20 September 2018
EMA/691796/2018
Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Exondys

International non-proprietary name: eteplirsen

Procedure No. EMEA/H/C/004355/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

CQA	Critical Quality Attribute
EU	European Union
GC	Gas Chromatography
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPC	In process control
IP	Ion pair
IV	Intravenous
LCMS	Liquid chromatography mass spectrometry
NMR	Nuclear Magnetic Resonance
PBS	Phosphate buffered saline
Ph. Eur.	European Pharmacopoeia
PMO	Phosphoridiidate morpholino oligomer
PP	Polypropylene
PTFE	Polytetrafluoroethylene
SCX	Strong Cation Exchange chromatography
SPOS	Solid-phase oligomer synthesis

1. Background information on the procedure

1.1. Submission of the dossier

The applicant AVI Biopharma International Ltd submitted on 30 November 2016 an application for marketing authorisation to the European Medicines Agency (EMA) for Exondys, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 17 December 2015.

Exondys was designated as an orphan medicinal product (EU/3/08/586) on 3 December 2008 in the following condition: treatment of Duchenne muscular dystrophy.

The applicant applied for the following indication:

Treatment of Duchenne muscular dystrophy (DMD) in adults, adolescents, and children aged 4 years and older who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0279/2016 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-001722-PIP01-14-M01 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Applicant's request for consideration

Conditional marketing authorisation

The applicant requested consideration of its application for a Conditional marketing authorisation in accordance with Article 14(7) of Regulation (EC) No 726/2004.

New active Substance status

The applicant requested the active substance eteplirsen contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Protocol assistance

The applicant received Protocol assistance from the CHMP:

Scientific advice	**date**	**Area**
EMEA/H/SA/2892/1/2014/PED/SME/III	18 December 2014	non-clinical and clinical
EMEA/H/SA/2892/1/FU/1/2015/PA/SME/II	17 December 2015	clinical

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Concepcion Prieto Yerro Co-Rapporteur: Kristina Dunder

The application was received by the EMA on	30 November 2016
The procedure started on	23 December 2016
The Rapporteur's first Assessment Report was circulated to all CHMP members on	15 March 2017
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	10 March 2017
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	24 March 2017
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	21 April 2017
The applicant submitted the responses to the CHMP consolidated List of Questions on	12 October 2017
A GMP inspection at 1 site: manufacturing site in the US between 27 March 2017 to 31 March 2017. The outcome of the inspection carried out was issued on	7 June 2017
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	21 November 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	30 November 2017
The Rapporteurs circulated the updated Joint Assessment Report on the responses to the List of Questions to all CHMP members on	7 December 2017

The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	14 December 2017
The applicant submitted the responses to the CHMP List of Outstanding Issues on	19 March 2018
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	12 April 2018
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	26 April 2018
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Exondys on	31 May 2018

1.3. Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Dr Tomas Boran Co-Rapporteur: Dr Greg Markey

The Applicant submitted written notice to the EMA, to request a re-examination of Exondys CHMP opinion of 20 September 2018 on	1 June 2018
The CHMP appointed Tomas Boran as Rapporteur and Greg Markey as Co-Rapporteur on	28 June 2018
The Applicant submitted the detailed grounds for the re-examination on	23 July 2018
The re-examination procedure started on	24 July 2018
The Rapporteur's re-examination assessment report was circulated to all CHMP members on	28 August 2018
The Co-Rapporteur's assessment report was circulated to all CHMP members on	28 August 2018
The Rapporteurs circulated the Joint Assessment Report on the detailed grounds for re-examination to all CHMP members on	11 September 2018
A SAG (Scientific Advisory Group) was convened to address questions raised by the CHMP on	7 September 2018
The detailed grounds for re-examination were addressed by the applicant during an oral explanation before the CHMP on	18 September 2018
The CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application did not satisfy the criteria for authorisation and did not recommend the granting of the conditional marketing authorisation on	20 September 2018

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Exondys is intended to be indicated for the treatment of Duchenne muscular dystrophy (DMD) in ambulatory patients 4 years and older who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping.

2.1.2. Epidemiology

Duchenne muscular dystrophy is a severe, progressive paediatric neuromuscular disorder that is ultimately lethal. It occurs almost exclusively in males (X-linked recessive disorder) with a global incidence of up to 1 in 3500 male births. The estimated prevalence in the EU is approximately 15,000 cases, with an additional 18,000 in the US.

2.1.3. Biologic features

The most common cause of DMD is deletion mutations of one or more DMD exons. Exon 51-skipping amenable mutations occur in approximately 13% of DMD boys, resulting in a prevalence 1950 boys in the EU and 2340 boys in the US. It is caused by the relative absence of functional dystrophin protein due to mutations in the dystrophin gene, most frequently exon deletions.

2.1.4. Clinical presentation, diagnosis and stage

Dystrophin has a structural role as a cytoskeletal stabilisation protein protecting muscle fibres against contraction-induced damage, but also a signalling role including mechano-transduction of forces and localisation of signalling proteins. Lack of dystrophin results, through mechanisms not precisely understood, in degeneration of muscle fibres, attracting inflammatory cells and ultimately replacement by fibrotic tissue and adipose tissue.

The progression of muscle degeneration in DMD is well documented, showing a proximal-to distal progression of muscle weakness leading to progressive functional decline with eventual loss of ambulation, loss of upper limb function, decreased respiratory function, cardiomyopathy, and ultimately death. Untreated, muscle strength deteriorates and boys require the use of a wheelchair before their teens. Respiratory, orthopaedic and cardiac complications emerge, and without intervention the mean age at death is around 19 years.

Historically, diagnosis of DMD had to be confirmed by muscle biopsy; however, genetic testing for DMD has become a common part of the diagnostic process in Europe as well as in the US, thereby reducing the need for muscle biopsies. The use of newer methods of testing, such as next generation sequencing, has greatly improved the sensitivity and accuracy of genetic testing for DMD and ensures that patients amenable to exon 51 skipping can be readily and reliably identified (Wei 2014; Bovolenta 2012).

2.1.5. Management

There are no approved treatments to cure or stop the ultimately fatal progression of DMD. As a result, supportive care (e.g., physiotherapy) and glucocorticoids are currently the primary means to help improve the quality of life of affected boys. Glucocorticoids have been shown to delay the loss of ambulation and proper orthopaedic care including regular physical therapy and use of orthotic devices support continued ambulation. Aside from glucocorticoids, none of these interventions have been shown to impact loss of ambulation. Even with the introduction in the 1990s of assisted ventilation in the later stages of the disease, the mean age of survival (for those ventilated patients who do not develop early and severe cardiomyopathy) is still only 24 years.

Despite improvements in the standard of care, including steroids and other supportive care, these measures do not address the underlying absence of dystrophin. For treatment of DMD patients with exon 51 deletion mutations, there are no approved specific treatments for this subset of DMD patients in the European Union. Translarna (ataluren) has received conditional marketing authorization in the EU but in DMD patients with nonsense mutations. Therefore, an unmet medical need remains for DMD patients with exon 51 deletion.

About the product

Eteplirsen is a phosphorodiamidate morpholino oligomer (PMO) that represents a new chemistry, structurally and biologically distinct from other synthetic antisense ribonucleic acid (RNA) therapeutics, such as phosphorothioates. Eteplirsen hybridizes with pre-mRNA transcripts of the DMD gene in a sequence-specific manner so that exon 51 is specifically excluded or skipped from mRNA. Skipping exon 51 restores the reading frame and induces production of an internally shortened functional dystrophin protein in patients with genetic mutations that are amenable to exon 51 skipping.

DMD mutations amenable to skipping exon 51 include deletions of exons contiguous to exon 51 (such as deletion mutations of exons 45-50, 47-50, 48-50, 49-50, 50, 52, or 52-63).

The scientific rationale for eteplirsen is that production of de novo dystrophin protein, which is essential for muscle function and stability, will delay progression of DMD.

Type of Application and aspects on development

AVI Biopharma International considered that seeking a Conditional Marketing Authorisation (CMA) was appropriate as according to their statement eteplirsen met the criteria for CMA:

• The benefit-risk balance of eteplirsen was seen as positive by the Applicant, as evidenced by the demonstration of efficacy in multiple clinical and pharmacodynamic endpoints with a well-tolerated safety profile.

• There is a high unmet medical need with no other approved treatments within the EU.

• DMD is an orphan disease that is seriously debilitating and life threatening. Eteplirsen has been designated as an Orphan Medicinal Product in the EU for the treatment of DMD.

• Comprehensive clinical data were committed to be provided from the ongoing confirmatory studies (PROMOVI and ESSENCE).

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as concentrate for solution for infusion containing 50 mg/ml of eteplirsen as active substance. The finished product is presented in two different configurations: a 2-ml vial and a 10-ml vial.

Other ingredients are: sodium chloride, potassium chloride, potassium dihydrogen phosphate, anhydrous disodium phosphate, water for injections, hydrochloric acid, and sodium hydroxide.

The product is packed in a Type I glass vial with chlorobutyl rubber stopper and an aluminium cap with a flip-off seal.

2.2.2. Active Substance

General information

The chemical name of eteplirsen is RNA, [*P*-deoxy-*P*-(dimethylamino)] (2',3'-dideoxy-2',3'-imino-2',3'-seco) (2'a→ 5')(C-m5U-C-C-A-A-C-A-m5U-C-A-A-G-G-A-A-G-A-m5U-G-G-C-A-m5U-m5U-m5U-Cm5U-A-G), 5'-[P-[4-[[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]carbonyl]-1-piperazinyl]-N,N-dimethylphosphonamidate] corresponding to the molecular formula $C_{364}H_{569}N_{177}O_{122}P_{30}$. It has a relative molecular mass of 10300.59 g/mol and the following structure:

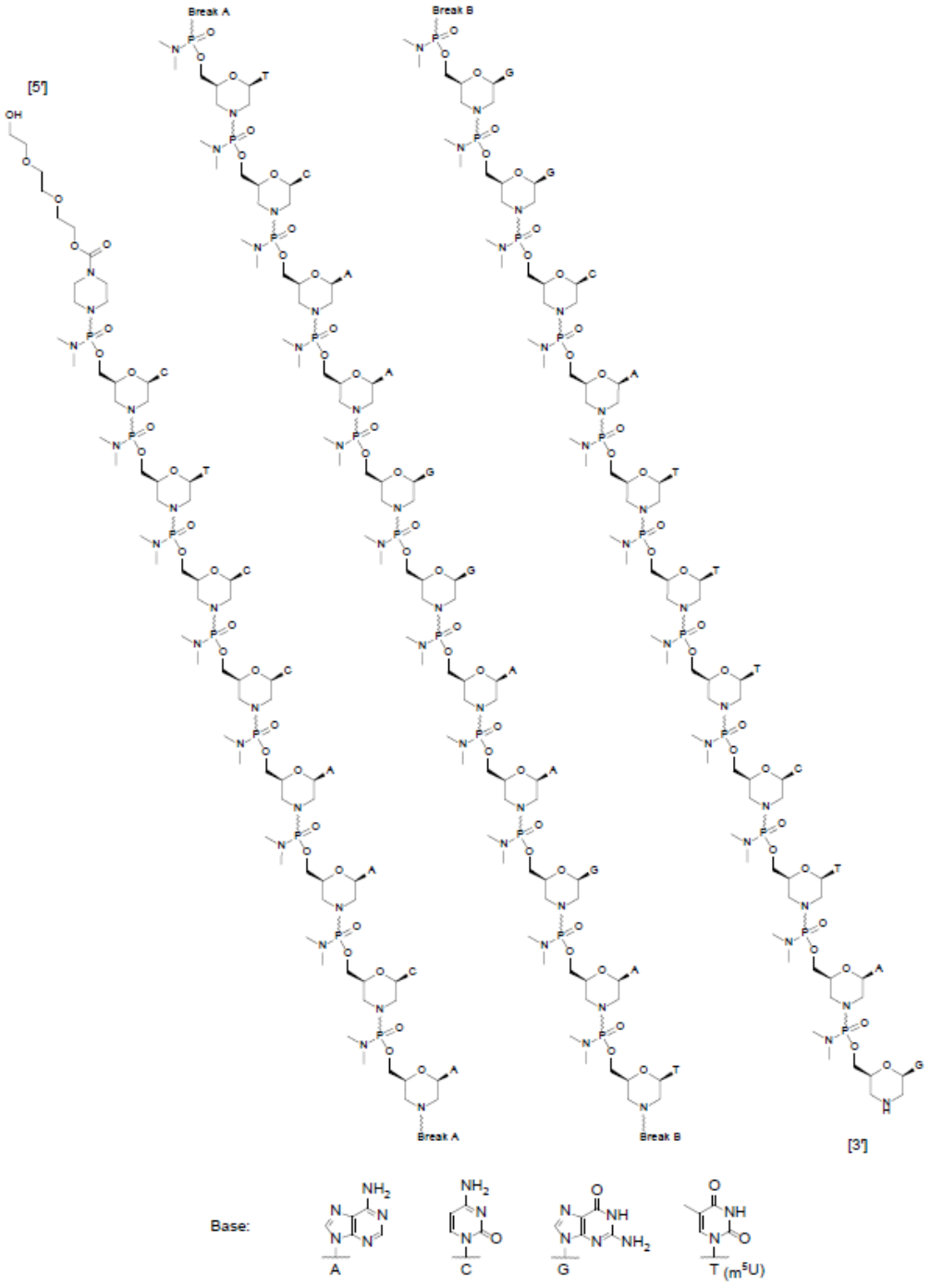


Figure 1: Active substance structure

Due to the molecular complexity of eteplirsen, the confirmation for the structure of the active substance includes not only spectral analyses but also information for the synthetic route. The spectral analysis included: proton NMR (1H NMR) spectroscopy, Carbon-13 NMR (13C NMR) spectroscopy, Phosphorus-31 NMR (31P NMR) spectroscopy, LC/MS for molecular weight, acid hydrolysis with LC/MS analysis of fragments for proof of sequence. The active substance is an amorphous white to off-white hygroscopic powder soluble in water and phosphate buffered saline.

Eteplirsen exhibits stereoisomerism due to the presence of 90 chiral centres. The stereochemistry of the chiral centres in the morpholine rings of eteplirsen is the same absolute configuration as in the ribose from which they are derived.

Manufacture, characterisation and process controls

Detailed information about the manufacturing process and process validation has been provided.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.
The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. Changes introduced have been presented in sufficient detail and have been justified.

The active substance is packaged in glass bottles (clear Type III soda-lime glass) with screw caps (polypropylene, PP, cap with a polytetrafluoroethylene, PTFE, liner). The glass is described in Ph Eur and the PP and PTFE conform to EU Regulation No 10/2011.

Specification

The active substance specification includes tests for appearance (visual), identification, molecular weight (LC/MS), identification, proof of sequence (mass spectrometry), assay (HPLC), purity (HPLC), impurities (HPLC), residual solvents (GC, HPLC), water content (Ph Eur), residue on ignition (Ph Eur), pH of a 1% solution (Ph Eur), bacterial endotoxins (Ph Eur), and microbial limit test (Ph Eur).

Impurities have been appropriately characterised and are controlled by the active substance specifications.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used has been presented.

Batch analysis data on several pilot and commercial scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from 3 commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 36 months under long term conditions (5±3 ºC) and for up to 6 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided.

The following parameters were tested*:* appearance, pH of 1% solution, water content, assay and impurities. The analytical methods used were the same as for release and were stability indicating. Bacterial endotoxins and microbial limit were tested at 0 and 36 months in samples stored under long term conditions.

No significant trends were observed with respect to the material stored under long-term storage or accelerated conditions.

Photostability testing following the ICH guideline Q1B was performed on two batches. No significant changes in the results for appearance, pH, assay by IP-HPLC, purity by IP-HPLC and impurities by SCX tests were observed on the light exposed samples stored at both 5 °C and at 25 °C/60% RH. The impurity analysis by IP-HPLC showed significant change in the results at 25 °C/60% RH. Based upon the photodegradation studies, the active substance should be stored in the original container until ready for use.

Results on stress conditions (40 °C/75% RH) for 6 months were also provided on one batch. With the exception of impurities, all stability indicating tests were within the specification acceptance criteria for the duration of the 6-month study.

A forced degradation study was conducted. Samples of the active substance were subjected to acid (pH 3 citric acid) and base (pH 13 NaOH) hydrolysis, thermal (Inert Nitrogen), and oxidative (air) atmosphere: 80 °C) and humidity stress conditions (40 °C/75% RH). Degradation was only observed under hydrolysis (both acid and base) and thermal stress conditions. The results demonstrate that the IP-HPLC and SCX Chromatography methods are suitable for the intended use.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period when stored at the recommended storage conditions in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The finished product is supplied as a clear to slightly opalescent colourless, sterile, isotonic, phosphate-buffered (pH 7.5) preservative-free solution of eteplirsen active substance at 50 mg/ml in single-use vials. The finished product is a concentrate for solution for infusion intended for dilution into 0.9% sodium chloride solution followed by intravenous administration.

The finished product consists of the active substance dissolved in a solution of phosphate buffered saline (PBS). No interactions between the active substance and any component of the finished product have been observed. This absence of interaction has been confirmed by finished product stability data. The physicochemical characteristics of the active substance that affect the manufacturability and/or performance of the finished product are its solubility and electrostatic nature. Additionally, the active substance is soluble at 50 mg/ml in PBS at 2 – 8 °C, the designated storage condition for the finished product.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in paragraph 2.2.1 of this report.

The finished product is a sterile injection for intravenous administration in two configurations: 2.0 ml and 10.0 ml, packaged in borosilicate glass vials having stopper and a seal that consists of an aluminium shell (ferrule) with a cap overseal. The critical quality attributes (CQAs) are identity, assay, purity, impurity profile, bacterial endotoxins and sterility.

The finished product formulation development was based on the chemistry of the active substance, previous experience with other PMO drug candidates and the suitability of diluents for intravenous delivery.

Each vial contains an overfill of sufficient volume to ensure that a full 2.0 or 10.0 ml dose of the 50 mg/ml can be withdrawn.

The manufacturing process was developed based upon previous manufacturing experience with predecessor PMO based products. The critical parameters for manufacturing are sterility assurance, active substance concentration and tonicity. Since eteplirsen is hygroscopic and electrostatic in nature, the applicant decided to first prepare a concentrated solution (prepared to a predetermined concentration) that is then diluted to obtain the bulk finished product at the target concentration of 50 mg/ml. The focus of the procedural aspects of manufacturing development was to assure that the preparation of the concentrate solution and its subsequent dilution is carried out correctly.

The choice for the method of preparation for the sterile finished product followed the concepts presented in Ph. Eur. 5.1.1 and EMA Note for Guidance on Development Pharmaceutics and EMA Decision Trees for the Selection of Sterilisation Methods. A feasibility study to evaluate the impact of terminal sterilization by heat on the finished product was conducted. The results of the study demonstrate that terminal sterilization leads to unacceptable product degradation and, therefore, the practice of sterile filtration (using a bacterial-retentive filter membrane) with aseptic processing is the method of choice for the sterile manufacture of the finished product.

The primary packaging is clear Type I glass vial with chlorobutyl rubber stopper and an aluminum cap with a flip-off seal. The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of 5 main steps: PBS solution formulation, finished product formulation, aseptic filling, inspection and bulk packaging. The process is considered to be a non-standard manufacturing process.

The finished product manufacturing process validation has been performed in accordance with current regulatory standards and guidelines.

The process validation protocol acceptance criteria relating to the IPC tests and the finished product specification were met for all batches. The finished product manufacturing process demonstrated process reliability, repeatability and consistency for the manufacturing process. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form appearance (visual), identification (LC/MS), assay (HPLC), purity (HPLC), impurities (HPLC), impurity N-Tail (SCX), elemental impurities (Ph Eur), volume in container (Ph Eur), pH (Ph Eur), osmolality (Ph Eur), sterility (Ph Eur), bacterial endotoxins (Ph Eur), and particulate matter (Ph Eur).

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. The reference standard that is used for the testing of the active substance is also used for the testing of the finished product.

Batch analysis results are provided for twenty two pilot scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data from six commercial scale batches (three batches from each fill configuration) of finished product stored for up to 36 months under long term conditions (5 ± 3 °C) and for up to 6 months under accelerated conditions (25 ± 2 °C/60 ± 5% RH) according to the ICH guidelines were provided. The vials were stored in the inverted position on stability.The batches of the medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for: appearance, assay, purity by IP-HPLC and SCX, impurities by IP-HPLC and SCX, pH, sterility, bacterial endotoxins, and particle matter. The analytical procedures used are stability indicating. The evaluation showed that the results meet the acceptance criteria for all quality attributes tested under long term and accelerated conditions and no trends were observed.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products in its primary packaging. No significant change in results for appearance, pH, assay, purity by IP-HPLC and impurities by SCX tests were observed for the light exposed samples stored at both 5 °C and at 25 °C/60% RH. The impurity analysis by IP-HPLC showed slight changes in results for impurity Regions B and F and a significant change in results for Impurity Region H. Based upon the photodegradation studies, the finished product should be stored in the original container and carton until ready for use.

As for the active substance, a forced degradation study was conducted as a characterization test to determine the suitability of the IP-HPLC and SCX Chromatography methods for use in the analysis of finished product stability samples. The forced degradation studies showed that the IP-HPLC method is suitable for assay, purity and impurity analysis of stability samples and that the SCX Chromatography method is suitable for N-Tail analysis of stability samples.

Since the finished product is a sterile concentrate which should be diluted in 0.9% sodium chloride solution for infusion in-use stability data was provided which demonstrated compatibility with 0.9% sodium chloride solution and confirmed that the diluted drug product is stable for up to 4 hours at room temperature and up to 24 hours at refrigerated temperature.

A freeze-thaw study was conducted to evaluate the physical and chemical changes that may occur during the temperature cycling (freeze/thaw) from -20 °C to 25 °C. No significant changes were observed for the drug product samples.

Based on available stability data, the proposed shelf-life and storage conditions were deemed acceptable.

Chemical in use stability has been demonstrated for up to 4 hours at 25 °C or for 24 hours at 2 °C to 8 °C. From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2 to 8 °C, unless dilution has taken place in controlled and validated aseptic conditions.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the proposed SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendation(s) for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Pharmacology

In vitro and ex vivo studies in normal human muscle cells and muscle cells or tissues from DMD patients showed that eteplirsen is able to induce exon 51 skipping. These experiments also demonstrated the induction of dystrophin expression in muscle cells from a DMD patient with a deletion of exon 50, a mutation amenable to exon 51 skipping. Exon skipping activity of eteplirsen was confirmed in vivo in hDMD mice, a transgenic mouse model which expresses the wild type human dystrophin gene.

In addition, in healthy monkeys treated with eteplirsen, dose-dependent exon 51 skipping was observed in quadriceps, heart and diaphragm, justifying selection of this species as the non-rodent species for nonclinical safety studies. A low exon splicing effect of eteplirsen was evident from the studies in NHPs, suggesting that relatively few mRNA copies with the correct reading frame amenable for protein translation is produced. Mammalian cells responding to various treatments often disagree substantially with regards to relative protein and mRNA expression. While the levels of mRNA in tissues are informative, and suggestive of the intended exon splicing, the potential therapeutic effect is dependent on incorporation of the truncated dystrophin protein. However, no effort has been made to isolate and quantify the dystrophin protein, despite the relative ease with which both mRNA and protein can be isolated from any given tissue. Based on the justification provided by the applicant it was agreed that it is not possible to measure expression of an internally shortened dystrophin protein by Western blot or any other method. In addition, studies in *mdx*-mice have shown that greater duration of therapy with AVI-4225 (an exon skipping PMO analogous to eteplirsen, but with specificity for the mouse dystrophin exon 23) generally increased the number of dystrophin positive fibres in skeletal muscle (except for triceps) and that dystrophin intensity in the dystrophin positive skeletal muscle fibres generally increased in a dose- and time-dependent manner.

Supportive data for eteplirsen mechanism of action in vivo is available from general toxicity studies and in the literature and was obtained with surrogate PMO sequences targeted to the exons appropriate for the two available DMD animal models: *mdx* mice, with a nonsense mutation in the dystrophin gene amenable to skipping exon 23 and a significant muscle pathology; and CXMD beagle dogs, with a phenotype more similar to human

DMD patients and whose dystrophin open reading frame can be restored by skipping both exons 6 and 8 (multi-exon skipping) with a combination of dog-specific PMOs targeted to canine exon 6 and exon 8.

