration.

All homogeneity and concentration samples were within 10% of target concentrations. No significant levels of drug were found in control samples.

Toxicokinetics

Blood samples were collected from nonfasted animals via a femoral or saphenous vein on Day 1 and on the last day of dosing at the following timepoints: predose, and 0.25, 0.5, 1, 2, 4, 12, and 36 hours postdose.

As shown in the sponsor's summary tables below, eteplirsen plasma exposures generally increased dose-proportionately; accumulation in plasma with repeated dosing was not observed. Mean V_d at the HD (810 mL/kg in Week 39) was only slightly greater than the total body water in cynomolgus monkey (693 mL/kg), suggesting that eteplirsen may not be highly distributed into the tissues.

Summary of the Mean Toxicokinetic Parameters for AVI-4658 in Cynomolgus Monkey Plasma: Day 1

Dose Group	Dose Level (mg/kg)		C_0 (ng/mL)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} [(ng•hr/mL)/(mg/kg)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	V_d (mL/kg)	CL (mL/hr/kg)	MRT (hr)
2	5	Mean	70059	32525	6505	0.250	38035	7607	38250	1.93	380	137	1.23
		SD	18319	8802	1760	0	9668	1934	9761	0.14	78	29	0.17
		N	8	8	8	8	8	8	8	8	8	8	8
3	40	Mean	589547	268250	6706	0.254	346523	8663	346691	4.02	698	120	1.56
		SD	82234	55107	1378	0.012	84991	2125	85022	0.14	147	24	0.13
		N	8	8	8	8	8	8	8	8	8	8	8
4	320	Mean	4197411	1847500	5773	0.250	2730176	8532	2731652	3.94	743	129	1.92
		SD	689521	335250	1048	0	972815	3040	973609	0.31	264	38	0.69
		N	8	8	8	8	8	8	8	8	8	8	8

Table 2
Summary of the Mean Toxicokinetic Parameters for AVI-4658 in Cynomolgus Monkey Plasma: Week 39

Dose Group	Dose Level (mg/kg)		C_0 (ng/mL)	C_{max} (ng/mL)	DN C_{max} [(ng/mL)/(mg/kg)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} [(ng•hr/mL)/(mg/kg)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	V_d (mL/kg)	CL (mL/hr/kg)	MRT (hr)	AR AUC_{0-t}
2	5	Mean	83655	39438	7888	0.250	48494	9699	NA	2.35	335	102	NA	1.33
		SD	24064	13898	2780	0	19202	3840	NA	1.16	146	32	NA	0.63
		N	8	8	8	8	8	8	0	7	7	7	0	8
3	40	Mean	745916	348125	8703	0.250	433848	10846	NA	4.61	692	101	1.61	1.26
		SD	138728	98667	2467	0	144782	3620	NA	0.48	314	37	0.25	0.33
		N	8	8	8	8	8	8	0	7	7	7	7	8
4	320	Mean	4184465	1843750	5762	0.281	2551895	7975	NA	4.24	810	132	1.95	1.00
		SD	1754318	508638	1589	0.088	608503	1902	NA	0.17	218	31	0.61	0.35
		N	8	8	8	8	8	8	0	8	8	8	8	8

NA Not applicable.

(page 9 of Final Toxicokinetic Report; t = 36 hrs)

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Bacterial Reverse Mutation Assay

Study number:	AVI-4658-GLP-0701
	(b) (4) Study AC07WC.503.BTL
Study report location:	EDR SDN1 May 20, 2015
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 4, 2007
GLP compliance:	Yes; except for analyses of dose formulations, which were in compliance with GMP
QA statement:	Yes
Drug, lot #, and % purity:	AVI-4658 Lot # 37GD-LY01(XX01) (Purity, 96%)

Key Findings:

In the in vitro bacterial reverse mutation assay with the plate incorporation method, eteplirsen was negative for genotoxicity in all strains tested, in the presence and absence of Aroclor-induced rat liver S9 metabolic activation.