In *mdx* mice, dystrophin production and muscle function improvement increased progressively over 50 weeks of PMO treatment, suggesting that longer durations of therapy may have therapeutic benefit. However, discrepancies among the different studies were found regarding the amount of dystrophin required to restore muscle strength.

A 26-week toxicity study with the murine surrogate of eteplirsen also showed a reduction of muscle damage in *mdx* mice, with reduced incidence and severity of myofiber degeneration in most muscles examined, as well as an improvement in muscle damage biomarkers AST, ALT and CK.

In CXMD dogs, PMO treatment induced therapeutic levels of dystrophin, though considerable inter- and intramuscular variation in dystrophin levels was observed. This was accompanied by reduced inflammatory signals, improved or stabilized timed running tests, and clinical symptoms.

No evidence of off-target interactions of eteplirsen with the human genome was observed in silico.

The safety pharmacology in vivo studies conducted with eteplirsen or its analogue AVI-4225 in monkeys did not show effects on cardiovascular, respiratory, neurological, renal or liver functions. In repeat-dose toxicity studies (see section 4.2 of this AR), the highest dose of 320 mg/kg represents approximately 20-fold greater plasma exposures compared to human exposures at the proposed clinical dose of eteplirsen (30 mg/kg). In repeat-dose toxicity studies in monkeys, eteplirsen did not have any effect on cardiovascular parameters such as HR, R-R or P-R intervals, QRS duration, QT and QTc intervals.

Nonclinical pharmacodynamic interaction studies were not conducted with eteplirsen. Regarding medications used concomitantly in DMD patients, such as corticosteroids, literature data shows that prednisolone does not interfere with 2'-O-methyl phosphorothioate antisense oligomers, although eteplirsen does not belong to this class of oligomers. Therefore, no conclusion can be drawn from this information and interactions of eteplirsen with corticosteroids cannot be ruled out.

2.3.2. Pharmacokinetics

In mice, after a single eteplirsen dose, concentrations in plasma and blood declined BQL by 48 and 24 hours postdose, respectively. This dose produced plasma exposure of 2490 µg-equivalents eteplirsen/g (C0) and 345 µg-equivalents eteplirsen·h/ml (AUC0-∞).The plasma half-life was 6.03 h, the volume of distribution 175 mL/kg and the clearance was 348 ml/h/kg. TK parameters in juvenile rats and adult monkeys show that exposure increases in an approximately dose-proportional manner. No plasma accumulation was observed in any species.

Tissue distribution of eteplirsen after IV administration was evaluated in *mdx* mice by LSC and autoradiography. Peak concentrations of eteplirsen-derived radioactivity were observed generally at the first sample point, with highest concentrations in the kidneys due to renal excretion. Of the evaluated muscles, the hindlimb biceps femoris, diaphragm and heart had the highest peak concentrations. Minimal distribution of radioactivity was observed in the bone, brain, spinal cord.

An in vitro protein binding study showed that [^{14}C]eteplirsen had low protein binding in mouse, rat, monkey and human plasma.

Only in vitro metabolism studies were conducted with eteplirsen. The extent of eteplirsen metabolism was determined in vitro in hepatic microsomes from mice, rats, monkeys and human subjects. Eteplirsen was found

to be metabolically stable for up to 2 hours in microsomes of all species tested. In vivo metabolism data would have been helpful to fully characterize the metabolic profile of eteplirsen; however, considering the lack of in vitro metabolites, the use of animals for in vivo studies seems unethical at this point of the product development.

The potential for inhibition or induction of human CYP enzymes was determined in vitro using human hepatic microsomes and cryopreserved human hepatocyte suspension, respectively. Significant drug-drug interactions (DDIs) related to the potential for transient, low-level CYP2C9 or CYP2C19 inhibition by eteplirsen observed in vitro are not expected to occur.

The in vitro induction studies showed no induction via PXR (CYP2B6 and 3A4); therefore no in vivo studies are required. Some evidence of CYP1A2 induction was observed for eteplirsen. However, the level of induction of this enzyme was not consistent across the three donors and was lower than that of the positive control omeprazole.

Renal excretion was the major elimination pathway in *mdx* mice.

Eteplirsen was not a substrate of OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, or BSEP. Eteplirsen showed weak inhibition of OCT1 and OATP1B1, but not of OAT1, OAT3, OCT2, OATP1B3, P-gp, BCRP, MRP2, or BSEP.

2.3.3. Toxicology

No single dose studies were carried out for the nonclinical development of eteplirsen. Repeated dosing by either SC or IV route was carried out on in *mdx* and non-*mdx* mice (up to 12 weeks), rats (up to 13 weeks) and cynomolgus monkeys (up to 39 weeks) for the assessment of eteplirsen. In addition a murine surrogate of the product was evaluated as well in mice. This surrogate (AVI-4225) was mice specific for skipping exon 23 in the *mdx* murine model.

The main common adverse finding common to all studies performed was kidney toxicity, which is not an unexpected finding for medicines of the same class. The findings were dose dependent and were seen in both SC and IV administration. Kidney findings were also evident in AVI-4225 studies, with the product surrogate for eteplirsen in mice, thus adding further confirmation that the kidney was the main target of toxicity of the product. Reversibility of the findings was not completely achieved but trends for reversibility were reported in all animal species assessed. Interestingly findings were more severe in the non-*mdx* model than in the *mdx* mice model probably related to the disease and therefore adding further confirmation to the kidney toxicity and the human target population. Kidney findings were reported as increased absolute kidney weights and microscopically as basophilic granules and/or vacuolated tubules and tubular dilation in the kidneys. Findings were seen in a dose dependent manner generally at all dose levels.

Urinary bladder findings were also seen and reported as basophilic granules, in rodents but not in NHP. In a 13 week study in rats in the urothelium of the urinary bladder, the data shows that minimal to mild intracytoplasmic basophilic granules, as well as associated areas of cellular hypertrophy with atypical, irregularly shaped, large cells with swollen nuclei and cytoplasm were seen at the dose level of 300mg/kg [C_{max} 2,180 µg/ml and $AUC_{0\text{-}24hr}$ 1,020 µg·hr/ml]. Urothelial hypertrophy might be a relevant early biological signal of bladder carcinogenicity and therefore it was considered as adverse. The in-life phases of two carcinogenicity studies (26-week study in Tg.rasH2 mice and 2-year rat study) are currently ongoing using only male animals. Basophilic granules were also reported in the injection sites by both routes of administration being more evident by SC administration. This is also consistent with findings reported with analog products of the same class.

Eteplirsen seems to generate dilatation of the lateral ventricles of the brain of 26w AVI-4225 exposed mdx mice which the applicant interprets as being mdx-AVI-4225 specific. Overall the information provided by the applicant have addressed the issue sufficiently and support the view that eteplirsen is unlikely to generate ventricular dilation-like effects.

In cynomolgus, after 39w exposure, there were changes in lung lobe adhesion but no difference in organ weight. It is not clear whether these macroscopic findings were correlated with any changes at the microscopic level. The Applicant was requested to provide information regarding correlating histopathology; submit historical control data from the laboratory where the study was conducted and/or published literature to support the notion that lung lobe adhesion may be regarded as a spontaneous background finding; and to discuss the clinical relevance of this finding. The document of responses included data on macroscopic lung lobe adhesion which was present in 22% (7 out of 32) of the exposed cynomolgus animals and in none of the control animals plus that 2 out of 7 lung lobe adhesion animals also demonstrated some microscopic signs of minimal fibrosis. Historical control data gave a range around 2% and additional data on a related focal pleural/sub-pleural fibrosis outcome was reported at 6%. It was unclear to what extent the microscopic pleural/sub-pleural signs are clinically relevant. The Applicant dismissed a clinical relevance based on the control data presence, on reports of historical control data for a related microscopic fibrosis and based on that there is no dose response–like relation (there were more affected animals in the middle dose group than the high dose group). Considering the small group size of non-human primate studies, it may indeed be possible that the absence of macroscopic and microscopic lung lobe and fibrosis signs was due to chance. Nevertheless, the extent of the presence/potentiation of lung lobe adhesions with or without microscopic correlates in all experimental exposure groups (including the recovery group) is still far greater than one would expect based on the average prevalence in the control data. The absence of a clear dose-response relation is less relevant considering the group sizes and is therefore not a relevant counter-argument. The Applicant's clarification has failed to confirm that the nature of the lung adhesions can be considered as non-adverse. The applicant stated in the RMP that the adhesion affects in cynomolgus are not considered adverse. There are no grounds for that conclusion and therefore statements in the SmPC and RMP are considered necessary in order to reflect this issue. While the lung adhesion issue cannot be removed or simply considered non-adverse, it is agreed by the CHMP that *in case of* it being a result of chronic inflammation, that it is reasonable to speculate that the immunosuppression treatment may help reduce its manifestation in patients.

The 39w cynomolgus study and the juvenile rat study both demonstrated changes in leukocytes/lymphocyte levels. Cynomolgus demonstrated a reduced leukocyte count at the middle dose with low levels even after 8w recovery. In juvenile rats, there was an increase in neutrophils, monocytes and cytotoxic T-cells and a decrease in NK cells (the cytotoxic T-cell effect was only apparent after the recovery period, possibly indicating a developmentally propagated effect). Considering the presence of inflammatory processes in DMD, the Applicant was requested to discuss the relevance of these findings. The justification provided by the Applicant indicates that the variation reported in the cynomolgus study was within the bounds of natural variability which is also reported in the rat data. It should be also considered the unlikely relevance of the AEs reported in the clinical scenario according to the provided data.

Genotoxicity assessment showed that eteplirsen is not a potential genotoxic product.

The Applicant proposed to carry out carcinogenicity studies post approval. The Applicant committed to conducting post-marketing approval studies for carcinogenicity (declared to be initiated during 2017). This was supported considering that there were urothelial hypertrophy effects (30% of animals) at 600 and 900 mg/kg in the 13w repeat-dose toxicity rat study (SR-15-048). Based on 3R considerations, it is proposed that only male

animals are used in these studies. The in-life phases of two carcinogenicity studies (26-week study in Tg.rasH2 mice and 2-year rat study) are currently ongoing using only male animals.

Taking into account the intended target population, the Applicant's approach to assess only male fertility in toxicity studies was deemed acceptable. No relevant toxicity to the male reproductive parameters has been identified in any study.

The effects of eteplirsen in juvenile studies were assessed in rats in a preliminary study in which animals were single dosed the test product. Animals received a dose of 600 mg/kg at 14 post-natal date (PND) or a dose of 960 mg/kg at 77 PND. Single dosing resulted in kidney macroscopic and microscopic findings which correlated with clinical chemistry changes in BUN values up to 2.2-fold increases. Urine protein concentrations and protein:creatinine ratio were also higher (up to 2.9 fold). Microscopic changes where similar to those reported in repeated dose toxicity.

In the pivotal juvenile study adverse findings again included the kidney and at 900 mg/kg with increases in BUN, creatinine (2.38- and 1.37-fold, respectively) with alterations other clinical parameters which by the end of the reversibility period were still evident but with lower severity. Eteplirsen related pathology findings were observed at all dose levels in the kidneys, generally with a dose-related incidence and/or severity. There was reversibility of changes at the injection site and of a few renal findings (i.e., hyaline casts, tubular necrosis and hemorrhage) however, the other changes in the kidneys (increased weight, enlargement, tubular vacuolation and basophilia, tubular dilatation, basophilic casts, intravascular basophilic material, tubular mineralization and/or interstitial inflammation) persisted but generally had decreased in severity at 900 mg/kg. Increases in systemic exposure of the product were generally proportional with the increase in dose from 100 to 900 mg/kg. No evidence of plasma accumulation of the product was reported. In this regard the claim that the renal findings in adult animals are non-adverse (partly based on the argument that the measured blood biomarker changes are non-significant) was not acceptable. Nor is the associated claim that the max-doses in the repeat-dose toxicity studies are the NOAEL for renal endpoints. The Applicant was requested to modify the proposed text in SmPC section 5.3 and RMP regarding the renal toxicity aspects and NOAEL-value dependent statements**.** The Applicant emphasized mouse and cynomolgus where there were very weak clinical pathology findings (i.e. <10% changes in Na, Cl, Ca-ion levels) but not rat (where one had >10% changes in potassium [27% reduction] and phosphorus [16% reduction]) before stating that there are no indications of functional changes based on traditional renal markers such as creatinine. Considering the overall renal profile, the fact that the adult rat study is non-GLP is not sufficient to ignore the findings. The value of this argument (i.e. about total absence of creatinine changes) is also weakened by the discussion that such creatinine effects where seen in juvenile rats (which received only one or two high doses at ≥ 600mg/kg and no NOAEL could be determined). The claimed 20x margin of safety in cynomolgus is not accepted as the 39w cynomolgus NOAEL is 40mg/kg (giving an AUC of 433.9h x ug/mL against clinical "Study 201 (12w)" AUC of 91h x ug/ml alternatively "Study 202 (152w)" AUC of 127h x ug/ml --> roughly 3x-5x margin (3.4x-4.8x). The proposed SmPC should state that repeat-dose toxicity studies identified the kidney as a target organ (organ weight increase, multifocal, basophilic cytoplasm in renal tubules and minimal to slight tubular degeneration) in all species tested (i.e., mice, rats, and cynomolgus monkeys). Renal effects were seen in both adult and juvenile rats (3x-4x NOAEL based margin of exposure for adult rat and 6x-8.4x for juvenile rat). Most, but not all effects in adult animals were reversible after the end of treatment (slight renal tubular dilation and degeneration remained after 8w recovery in 39w cynomolgus exposure study at a systemic dose margin of ~3x-5x to the human recommended dose). Mild increases in neutrophils and monocytes, a mild decrease in natural killer (NK) cells, and an increase in cytotoxic T-cells that occurred only after the recovery period were observed in juvenile rats. The majority of the non-clinical safety data is based on studies using male animals. In particular, the claim about the max dose (320mg/kg, giving a safety margin of 20x to human dose of 30mg/kg) in the 39w monkey study being the

NOAEL has to be removed/changed as this value is rejected and the NOAEL is instead considered to be 40mg/kg. Any associated RMP text linking to the NOAEL claim (such as high dose AUC and Cmax information and safety margins) needs to be corrected for the middle dose.

There were no eteplirsen related changes in the T-cell dependent antibody response or biologically meaningful changes in circulating cell populations identified by immunophenotyping.

The lack of formal local tolerance studies is considered acceptable as the main local toxicity findings observed were limited and sufficiently addressed in toxicity studies. Assessment of the injection sites has shown that effects by SC were more severe than those reported by IV route. Findings revealed infiltrations of macrophages with basophilic, granular to foamy cytoplasm in the subcutis and dermis, which were often not reversible.

The impurities specification limits were tightened by the applicant and deemed acceptable

2.3.4. Ecotoxicity/environmental risk assessment

During the environmental risk assessment (ERA), eteplirsen PEC surface water value was found to be below the action limit of 0.01 µg/L. The compound is not a PBT substance as log Kow does not exceed 4.5. Therefore, the eteplirsen is not expected to pose a risk to the environment and to stop the environmental risk assessment in Phase I is therefore acceptable.

Summary of main study results

Substance (INN/Invented Name): Eteplirsen			
CAS-number (if available): 1173755-55-9			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	< -2.5	Not potential PBT
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	-2.5	not B
	BCF		not B
Persistence	DT50 or ready biodegradability		not P
Toxicity	NOEC or CMR		not T
PBT-statement :	The compound is not considered as PBT nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{surfacewater}$, refined F_{pen} (prevalence)	0.00155 µg/L	µg/L	> 0.01 threshold: No
Other concerns (e.g. chemical class)			None

2.3.5. Discussion on non-clinical aspects

Pharmacology

The rationale behind the intended use of eteplirsen is to express a truncated protein in DMD patients in whom no or very little full-length dystrophin protein is expressed. In the clinical dossier, this truncated protein has been semi-quantified in DMD patients receiving eteplirsen using Western blot and Immunohistochemistry. Skipping exon 51 in healthy monkeys should create a similar protein amenable to quantification or at least (as in the clinical studies) detection. The Applicant was thus asked to explain in what way the protein expressed in healthy animals would differ from the protein expressed in the clinical studies, and why it cannot (and has not) be detected using e.g. western blot, immunohistochemistry or MS-analysis in tissues from the NHPs used in the 12-week repeated-dose toxicology study. This is especially pertinent in light of the less convincing quantifications of the truncated dystrophin protein presented in the clinical studies. The Applicant's response described that exposure of healthy NHPs to eteplirsen is expected to result in skipping of exon 51, and that deletion (skipping) of exon 51 in healthy animals results in an unstable protein that is also targeted for degradation by the intracellular enzymes. It is therefore agreed by the CHMP that it is not possible to measure expression of an internally shortened dystrophin protein by Western blot or any other method. In addition, studies in *mdx*-mice have shown that greater duration of therapy with AVI-4225 (an exon skipping PMO analogous to eteplirsen, but with specificity for the mouse dystrophin exon 23) generally increased the number of dystrophin positive fibres in skeletal muscle (except for triceps) and that dystrophin intensity in the dystrophin positive skeletal muscle fibres generally increased in a dose- and time-dependent manner (Malerba, 2011). This has not been shown for eteplirsen, and while not presenting as a formal deficiency, it would have been assuring to see the eteplirsen exon 51 splicing efficiency in the tissues of the NHPs treated for 39w in the repeated-dose toxicology studies.

Toxicology

No satisfactory explanation has been provided for the relatively high level of lung lobe adhesion in 39w cynomolgus (22% of exposed animals including recovery animals against 0 animals in controls and with a background historical control level of 2-6%). Also, the clinical relevance remains unclear. As such, the findings should be included in the SmPC and discussed for its relevance for RMP. Regarding the findings of renal tubular degeneration, the NOAEL should be noted at lower doses than those proposed by the Applicant (and then proposed to be used in the SmPC section 5.3 and RMP. Consequently, as discussed above, further modifications on the SmPC and RMP are warranted.

2.3.6. Conclusion on the non-clinical aspects

Eteplirsen, a phosphorodiamidate morpholino oligomer (PMO), hybridizes with pre-mRNA transcripts of the DMD gene in a sequence-specific manner so that exon 51 is specifically excluded or skipped from mRNA. Skipping exon 51 restores the reading frame and induces production of an internally shortened functional dystrophin protein in patients with genetic mutations that are amenable to exon 51 skipping.

In vitro and ex vivo studies in normal human muscle cells and muscle cells or tissues from DMD patients showed that eteplirsen is able to induce exon 51 skipping. These experiments also demonstrated the induction of

dystrophin expression in muscle cells from a DMD patient with a deletion of exon 50, a mutation amenable to exon 51 skipping. Exon skipping activity of eteplirsen was confirmed in vivo in hDMD mice, a transgenic mouse model which expresses the wild type human dystrophin gene. In addition, the in vivo activity of eteplirsen was confirmed in healthy NHPs following IV injection.

Repeat-dose studies identified kidney as the target organ of toxicity in all species tested.

Genotoxicity assessment showed that eteplirsen is not a potential genotoxic product. Carcinogenicity studies with eteplirsen have not been completed.

The CHMP considers that the two toxicology issues related to high level of lung lobe adhesion and NOAEL data still need to be addressed.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant

This application for eteplirsen is supported by efficacy data derived from 4 interventional clinical studies, an external control cohort, and a review of literature describing the natural history of DMD.

- ***Tabular overview of clinical studies***

Table 1 Description of Eteplirsen Studies Included in the Summary of Clinical Efficacy

Descriptor	Study Number			
	Pivotal		Supportive	
	Study 201	Study 202	Study 28	Study 33
Study Design	Randomized, double- blind, placebo-controlled, multiple-dose, single-center (US) study	Multicenter (US), open-label, multiple-dose extension study	Dose-ranging study Open-label, multiple-dose, (UK)	Proof of concept Single-blind, placebo-controlled, single-dose, investigator-sponsored, (UK)
Dosing Regimen	Eteplirsen 30 or 50 mg/kg/week, or placebo (weekly IV infusion) Weeks 1-24, then eteplirsen 30 or 50 mg/kg Weeks 25-28	Eteplirsen 30 or 50 mg/kg/week (weekly IV infusion)	Eteplirsen 0.5, 1.0, 2.0, 4.0, 10.0, or 20.0 mg/kg/week (weekly IV infusion)	Eteplirsen 0.09 or 0.9 mg IM in the EDB of 1 foot and placebo (IM) in the EDB of the opposite foot

PD Endpoints	**Primary:** Change from BL in PDPF at Week 12 (50 mg/kg group) and at Week 24 (30 mg/kg group). **Other:** Exon skipping (RT-PCR) and dystrophin PDPF and intensity in biopsied muscle	**Primary:** Change from BL (of Study 201) to Week 48 (combined) in PDPF **Other:** Exon skipping (RT-PCR), change from BL in PDPF (Week 12 and 24); change from BL in dystrophin intensity at Week 48; differences from untreated controls in PDPF, dystrophin intensity, and dystrophin quantity by Western blot at Week 180	**Exploratory:** Change from BL to Week 14 in dystrophin PDPF; Change from BL to Week 14 in dystrophin intensity (IHC) and protein levels (Western blot)	**Exploratory:** Restoration of dystrophin protein expression and the DAPC at Week 2-4, Exon Skipping (RT-PCR)
Clinical Endpoints	**Primary:** 6MWT, LOA, NSAA, rise time and PFTs	**Primary:** Change from BL in 6MWT through Week 240 (combined), LOA, NSAA, rise time and PFTs	**Primary:** Safety and tolerability	**Primary:** Safety
Required Age at Entry (yrs)	7-13		5-15	10-17
Study Status	Completed	Completed[a]	Completed	Completed

Descriptor	**Study Number**			
	Pivotal		**Supportive**	
	Study 201	**Study 202**	**Study 28**	**Study 33**
No. Enrolled	12		19[b]	7
No. Completed	12	12	18[b]	7
Study Period	Jul 2011 – Feb 2012	Feb 2012 – Apr 2016 (for Efficacy)	Jan 2009 – Jun 2010	Oct 2007 – Apr 2009
Study Duration	28 Weeks	212 Weeks (240 weeks combined Study 201/202)	12 Weeks	Single Dose

Abbreviations: 6MWT = 6-Minute Walk Test; BL = Baseline; DAPC = dystrophin-associated protein complex; EDB = extensor digitorum brevis muscle; IM = intramuscular; IV = intravenous; LOA = Loss of Ambulation; No. = number; NSAA = North Star Ambulatory Assessment; PD = pharmacodynamic; PDPF = percent dystrophin-positive fibers; PFT = pulmonary function testing; RT-PCR = reverse transcriptase-polymerase chain reaction; UK = United Kingdom; US = United States; Wk = week; yrs = years.
a Patients continue to be dosed and followed for safety until they transition to commercial drug.
b Two patients did not have both pretreatment and post-treatment biopsies for analysis; therefore, data for 17 patients were used in the pharmacodynamic analyses.

In addition to the completed studies, safety data are provided from an additional 112 eteplirsen-treated patients from 3 ongoing clinical studies, for a total of 150 patients included in the eteplirsen safety assessment for this MAA:

• Study 4658-301 (Study 301 [PROMOVI]) is a confirmatory 96-week, open-label Phase 3 study of eteplirsen (30 mg/kg by weekly IV infusion) in ambulatory patients (N = 120) with DMD, ages 7 to 16 years old compared an untreated control group of patients with DMD amenable to skipping of any exon, with the exception of exon 51.Enrollement of this study is complete; 79 in the eteplirsen-treated group and 29 in the untreated control group.

• Study 4658-203 (Study 203) is a 96-week, open-label Phase 2 study to evaluate safety and pharmacokinetics of eteplirsen (30 mg/kg by weekly IV infusion) in younger patients (N = 40) with DMD, aged 4 to 6 years old. Study 203 is currently enrolling patients; 26 eteplirsen-treated patients and 7 untreated controls were enrolled at the time of the MAA safety data cutoff.

• Study 4658-204 (Study 204) is a 96-week, open-label Phase 2 study primarily to evaluate safety of eteplirsen (30 mg/kg by weekly IV infusion) in patients (N = 24) with advanced stage DMD (including non-ambulatory patients), ages 7 to 21 years old. Enrollment in Study 204 has been completed.

2.4.2. Pharmacokinetics

The eteplirsen clinical pharmacology program includes the evaluation of the primary PD effect of eteplirsen injection (exon skipping) and the characterization of the human PK profile based on data obtained from patients with DMD treated with eteplirsen in the 4 clinical studies completed to date.

1. Study AVI-4658-33 (Study 33), a proof-of-concept clinical trial performed in the United Kingdom (UK) in which boys with DMD due to dystrophin mutations amenable to exon 51 skipping were given a single dose of eteplirsen by intramuscular (IM) injection (0.09- or 0.9-mg dose levels).
2. Study AVI-4658-28 (Study 28), a dose ranging study of eteplirsen 0.5 mg/kg up to 20 mg/kg IV over 12 weeks to induce dystrophin expression in DMD patients. The safety of escalating doses of eteplirsen as well as the PK and efficacy of eteplirsen after 12 weekly doses were also evaluated.
3. Study 4658-us-201 (Study 201), a randomized, double-blind, placebo-controlled, multiple-dose efficacy, safety, tolerability, and PK study of eteplirsen 30 and 50 mg/kg IV administered over 28 weeks in the treatment of ambulant subjects with DMD.
4. Study 4658-us-202 (Study 202), an open-label, multiple-dose, efficacy, safety, and tolerability extension study of eteplirsen 30 and 50 mg/kg IV in subjects with DMD who participated in Study 201 with approximately 5 years of combined study experience.

Analytical methods

Validated anion exchange high performance liquid chromatography (HPLC) with fluorescence detection (FL) was used to quantify eteplirsen in K_3EDTA human plasma and urine samples.

Pharmacokinetic analysis

Conventional non-compartmental methods have been used. However, population pharmacokinetic analysis including investigation of the effect of demographic covariates is planned at the time that data from the ongoing eteplirsen clinical trials (Studies 301, 203, and 204) becomes available.

Formulation differences

Eteplirsen is administered i.v. an intramuscular (IM) formulation was used in the first clinical study (Study 4658-33) and an intravenous (IV) formulation was used in the subsequent clinical studies (100 mg/ml formulation: 4658-28, 50 mg/ml formulation: 4658-us-201, 4658-us-202, 4658-301, 4658-203 and 4658-204).

Absorption

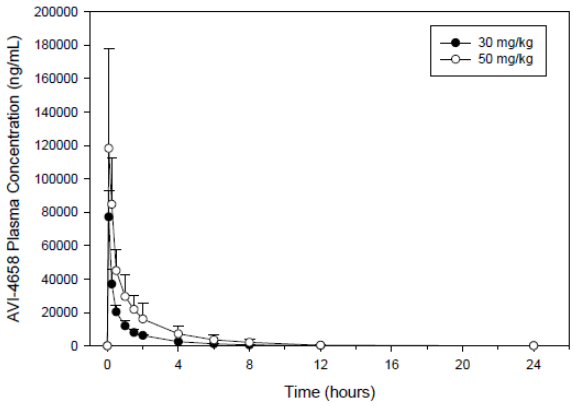
Tmax occurred at the first time point post-end of infusion. Cmax averaged between 1,360 and 39,000 ng/ml at doses from 0.5 to 20.0 mg/kg/wk after the 60-minute IV infusion (Study 28). Plasma concentrations declined in a multiphasic manner and were below the quantitation limit (BLQ, 10 ng/mL) by 12 hours following the 0.5- and 1.0-mg/kg doses and generally above BLQ at 24 hours following the 2.0- through 20-mg/kg doses.

Exposures at different infusion times

The applicant applies for an infusion time of 35 to 60 minutes. The highest mean concentration during 30 mg/kg treatment (60 minutes infusion) was 88100 ng/ml. (Study 201, 5 minutes post infusion, week 25.) An AUC of 91200 h*ng/ml was observed after 12 weeks of treatment with 30 mg/kg eteplirsen. There is limited data available from 35 minutes infusions. The mean Cmax was 140000 ng/ml and the $AUC_{0\text{-last}}$ was 161000 hr*ng/ml (Study 202, week 240).

Figure 2 Mean (± SD) AVI-4658 Plasma Concentrations, Visit 13, Week 12

A. Linear Plot



B. SemiLog Plot

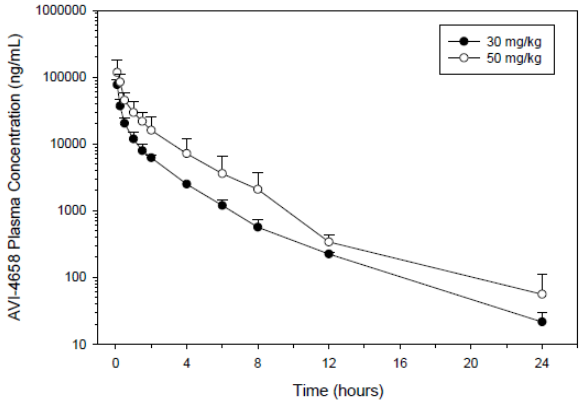


Table 2 Plasma Pharmacokinetic Parameters for Eteplirsen at Week 12 (Study 201)

Treatment Group	Statistic	T_{max} hr	C_{max} ng/mL	AUC_{0-24} hr*ng/mL	$AUC_{0-\infty}$ hr*ng/mL	CL_{PL} mL/hr/kg	V_{ss} mL/kg	$t_{½}$ hr
Eteplirsen 30 mg/kg	N	4	4	4	4	4	4	4
	Mean	1.08	77,200	91,000	91,200	339	601	3.30
	SD	0.0136	15,600	16,700	16,800	75.8	157	0.341
	CV%	1.26	20.2	18.4	18.4	22.3	26.1	10.3
Eteplirsen 50 mg/kg	N	4	4	4	4	4	4	4
	Mean	1.14	125,000	181,000	181,000	319	638	3.17
	SD	0.0752	54,900	87,700	88,000	125	224	0.249
	CV%	6.58	44.1	48.5	48.6	39.1	35.1	7.85

AUC0-24 = area under the plasma concentration-time curve from time 0 to 24 hours; AUC0-∞ = area under the plasma concentration-time curve from time 0 to infinite time; CLPL = total clearance of drug after intravascular administration; Cmax = observed maximum plasma concentration; CV% = coefficient of variation; SD = standard deviation; t½ = elimination half-life; Tmax = time to the observed maximum plasma concentration; Vss = apparent volume of distribution at steady state.

Table 3 Plasma Pharmacokinetic Parameters for Eteplirsen at Week 240 (Study 202)

Treatment Group	Statistic	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-last} (hr*ng/mL)	CL_{PL} (mL/hr/kg)
Eteplirsen 30 mg/kg	N	6	6	6	6
	Mean	0.744	140,000	161,000	218
	SD	0.0554	53,000	55,200	114
	CV%	7.45	37.8	34.4	52.1
Eteplirsen 50 mg/kg	N	6	6	6	6
	Mean	0.797	206,000	275,000	205
	SD	0.224	81,500	120,000	63.8
	CV%	28.0	39.6	43.8	31.2

AUC0-last = area under the plasma concentration time curve from time 0 to the last time point with measurable concentrations; CLPL = total clearance of drug after intravascular administration; Cmax = observed maximum plasma concentration; CV% = coefficient of variation; Tmax = time to the observed maximum plasma concentration; SD = standard deviation.
Note: Parameters are estimates based on only 3 postdose time points rather than the 11 to 12 time points at earlier weeks. Note: CLPL is an estimate based on AUC0-last, rather than AUC0-∞.

Because the first time point sample was collected at over a range from 5-10 minutes post-end of infusion rather than at a target time of 5 minutes, as at prior weeks, Cmax was not directly comparable to values at prior weeks. Plasma concentrations at Week 240 were greater than those at prior weeks likely due to the shorter infusion duration (35 versus 60 minutes).

- **Distribution**

The volume of distribution at steady state is approximately 600 ml/kg. The protein binding ranged from 6.1% to 16.5% in human plasma. The binding was not concentration dependent.

The mass balance and tissue distribution study with 14C-eteplirsen in mdx mice demonstrated widespread distribution of eteplirsen-derived radioactivity in blood, plasma, and tissues, with peak concentrations occurring 4 to 24 hours post-dose. The highest levels of radioactivity were found in urine and kidney, consistent with renal clearance being the predominant route of excretion. Human data with rapid plasma clearance and a large volume of distribution at all dose levels tested (0.5 to 50 mg/kg) are consistent with these animal data.

In studies 201/202 the distribution of eteplirsen to the target tissue was not assessed since, samples were not available for these evaluations. The Applicant has committed to measuring concentrations in future studies, and this was accepted.

- **Elimination**

The terminal half-life of eteplirsen is ca. 3.5 hrs. Results from a mass-balance study (Study 4658-101) conducted in eight healthy male subjects have been provided in the Responses to the list of questions. Eteplirsen was shown to be minimally metabolized. Urinary excretion was the primary elimination pathway for eteplirsen (accounting for 99.2% of the overall administered dose). The renal clearance is similar to the expected creatinine clearance. There are no signs of active secretion being involved in the renal clearance. Eteplirsen is not a substrate of OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, P-gp, BCRP, MRP2, or BSEP in vitro. The investigations of the potential transport of eteplirsen have been conducted at relevant concentrations. However, the concentration dependency in eteplirsen Caco-2 cell permeability does indicate that an efflux transporter was saturated at 800 ug/ml. This is not further pursued as it is likely not to have an impact of labelling.

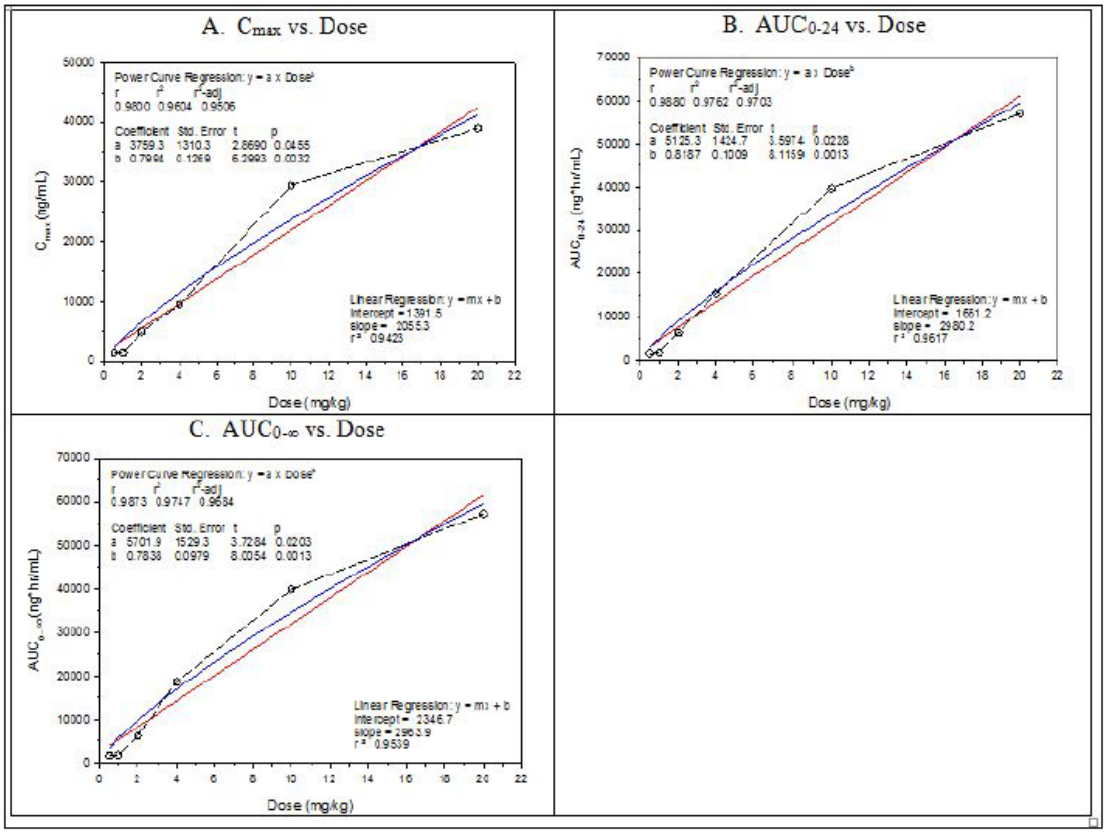
Chirality

Eteplirsen is a mixture of 230 diastereomers. The applicant did not perform any enantioselective analysis.

2.4.3. Dose proportionality and time dependency

The pharmacokinetics of eteplirsen appears reasonably dose-proportional in the therapeutic dose range. At lower doses there may be a tendency to somewhat less than proportional increase in exposure when increasing the dose. In contrast, based on urine data, a lower recovery (as % of dose) was observed in the low dose range. This finding could have analytical reasons.

Figure 3 Dose-Proportionality Plots of Cmax, AUC0-24, and AUC0-Inf Versus Dose Mean Values Averaged Across Weeks 1, 6, and 12 (Study 28)



Notes: Dashed line is point-to-point connection; solid red line is linear regression line; solid blue line is power-curve regression line.

- **Time dependency**

Concentration versus time profiles showed no relevant accumulation between study weeks.

Table 4 Plasma Pharmacokinetic Parameters for Eteplirsen at Week 152 (Study 202)

Treatment Group	Statistic	T_{max} hr	C_{max} ng/mL	AUC_{0-24} hr*ng/mL	$AUC_{0-\infty}$ hr*ng/mL	CL_{PL} mL/hr/kg	V_{ss} mL/kg	$t_{1/2}$ hr
Eteplirsen 30 mg/kg	N	6	6	6	6	6	6	6
	Mean	1.12	85,100	127,000	128,000	244	526	3.54
	SD	0.0816	15,900	25,800	25,900	54.9	91.5	0.643
	CV%	7.31	18.7	20.2	20.3	22.5	17.4	18.2
Eteplirsen 50 mg/kg	N	6	6	6	6	6	6	6
	Mean	1.11	126,000	193,000	193,000	322	690	3.77
	SD	0.0648	64,600	107,000	107,000	150	340	0.628
	CV%	5.84	51.4	55.5	55.6	46.6	49.2	16.6

AUC0-24 = area under the plasma concentration-time curve from time 0 to 24 hours; AUC0-∞ = area under the plasma concentration-time curve from time 0 to infinite time; CLPL = total clearance of drug after intravascular administration; Cmax = observed maximum plasma concentration; CV% = coefficient of variation; SD = standard deviation; t½ = elimination half-life; Tmax = time to the observed maximum plasma concentration; Vss = apparent volume of distribution at steady state.

Table 5: Plasma Pharmacokinetic Parameters for Eteplirsen at Week 240 (Study 202)

Treatment Group	Statistic	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL)	CL_{PL} (mL/hr/kg)
Eteplirsen 30 mg/kg	N	6	6	6	6
	Mean	0.744	140,000	161,000	218
	SD	0.0554	53,000	55,200	114
	CV%	7.45	37.8	34.4	52.1
Eteplirsen 50 mg/kg	N	6	6	6	6
	Mean	0.797	206,000	275,000	205
	SD	0.224	81,500	120,000	63.8
	CV%	28.0	39.6	43.8	31.2

AUC0-last = area under the plasma concentration time curve from time 0 to the last time point with measurable concentrations; CLPL = total clearance of drug after intravascular administration; Cmax = observed maximum plasma concentration; CV% = coefficient of variation; Tmax = time to the observed maximum plasma concentration; SD = standard deviation.
Note: Parameters are estimates based on only 3 postdose time points rather than the 11 to 12 time points at earlier weeks. Note: CLPL is an estimate based on AUC0-last, rather than AUC0-∞.

Because the first time point sample was collected at over a range from 5-10 minutes post-end of infusion rather than at a target time of 5 minutes, as at prior weeks, Cmax was not directly comparable to values at prior weeks. Plasma concentrations at Week 240 were greater than those at prior weeks likely due to the shorter infusion duration (35 versus 60 minutes).

There are no indications of time-dependent changes in eteplirsen elimination.

- **Intra- and inter-individual variability**

No estimation of the intra-individual variability has been found but this information should be possible to obtain from the dataset. The inter-individual variability of the eteplirsen AUC was approximately 20 and 50% on the low and high dose, respectively. One subject in study at the 50 mg/kg dose level had higher concentrations than for the other subjects treated at this dose level, indicating that there may be “outliers”.

- **Pharmacokinetics in target population**

All clinical studies have been performed in DMD subjects. The age range of the dataset with PK data was 4-13 years. Age range applied for is children aged 4 years and older. The exposure in patients older than 13 years and having higher body weights is thus unknown.

Eteplirsen exposure and CL was evaluated as a function of body weight across studies AVI-4658-28, AVI-4658-us-201 and AVI-4658-us-202 which are finalized and Study AVI-4658-203. Although the inter-individual variability is large, bodyweight based dosing appears to reasonably normalise eteplirsen exposure in the weight range 15 to 40 kg. The AUC resulting from the weight normalised dosing was tended to be to some extent higher in the higher weight range.

Pharmacokinetic parameters Cmax, AUC, and CLtotal were plotted versus weight. A linear regression was performed and the correlation was assessed by the goodness of fit parameter, R2 (correlation coefficient). Plots of exposure parameters Cmax and AUC versus weight were created for the 30 mg/kg dose level. Plots of CLtotal

versus weight were created across all dose levels, since eteplirsen has demonstrated dose proportionality. In these plots, data from different study weeks in the same patient are included.

Figure 4 AUC0-∞ versus weight at the 30 mg/kg dose level

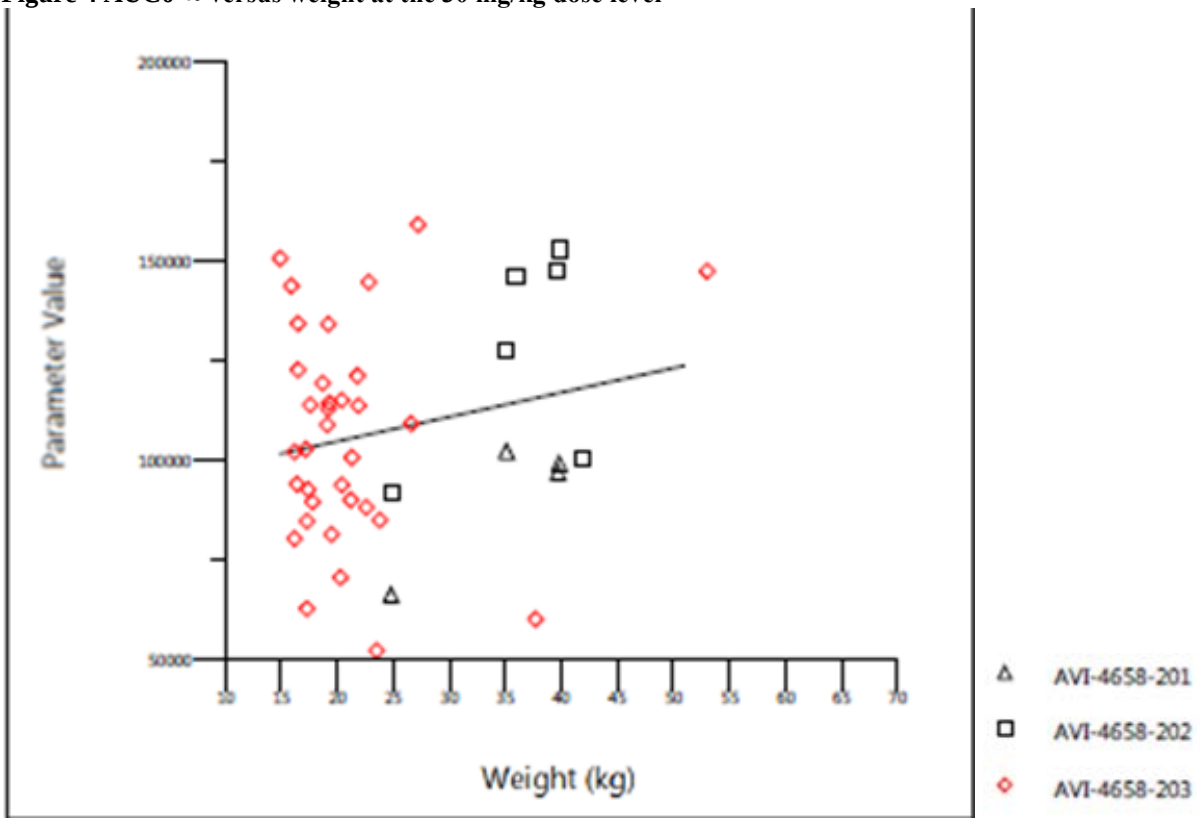
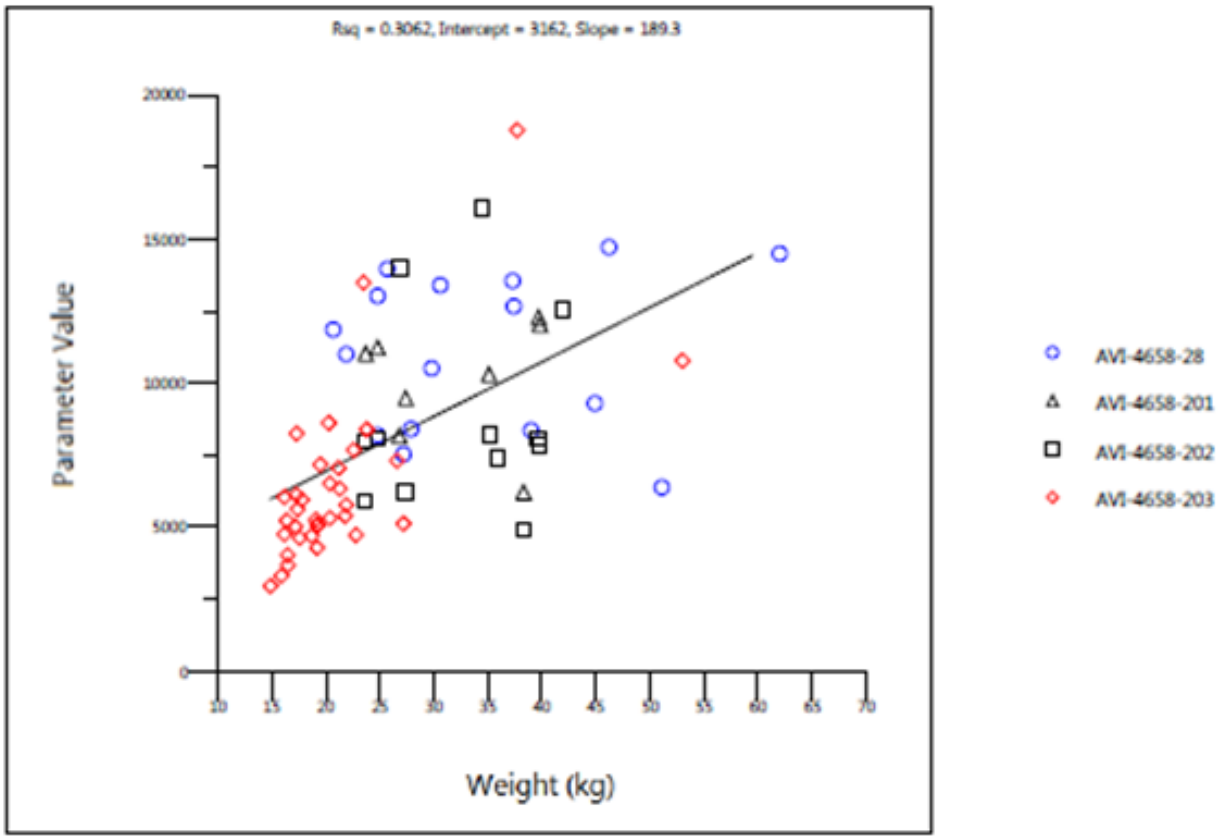


Figure 5 CLtotal (mL/hr) versus weight (all studies)



- **Special populations**

As eteplirsen is eliminated through renal excretion, renal impairment is expected to have significant influence on eteplirsen exposure. At Day 180 the Applicant submitted the final report of a renal impairment study (AVI-4658-103). This study included subjects with normal renal function (CLcr >90 ml/min; n=9), mild renal impairment (CLcr >60 to <90 ml/min, n=8) and moderate renal impairment (CLcr >30 to <60 ml/min, n=8). After administering a single dose of eteplirsen (30 mg/kg) the increase in exposure (AUC) in subjects with mild renal impairment was approximately 1.35 fold and is not clinically relevant. In subjects with moderate renal impairment the increase was approximately 2.36 fold and a 50% dose reduction is recommended. The effect of severe renal impairment or end-stage renal disease on eteplirsen PK and safety has not been studied. Large increases in exposure can be expected in these populations.