Methods

Strains:	*S. typhimurium* tester strains TA98, TA100, TA1535, and TA1537; *E. coli* tester strain WP2 *uvr*A
Concentrations in confirmative assay:	50, 150, 500, 1500, 5000 µg/plate eteplirsen ± S9
Basis of concentration selection:	Absence of mutagenic responses, bacterial lawn toxicity, or precipitates observed in an initial assay at up to 5000 µg/plate ± S9.
Negative control:	Sterile water for injection
Positive controls:	-S9: sodium azide (TA1535, TA100), 2-nitrofluorene (TA98), 9-aminoacridine (TA1537), and methyl methanesulfonate (WP2 *uvr*A). +S9: 2-aminoanthracene (all tester strains)
Formulation/Vehicle:	Sterile water for injection
Incubation & sampling time:	48-72 hours at 37°C. Initial plate incorporation (duplicate). Confirmatory plate incorporation (triplicate). Metabolic activation: liver S9 from Aroclor 1254-induced male Sprague-Dawley rats.

Study Validity

Selection of bacterial tester strains was adequate based upon current guidelines. Positive and negative controls produced expected responses. Dose selection was adequate based upon use of the limit concentration 5000 µg/plate. The S9 concentration (10%) was within acceptable limits. 2-aminoanthracene was used as the positive control for all tester strains in the presence of metabolic activation, and the activity of the S9 preparation was adequately characterized by the sponsor, as recommended by OECD guidelines. Analysis of pre-dose and post-dose samples of bulk formulations confirmed that concentrations of the test article were within 10% of nominal concentrations.

Results

Criteria for a positive response were provided in the study report. Positive findings had to show increases in the mean number of revertants per plate that were dose-related over at least two increasing concentrations. For tester strains, TA98, TA100, and WP2uvrA, the test article was considered positive if it produced at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. For tester strains TA1535 and TA1537, the test article was considered positive if it produced at least a 3-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control.

No positive mutagenic responses were observed with any of the tester strains in the presence or absence of metabolic activation in the initial or confirmatory plate incorporation study. No cytotoxicity or precipitation was observed.

Conclusion

All criteria for a valid study were met. Eteplirsen was negative for mutagenicity in the in vitro bacterial mutation assay in the presence and absence of metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

In Vitro Mammalian Chromosome Aberration Test

Study no.:	AVI-4658-GLP-0703
	(b) (4) Study AC07WC.331.BTL
Study report location:	EDR SDN1 May 20, 2015
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 26, 2007
GLP compliance:	Yes; except for analyses of dose formulations, which were in compliance with GMP
QA statement:	Yes
Drug, lot #, and % purity:	AVI-4658 Lot # 37GD-LY01(XX06) (Purity, 96%)

Key Findings:

In the in vitro chromosome aberration assay using CHO cells, eteplirsen was negative in the presence and absence of rat liver S9 metabolic activation.

Methods

Cell line:	Chinese hamster ovary (CHO) cells
Concentrations in definitive study:	1250, 2500, 5000 µg/mL eteplirsen for all treatments
Basis of concentration selection:	preliminary cytotoxicity assessment
Negative control:	water for injection
Positive control:	-S9: mitomycin C; +S9: cyclophosphamide
Formulation/Vehicle:	water for injection
Incubation time:	-S9: 4 and 20 hours; +S9: 4 hours
Sampling time:	20 hours after start of incubation
Metabolic activation system:	Aroclor 1254-induced male S-D rat liver S9

Study Validity

The following criteria for a valid study were listed in the study report, and were met: 1) “The frequency of structural chromosome aberrations in the solvent control must be within the range of the historical solvent control”; and 2) “The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher’s Exact test) relative to the solvent control.” Dose selection was adequate, based upon use of the limit dose for this assay, 5000 µg/mL. The S9 concentration used (2%) was within acceptable limits. The study was performed using standard procedures. Analysis of pre-dose and post-dose samples of the stock 50 mg/mL eteplirsen formulation confirmed that concentrations of eteplirsen were within 10% of nominal concentrations.

Results

A positive response was defined in the study report as one in which “the percentage of cells with aberrations was increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$).” A negative response was defined as one in which the test article did not demonstrate a statistically significant increase in chromosome aberrations.