With respect to the monitoring of the renal function, the Applicant was requested to propose an alternative method to serum creatinine (which leads to overestimation of kidney function). Cystatin C has been proposed as marker (already included in eteplirsen clinical studies). Estimated GFR based on cystatin C has been shown to more closely approximate measured GFR ([mGFR]; based on 51Cr-EDTA), than eGFR based on serum creatinine, which overestimated GFR by 300% relative to mGFR (Braat 2015). It seems a suitable alternative but still needs further evaluation in larger studies (Braat 2015). An option could be the inclusion of both Cystatin C and 51Cr-EDTA in a future confirmatory trial.

- **Interactions**

The applicant has submitted studies in the potential for eteplirsen to inhibit and induce CYPs and transporters.

No inhibition was found on CYP1A2, CYP2B6, 2D6, 2C8 and 3A4/5 applying concentrations up to 6.6 mg/ml. Based on the 30 mg/kg cut-off (7 mg/ml), all the observed inhibition (2C9 and 2C19, Ki:s 0.68 and 0.55 mg/ml, respectively) are in vivo relevant.

No inhibition of OAT1, OAT3, OCT2, OATP1B3, P-gp, BCRP, MRP2, or BSEP was observed at suboptimal concentrations (0.8 mg/ml). A decrease in OCT-1 and OATP1B1 activity is observed at the highest studied concentration. The inhibition may be relevant in vivo.

Due to the transient exposure of eteplirsen after dose, the inhibition of enzyme and transporters is probably very short.

In the in vitro induction study, the mRNA levels (encoding for enzymes) are highly variable interfering with the assessment of concentration dependency. Induction of CYP1A2 and 2B6 is concluded based on the individual donor results and considered potentially clinically relevant. An increase in concentrations at later times during the hepatocyte culturing (in vitro induction study) indicates that there is interference by metabolites in the assay.

2.4.4. CHMP overall conclusions on pharmacokinetics

Eteplirsen is intended for use in patients 4 years and older. Pharmacokinetics of eteplirsen was studied in DMD patients who were amenable to exon 51 skipping. Studies were conducted in ambulant subjects aged 6 to 13 year and who were able to walk independently for at least 25 m. The pharmacokinetics is mainly descriptive. A study was evaluating the PK profile in patients 4-6 years old (Study 4658-203).

Following IV injection, peak concentrations occurred at the first time point post-end of infusion (i.e., 5 minutes after the end of the 60-minute infusion). Cmax averaged 77,200 ±15,568 ng/ml after repeated weekly dosing with 30 mg/kg (the recommended dose). Afterwards plasma concentrations declined in a multiphasic manner. Half-life was approximately 3-4 hours, with no significant accumulation in plasma.

Eteplirsen exposure increased with dose, and exhibits an approximate dose-proportionality across the studied dose range of 0.5 mg/kg/wk to 50 mg/kg. Whereas Cmax appear to increase in a proportional manner with dose, AUC increased in a greater than proportional manner. Due to the high number of enantiomers (230 diastereomers), exposure data on specific enantiomers is not requested.

Distribution to the target i.e. muscle tissue has not been investigated in the muscle biopsies so that tissue concentrations achieved in subjects treated with eteplirsen is unknown. Such distribution can be relevant since it is believed that the efficacy of AONs depends partly on the amount of AON that reaches its target, i.e. the muscle fibre nuclei[1]. The Applicant have committed to measuring tissue concentrations in future studies, and this was agreed.

A number of assumptions are based on *in vitro* and non-clinical studies. Considering the nature of the product and the microsomal metabolism study results hepatic metabolic does not appear of relevance.

[1] Ingrid E. C. Verhaart and Annemieke Aartsma-Rus (2012). AON-Mediated Exon Skipping for Duchenne Muscular Dystrophy, Neuromuscular Disorders, Dr. Ashraf Zaher (Ed.), InTech, DOI: 10.5772/33938. Available from: http://www.intechopen.com/books/neuromuscular-disorders/aon-mediated-exon-skipping-for-duchenne-muscular-dystrophy

The observed inhibition of CYP1A2, 2C9 and 2C19 is *in vivo* relevant and should be reflected in the proposed SmPC. However, the inhibition is temporary and only relevant the first 12 hours after the first dose. Induction of CYP1A2 and 2B6 is concluded based on the individual donor results and considered potentially clinically relevant. A decrease in OCT-1 and OATP1B1 activity is observed at the highest studied concentration. This information has been reflected in the SmPC.

Eteplirsen is eliminated through renal excretion and thus, renal impairment has marked effects on eteplirsen exposure. Specific treatment recommendations are thus needed. A 50% dose reduction is proposed in moderate renal impairment (RI). Eteplirsen is not recommended in patients with severe renal impairment with respect to the monitoring of the renal function, the Applicant was requested to propose an alternative method to serum creatinine (which leads to overestimation of kidney function). Cystatin C has been proposed as marker (already included in eteplirsen clinical studies). Estimated GFR based on cystatin C has been shown to more closely approximate measured GFR ([mGFR]; based on 51Cr-EDTA), than eGFR based on serum creatinine, which overestimated GFR by 300% relative to mGFR (Braat 2015). It seems a suitable alternative but still needs further evaluation in larger studies (Braat 2015). An option could be the inclusion of both Cystatin C and 51Cr-EDTA in a future confirmatory trial.

No dedicated drug-drug interactions studies have been conducted. There are signals from in vitro studies on enzyme and transporter inhibition as well as induction. The effects are probably transient due to the short exposure after an eteplirsen dose.

In addition a population PK analysis will be done across all eteplirsen studies and will investigate the effect of demographic covariates on PK.

2.4.5. Pharmacodynamics

Eteplirsen belongs to a distinct class of novel synthetic antisense ribonucleic acid (RNA) therapeutics called phosphorodiamidate morpholino oligomers (PMOs), which are a redesign of the natural nucleic acid structure.

The precise mechanism of action of eteplirsen is exon 51 skipping during mRNA processing. Exon skipping by eteplirsen is achieved through its sequence-specific hybridization with dystrophin pre-mRNA, which interferes with formation of the pre-mRNA splicing complex at the target site and prevents inclusion of exon 51 into the mature mRNA. In DMD patients with amenable mutations, exon 51 skipping restores the open reading frame which results in the production of internally shortened, functional dystrophin protein.

The application contained 4 completed studies with biological (muscle biopsy) measurement defined as primary efficacy endpoint in study 201, as primary pharmacodynamics endpoint in study 202 (in addition to a primary functional efficacy endpoint) (extension study of study 201), a proof of concept study (study 33) and a dose-ranging study (study 28).

Exon skipping

In the eteplirsen clinical program, the evaluation of exon skipping was accomplished by nested reverse transcription polymerase chain reaction (RT-PCR) analysis followed by sequencing of the polymerase chain reaction (PCR) product, demonstrating the proof of principle for the mechanism of action of eteplirsen in DMD patients.

Exon skipping of exon 51 as assessed using RT-PCR was observed uniformly across clinical studies, indicative of the primary PD effect of eteplirsen.

- In Study 33, the proof of concept evaluation eteplirsen (administered as a single-dose IM), the high dose (0.9 mg) was observed to induce skipping of exon 51, as determined by RT-PCR, in the 5 subjects with DMD in muscle biopsy specimens from the eteplirsen-treated feet. For the low dose group, a single IM dose of 0.09 mg of eteplirsen induced low-level exon skipping in the 2 subjects when increased polymerase chain reaction (PCR) amplification was used.
- In Study 28, the dose ranging evaluation of 6 dose levels of eteplirsen administered once weekly as an IV infusion, exon 51 skipping (as detected by RT-PCR and confirmed with DNA sequencing) was observed in all 17 (100%) evaluable patients with pre- and post-treatment biopsies. Exon skipping was most easily and reliably detected in those patients within the 2 highest dose groups (10.0 and 20.0 mg/kg).
- The RT-PCR method was used to confirm exon skipping in Study 201 where all 4 of the 50 mg/kg/week eteplirsen patients biopsied at Week 12 and all 4 of the 30 mg/kg/week eteplirsen patients biopsied at Week 24 demonstrated exon skipping. Exon skipping was observed in Study 202 at Week 48 in all 12 eteplirsen-treated patients and at Week 180, with sequencing confirmation, in all 11 patients tested.

Dystrophin production

In Study 33 increased truncated dystrophin expression and percentage of dystrophin-positive fibers in EDB muscle was measured in patients with DMD, indicating proof of principle.

In the dose-ranging study 28 induction of truncated dystrophin protein expression by eteplirsen was shown with most consistent results observed for the higher dose levels of 10 and 20 mg/kg, indicating a dose dependent effect of eteplirsen.

Dystrophin production after treatment was evaluated in muscle biopsy tissue obtained from patients in Studies 201/202 following 12, 24 or 48 weeks (cumulative) of treatment. Three different methods were used, as they provide complementary evaluation.

a) Western blot was used to quantify dystrophin following extraction of protein from muscle tissue.

b) BIOQUANT was used to assess the fluorescence signal of dystrophin fiber intensity following indirect immunofluorescence staining with different anti-dystrophin antibodies.

c) IHC images were used to assess the percent dystrophin-positive fibers following indirect immunofluorescence staining with different anti-dystrophin antibodies, providing information on sarcolemmal localization and distribution of dystrophin in muscle fibers.

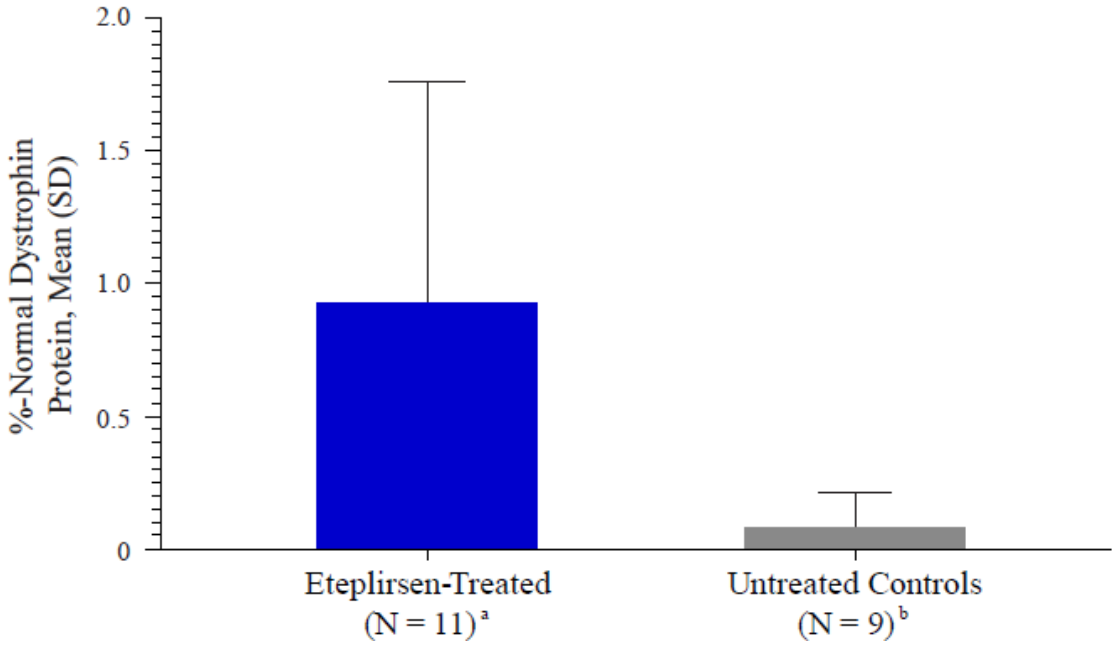
Muscle biopsy tissues were obtained from patients in Study 201/202 following 12, 24 or 48 weeks (cumulative) of treatment. Eleven of the 12 patients agreed to provide muscle biopsies at Week 180. These biopsies were compared to baseline samples from 3 untreated patients in Study 201/202 and samples from 6 untreated control patients from the ongoing confirmatory Study 301 (PROMOVI). The tissue samples were obtained from 9 patients who were highly comparable to the 11 patients in Study 201/202.

Dystrophin Quantification by Western Blot

In Western blot analysis, 9 of 11 biopsied eteplirsen-treated patients had an observable dystrophin band. Western blot analysis showed that eteplirsen-treated patients demonstrated a statistically significant ($p = 0.007$) higher mean dystrophin expression level compared to untreated controls. The mean dystrophin protein

level in eteplirsen-treated patients at Week 180 was 0.93% of normal compared to 0.08% in untreated controls (Figure 6), demonstrating a statistically significant (p = 0.007) increase of treated over untreated samples.

Figure 6 Mean Percent Normal Dystrophin in Eteplirsen-Treated Patients (Week 180, Study 201/202) (N = 11) vs. Untreated DMD Controls (Western Blot) (N = 9)



a 1 eteplirsen-treated patient did not consent to a biopsy at Week 180
b Untreated controls comprised of 3 patients from Study 201/202 and 6 patients from PROMOVI

In the responses to the CHMP questions the applicant has provided the interim analysis of dystrophin production for 12 patients after 48 weeks of treatment in study 301 (PROMOVI). The results show that only 0.44% of normal dystrophin was produced. This level is even lower that that observed in study 201 (0.93%). When individual results are examined, 10/12 patients had values lower than 0.5% and only 2 patients showed values higher than 1 (although less than 2%). Four patients did not show an increase or only a minimal increase in dystrophin production (patients 301-02, 301-06, 301-10, 301-13) (see table below).

Table 6 Study 301 individual patient western blot data

Patient ID	Baseline % mean of NC	Week 48 % mean of NC
301-01	0.13	0.26
301-02	0.35	0.36
301-03	0.06	0.37
301-04	0.04	0.10
301-05	0.17	1.02
301-06	0.37	0.30
301-07	0.17	0.42
301-09	0.24	1.57
301-10	0.11	0.12
301-11	0.05	0.47
301-12	0.02	0.09
301-13	0.18	0.21

Immunofluorescence and BioQuant® Assay Methods

In *Study 201*, statistically significant increases over baseline were observed at Week 12 (n = 4; eteplirsen 50 mg/kg, p = 0.004) and Week 24 (n = 4; eteplirsen 30 mg/kg, p = 0.012) using MANDYS106 antibody. Assessment using the DYS2 antibody did not reach statistical significance for either Week 12 or 24.

In *Study 202*, the Week 48 data were supportive of the Week 24 findings. In the eteplirsen-treated group, change from Baseline in dystrophin intensity per fiber (as measured by IHC with antidystrophin antibody) increased over time from 10.57% of normal at Baseline to 25.98% of normal at Week 48. In the placebo-to-eteplirsen group, dystrophin intensity per fiber also increased substantially from 9.11% of normal at Baseline to 23.43% of normal at Week 48, i.e., after initiation of eteplirsen after Week 24. Week 48 results were limited by the inability to directly compare on-treatment samples to baseline samples since baseline samples were not processed and/or scored at the same time as Week 48 samples.

Percent Dystrophin-Positive Fibers

The primary pharmacodynamic endpoint was the percent dystrophin-positive fibers, assessed by determination of the percentage of dystrophin-positive fibers in muscle tissue samples obtained pre- and post-treatment using IHC detection with the different anti-dystrophin antibodies with immunofluorescent staining.

In Study 201, the primary endpoint in the 24-week placebo-controlled portion of Study 201 was the change from baseline in percent dystrophin-positive fibers. At Week 25, the placebo patients began open-label treatment and all patients continue to receive eteplirsen treatment in the ongoing extension study.

Treatment with 50 mg/kg did not demonstrate a significant increase in the amount of mean percent dystrophin-positive fibers at Week 12. However, treatment with 30 mg/kg eteplirsen (N = 4) for 24 weeks significantly increased the mean percent dystrophin-positive fibers from a baseline of 18.19% to 41.14% resulting in an absolute increase of 22.95% baseline using the MANDYS106 primary antibody.

Table 7 Effect of Eteplirsen on Dystrophin-Positive Fibers Detected by IHC with MANDYS106 (Full Analysis Population)

Time point		Placebo N = 4	30 mg/kg/wk Eteplirsen N = 4	50 mg/kg/wk Eteplirsen N = 4
Baseline	Mean	15.64	18.19	11.00
	Median	15.58	17.80	11.51
	SD (SE)	10.742 (5.371)	5.501 (2.751)	4.668 (2.334)
	Min, Max	3.2, 28.2	11.9, 25.3	5.4, 15.6
On-Treatment[b]	Mean	11.59	41.14	11.79
	Median	9.44	38.77	11.81
	SD (SE)	7.130 (3.565)	10.097 (5.049)	4.456 (2.228)
	Min, Max	5.7, 21.7	32.7, 54.3	6.4, 17.2
Change from Baseline	Mean	-4.05	22.95[c]	0.79
	Median	-6.13	23.46	2.52
	SD (SE)	5.834 (2.917)	5.792 (2.896)	7.099 (3.549)
	Min, Max	-8.5, 4.5	15.9, 29.0	-9.3, 7.4

Source: Table 14.2.1.1.1; Table 14.2.1.1.2

[a]Results are expressed as a percentage of total fibers counted. As normal muscle samples have 100% dystrophin-positive muscle fibers, percent total dystrophin-positive muscle fibers can also be expressed as a percentage of normal.

[b]On-treatment samples are from Week 12 for all 4 patients in the 50 mg/kg/wk eteplirsen group and 2 patients in the placebo group, or from Week 24 for all 4 patients in the 30 mg/kg/wk eteplirsen group and 2 patients in the placebo group.

[c] p = 0.002 for 30 mg/kg/wk eteplirsen vs. placebo based on ANCOVA model for ranked data with treatment (placebo, 30 mg/kg/wk, 50 mg/kg/wk) as a fixed effect and baseline value and time since DMD diagnosis as covariates.

Abbreviations: max = maximum; min = minimum; SD = standard deviation; SE = standard error.

However, the percentage of dystrophin-positive fibers assessed via IHC using other anti-dystrophin antibodies Dys2, and Dys3 did not show any statistical differences between patients treated with eteplirsen and placebo.

In study 202 change from baseline to Week 48 (cumulative study period for Studies 201/202) in the percentage of dystrophin positive fibers as measured by immunohistochemistry (IHC) using different anti-dystrophin antibodies was specified in the protocol as the primary biological endpoint.

Eteplirsen treatment significantly increased the mean percentage of dystrophin positive fibers from baseline to Week 48 for both the placebo-to-eteplirsen group (n = 4; p = 0.009) and the all eteplirsen group (n = 8; p <0.001) (Table X). In the placebo-to-eteplirsen group (n = 4), the mean percentage of dystrophin positive fibers increased from 15.6% of normal at baseline to 53.4% of normal at Week 48. In the all eteplirsen group (n = 8), the mean percentage of dystrophin positive fibers increased from 14.6% of normal at baseline to 61.9% of normal at Week 48.

Table 8 Change from Baseline to Week 48 in Percent Dystrophin Positive Fibers Detected Using MANDYS106 Antibody (ITT Population, original evaluation)

Parameter	Placebo-to-eteplirsen N = 4	Eteplirsen		
		30 mg/kg N = 4	50 mg/kg N = 4	All Eteplirsen N = 8
Baseline, n	4	4	4	8
Mean (SD)	15.64 (10.742)	18.19 (5.501)	11.00 (4.668)	14.60 (6.088)
Median	15.58	17.80	11.51	14.77
Min, Max	3.2, 28.2	11.9, 25.3	5.4, 15.6	5.4, 25.3
Week 48 actual values, n	4	4	4	8
Mean (SD)	53.35 (11.802)	69.89 (11.489)	53.93 (13.217)	61.91 (14.289)
Median	53.16	67.34	52.81	60.83
Min, Max	40.0, 67.0	60.2, 84.7	40.7, 69.4	40.7, 84.7
Change from baseline to Week 48	4	4	4	8
Mean (SD)	37.70 (12.602)	51.69 (7.089)	42.93 (13.433)	47.31 (10.992)
Median	33.67	51.88	40.83	47.29
Min, Max	28.4, 55.1	43.6, 59.4	29.8, 60.3	29.8, 60.3
p-value	0.009	<0.001	0.008	<0.001

Max = maximum; Min = minimum; SD = standard deviation.
Note: P-value was from a paired t-test for difference from baseline.
Source: Table 14.2.10.1.1.1

However, the applicant points out that interpretation of the Week 48 dystrophin results was limited by the inability to directly compare on-treatment to baseline samples since baseline samples were not processed and/or scored at the same time as Week 48 samples. Therefore the additional Week 180 (fourth biopsy) was performed.

The results from a reassessment of baseline, Week 12 and Week 24 images samples from study 201 by three trained pathologists overall indicated an increase of dystrophin-positive fibers in the eteplirsen group exposed for the longest time-period 24 weeks, even though the actual values showed some differences from the original evaluation.

At the time of analysis of the Week 180 biopsy samples, frozen, archived baseline muscle biopsy tissue from Study 201 was available for re-analyses from only a limited number of patients, resulting in baseline values for only 3 patients for each of the 3 dystrophin parameters. Since baseline tissue was not available for all patients, samples were supplemented with tissue from untreated control patients amenable to exon 51 skipping not enrolled in Study 201/202, in order to provide a total of 9 untreated samples as a comparator group. The additional 6 untreated control samples for each assay were from confirmatory Study301.

In the comparison of Week 180 biopsies of eteplirsen-treated patients to the biopsies of untreated controls, the mean percent dystrophin-positive fibers in the eteplirsen-treated patients at Week 180 (37.33%), as determined by a blinded analysis of digital images performed by a single expert, showed a difference of 32.29% between the eteplirsen-treated patients and the untreated controls ($p < 0.001$; Report SR-CR-15-008). Confirmation of this finding was provided on the identical digital images by 3 blinded pathologists (Flagship

Biosciences) performing independent analysis, with mean differences between the eteplirsen-treated patients and the untreated controls ranging from 14.15% to 19.99% for the 3 raters (all p-values <0.001).

Table 9 Mean Percent Dystrophin-Positive Fibers in Eteplirsen-Treated Patients (Week 180, Studies 201/202) (N = 11) vs. Untreated DMD Controls (N = 9)

	Single-Rater Assessment	Multirater Assessment			
	NCH Pathologist	Pathologist 1	Pathologist 2	Pathologist 3	Composite*
Eteplirsen, mean (SD)	37.33 (14.267)	15.67 (9.846)	21.30 (12.219)	15.20 (8.442)	17.39 (9.999)
Untreated, mean (SD)	5.04 (5.855)	1.02 (1.293)	1.31 (1.294)	1.05 (1.371)	1.12 (1.312)
Mean Diff. (95% CI) p-value	32.29 (22.15, 42.43) p <0.001	14.66 (8.01, 21.30) p <0.001	19.99 (11.75, 28.22) p <0.001	14.15 (8.44, 19.87) p <0.001	16.27 (9.51, 23.02) p <0.001

Abbreviations: CI = confidence interval; DMD = Duchenne muscular dystrophy; NCH = Nationwide Children's Hospital; SD = standard deviation.
* Composite is the average of the 3 pathologists in the multirater assessment.