No statistically significant increases in structural or numerical chromosomal aberrations (relative to solvent control) were observed in CHO cells incubated with eteplirsen at up to 5000 µg/mL in the presence or absence of rat liver S9 metabolic activation. No precipitate or toxicity was observed up to the highest concentration tested, 5000 µg/mL, which was associated with reductions in mitotic index (relative to solvent control) of 8%, 17%, and 2% in the 4-hour assay without S9, the 20-hour assay without S9, and the 4-hour assay with S9, respectively.

Conclusion

All criteria for a valid study were met. Eteplirsen was negative in the in vitro chromosomal aberration assay in CHO cells in the presence and absence of metabolic activation.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Mouse Bone Marrow Erythrocyte Micronucleus Test following Intravenous Administration of Eteplirsen

Study no:	AVI-4658-GLP-0702
	(b) (4) Study AC07WC.123.BTL
Study report location:	EDR SDN1 May 20, 2015
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 7, 2008
GLP compliance:	Yes; except for analyses of dose formulations, which were in compliance with GMP
QA statement:	Yes
Drug, lot #, and % purity:	AVI-4658 Lot # 38GD-LY01(XX01) (Purity, 96%)

Key Findings:
Eteplirsen was negative in the in vivo mouse bone marrow micronucleus assay.

Methods

Doses in definitive study:	24-hr: 0, 500, 1000, 2000 mg/kg eteplirsen 48-hr: 0, 2000 mg/kg eteplirsen
Frequency of dosing:	single dose
Route of administration:	IV injection into the lateral tail vein
Dose volume:	5, 10, or 20 mL/kg
Formulation/Vehicle:	PBS
Species/Strain:	ICR mice
Number/Sex/Group:	5
Satellite groups:	5/sex replacement mice, 2000 mg/kg eteplirsen
Basis of dose selection:	dose range-finding study: only piloerection was observed in 5/5 M & F at 2000 mg/kg
Negative control:	PBS
Positive control:	cyclophosphamide monohydrate, 50 mg/kg

Study Validity

The study was deemed valid for the following reasons: 1) previous pharmacokinetic assessments demonstrated systemic exposure in mice after IV injection of eteplirsen; 2) dose selection was adequate based on the use of the limit dose for this assay, 2000 mg/kg; 3) preparation and administration of the test substance was acceptable, based on analyses of the bulk dosing solution; 4) the species and number of animals/sex/group were acceptable; 5) tissue sampling and analysis was acceptable; and 6) the proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

The following additional criteria for a valid study were listed in the study report, and were met: 1) the positive control must induce a statistically significant increase in the incidence of micronucleated polychromatic erythrocytes compared to vehicle controls; 2) the incidence of micronucleated polychromatic erythrocytes observed in the vehicle control group should not exceed the historical vehicle control range.

Results

A positive response was defined in the study report as one in which “a dose-responsive increase in the incidence of micronucleated polychromatic erythrocytes is observed and one or more doses are statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time.” A negative result was defined as one in which “no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the test article groups above the concurrent negative (vehicle) controls is observed at any sampling time” *(page 14 of Study Report)*.

Single doses of up to 2000 mg/kg IV eteplirsen did not induce significant increases (compared to vehicle control) in the incidence of micronucleated polychromatic erythrocytes in bone marrow of male or female ICR mice. Reductions in mean PCE/EC ratios were limited to ≤13% in drug-treated groups, suggesting that erythropoiesis was not inhibited by treatment with a single IV dose of eteplirsen.

Conclusion

All criteria for a valid study were met. Eteplirsen was negative in the in vivo mouse bone marrow micronucleus assay.

8 Carcinogenicity

No carcinogenicity studies have been conducted. A Request for Deferral of Nonclinical Assessment of Eteplirsen Carcinogenicity was provided with the NDA submission, citing the agency's determination that the assessment of carcinogenicity for eteplirsen may be conducted as a post-marketing requirement (End of Phase 2 Meeting Minutes dated 12 April 2013). The sponsor provided the following justifications for the request for deferral:

1) Eteplirsen was not genotoxic in the ICH standard battery of assays: in vitro bacterial mutation and mammalian chromosome aberration, and in vivo mouse bone marrow micronucleus.
2) No pre-neoplastic or other proliferative lesions were observed in the 39-week toxicity study in monkey.
3) No treatment-related clinically significant adverse events have been observed in clinical studies of > 3 years of once-weekly IV administration of eteplirsen.
4) DMD is a life-threatening and severely disabling disease for which no satisfactory alternative therapy exists.

The sponsor's justification is acceptable.

The sponsor plans to conduct a 26-week study in transgenic mice and a 2-year study in rats to assess the carcinogenicity of eteplirsen. The proposed date for SPA submission of carcinogenicity protocols and supporting dose range-finding data is the first quarter of 2016, at the earliest, with dosing projected to begin in the second quarter of 2016; final reports are projected to be available for submission by the fourth quarter of 2017 (Tg mouse) and the first quarter of 2019 (rat).

9 Reproductive and Developmental Toxicology

Assessments of the effects of eteplirsen on female fertility, embryofetal development, and early postnatal development were not conducted because the DMD patient population is almost entirely male. No drug-related effects on the male reproductive system were observed in the 39-week monkey and 10-week juvenile rat studies of eteplirsen or in the 26-week *mdx* and non-*mdx* mouse studies of AVI-4225. Testicular histology in the monkey study was conducted in a stage-aware manner. No drug-related effects were observed on developmental parameters or learning and memory performance included in the 10-week toxicity study in juvenile rat (day of preputial separation, auditory startle habituation, motor activity, water maze, functional observation battery, bone dimensions) with one exception: dose-dependent reductions were observed in bone length, width, area, mineral content, and mineral density.

11 Integrated Summary and Safety Evaluation

Eteplirsen is a 30-base PMO antisense oligonucleotide designed to interact specifically with the pre-mRNA of the human dystrophin gene resulting in the exclusion ("skipping") of exon 51, restoration of an open reading frame in the mRNA, and production of an internally truncated (but still functional, if less so than full-length) dystrophin protein in the 13% of DMD patients with mutations causing deletions in exons 45-50, 47-50, 48-50, 49-50, 50, 52, or 52-63. The proposed therapeutic dosing regimen is 30 mg/kg once weekly via IV infusion.

The ability of eteplirsen to induce exon 51 skipping in human dystrophin mRNA has been demonstrated in the following: human muscle cell cultures; muscle explants from a DMD patient; gastrocnemius muscle of transgenic hDMD mice (after direct IM injection); quadriceps muscle, heart, and diaphragm of cynomolgus monkey (after 12 weeks of once weekly IV infusions); and EDB muscle of 7 DMD patients (after direct IM injection). Induction of dystrophin protein by treatment with eteplirsen was shown by Western blot in muscle cell cultures derived from a DMD patient with a deletion of exon 50 and in EDB muscle of 5 DMD patients directly injected; the latter also showed dystrophin immunoreactivity by IHC in 44-79% of EDB muscle fibers.

The sponsor summarized several published studies investigating the feasibility of exon skipping to restore production of dystrophin protein using species-specific PMOs in animal models of DMD (AVI-4225 targeting exon 23 in *mdx* mice, and a cocktail of 3 PMOs targeting exons 6 and 8 in CXMD dogs). These studies generally showed induction of exon skipping by PCR and expression of dystrophin protein by IHC and/or Western blot in skeletal muscles, but little or none in heart. Reductions in muscle pathology and/or impairment of muscle function were also demonstrated, but results varied depending on the specific assays and dosing regimens used. Because of the small number of animals used (4-7 animals per group in most) and the lack of detailed data presented in these published studies, the claims of functional improvement remain to be definitively established. The most consistent finding among these studies was that the extent of PMO-induced expression of dystrophin protein varied widely within each muscle examined, between samples from the same type of muscle in the same or different animals, and between different types of muscle. Therefore, caution should be used in generalizing from the results of muscle biopsies from only a few sites, muscles, and/or patients.