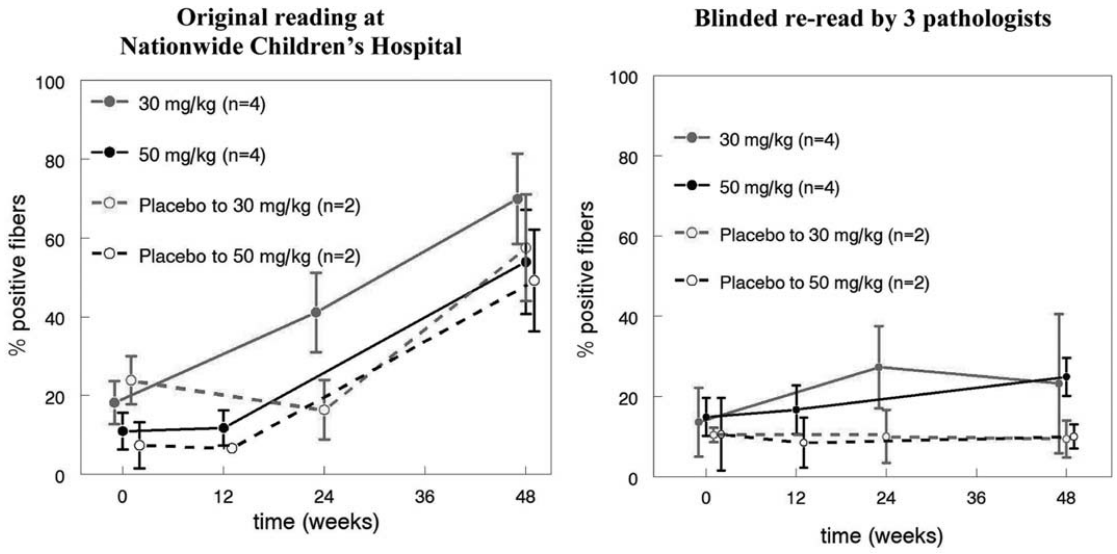
These results suggest a production of a (truncated) dystrophin and, in addition, might indicate that eteplirsen-induced increase of truncated dystrophin is delayed up to several months from start of treatment. However, nothing is known regarding the effectiveness of the truncated dystrophin. The amount produced seems very limited according to the Western blot results.

However, there are still some outstanding questions regarding the measured dystrophin levels/% positive fibers/intensity, as some of the ab used did not show any significant effect, and many analyses were not pre-specified, as understood by the CHMP.

In addition, the variability of results due to methodological differences is clearly illustrate by the two very different results for dystrophin-positive fiber levels resulting from different methodology by different evaluators, during the first 48 weeks of study 202 (Figure A). This variability is referred to by two representatives of FDA (Unger et al. Annals of Neurology, 81,1,2017), where they express "the numerous methodological shortcomings (in studies 201/202) should be noted to assist others who may be involved in producing evidence of a quality needed for regulatory submissions". They also noted that in the 3 pathologists' analysis the patients who switched from placebo to eteplirsen at Week 24, there was no response between Weeks 24 and 48 which is not in line with an expected increase based on the first 24 weeks results from the patients initially receiving eteplirsen. This variability of results adds to the uncertainties regarding the muscle biopsy results.

In this context it could also be noted that in study 33 a suggested effect on dystrophin production was already seen after one i.m. injection, and in study 28 a suggested dystrophin production was already seen after 14 weeks, which is not in line with no increase of dystrophin-positive fibers in the 50 mg/kg group after 12 weeks of eteplirsen exposure.

Figure 7 Percent of dystrophin-positive fibers



From Unger et al. Annals of Neurology, 81,1,2017

The differences observed between both assessments have been explained by the fact that (as suggested by the FDA) a revised, more stringent protocol for assigning positivity was used in the retesting assessment. This new protocol represented a different counting procedure as excluded fibers partially positive for dystrophin expression (Muntoni et al. Nature Biothecnology 2017; 35(3): 207-209). In any case both methodologies confirm dystrophin expression after eteplirsen treatment. However, the main concern has to do with how the dystrophin production translates into a clinical effect.

The amount of dystrophin expression is lower than it was expected when the trials were initiated, very likely due to delivery problems which seem to be shared by many antisense oligonucleotides (Godfrey et al EMBO Mol Med 2017), as mechanism of action (RNA exon skipping) has also been demonstrated.

The clinical relevance of this low amount of dystrophin is unknown: some studies report than 30% of dystrophin is enough for some Becker muscular dystrophy (BMD) patients to have a much milder phenotype (Neri Neuromuscular Disorders 2007). Also, dystrophinopathy patients of intermediate clinical severity have been associated with dystrophin levels of between 10 and 25% of normal levels while in-frame deletions in BMD patients with severe DMD phenotype have been associated with less than 10% dystrophin (Lu Q Molecular Therapy—Nucleic Acids 2014). The argument that BMD patients have expressed that amount of dystrophin from birth indicates that a higher amount of newly expressed dystrophin may be necessary. In this scenario the amount of dystrophin expressed after treatment with eteplirsen will be clearly insufficient. However, other studies (van Putten 2012) report that the new expression of less than 4% of dystrophin in mouse models is enough to clinically benefit them and DMD patients that naturally present dystrophin traces and revertant fibres present also a milder phenotype (Anthony JAMA Neurlo 2014). Although those studies present mouse data and data from very few patients, a minimum beneficial amount of dystrophin expression has not been established, and according to this data, it is unknown whether the amount of dystrophin expressed after treatment with eteplirsen could be beneficial to patients.

2.5. Clinical efficacy

This application for eteplirsen is supported by efficacy data derived from 4 interventional clinical studies, an external control cohort, and a review of literature describing the natural history of DMD.

Table 10 Description of Eteplirsen Studies Included in the Summary of Clinical Efficacy

Descriptor	Study Number			
	Pivotal		Supportive	
	Study 201	Study 202	Study 28	Study 33
Study Design	Randomized, double-blind, placebo-controlled, multiple-dose, single-center (US) study	Multicenter (US), open-label, multiple-dose extension study	Dose-ranging study Open-label, multiple-dose, (UK)	Proof of concept Single-blind, placebo-controlled, single-dose, investigator-sponsored, (UK)
Dosing Regimen	Eteplirsen 30 or 50 mg/kg/week, or placebo (weekly IV infusion) Weeks 1-24, then eteplirsen 30 or 50 mg/kg Weeks 25-28	Eteplirsen 30 or 50 mg/kg/week (weekly IV infusion)	Eteplirsen 0.5, 1.0, 2.0, 4.0, 10.0, or 20.0 mg/kg/week (weekly IV infusion)	Eteplirsen 0.09 or 0.9 mg IM in the EDB of 1 foot and placebo (IM) in the EDB of the opposite foot
PD Endpoints	**Primary:** Change from BL in PDPF at Week 12 (50 mg/kg group) and at Week 24 (30 mg/kg group). **Other:** Exon skipping (RT-PCR) and dystrophin PDPF and intensity in biopsied muscle	**Primary:** Change from BL (of Study 201) to Week 48 (combined) in PDPF **Other:** Exon skipping (RT-PCR), change from BL in PDPF (Week 12 and 24); change from BL in dystrophin intensity at Week 48; differences from untreated controls in PDPF, dystrophin intensity, and dystrophin quantity by Western blot at Week 180	**Exploratory:** Change from BL to Week 14 in dystrophin PDPF; Change from BL to Week 14 in dystrophin intensity (IHC) and protein levels (Western blot)	**Exploratory:** Restoration of dystrophin protein expression and the DAPC at Week 2-4, Exon Skipping (RT-PCR)
Clinical Endpoints	**Primary:** 6MWT, LOA, NSAA, rise time and PFTs	**Primary:** Change from BL in 6MWT through Week 240 (combined), LOA, NSAA, rise time and PFTs	**Primary:** Safety and tolerability	**Primary:** Safety
Required Age at Entry (yrs)	7-13		5-15	10-17
Study Status	Completed	Completed[a]	Completed	Completed

No. Enrolled	12		19[b]	7
No. Completed	12	12	18[b]	7
Study Period	Jul 2011 – Feb 2012	Feb 2012 – Apr 2016 (for Efficacy)	Jan 2009 – Jun 2010	Oct 2007 – Apr 2009
Study Duration	28 Weeks	212 Weeks (240 weeks combined Study 201/202)	12 Weeks	Single Dose

Abbreviations: 6MWT = 6-Minute Walk Test; BL = Baseline; DAPC = dystrophin-associated protein complex; EDB = extensor digitorum brevis muscle; IM = intramuscular; IV = intravenous; LOA = Loss of Ambulation; No. = number; NSAA = North Star Ambulatory Assessment; PD = pharmacodynamic; PDPF = percent dystrophin-positive fibers; PFT = pulmonary function testing; RT-PCR = reverse transcriptase-polymerase chain reaction; UK = United Kingdom; US = United States; Wk = week; yrs = years.
a Patients continue to be dosed and followed for safety until they transition to commercial drug.
b Two patients did not have both pretreatment and post-treatment biopsies for analysis; therefore, data for 17 patients were used in the pharmacodynamic analyses.

In addition to the completed studies, safety data are provided from an additional 112 eteplirsen-treated patients from 3 ongoing clinical studies, for a total of 150 patients included in the eteplirsen safety assessment for this MAA:

• Study 4658-301 (Study 301 [PROMOVI]) is a confirmatory 96-week, open-label Phase 3 study of eteplirsen (30 mg/kg by weekly IV infusion) in ambulatory patients (N = 120) with DMD, ages 7 to 16 years old compared an untreated control group of patients with DMD amenable to skipping of any exon, with the exception of exon 51.Enrollement of this study is complete; 79 in the eteplirsen-treated group and 29 in the untreated control group.

• Study 4658-203 (Study 203) is a 96-week, open-label Phase 2 study to evaluate safety and pharmacokinetics of eteplirsen (30 mg/kg by weekly IV infusion) in younger patients (N = 40) with DMD, aged 4 to 6 years old. Study 203 is currently enrolling patients; 26 eteplirsen-treated patients and 7 untreated controls were enrolled at the time of the MAA safety data cutoff.

• Study 4658-204 (Study 204) is a 96-week, open-label Phase 2 study primarily to evaluate safety of eteplirsen (30 mg/kg by weekly IV infusion) in patients (N = 24) with advanced stage DMD (including non-ambulatory patients), ages 7 to 21 years old. Enrollment in Study 204 has been completed.

2.5.1. Dose-response studies and main clinical studies

Dose selection

The doses of eteplirsen administered in the Studies 201/202, 30 or 50 mg/kg/wk, were based on preclinical data in non-human primates and mice in which maximum feasible doses (320 mg/kg/wk and 960 mg/kg/wk, respectively) were well tolerated when administered for 12 weeks. Since the maximum tolerated dose had not been identified in the dose-ranging study (Study AVI-4658-28), higher doses of eteplirsen, 30 and 50 mg/kg weekly IV infusion, were selected for Study 201.

2.5.2. Main studies

Study 4658-US-201

"A Randomized, Double-Blind, Placebo-Controlled, Multiple Dose Efficacy, Safety, Tolerability, and Pharmacokinetics Study of AVI-4658 (Eteplirsen), a Phosphorodiamidate Morpholino Oligomer, Administered Over 28 Weeks in the Treatment of Ambulant Subjects with Duchenne Muscular Dystrophy"

The number of patient included in the study 201 was very limited, and included only 12 patients. It could also be noted that only investigative site was located in US.

Patients were randomized to 1 of 3 treatment groups: 30 mg/kg eteplirsen (n = 4), 50 mg/kg eteplirsen (n = 4), or placebo (n = 4) administered as weekly intravenous (IV) infusions. After 24 weeks, all patients received open-label eteplirsen treatment through the last visit in Study 201 (Study Week 28), where patients originally randomized to placebo began open-label treatment with 30 or 50 mg/kg/wk eteplirsen (n = 2 per dose level) and patients originally randomized to eteplirsen continued treatment at the same dose.

All patients who completed this study were eligible to continue eteplirsen treatment in an open-label extension study, Study 4658-us-202.

All patients received a pre-treatment biopsy of the biceps muscle within 4 weeks prior to the first administration of study drug.

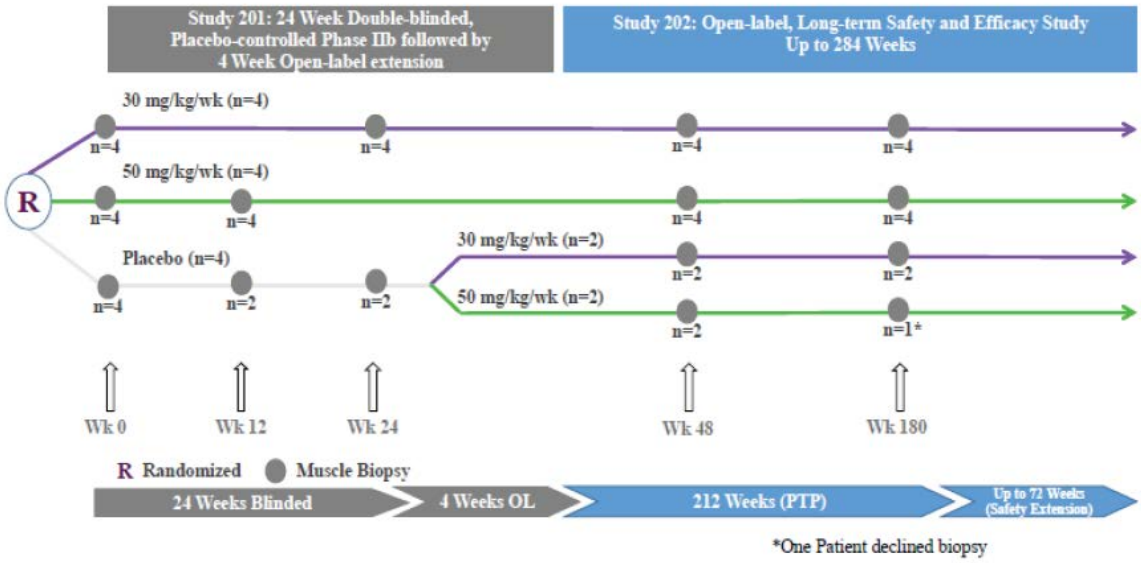


Figure 8 Schematic of Study Flow for Pivotal Studies 201/202

Abbreviations: PTP =Primary Treatment Period.

Study participants

Study 201 inclusion and exclusion criteria were designed to select a homogeneous population of DMD boys, with genetically confirmed deletion mutation amenable to exon 51 skipping that would be expected to experience a

predictable decline in 6MWT over the course of the study. Selection of this narrow population was considered the best group to evaluate whether stabilization of function would occur with eteplirsen intervention. Accordingly, the inclusion criteria specified boys aged 7-13 years.

Table 11 Key Entry Criteria for Pivotal Study 201

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

Abbreviations: 6MWT = 6-minute walk test; DMD = Duchenne muscular dystrophy; FVC = forced vital capacity; LVEF = left ventricular ejection fraction.

Exclusion Criteria

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

1. Use of any pharmacologic treatment, other than corticosteroids, that might have an effect on muscle strength or function within 12 weeks before study entry (e.g., growth hormone, anabolic steroids).

2. Previous treatment with the experimental agent eteplirsen, BMN-195, or PRO051.

3. Previous treatment with any other experimental agents or participation in any other DMD interventional clinical study within 12 weeks before entry into this study; including use of the shock training system or "STS," or planned use during this study.

4. Surgery within 3 months before study entry or planned surgery at any time during this study.

5. Presence of other clinically significant illness at the time of study entry, including significant renal dysfunction (as measured by urinary cystatin C, KIM-1, or urinary total protein), or average heart rate during screening Holter monitoring in excess of 110 bpm (unless subsequently treated and confirmed controlled and stable on a β-blocker) or QTc >450 ms.

6. Use of any aminoglycoside antibiotic within 12 weeks before the screening visit (Visit 1) or need for use of an aminoglycoside antibiotic during the study (unless discussed and agreed with the Principal Investigator and Medical Monitor).

7. Prior or ongoing medical condition that, in the Investigator's opinion, could adversely affect the safety of the patient or that makes it unlikely that the course of treatment or follow-up would be completed or could impair the assessment of study results.

The primary efficacy endpoint was the change from baseline in the percentage of dystrophin-positive fibers as measured in muscle biopsy tissue using immunohistochemistry (IHC) at Week 12 for the 50 mg/kg/wk

eteplirsen and matching placebo groups (Groups 1 and 3a) and at Week 24 for the 30 mg/kg/wk eteplirsen and matching placebo groups (Groups 2 and 3b).

Functional Efficacy Endpoints

Change from baseline to week 24 in the:

- 6-Minute Walk Test (6MWT)
- Timed 4-Step Test
- Maximum Voluntary Isometric Contraction Test (MVICT)
- North Star Ambulatory Assessment (NSAA) total score, and NSAA components including the Timed 10-Meter Run and rise time
- 9-Hole Peg Test
- Pulmonary Function Testing (PFT) including forced vital capacity (FVC), percent predicted FVC (%FVC), forced expiratory volume in 1 second (FEV1), percent predicted FEV1 (%FEV1), FEV1/FVC ratio; maximal inspiratory pressure (MIP), and maximal expiratory pressure (MEP)
- Pediatric Quality of Life Inventory (PedsQL)

Statistical methods

The following analysis populations were defined:

• Safety Population: included all randomized patients who received any amount of study drug. Analyses performed on the safety population were done according to the treatment actually received.

• Full Analysis Population: the same as the safety population. Given the small sample size, analyses performed on the full analysis population were done according to the treatment actually received.

• Modified Intent-to-Treat Population (mITT): The mITT is similar to the Full Analysis Population but excluded 2 patients who showed rapid disease progression during the first few weeks of this study.

• PK Population: included all randomized patients for whom there were adequate PK samples from which to estimate PK parameters. Analyses performed on the PK samples were done according to the treatment actually received.

No formal sample size calculations were performed. A sample size of 12 total patients was selected with 4 patients in each of the 3 treatment groups: 50 mg/kg/wk eteplirsen, 30 mg/kg/wk eteplirsen, and placebo.

Analysis of change from baseline to Week 24 in the 6MWT, Timed 4-Step Test, MVICT, NSAA total score, and the Timed 10-Meter Run was based on a restricted maximum likelihood (REML)-based mixed model repeated measures (MMRM) with treatment (placebo, 30 mg/kg/wk, 50 mg/kg/wk), time, and treatment-by-time interaction terms as fixed effects, patient nested within treatment as a random effect, and with the baseline value and time since DMD diagnosis as covariates. A first-order autoregressive (AR1) covariance structured matrix was used. The treatment comparison was made between each of the active treatments and placebo at Week 24 and at each of the other post-baseline visits. This procedure did not replace missing data, all available on-treatment assessments were used in the mixed model. In this analysis, in which the MMRM is fitted to all post-baseline data, patients in the full analysis population who did not have complete data still contributed to the estimates at Week 24, but had less weight in the analysis than those patients with complete data. An analysis

of observed scores available at each visit was also performed. Estimates for changes from baseline at each time-point in each treatment group and for treatment difference were provided with 95% confidence intervals (CIs) and p-values using the least significant difference contrasts from the model. The same MMRM analysis described above was repeated to compare the combined eteplirsen group to placebo.

Sensitivity analyses of each of the clinical assessment parameters were to be performed and included an analysis of covariance for repeated measures (ANCOVAR) model with treatment, time, and treatment-by-time interaction terms as fixed effects, patient nested within treatment as a random effect, and with baseline values and time since DMD diagnosis as covariates. In this analysis, patients without a post-baseline value had a value imputed from an earlier post-baseline score using the last-observation-carried-forward (LOCF) method (baseline values will not be carried forward).

If there was strong evidence suggesting that any of these endpoints deviated from normal distribution, as judged by the p-value for the Shapiro-Wilk test, then ANCOVA for ranked data (Stokes 2000) was utilized.

The data for the NSAA Rise Time, and time to complete the 9-Hole Peg Test using dominant hand were analyzed using the MMRM analysis described above.

All remaining discrete functional outcome variables, which include components of the NSAA and the 9-Hole Peg Test, were summarized with descriptive statistics by treatment group and visit. Week 24 data was also compared to baseline data using the Cochran-Mantel Haenszel (CMH) statistics or other cross-tabulation procedures as appropriate based on the distribution of the observed data.

Pulmonary function testing parameters including FVC, %FVC, FEV1, %FEV1, FEV1/FVC ratio, MIP, and MEP were summarized using descriptive statistics by treatment group (placebo, 30 mg/kg/wk eteplirsen, 50 mg/kg/wk eteplirsen, combined eteplirsen group) and visit using the observed, on-treatment change from baseline, and the percentage of on-treatment change from baseline. The MMRM analysis described above was also utilized for these data.

The PedsQL core scale scores (physical, emotional, social, and school functioning), the psychosocial health summary score (combination of emotional, social, and school functioning scales), and the total scale score were summarized using descriptive statistics by treatment group (placebo, 30 mg/kg/wk eteplirsen, 50 mg/kg/wk eteplirsen, combined eteplirsen group) and visit using the observed, on-treatment change from baseline, and the percent of on-treatment change from baseline. The NMM was scored and summarized in a similar fashion, and an MMRM analysis, as described above, was also utilized.

The analysis plan in the study protocol was updated in version 7.0, but in the final SAP, the last version of the protocol is not adhered to, instead it is based on an earlier version of the CSP. The analysis plan followed is according to the SAP.

Results

The first subject was randomized into the study on 18 July 2011. The date of last subject last visit was 29 February 2012.

A total of 12 patients were randomized into the groups. All 12 patients received all scheduled infusions of study medication and completed the study as planned.

Baseline data and demographic characteristics

All patients were male and, except for one patient of Asian descent, all were white. At baseline, patients had a mean age of 8.8 years. Compared to the other groups, patients in the 30 mg/kg/wk group were slightly older, heavier, and taller at baseline, and they achieved a shorter distance on the 6MWT.

Table 12: Summary of Demographic Characteristics (Safety Population)

			Eteplirsen			
Parameter		**Placebo N = 4**	**30 mg/kg/wk N = 4**	**50 mg/kg/wk N = 4**	**All Eteplirsen N = 8**	**All Patients N = 12**
Gender n(%)	Male	4 (100)	4 (100)	4 (100)	8 (100)	12 (100)
Age, years	Mean	8.5	9.3	8.5	8.9	8.8
	Median	8.5	9.0	8.5	9.0	9.0
	SD	1.73	0.50	1.29	0.99	1.22
	Min, Max	7, 10	9, 10	7, 10	7, 10	7, 10
Height, cm	Mean	119.3	130.5	121.3	125.9	123.7
	Median	118.5	133.5	117.5	124.5	118.5
	SD	3.40	9.47	7.85	9.45	8.40
	Min, Max	116, 124	117, 138	117, 133	117, 138	116, 138
Weight, kg	Mean	30.65	34.85	29.05	31.95	31.52
	Median	32.15	37.40	27.10	31.25	32.15
	SD	6.035	7.050	6.376	6.952	6.411
	Min, Max	22.1, 36.2	24.8, 39.8	23.7, 38.3	23.7, 39.8	22.1, 39.8
BMI, kg/m^2	Mean	21.51	20.23	19.57	19.90	20.44
	Median	22.02	20.68	19.80	20.23	20.47
	SD	3.980	1.470	1.918	1.622	2.573
	Min, Max	16.4, 25.6	18.1, 21.5	17.0, 21.7	17.0, 21.7	16.4, 25.6
Race, n(%)	Asian	0	1 (25)	0	1 (12.5)	1 (8.3)
	White	4 (100)	3 (75)	4 (100)	7 (87.5)	11 (91.7)

Abbreviations: BMI = body mass index; max = maximum; min = minimum; SD = standard deviation.

Table 13: Baseline Disease Characteristics (Safety Population)

Parameter		Placebo N = 4	Eteplirsen 30 mg/kg/wk N = 4	Eteplirsen 50 mg/kg/wk N = 4	All Eteplirsen N = 8	All Patients N = 12
Mutation	45-50 n (%)	0	2 (50)	1 (25)	3 (37.5)	3 (25)
	48-50 n (%)	0	1 (25)	0	1 (12.5)	1 (8.3)
	49-50 n (%)	3 (75)	0	2 (50)	2 (25)	5 (41.7)
	50 n (%)	1 (25)	0	0	0	1 (8.3)
	52 n (%)	0	1 (25)	1 (25)	2 (25)	2 (16.7)
Time Since DMD Diagnosis, months	Mean	50.3	52.5	66.5	59.5	56.4
	Median	51.0	57.0	68.0	57.0	57.0
	SD	13.74	14.06	44.29	31.33	26.40
	Min, Max	36, 63	32, 64	18, 112	18, 112	18, 112
Duration of Steroid Use, months	Mean	44.875	49.875	52.825	51.350	49.192
	Median	45.550	53.800	52.050	53.800	53.800
	SD	21.6297	13.4812	35.3952	24.8455	23.0344
	Min, Max	21.7, 66.7	30.4, 61.5	15.5, 91.7	15.5, 91.7	15.5, 91.7
Holter Monitor Average Heart Rate, bpm	Mean	96.8	96.8	93.8	95.2	95.8
	Min, Max	91, 102	86, 102	86, 102	86, 102	86, 102
6MWT[a], meters	Mean	394.5	355.3	396.0	375.6	
	Median	379.0	359.0	395.0	380.5	
	SD	42.25	74.78	26.61	56.34	
	Min, Max	364, 456	261, 442	365, 429	261, 442	
%FEV_1 (%)	Mean	111.000	92.750	94.000	93.375	
	Median	109.500	92.000	98.500	95.500	
	SD	11.9722	7.7190	23.2236	16.0351	
	Min, Max	98, 127	85, 102	62, 117	62, 117	
%FVC (L)	Mean	116.3	95.3	92.3	93.8	
	Median	119.0	98.0	88.0	93.5	
	SD	15.44	7.80	11.59	9.29	
	Min, Max	96, 131	84, 101	84, 109	84, 109	

Outcomes and estimation

Change from Baseline in 6MWT

As shown in the table below, from baseline to Week 24, placebo-treated patients experienced a mean decline of 17.3 meters, while patients in the 30 and 50 mg/kg/wk eteplirsen groups showed mean declines of 134.8 and 2.3 meters, respectively.

The large decline in the 30 mg/kg/wk eteplirsen group was directly attributable to Patients 009 and 010; when these 2 patients were excluded from the analysis, the mean change from baseline to Week 24 was a decline of 12.5 meters.

Table 14: Summary and Change from Baseline in 6MWT Results (Full Analysis and mITT Populations)

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[b]				
Mean	394.5	355.3	407.0	396.0
Median	379.0	359.0	407.0	395.0
SD (SE)	42.25 (21.12)	74.78 (37.39)	49.50 (35.00)	26.61 (13.30)
Min, Max	364, 456	261, 442	372, 442	365, 429
Week 24[b]				
Mean	377.3	220.5	394.5	393.8
Median	377.5	204.0	394.5	403.5
SD (SE)	19.00 (9.50)	203.14 (101.57)	51.62 (36.50)	53.67 (26.84)
Min, Max	354, 400	43, 431	358, 431	325, 443
Change at Week 24				
Mean	-17.3	-134.8	-12.5	-2.3
Median	-12.0	-116.0	-12.5	1.5
SD (SE)	28.06 (14.03)	144.71 (72.36)	2.12 (1.50)	29.89 (14.95)
Min, Max	-56, 11	-296, -11	-14, -11	-40, 28

a mITT excludes Patients 009 and 010.
b 6MWT value for each patient is the maximum distance achieved on days 1 and 2.
Abbreviations: 6MWT = 6-Minute Walk Test; max = maximum; min = minimum; mITT = modified intent to treat population; SD = standard deviation; SE = standard error.

Change from Baseline in the Timed 4-Step Test

Mean scores for the placebo and 50 mg/kg/wk eteplirsen groups decreased slightly from baseline to Week 24, while mean scores for the 30 mg/kg/wk eteplirsen group were 3 times higher at Week 24 than at baseline.

Table 15 Summary and Change from Baseline in Timed 4-Step Test (Full Analysis and mITT Populations)

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[b]				
Mean	5.30	4.88	3.75	3.50
Median	4.35	4.80	3.75	3.35
SD (SE)	1.934(0.967)	1.355(0.677)	0.354(0.250)	1.074(0.537)
Min, Max	4.3, 8.2	3.5, 6.4	3.5, 4.0	2.4, 4.9
Week 24[c]				
Mean	4.08	14.73	3.70	3.35
Median	4.15	10.15	3.70	3.15
SD (SE)	0.685(0.342)	15.069(7.535)	0.990(0.700)	1.240(0.620)
Min, Max	3.3, 4.7	3.0, 35.6	3.0, 4.4	2.1, 5.0
Change at Week 24				
Mean	-1.22	9.85	-0.05	-0.15
Median	-0.80	5.35	-0.05	-0.05
SD (SE)	1.597(0.798)	13.797(6.898)	0.636(0.450)	1.115(0.558)
Min, Max	-3.5, 0.2	-0.5, 29.2	-0.5, 0.4	-1.6, 1.1

a mITT excludes patients 009 and 010.
b Baseline is the last non-missing value before first dose.

c Week 24 is the best time achieved on days 1 and 2 of that visit.
Abbreviations: max = maximum; min = minimum; mITT = modified intent to treat population; SD = standard deviation; SE = standard error.

Change from Baseline in the North Star Ambulatory Assessment Total Score

While individual performance on the NSAA varied considerably, mean NSAA scores were relatively stable from baseline to Week 24 in the placebo and 50 mg/kg/wk eteplirsen groups (Table 15). In

Table 16 Summary and Change from Baseline in NSAA Total Scores

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[b]				
Mean	23.3	20.8	22.5	29.0
Median	22.0	19.0	22.5	29.0
SD (SE)	3.30 (1.65)	5.19 (2.59)	7.78 (5.50)	2.31 (1.15)
Min, Max	21, 28	17, 28	17, 28	27, 31
Week 24[c]				
Mean	26.5	14.8	23.5	26.8
Median	26.5	13.5	23.5	27.0
SD (SE)	4.04 (2.02)	10.53 (5.27)	4.95 (3.50)	5.12 (2.56)
Min, Max	23, 30	5, 27	20, 27	21, 32
Change at Week 24				
Mean	3.3	-6.0	1.0	-2.3
Median	2.0	-5.5	1.0	-2.0
SD (SE)	2.50 (1.25)	8.60 (4.30)	2.83 (2.00)	2.99 (1.49)
Min, Max	2, 7	-16, 3	-1, 3	-6, 1

a mITT excludes patients 009 and 010.
b Baseline is the last non-missing value before first dose.
c Week 24 is the best score achieved on days 1 and 2 of that visit.
Abbreviations: max = maximum; min = minimum; mITT = modified intent to treat population; NSAA = North Star Ambulatory Assessment; SD = standard deviation; SE = standard error.

Change from Baseline on the Timed 10-Meter Run

Table 17 Summary and Change from Baseline in 10-Meter Run Scores

Time point	Placebo (N = 4)	30 mg/kg/wk (N = 4)	30 mg/kg/wk (mITT)[a] (N = 2)	50 mg/kg/wk (N = 4)
Baseline[b]				
Mean	6.43	6.95	5.50	5.30
Median	6.55	7.60	5.50	4.90
SD (SE)	1.011(0.506)	2.138(1.069)	2.263(1.600)	1.111(0.555)
Min, Max	5.1, 7.5	3.9, 8.7	3.9, 7.1	4.5, 6.9
Week 24[c]				
Mean	5.78	13.10	4.35	4.78
Median	5.70	9.40	4.35	4.30
SD (SE)	0.150(0.075)	11.836(5.918)	0.212(0.150)	1.839(0.920)
Min, Max	5.7, 6.0	4.2, 29.4	4.2, 4.5	3.1, 7.4
Change at Week 24				
Mean	-0.65	6.15	-1.15	-0.53
Median	-0.70	3.25	-1.15	-0.55
SD (SE)	0.985(0.492)	10.368(5.184)	2.051(1.450)	0.866(0.433)
Min, Max	-1.8, 0.6	-2.6, 20.7	-2.6, 0.3	-1.5, 0.5

a mITT excludes patients 009 and 010.
b Baseline is the last non-missing value before first dose.
c Week 24 is the best score achieved on days 1 and 2 of that visit.
Abbreviations: max = maximum; min = minimum; mITT = modified intent to treat population; NSAA = North Star Ambulatory Assessment; SD = standard

Change from Baseline in Rise-Time

Rise time (a component of the NSAA) scores were variable across patients and over time. While mean times increased noticeably from baseline to Week 24 in the 30 mg/kg/wk eteplirsen group, this was again due to the performance of Patients 009 and 010. When these 2 patients were excluded, the mean time to rise from the floor decreased by 3 seconds. Mean time at 24 weeks increased 4.55 seconds in the 30 mg/kg/wk eteplirsen group compared and decreased 0.70 sec in the placebo group.

Change from Baseline on the 9-Hole Peg Test

Performance on the 9-Hole Peg Test was generally stable for all 3 treatment groups over the 24-week treatment period. All 12 patients were right-handed. Consistent with this, no significant between-group differences were observed at any time point in the full analysis population using the MMRM analysis.

Change from Baseline on Pulmonary Function Test Measurements

For all 3 treatment groups, PFT values tended to decline over the 24-week period assessed; however, %FVC and %FEV1 generally remained above 80%. Exceptions to this were observed in 2 placebo patients (Patient 007 had a %FEV1 of 63 at Week 24 and Patient 013 had a %FEV1 of 73 at week 12), 1 patient in the 30 mg/kg/wk eteplirsen group (Patient 009 had a %FEV1 of 78 at Week 24), and 1 patient in the 50 mg/kg/wk eteplirsen group (Patient 012 had a %FEV1 of 62 and 77 at baseline and Week 24, respectively).

Change from Baseline on the Pediatric Quality of Life Inventory

In general, small changes, both declines and improvements, were observed over the course of the study on both the child and parent versions of the PedsQL, including the Neuromuscular Module. Analysis of the full analysis population did not reveal any statistically significant differences between the treatment groups at any time point other than a small, but statistically significant difference between the placebo and 30 mg/kg/wk eteplirsen groups on the School Functioning subscale of the parent-reported PedsQL at Week 13 ($p = 0.026$). The PedsQL data were not analyzed for the mITT population.

Study 4658-US-202

"Open-Label, Multiple-Dose, Efficacy, Safety, and Tolerability Study of Eteplirsen in Patients with Duchenne Muscular Dystrophy Who Participated in Study 4658-us-201"

This study was conducted at 12 sites located in the United States

Study 202 was an extension of Study 201 in order to assess the long-term efficacy, safety, and tolerability of weekly intravenous (IV) infusions of either 30 mg/kg or 50 mg/kg eteplirsen, at the same dose they were receiving at their completion of Study 201. Eligible patients were enrolled in this study during the last visit of Study 201 (Study Week 28).

The placebo-to-30 mg/kg and placebo-to-50 mg/kg groups were pooled together for analysis (placebo-to-eteplirsen group). Treatment groups analyzed were as follows: placebo-to-eteplirsen (n = 4), eteplirsen 30 mg/kg (n = 4, or n = 6 when the eteplirsen experience for placebo-to-30 mg was included), and eteplirsen 50 mg/kg (n = 4, or n = 6 when the eteplirsen experience for placebo-to-50 mg was included). The

All Eteplirsen group (n = 8) includes the 8 patients originally randomized to eteplirsen 30 or 50 mg/kg/wk. The All Patients group (N = 12) includes the eteplirsen experience of all patients plus the placebo experience from the placebo-to-eteplirsen patients.

Upon completion of the study period (i.e., last patient, last Study Week 240 visit for efficacy assessments), patients originally randomized to placebo had each received approximately 216 weeks of eteplirsen treatment, and patients originally randomized to eteplirsen had each received approximately 240 weeks of eteplirsen treatment.

Efficacy was assessed by evaluating muscle/motor function and strength using the 6MWT, the Timed 4-Step Test, the NSAA, the 9-Hole Peg Test the maximum voluntary isometric contraction test (MVICT), and PFTs. In addition, quality of life was assessed using the PedsQL.

Muscle biopsies were obtained at Study Week 48 for analysis of exon skipping, dystrophin expression, and inflammatory markers. In addition, 11 out of 12 patients opted to participate in a voluntary fourth biopsy at approximately Study Week 180 for analysis of long-term exon skipping and dystrophin expression.

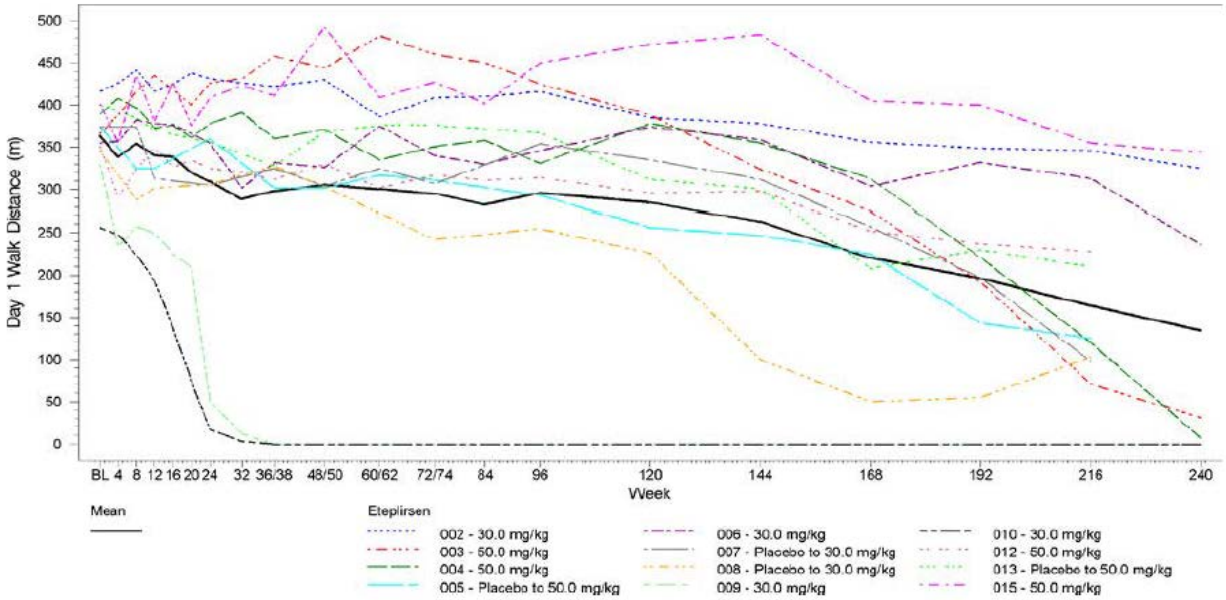
Patients were analyzed according to the actual dose of eteplirsen they received. No patients discontinued participation in the study or were withdrawn for any reason.

Efficacy Results

Primary Functional Efficacy Analysis – 6-Minute Walk Test

The primary functional efficacy analysis was change from baseline in 6MWT by Treatment Week. The analyses by Treatment Week standardized the baseline for all patients (N = 12) as being the last assessment prior to the start of eteplirsen treatment; this facilitated evaluation of the effect of eteplirsen treatment over time for all patients combined.

Figure 9 Individual Patient 6-Minute Walk Test Distance (Meters) and Population Mean Over Time by Treatment Week, Day 1 Values, ITT Population



ITT = intent-to-treat

Note: Treatment Week is derived from the first dose of the given study drug. For patients originally randomized to eteplirsen, Treatment Week is equal to Study Week. For patients originally randomized to placebo, the eteplirsen Treatment Week is 24 weeks less than Study Week, and baseline was the last value prior to initiation of eteplirsen.

Note: All 12 patients have data through Treatment Week 216 and 7 patients have data through Treatment Week 240. The 4 patients originally randomized to placebo had not reached Treatment Week 240 at the time of completion of the study period. In addition, Patient 012 (originally randomized to 50 mg/kg eteplirsen) did not perform this assessment at Study/Treatment Week 240 because of an injury (femur fracture) that prevented the patient from being able to travel to the central study site. Thus the n = 7 at Treatment Week 240

Table 18: 6-Minute Walk Test Distance (Meters) Over Time by Treatment Week, Day 1 Values, ITT Population

Time Point	Eteplirsen		
	30 mg/kg[a] (N = 6)	50 mg/kg[a] (N = 6)	All Eteplirsen[a] (N = 12)
Baseline, n	6	6	12
Mean	346.2	380.2	363.2
Median	350.5	381.5	370.0
SD (SE)	53.14 (21.70)	19.97 (8.15)	42.19 (12.18)
Min, Max	256, 416	351, 401	256, 416
Treatment Week 48/50, n	6	6	12
Mean	227.2	384.5	305.8
Median	303.5	369.5	328.0
SD (SE)	182.08 (74.34)	71.17 (29.06)	155.32 (44.84)
Min, Max	0, 430	302, 492	0, 492
Treatment Week 96, n	6	6	12
Mean	228.5	363.3	295.9
Median	300.5	349.0	338.5
SD (SE)	184.30 (75.24)	62.85 (25.66)	148.98 (43.01)
Min, Max	0, 416	293, 450	0, 450
Treatment Week 144, n	6	6	12
Mean	191.5	334.7	263.1
Median	206.0	312.5	306.5
SD (SE)	178.37 (72.82)	80.86 (33.01)	151.74 (43.80)
Min, Max	0, 378	247, 483	0, 483
Treatment Week 192, n	6	6	12
Mean	155.5	237.2	196.3
Median	126.0	225.5	209.0
SD (SE)	160.49 (65.52)	86.87 (35.47)	130.22 (37.59)
Min, Max	0, 349	143, 400	0, 400

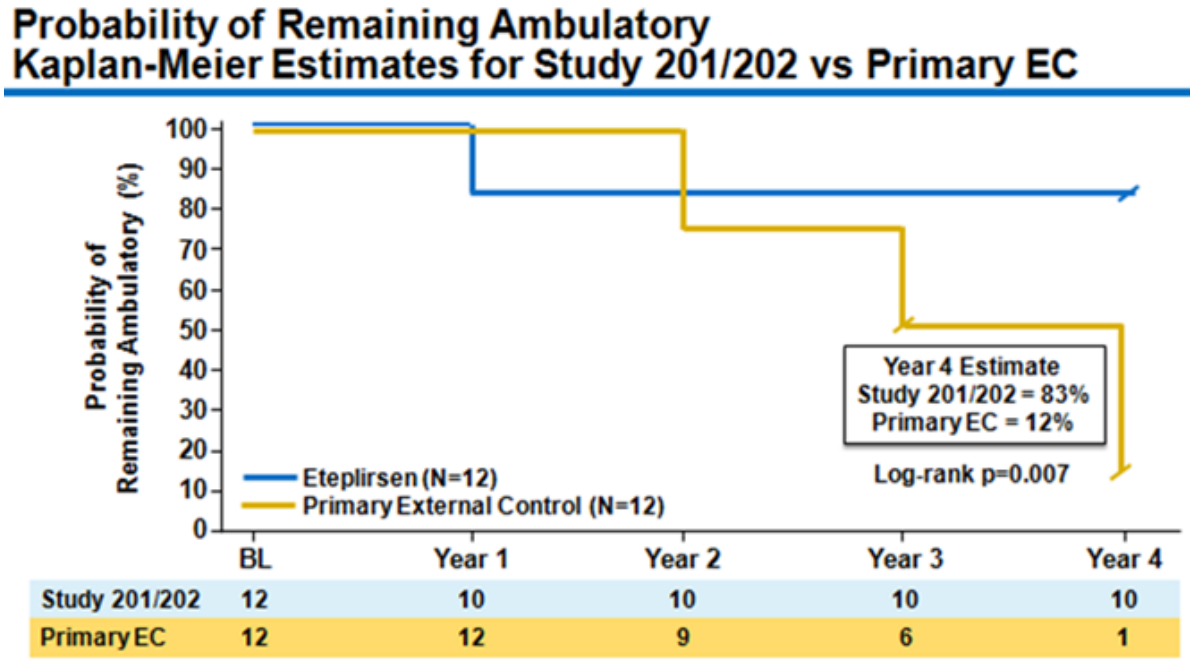
Treatment Week 216, n	6	6	12
Mean	143.3	184.8	164.1
Median	100.5	167.5	122.5
SD (SE)	151.41 (61.81)	102.17 (41.71)	125.04 (36.10)
Min, Max	0, 346	71, 355	0, 355
Treatment Week 240[b], n	4	3[b]	7[b]
Mean	140.3	127.3	134.7
Median	118.0	31.0	31.0
SD (SE)	165.97 (82.99)	188.02 (108.55)	160.02 (60.48)
Min, Max	0, 325	7, 344	0, 344

Max = maximum, Min = minimum, SD = standard deviation; SE = standard error.
Note: Treatment Week is derived from the first dose of the given study drug. For patients originally randomized to eteplirsen, Treatment Week is equal to Study Week. For patients originally randomized to placebo, the eteplirsen Treatment Week is 24 weeks less than Study Week.
a Placebo-to-eteplirsen patients are included in the appropriate eteplirsen group with baseline as the last value prior to initiation of eteplirsen.
b The 4 patients originally randomized to placebo had not reached Treatment Week 240 at the time of completion of the study period. In addition, Patient 012 (originally randomized to 50 mg/kg eteplirsen) did not perform this assessment at Study/Treatment Week 240 because of an injury (femur fracture) that prevented the patient from being able to travel to the central study site. Thus the n = 7 at Treatment Week 240.

Loss of Ambulation

Two patients (Patient 009 and Patient 010) in the 30 mg/kg eteplirsen group lost ambulation at approximately Study/Treatment Week 36. No additional patients lost ambulation through Study Week 240. Figure below shows the probability of remaining ambulatory in studies 201/202 for eteplirsen group and patients from Primary EC.

Figure 10 Probability of Remaining Ambulatory Kaplan-Meier Estimates for Study 201/202 vs Primary EC



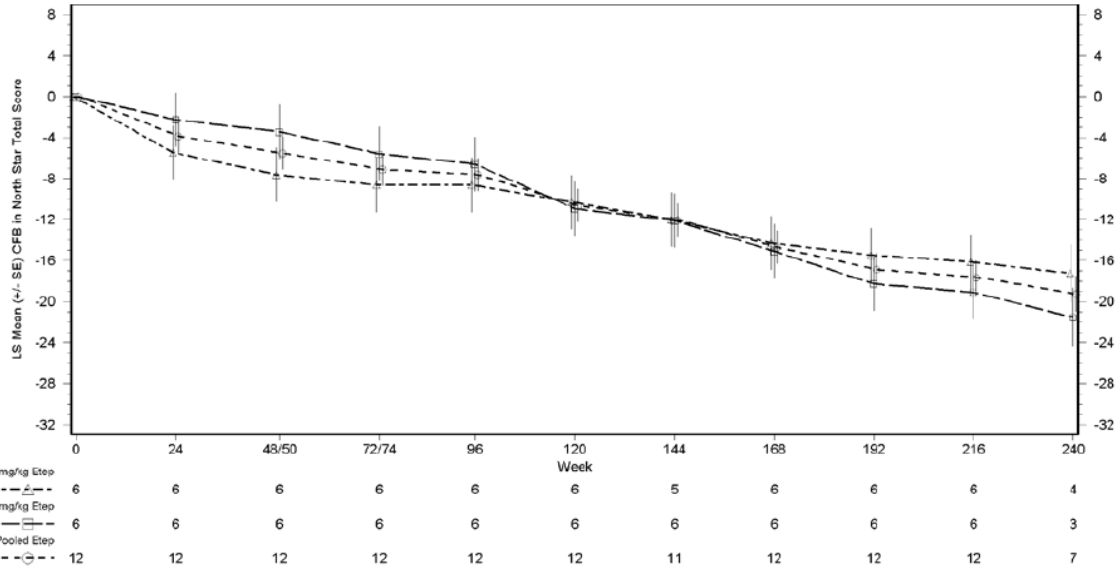
Lower limb fractures

Four ambulatory patients and one non-ambulatory patient had lower limb fractures over the course of the study period.

NSAA Total Score

The mean NSAA Total Score at baseline was 24.9 for the ITT population and the mean NSAA Total Score steadily decreased over time. The disease progression trajectories were similar for the 30 mg/kg and 50 mg/kg eteplirsen groups.