No toxicologically important effects were observed in a safety pharmacology study in cynomolgus monkeys given single injections of eteplirsen at up to 320 mg/kg SC or IV.

An absorption, distribution, metabolism, and excretion study of IV ^{14}C-eteplirsen in adult male *mdx* mice showed rapid clearance ($t_{½}$ = 6.03 hrs) from plasma, highest concentration in kidneys, low concentration in brain, and excretion primarily via urine (~60%). Plasma protein binding of eteplirsen was low (0.2-25.4%) in mouse, rat, monkey, and human. ^{14}C-eteplirsen was not significantly metabolized by liver microsomes in in vitro studies. Eteplirsen (up to 7 mg/mL) showed slight induction of

CYP1A2 in in vitro studies of primary human hepatocytes (2.5- to 6-fold for mRNA, 4- to 7.5-fold for enzyme activity), and significant inhibition of CYPs 1A2, 2C9, and 2C19 in human liver microsome preparations (IC_{50} = 6.52, 2.75, and 1.16 mg/mL, respectively). In vitro studies of the ability of eteplirsen to act as a substrate or inhibitor of human drug transporters showed weak inhibition of OCT1 and OATP1B1.

The toxicology of eteplirsen was assessed in *mdx* and non-*mdx* (normal) C57BL mice (12-week IV and SC Q1W), juvenile Sprague-Dawley rats (single dose IV and 10-week IV Q1W), adult Sprague-Dawley rats (single dose), and cynomolgus monkeys (12-week IV and SC Q1W and 39-week IV Q1W). Only males were included in most studies because DMD is an X-linked disease presenting almost exclusively in males. In all studies, the primary target organ was the kidney. In the pilot single-dose study, male juvenile and adult rats showed increased BUN, tubular vacuolation/basophilia, and hemorrhage in renal papilla at the HD of 960 mg/kg IV eteplirsen; adult HDM rats also showed increased urinary protein, urine protein/creatinine ratio, tubular dilatation, basophilic and hyaline cast, and minimal to slight tubular necrosis. In the pivotal 10-week study in juvenile rat, dose-related kidney histopathology findings (tubular vacuolation/basophilia/dilatation/necrosis) were correlated at the HD (900 mg/kg) with clinical pathology changes consistent with impairment in renal function in blood (increased BUN and creatinine) and urine (decreased creatinine clearance, cystatin C/creatinine ratio, pH, and chloride; increased protein/creatinine ratio and incidence of blood in urine); the NOAEL was the MD of 300 mg/kg. Dose-related reductions were observed in juvenile rats in bone length, width, area, mineral content, and mineral density, achieving statistical significance at the HD and showing dose-related trends in some parameters at the MD and/or LD.

In a 3-month study, vacuolation/basophilia and degeneration/necrosis in kidney were observed at the HD of 960 mg/kg IV eteplirsen in *mdx* and/or non-*mdx* mice, as well as increased lymphoid necrosis in spleen and thymus.

In a 3-month study of eteplirsen in cynomolgus monkey, renal tubular vacuolation/basophilia and basophilic granules were observed at the HD of 320 mg/kg IV and SC. In the pivotal 39-week study in monkey, dose-related increases were observed in renal tubular basophilia and HDM recovery animals also showed minimal to slight tubular dilatation, degeneration, and mononuclear inflammation. The NOAEL was the HD of 320 mg/kg IV eteplirsen based on the mild severity of the renal changes and the absence of effects on clinical pathology parameters typical of impaired renal function.