Figure 11 LS Mean Change from Baseline in NSAA Total Score by Treatment Week, Day 1 Values, ITT Population



CFB = change from baseline; Etep = eteplirsen; ITT = intent-to-treat; LS = least squares; SE = standard error.
Note: Treatment Week is derived from the first dose of the given study drug. For patients originally randomized to eteplirsen, Treatment Week is equal to Study Week. For patients originally randomized to placebo, the eteplirsen Treatment Week is 24 weeks less than Study Week.
Note: Placebo-to-eteplirsen patients are included in the appropriate eteplirsen group with baseline as the last value prior to initiation of eteplirsen.
Note: All 12 patients have data through Treatment Week 216 and 7 patients have data through Treatment Week 240. The 4 patients originally randomized to placebo had not reached Treatment Week 240 at the time of completion of the study period. In addition, Patient 012 (originally randomized to 50 mg/kg eteplirsen) did not perform this assessment at Study/Treatment Week 240 because of an injury (femur fracture) that prevented the patient from being able to travel to the central study site. Thus the n = 7 at Treatment Week 240.
Note: LS means and SEs are from a mixed model repeated measures with fixed effects for treatment, time, and treatment-by-time interaction, baseline value, age at start of steroid and age at start of study drug as covariates and patient nested in treatment as the random effect.

Ability to Independently Rise from Supine

At baseline, 11/12 patients (91.7%) were able to independently rise without external support or physical assistance (score of 1). At Treatment Week 216 (n = 12) (i.e., the last assessment on eteplirsen for the placebo-to eteplirsen patients) 1 patient was able to independently rise. At Treatment Week 240 (n = 7) (i.e., the last assessment on eteplirsen for those originally randomized to eteplirsen) 2 patients were able to independently rise.

The mean rise time at baseline was 8.23 seconds. The LS mean change from baseline to Treatment Week 216 (n = 12) was an increase of 20.05 seconds. The LS mean change from baseline to Treatment Week 240 (n = 7) was an increase of 18.99 seconds.

10-Meter Walk/Run Time

The mean 10-meter walk/run time at baseline was 6.18 seconds. The LS mean change from baseline to Treatment Week 216 was an increase of 11.47 seconds. The mean change from baseline to Treatment Week 240 was an increase of 13.92 seconds (n = 7).

Timed 4-Step Test

The mean time to complete the Timed 4-Step Test at baseline was 4.15 seconds. The LS mean change from baseline to Treatment Week 216 was an increase of 18.40 seconds. The LS mean change from baseline to Treatment Week 240 was an increase of 21.42 seconds.

9-Hole Peg Test

The mean time for the 9-Hole Peg Test at baseline was 21.9 seconds for the dominant hand and 24.8 seconds for the non-dominant hand. Values remained stable over time and showed improvement at some individual time points.

Maximum Voluntary Isometric Contraction Test

Knee extension strength - Baseline means were 5.527 and 5.945 kg for the left and right knee, respectively. The mean decrease in knee extension strength was approximately 2 kg at Treatment Week 216 and approximately 3 kg at Treatment Week 240.

- Knee flexion strength - Baseline means were 5.533 and 5.339 kg for the left and right knee, respectively. Mean decreases from baseline remained less than 1 kg until Treatment Week 240, at which time the mean decreases were approximately 1.5 kg for the left knee and 1.8 kg for the right knee.
- Elbow extension strength- Baseline means were 2.773 and 3.109 kg for the left and right elbow, respectively. For the left elbow, the largest mean decrease was observed at Treatment Week 240 (a decrease of 0.6 kg). For the right elbow, the largest mean decrease in elbow extension strength was observed at Treatment Week 168 (a decrease of approximately 0.4 kg).
- Elbow flexion strength - Baseline means were 3.686 and 3.858 kg for the left and right elbow, respectively. The decrease in mean elbow flexion strength was approximately 2.5 kg by Treatment Week 240.
- Hand grip strength - Baseline means were 7.633 and 7.984 kg for the left and right hand, respectively. Changes were minimal with no consistent pattern.

Pulmonary Function Tests:

- The mean FVC%p at baseline was 97.7% and a minimal decline below 90% in mean FVC%p was observed after Treatment Week 144. At Treatment Week 216, the mean FVC%p was 85.3%.
- The mean MEP%p at baseline was 80.7%; a minimal decline below 75% in mean MEP%p was observed after Treatment Week 144.
- The mean MIP%p at baseline was 91.7%; the mean values were generally sustained near that level throughout the study period.

PedsQL

Social domain scores and neuromuscular module scores increased by child assessment; school functioning domain scores increased by both parent and child assessments, neuromuscular module scores decreased by parent assessment; and physical domain scores decreased as rated by both parents and children. Other scores did not appear to show any trend.

Study 301 (PROMOVI)

During the process of assessment of the marketing authorization application, the Applicant submitted the preliminary results from the interim analysis of Study 301.

PROMOVI is an open-label, multicentre study performed in North America to evaluate the efficacy and safety of eteplirsen in DMD patients with genetically confirmed DMD with exon deletions amenable to exon 51 skipping. The comparison was done with an untreated control arm of DMD patients amenable to exon skipping of an exon other than exon 51.Clinical course of this population is variable and it is unknown the impact that this can have in the results.

Other inclusion criteria included age 7 to 16 years of age, stable pulmonary function (FVC% of predicted ≥50% and not require nocturnal ventilation), stable dose of oral corticosteroids for at least 24 weeks prior to Week 1 and the dose is expected to remain constant. Use of any pharmacologic treatment (other than corticosteroids) within 12 weeks of Week 1 was forbidden.

Approximately 110 patients were targeted for enrolment including approximately 90 patients with a Baseline 6MWT distance between 300 and 450 meters (~70 patients in the treated group and ~20 patients in the untreated control group) and approximately 20 patients with a Baseline 6MWT distance >450 meters (~10 patients in the treated group and ~10 patients in the untreated control group).

The primary analysis was performed in patients with a baseline 6MWT distance of 300 to 450 meters (i.e., the group most likely to decline during the study period). Efficacy endpoints include change in 6MWD from baseline at week 96 (primary endpoint), LOA, ability to rise independently, change in FVC % predicted, change in NSAA total score, change in dystrophin protein levels, safety and tolerability.

Patients in the treated group received eteplirsen 30 mg/kg as an IV infusion administered over a 35- to 60-minute period once weekly for at least 96 weeks in the treatment period, and for up to 48 weeks in the safety extension. Patients in the control group were untreated.

A predefined interim analysis was to be performed after approximately 35 eteplirsen-treated patients with a baseline 6MWT between 300 and 450 meters, inclusive, had completed their week 96 assessments. Data for the primary endpoint and all secondary objectives except the dystrophin objective are available.

Table 19 Summary of Demographics and Other Baseline Characteristics; Safety Set; Interim Analysis

		Untreated (N=29)	30 mg/kg (N=79)
Gender	Male	29 (100.0%)	79 (100.0%)
Age (years)	n	29	79
	Mean	8.9	9.1
	Median	8.0	9.0
	SD	1.87	2.04
	Min, Max	7, 13	7, 16
Race	American Indian or Alaska Native	0	0
	Asian	1 (3.4%)	5 (6.3%)
	Black or African American	0	2 (2.5%)
	Native Hawaiian or Other Pacific Islander	0	2 (2.5%)
	White	25 (86.2%)	67 (84.8%)
	Other	3 (10.3%)	3 (3.8%)
Ethnicity	Hispanic or Latino	6 (20.7%)	7 (8.9%)
	Not Hispanic or Latino	23 (79.3%)	71 (89.9%)
	Unknown	0	1 (1.3%)
Time Since DMD Diagnosis (months)	n	29	79
	Mean	53.2	53.3
	Median	49.4	49.4
	SD	31.06	33.26
	Min, Max	13, 115	6, 147
Previous Drisapersen Status	No	29 (100.0%)	63 (79.7%)
	Yes	0	16 (20.3%)
	Deflazacort	20 (69.0%)	22 (27.8%)

Corticosteroid Medication Name	Prednisone	9 (31.0%)	57 (72.2%)
Corticosteroid Schedule	Daily	24 (82.8%)	65 (82.3%)
	Intermittent	5 (17.2%)	14 (17.7%)
Genetic Mutation (exon number)	12-44	1 (3.4%)	0
	42-45	1 (3.4%)	0
	43	1 (3.4%)	0
	43-50	0	1 (1.3%)
	44	1 (3.4%)	0
	45	1 (3.4%)	0
	45-50	0	19 (24.1%)
	45-52	4 (13.8%)	0
	46-47	3 (10.3%)	0
	46-48	5 (17.2%)	0
	46-50	1 (3.4%)	0
	47-50	0	1 (1.3%)
	48-50	0	21 (26.6%)
	48-52	2 (6.9%)	0
	49-50	0	18 (22.8%)
	49-52	2 (6.9%)	0
	50	0	13 (16.5%)
	51	2 (6.9%)	0
	51-53	2 (6.9%)	0
	51-55	3 (10.3%)	0
	52	0	6 (7.6%)

Table 14.1.2.1

Results

In all, 29 untreated patients were enrolled but only 20 fulfilled the pre-specified criteria of age >7 years and >300 meters in 6MWT (Primary Efficacy Set). In this interim analysis only 7 patients reaching Week 96 and only 4 among those with baseline 6MWT distance between 300 and 450 meters. For eteplirsen 30 mg/kg arm 79 patients were enrolled.

Table 20 Summary of Patient Disposition; All Patients Enrolled; Interim Analysis

	Untreated (N=29)	30 mg/kg (N=79)
Number of Patients in Safety Population	29 (100.0%)	79 (100.0%)
Number of Patients in Efficacy Set	28 (96.6%)	71 (89.9%)
Number of Patients in Primary Efficacy Set[a]	20 (69.0%)	59 (74.7%)
Number of Patients in Efficacy Set with Baseline 6MWT >450 m	8 (27.6%)	12 (15.2%)

Source: Table 14.1.1.1

Baseline characteristics for the population of the primary analysis (6MWT between 300 and 450 meters) were similar except for DMD mutations that involved amenable exon 51 skipping mutations in eteplirsen treated group and no amenable to exon 51 skipping mutations in the untreated group.

The Applicant, considering the low number of untreated patients at weeks 72 and 96, presented only summaries of the results by treatment group with no statistical comparisons.

Primary endpoint

The primary endpoint was the change in 6MWT from baseline. No specific discussion on the results has been done by the Applicant. Table below shows the interim analysis. The mean 6MWT distances decreased over time in both groups with little difference between both groups. Although numbers are small and drawing sound conclusions is difficult data do not suggest a significant effect of eteplirsen on 6MWT.

Table 21 6MWT and change for baseline in the primary efficacy set (Interim analysis)

	Untreated group (n=20)		Eteplirsen 30 mg/kg group (n=59)	
	Observed (n)	Change from baseline	Observed (n)	Change from baseline
Baseline	382,6 (20)		376,5 (59)	
Wee 12	366,7 (20)	-16,0	364,8 (58)	-12,8
Week 24	363,9 (18)	-27,4	357,5 (55)	-19,3
Week 36	353,0 (17)	-35,0	341,0 (51)	-34,6
Week 48	348,2 (17)	-37,3	329,5 (50)	-47,5
Week 72	287,7 (7)	-80,7	328,6 (43)	-51,7
Week 96	247,3 (4)	-115,0	272,2 (33)	-108,6

Secondary endpoints

A majority of patients in the treated and untreated control group demonstrated the ability to rise independently from the floor at each visit through Week 72 (57,1% of untreated patients and 68,9% of etelirsen treated patients).

At the time of the data cut-off for the interim analysis, loss of ambulation had occurred in 5 out of 59 (8,5%) and no patients in the untreated control group (0%).

The mean NSAA Total Score at Week 96 for the eteplirsen treated group was 15.3, representing a 7.2 point decline from Baseline and for the untreated control group was 6.5, representing a 13.8 point decline from Baseline.

The mean FVC% at baseline was 91,344 and 86,573 for untreated and eteplirsen patients respectively. At week 96 the mean FVC%p was 94,853 and 85,285 respectively. The Applicant states that these data suggest stabilisation of pulmonary function at the end of the study given that a decline of 5%/year is expected in DMD population. However, pulmonary function keeps normal while children are ambulant and only deteriorates once patients become non-ambulant. Baseline FVC% as well as FVC% at week 96 shown in the table are within the normal ranges. These results do not allow for any sound conclusion.

Table 22 FVC% predicted and change for baseline in the primary efficacy set (Interim analysis)

	Untreated group (n=20)		Eteplirsen 30 mg/kg group (n=59)	
	Observed (n)	Mean change from baseline	Observed (n)	Mean change from baseline
Baseline	88,873 (20)		86,343 (59)	
Week 12	89,630 (20)	0,757	86,754 (59)	0,411
Week 24	90,117 (19)	1,005	85,017 (55)	-0,705
Week 36	91,344 (19)	2,233	86,573 (51)	0.636
Week 48	87,776 (17)	0,107	86,521 (51)	0.584
Week 72	91,514 (7)	3,518	87,968 (48)	2,176
Week 96	94,853 (5)	9,754	85,285 (36)	1,106

Clinical studies in special populations

No specific studies or analyses in special populations were conducted.

Analysis performed across trials (pooled analyses AND meta-analysis)

Given the short duration of 24 weeks for the placebo-controlled portion of Study 201/202, there was an absence of long-term concurrent placebo controlled data for comparison of clinical efficacy of eteplirsen. Therefore, as recommended by the US Food and Drug Administration (FDA), the applicant sought to identify appropriate external observational registries with longitudinal clinical outcome data.

Twelve candidate external DMD registries with clinical outcome data were identified; two external DMD registries were selected to be used as external control cohort used for comparison of long-term efficacy data. These databases had available, prospectively collected, 6MWT data, including baseline and at least 1 postbaseline value:

- Italian Telethon DMD Registry database (N = 97); Professor Eugenio Mercuri, MD, PhD (Catholic University in Rome); 11 participating tertiary care centres. Patients were recruited between age 2 and 18 years. Patients were seen at least once every 12 months.
- LNMRC database (N = 89); Professor Nathalie Goemans, MD (University Hospitals in Leuven, Belgium); single site. Patients enrolled at age < 17.5 years.

Although the 2 registries were chosen primarily based on availability of 6MWT outcomes, both registries had characteristics including entry criteria comparable to Study 201/202.

Individuals were identified for inclusion in the external control group(s) using the key prognostic entry criteria for Study 201. The key prognostic entry criteria (i.e., selection filters) were applied to the external registry data set in order identify external control patients.

From the 186 untreated DMD patients provided from the 2 external DMD registries two external control groups were identified for comparative analysis to eteplirsen-treated patients:

- Primary External Control (N = 13): External control group amenable to exon 51 skipping and the comparator for the primary analyses of clinical outcomes with eteplirsen. Given the exact genetic subtype, this is the most relevant comparator for the 4-year 6MWT data from Study 201/202 eteplirsen-treated patients. A subset of 10 external control patients from the Italian Telethon registry had 3-year NSAA data; NSAA Total Score data were not provided for patients in the LNMRC.
- Secondary External Control (N = 50): External control group amenable to any exon skipping comprises a secondary and a larger sized group for comparison of the 6MWT data for 3 years, albeit in a population of DMD with a mutation amenable to any kind of exon skipping. The secondary group also included 8 patients with DMD mutations amenable to skipping exon 44, which typically have a milder disease course than other genotypes of DMD.

Although the criteria for identification of the external control patients were predefined, the comparative analyses between eteplirsen-treated patients and external controls were not predefined before the data were collected and were considered post-hoc analysis; therefore, all p-values are for informational purposes only.

The eteplirsen treatment data for clinical outcomes included data from the patients originally randomized to placebo (N = 4) and data from the patients originally randomized to eteplirsen (N = 8). Therefore, in order to accurately portray functional assessment outcomes with respect to duration of eteplirsen treatment, alignment of the assessment time points for both groups required excluding the results for the 24-week placebo treatment

period for the 4 patients randomized to placebo in Study 201. Year 1, 2, 3, and 4 comparisons correspond to the following time-on-active treatment time points:

- Originally Randomized to Eteplirsen (N = 8): Week 0 (Baseline), Week 48, 96, 144, and 192
- Originally Randomized to Placebo (N = 4): Week 24 (Baseline), Week 74, 120, 168, and 216 (i.e. offset by 24 weeks from original study start)

As already noted all 12 patients from study 201/202 completed the studies. Of the 13 patients included in the primary external control all were included in the 2 years analysis, one patient was missing at the year 3 time point and 2 patients at the year 4 time point. Of the secondary external control all were included in the 1 year analysis, one patient was missing at the year 2 time point and 2 patient at the year 3 time point. The number of patient discontinuing over the evaluation periods was limited in all 3 groups, which give some strength to the data.

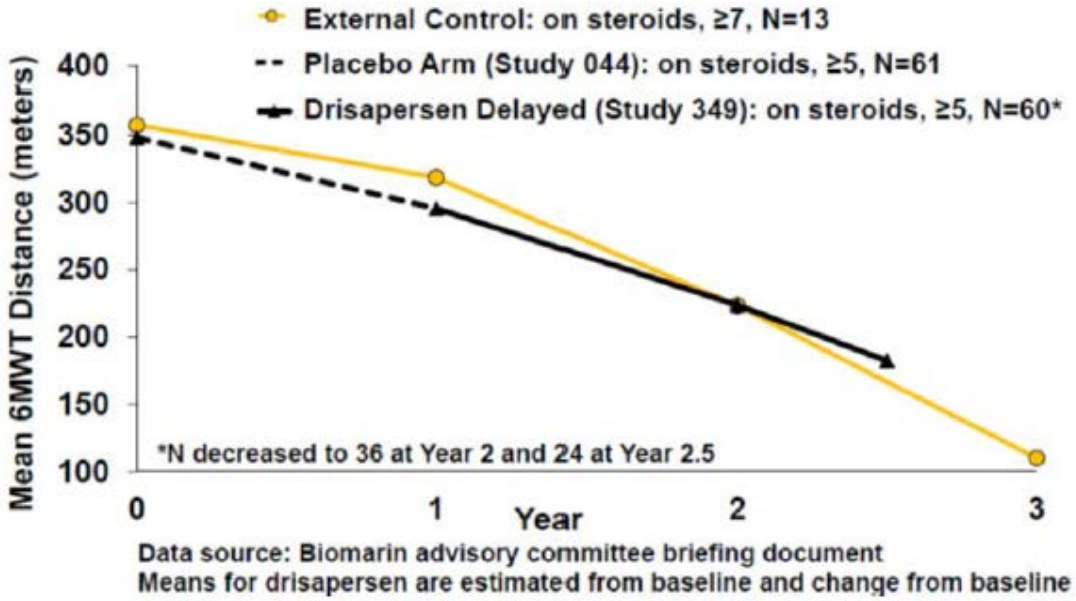
6MWT for External Control (N = 13) Over 2.5 Years Compared to Publicly Available Drisapersen Data

To evaluate the possibility that the external control group had a worse outcome than would have been expected in a cohort of patients from a randomized clinical trial, comparison of the 6MWT for the external control group to publicly available data from the drisapersen program was conducted by the applicant.

To evaluate the 6MWT trajectory for the primary external control group, the applicant utilized longitudinal data from the placebo arm of the randomized drisapersen trial Study 044 and those patients from Study 044 who entered the extension Study 349. As shown in Figure below in this dataset of boys who were >5 years of age, steroid-treated, and amenable to exon 51 skipping (N = 61), the decline of the 6MWT over a period of 2.5 years appeared similar to the primary external control group according to the applicant.

According to the applicant this supports the conclusion that the 6MWT 161 m difference observed for eteplirsen patients (N = 12) vs. the untreated external cohort (N = 13) at Year 4 is attributable to eteplirsen treatment, rather than the chance occurrence of an atypically rapid decline in the external control group. This material is publically available from the BioMarin FDA Advisory Committee Briefing Document for Drisapersen, Table 4.7.1 (Page 95) and Table 4.7.4.1.1 (Page 117).

Figure 12 Comparison of 6MWT Change from Baseline in External Controls (N = 13) vs. Placebo and Drisapersen Delayed Patients from a Randomized Trial



As described above the external control groups were recruited from two registries in Europe but the patients in studies 201/202 were clinically evaluated at one central study site in US. There are also some differences in inclusion criteria between study 201/202 and subjects from the registries, e.g. age range, glucocorticoid treatment initiation and dosing, 6MWT distance, differences in the definition related to amenable to exon 51 skipping therapy (secondary external control group). So, even though the patients from the registries seems in many ways comparable to the patients in the studies 201/202, the differences in inclusion criteria between the study population and the registries population, the fact that patients were evaluated in the context of a clinical study or in a registry, and the geographic difference, contribute to the uncertainties of any measured differences between the two populations. Also the very limited number of patients in the studies 201/202, and registries, could substantially influence the point estimates in the three populations by chance, i.e. there is a higher risk that the point estimates could substantially deviate from the “true” parameter in the same overall population, which in turn substantially have implications on the estimated differences between the three populations.

Baseline Characteristics

The Baseline characteristics for the eteplirsen-treated patients and that of the primary and secondary external controls were comparable on mean age, mean Baseline 6MWT distance, total NSAA scores, and ability to rise independently.

Figure 13 Summary of Baseline Characteristics

Parameter[a]	Combined 201/202 Studies (N = 12)	Primary External Control (N = 13)	Secondary External Control (N = 50)
Age (years)			
n	12	13	50
Mean (SD)	9.4 (1.18)	9.5 (1.45)	9.7 (1.52)
Median (Min, Max)	9.7 (7, 11)	9.0 (7, 12)	9.5 (7, 13)
Height (cm)			
n	12	13	NA
Mean (SD)	123.9 (8.37)	129.9 (7.55)	
Median (Min, Max)	119.0 (117, 138)	131.0 (107, 136)	
Weight (kg)			
n	12	13	NA
Mean (SD)	32.4 (6.75)	34.8 (9.74)	
Median (Min, Max)	34.6 (24, 41)	33.0 (17, 48)	
Deletion Mutations	45-50, 48-50, 49-50, 50, 52	45-50, 48-50, 49-50, 50, 52	Any skippable mutations[b]
6MWT Distance (meters)			
n	12	13	50
Mean (SD)	363.2 (42.19)	357.6 (66.75)	355.7 (87.28)
Median (Min, Max)	370.0 (256, 416)	373.0 (200, 458)	356.0 (100, 558)
NSAA (total score)			
n	12	10	34
Mean (SD)	24.9 (4.93)	22.0 (6.27)	22.7 (6.31)
Median (Min, Max)	25.5 (17, 31)	23.5 (10, 31)	23.5 (10, 32)
Able to rise from floor independently, n (%)[c]	11 (92%)	11 (85%)	28 (85%)
Rise Time (seconds)[d]			
n	12	12	32
Mean (SD)	8.2 (7.57)	9.8 (9.80)	9.4 (9.32)
Median (Min, Max)	5.5 (3, 30)	5.8 (2, 30)	5.7 (2, 30)

Abbreviations: 6MWT – 6-minute walk test; Max = maximum; Min = minimum; NSAA = North Star Ambulatory Assessment; SD = standard deviation.
a Day 1 values were used if assessed on 2 consecutive days
b See Appendix B for a list of mutation in the external control cohort
c NSAA Subscore item #2 (Italian Telethon) or separate assessment (LNMRC)
d for patients who were unable to rise independently, a rise time of 30 seconds was used

Table 23 Glucocorticoid Use at Baseline: Eteplirsen-Treated and Primary External Control Cohorts

Parameter	Eteplirsen Combined 201/202 Studies (N = 12)	Primary External Control (amenable to Exon 51 skipping) (N = 13)
Age at Steroid Start (years)		
Mean (SD)	5.2 (1.07)	6.5 (2.11)
Median (Min, Max)	5.5 (3, 7)	6.0 (4, 11)
Glucocorticoid used		
Deflazacort	8 (67%)	9 (69%)
Prednisone	4 (33%)	4 (31%)
Glucocorticoid regimen		
Continuous (daily)	11 (92%)	8 (62%)
Intermittent	1 (8%)	5 (38%)

Note: Prednisone recommended dose: 0.75 mg/kg; Deflazacort recommended dose: 0.9 mg/kg

Table 24 Physiotherapeutic Interventions: Eteplirsen-Treated Cohort (N = 12) and Primary External Control Cohort (N = 13)

Physical Therapy	PT Regimen	Eteplirsen Combined 201/202 Studies (N = 12) # of patients	Primary External Control (amenable to Exon 51 skipping) (N = 13) # of patients
Visits with trained physical therapist	4-6 days/week	2	5
	2-3 days/week	3	8
	1 day/week	4	0
	1 day/year	1	0
	None	2	0
Use of night splints (orthoses)	Use orthoses	11	11
	Orthoses not needed (TA <10)	0	2
	Orthoses not used	1	0

Results

Six-Minute Walk Test

Eteplirsen-Treated vs. Primary Control Cohort

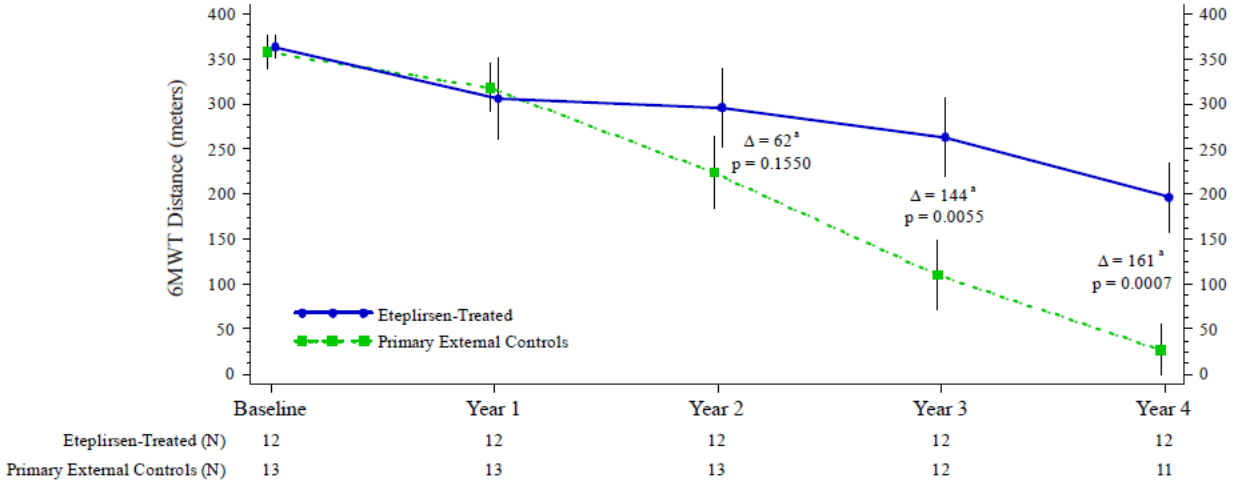
At Baseline, 6MWT distances were comparable between the eteplirsen-treated patients and the primary untreated control cohort with a mean difference of 5.6 meters. The eteplirsen and primary external control groups demonstrated a similar decline in disease progression through Year 1. Afterwards, the trajectory of disease progression diverged, favouring eteplirsen-treated patients.