The toxicology of AVI-4225, the mouse surrogate PMO targeting exon 23, was assessed in *mdx* and non-*mdx* mouse studies (12-week IV and SC Q1W, and 26-week IV Q1W). In both studies, dose-dependent reductions were observed in the incidence and severity of myofiber degeneration in muscles (larynx, biceps femoris, diaphragm, quadriceps, and tongue) of *mdx* mice, consistent with the anticipated pharmacodynamic action, increased expression of dystrophin protein. Both studies also showed minimal to slight renal tubular vacuolation/basophilia and macrophage infiltration of the injection

site at the HD of 960 mg/kg IV or SC AVI-4225. HD animals in the 26-week study also showed scattered minimal multifocal renal tubular degeneration, which was still evident after the 8-week recovery period. The renal changes are not considered adverse, based on their minimal severity. Unexpectedly, dose-dependent dilatation of the lateral ventricles in the brain was observed at the MD (minimal to mild) and HD (minimal to moderate) in *mdx* mice only in the 26-week study, and was considered adverse at the HD of 960 mg/kg IV AVI-4225 based on the moderate severity of the changes. The mechanism of this effect, and its relevance to humans treated with eteplirsen, is not clear. The NOAEL in this study was the MD of 120 mg/kg IV AVI-4225.

As shown in the table below, safety margins based on AUCs at the NOAELs in the pivotal toxicity studies in monkey and juvenile rat are 20-fold and 6-fold, respectively, compared to the mean AUC observed in DMD patients dosed once weekly with 30 mg/kg IV eteplirsen for 124 or 152 weeks (N=6; 2 were switched to this dose after 28 weeks on placebo).

Summary of Key Toxicities and Plasma Exposures in Pivotal Chronic Studies

Toxicity	Duration & Species	NOAEL (Q1W)	Mean $AUC_{0\text{-}24\ hr}$# (µg•hr/mL)	Safety Margin Based on AUC*
Renal tubular cytoplasmic basophilia/ vacuolation and tubular degeneration/ necrosis	10-Week Juvenile Rat	300 mg/kg eteplirsen	768	6-fold
	39-Week Cynomolgus Monkey	320 mg/kg eteplirsen	2552	20-fold
	26-Week *mdx* Mouse	960 mg/kg AVI-4225	2199	--
Moderate dilatation of lateral ventricles	26-Week *mdx* Mouse	120 mg/kg AVI-4225	284	--

*$AUC_{0\text{-}24\ hr}$ in human: 127 µg•hr/ml at 30 mg/kg IV eteplirsen Q1W, Week 152, Study 4658-US-202
#$AUC_{0\text{-}36\ hr}$ for the monkey study; however, $AUC_{24\text{-}36\ hr}$ was only ~1% of $AUC_{(0\text{-}36\ hr)}$

Eteplirsen was negative in a standard battery of valid genetic toxicology assays: in vitro bacterial mutation and CHO cell chromosomal aberration assays, and an in vivo mouse bone marrow erythrocyte micronucleus test.

The carcinogenicity of eteplirsen has not yet been assessed. The sponsor plans to conduct a 26-week carcinogenicity study in transgenic mice and a 2-year carcinogenicity study in rats. Based on the severity of the disease and the clear unmet medical need, these studies were not needed to support the NDA.

No reproductive and developmental toxicity studies of eteplirsen were required because the DMD patient population is almost entirely male. No effects on reproductive organs or developmental parameters were observed in the pivotal toxicity studies conducted in adult monkeys or juvenile rats, with the exception of reductions in bone length, width,

area, mineral content, and mineral density observed in juvenile rats at the HD, with dose-dependent trends noted in some parameters at lower doses.

Recommendations
The nonclinical data submitted adequately support the approval of eteplirsen for the treatment of DMD in patients with mutations amenable to exon 51 skipping therapies. Carcinogenicity studies in two species should be conducted as a post-marketing requirement.

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/s/

DAVID B HAWVER
01/22/2016

LOIS M FREED
01/22/2016

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206488 **Applicant:** Sarepta Therapeutics, Inc. **Stamp Date:** June 26, 2015

Drug Name: Eteplirsen **NDA Type:** Original NDA

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?		X	As stated in the Meeting Minutes dated April 12, 2013, and October 17, 2014, the assessment of carcinogenicity for eteplirsen may be conducted post-approval, "if the available nonclinical and clinical data support such a strategy."
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			CSS will address this issue.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			n/a

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

n/a

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

There are no review issues.

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/s/

DAVID B HAWVER
08/04/2015

LOIS M FREED
08/04/2015