Table 25 6MWT Distance Over 4 Years: Eteplirsen-Treated Cohort (N = 12) vs. Primary External Control Cohort (N = 13)

Groups Compared	Statistics	6MWT Baseline	6MWT Year 1	6MWT Year 2	6MWT Year 3	6MWT Year 4
Primary External Control Cohort (amenable to Exon 51 skipping)	N	13	13	13	12*	11**
	Mean (SD)/ Median	357.6 (66.75)/ 373.0	318.6 (94.20)/ 307	223.5 (145.43)/ 250	110.3 (136.21)/ 17.5	27.3 (90.45)/ 0.0
	Min, Max	200, 458	125, 495	0, 435	0, 362	0, 300
Eteplirsen-Treated	N	12	12	12	12	12
	Mean (SD)/ Median	363.2 (42.19)/ 370.0	305.8 (155.32)/ 328	295.9 (148.98)/ 338.5	263.1 (151.74)/ 306.5	196.3 (130.22)/ 209.0
	Min, Max	256, 416	0, 328	0, 450	0, 483	0, 400
Primary ANCOVA	Eteplirsen - External control difference in mean change from baseline (meters)		21	62	144	161
	p-value*		0.576	0.1550	0.0055	0.0007

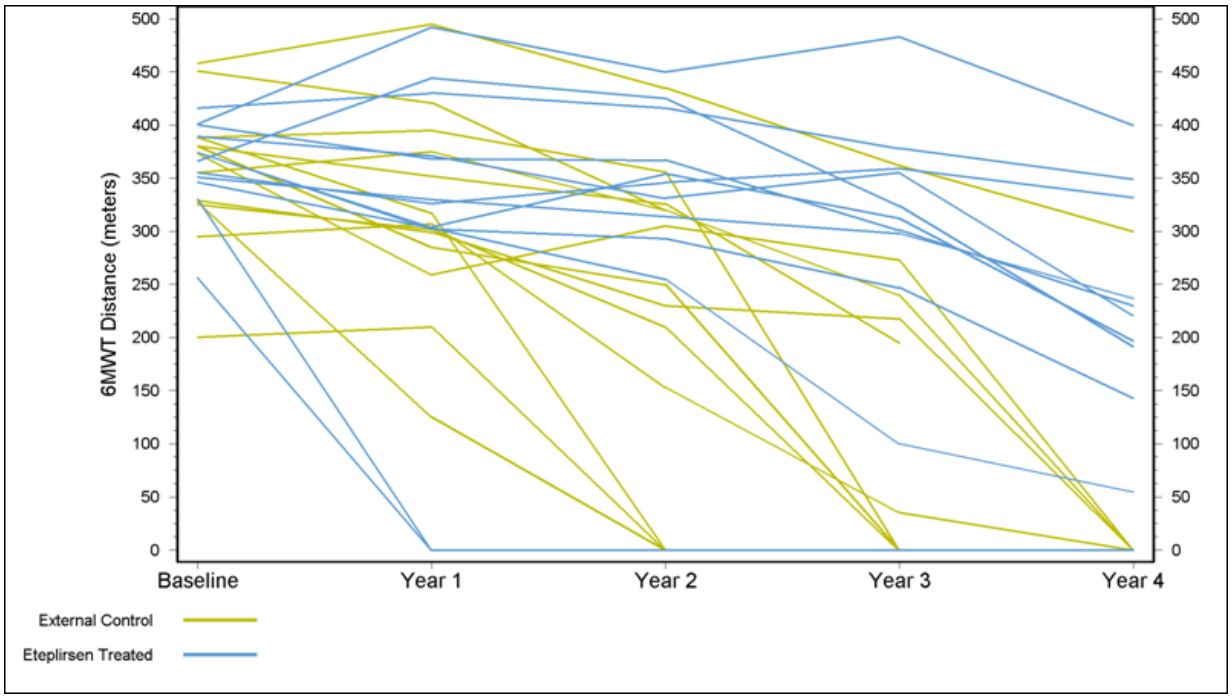
Abbreviations: 6MWT = 6-minute walk test; ANCOVA = analysis of covariance; SD = standard deviation.
* p-value is for baseline adjusted mean difference in change from baseline at the given time point.
** 1 Primary EC patient did not have data at Year 3 and 2 Primary EC patients did not have data at Year 4

Figure 14 Mean 6MWT Distance by Treatment Group (Eteplirsen-Treated Cohort [N = 12] vs. Primary External Control Cohort [N = 13])



Note: N represents the number of patients at the time point specified.
Abbreviations: 6MWT = 6-minute walk test; EC = external control; SD = standard deviation.
* the difference in mean change from baseline with associated p value from the primary ANCOVA model
** 1 patient did not have data at Year 3 and 2 patients did not have data at Year 4.

Figure 15 Individual Patient 6MWT Distance Over 4 Years: Eteplirsen-Treated Cohort (N = 12) and Primary External Control Cohort (N = 13)

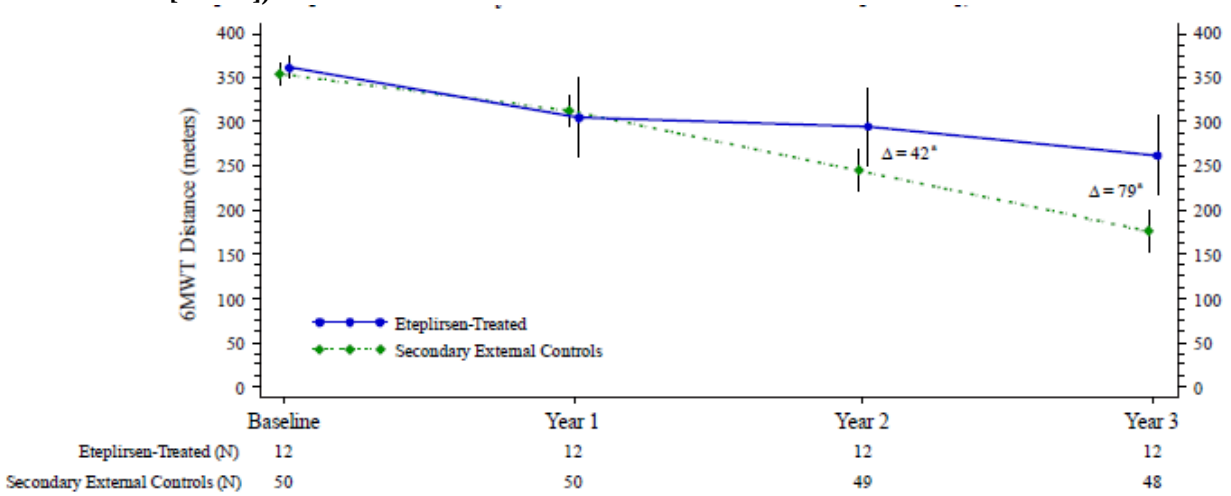


Abbreviations: 6MWT = 6-minute walk test.

Eteplirsen-Treated vs. Secondary Control Cohort

Data over 3 years were available for the secondary external control cohort (n = 50) and are compared to 3-year data in the eteplirsen-treated patients (n = 12). After Year 1 there is a divergence in the trajectory of disease progression favouring eteplirsen-treated patients, with a difference in the mean change from baseline of 6MWT at Year 2 of 42.5 meters and 79 meters by Year 3.

Figure 16 Mean 6MWT Distance by Treatment Group (Eteplirsen-Treated Patients [N = 12] vs. Secondary External Control Cohort [N = 50]) Over 3 Years



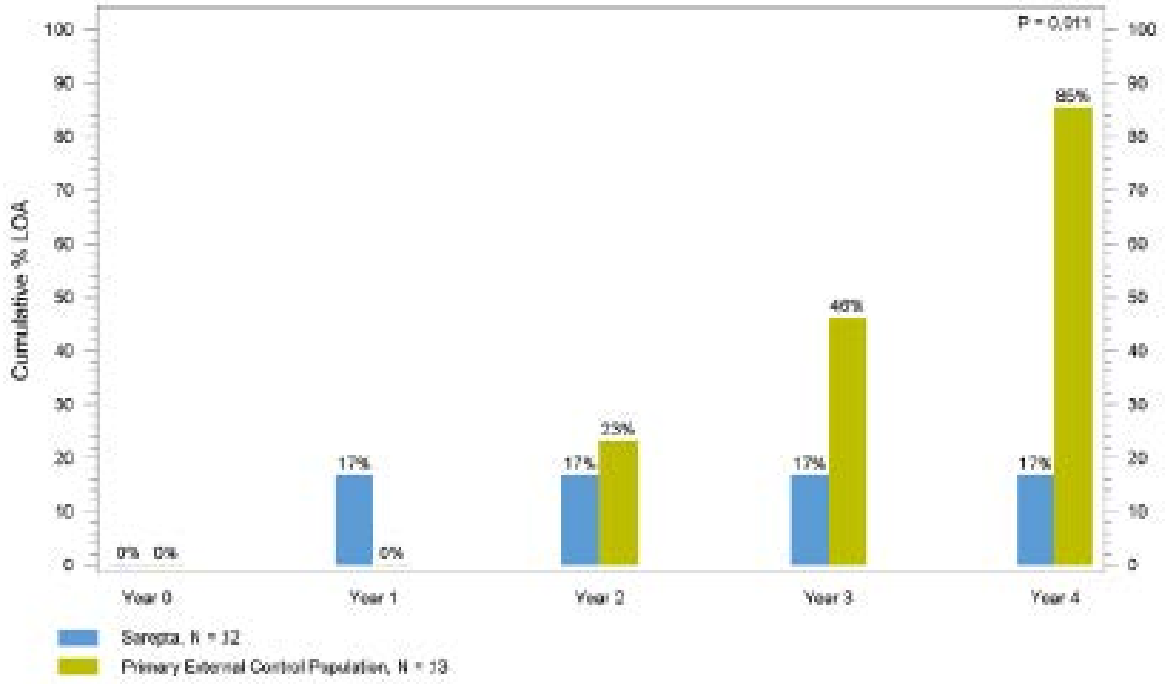
Note: N represents the number of patients at the time point specified.
Note: One patient did not have data at Year 2 and 2 patients did not have data at Year 3.
a the difference in mean change from baseline

Data from the figure above show how differences in the population selected as external control (Primary or Secondary) may result in different reference for comparison. At Year 3 the mean 6MWD was around 100 meters for the Primary External group and around 175 meters at Year 3 for the Secondary External control.

Loss of Ambulation

In the first year of study, 2 of the 12 (16.7%) eteplirsen-treated patients lost ambulation. These 2 patients entered Study 201/202 with the lowest 6MWT distances and experienced decline over the first 24 weeks with eventual loss of ambulation by Year 1. Thereafter, no additional eteplirsen-treated patients lost ambulation through the 4 years of study. In contrast, 10 of the 13 (76.9%) primary control patients had lost ambulation by Year 4. For the external control 2 of the 3 ambulatory boys had missing data at Year 4 and 1 boy was known to be ambulatory based on 6MWT at Year 4.

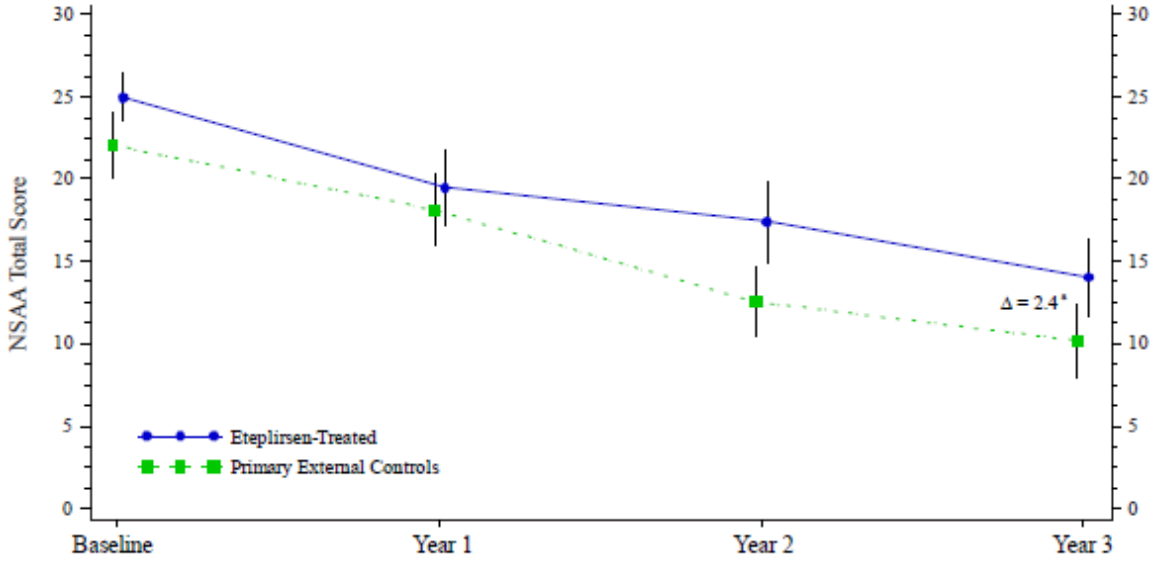
Figure 17 Kaplan-Meier Estimates of Loss of Ambulation Over 4 Years in Eteplirsen-Treated Patients (N = 12) vs. Primary External Control (N = 13) (Exon 51 Skippable)



North Star Ambulatory Assessment (NSAA)

Results of the NSAA total score and ability to rise independently component of the NSAA were analyzed over 3 years of treatment with eteplirsen (n = 12) and were compared to data at the corresponding time points from the primary external control cohort (exon 51 skippable patients, n = 13) and the secondary external control cohort (n = 50).

Figure 18 NSAA Total Score Over 3 Years: Eteplirsen-Treated (N = 12) vs. Primary External Controls (Exon 51 Skippable) (N = 10)



	Baseline	Year 1	Year 2	Year 3
Eteplirsen-Treated (N)	12	12	12	11
Primary External Controls (N)	10	10	10	8

* the difference in mean change from baseline

Ability to Rise Independently

At Baseline, 11 of 12 patients in the eteplirsen-treated cohort and 11 of 13 patients in the primary external control cohort were able to independently rise from supine to standing. By Year 3, the proportion of patients able to rise had declined to 8% in the external control cohort (N = 12), while 55% of eteplirsen-treated patients (6/11) retained the ability to rise independently without external support.

Pulmonary Function Tests

The analyses consistently show a slower decline in pulmonary function parameters in eteplirsen-treated patients compared to the expected annual decline in untreated DMD patients based on natural history data from the literature (i.e., a decrease of >5% for FVC% predicted and a decrease of 3%-4% for MEP% predicted and MIP% predicted).

In eteplirsen-treated patients, mean FVC% predicted decreased from 97.7% to 85.3% over 216 weeks, i.e., a decrease of 2.8% per year. In an analysis of FVC% predicted through Week 240 by age rather than time on study, a 2.3% decrease per year was demonstrated. This decrease of approximately 2.5% per year compares favorably with the expected ≥5% decrease in FVC% predicted that has been observed in natural history studies of DMD.

According to some publications as explained by the applicant FVC in DMD patients first increases steadily and then plateaus at approximately the age of 13-14 years, before decreasing due to the weakening of respiratory muscles. However, the applicant also notes that FVC% predicted has been reported to decrease in DMD patients after approximately 11-12 years of age by one author, but other authors suggest that FVC% predicted decreases almost as soon as pulmonary function can be reliably tested in patients with DMD. The majority, but not all, authors referred to by the applicant found a linear decrease of FVC% predicted in DMD patients of approximately 5% or more per year. In one study referred to by the applicant no decline in FVC% predicted was observed a in

a cohort treated with corticosteroids, while a decline was seen in untreated patients.

The different findings in different publications illustrate the difficulties to compare study results to published data. In addition the DMD patients in the publications referred to have a wider age span (Table below), and were not a specific group of DMD patients amenable to exon 51 skipping. There are likely also other differences between the subject included in the studies 201/202 and the subjects in the published literature.

Table 26 Literature-Reported Annual Decline in FVC % Predicted

Publication	Age Range (Years)	Number of Patients	FVC% Predicted Annual Decline
Mayer 2015	5-24	44 (patients with valid assessments)	5%
Buyse 2015	10-18	33	8.95% (Placebo arm)
Khirani 2014	6-19	48 (23 patients with successive assessments)	4.9%
Henricson 2013	7-18[a]	195 (340)[a]	Approx. 5.0% (Est. based on Table 8 in Henricson 2013)
Hahn 1997	7-25	51 (52)[b]	7.9%[c]
McDonald 1995	5-20+	39 (160)[b]	0.3% (7-10 years) n = 8 8.5% (10-20 years) n = 26 6.2% (>20 years) n = 5)
Miller 1988	8-21	147	Approx. 6% (Figure 2 in Miller 1988)

[a] For patients with available PFTs from age 7-18 years (total number in study)
[b] Number of patients with PFT available (total number in study)
[c] After FVC plateau (FVC% predicted,11-12 year group)

Taking the above in consideration, the CHMP concludes that even if the decrease in FVC% predicted in the eteplirsen-treated group of 2.3%-2.8% annually shows approximately half of the expected decrease in FVC% predicted (⩾5%) that has been observed in some natural history studies of DMD is promising, a none-biased conclusion regarding any effect of eteplirsen on FVC% predicted would have to be confirmed in a placebo-controlled study.

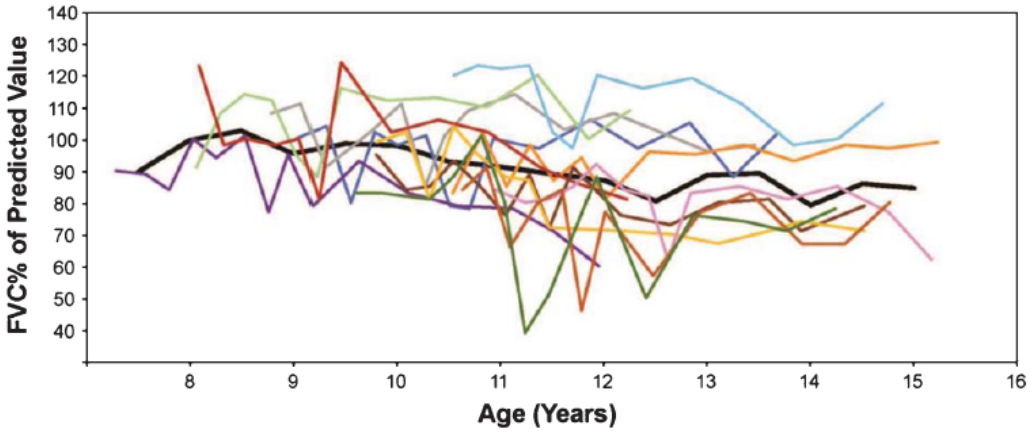
A decrease in MEP% predicted in the eteplirsen-treated group of 2.6% annually favours eteplirsen treatment when compared to the expected 3%-4% decrease in MEP% predicted observed in natural history studies. However, as also to some extent pointed out by the applicant and also outlined above in the previous comment by the CHMP, there are numbers of difficulties when comparing data from published natural history studies and actual study results making any interpretation impossible. Therefore, any effect of eteplirsen on MEP% predicted would have to be confirmed in a placebo-controlled study.

Also a difference in MIP% predicted favouring the eteplirsen-treated group compared to natural history studies was measured but is difficult to interpret due to the limitation outlined in the previous comments.

As the Study 201/202 External Control groups did not include patient-level data for PFTs a new comparison was included with a control group identified from patients who participated in the United Dystrophinopathy Project (UDP). Prospective data were collected from DMD patients in the Neuromuscular Clinic at The Children's Hospital of Philadelphia (CHOP; Philadelphia, PA) from 2005–2010. Both the eteplirsen and UDP Control patients were assessed using standardized pulmonary function testing performed by trained expert evaluators according to American Thoracic Society/European Respiratory Society guidelines. Assessments included FVC and FVC% predicted; however, MIP and MEP data were not available for UPD patients. The data from the UDP utilized for this comparison included patient number, age, height, weight, FVC and FVC%p at each visit.

A mixed model analysis was used to estimate the slope of FVC%p vs age in eteplirsen-treated patients in Study 201/202 and in the untreated cohort of patients (UDP; n=34). The patients selected from the UDP Control group were in an age group similar to Study 201/202 (7 to 15.5 years). The UDP Control group had an annual decline in FVC%p of 4.1% (95% CI; 1.9%, 6.3%) compared with an annual decline of 2.3% (95% CI; 1.2%, 3.4%) for the eteplirsen-treated patients. The high variability in individual trajectories precludes drawing a sound conclusion.

Table 27 Study 201/202.- FVC%p versus age



FVC%p versus age (rounded to nearest 0.5 year for mean line). Only assessments performed every 24 weeks are represented graphically although additional time points were assessed during the first 96 weeks. FVC%p, percent predicted forced vital capacity.
(Kinane TB et al. Journal of Neuromuscular Diseases 5 (2018) 47–58)

In addition, maximum expiratory pressure percent predicted (MEP%p) and maximum inspiratory pressure percent predicted (MIP%p) have declined more slowly in eteplirsen treated patients compared to natural history data obtained from published literature. An age-adjusted mixed model repeated-measures analysis indicated that eteplirsen patients enrolled in Study 201/202 experienced an annual decrease of 2.6% for MEP%p and an annual increase of 0.6% for MIP%p. Expected annual decreases based on published literature were at least 2.7% and 3.8%, respectively.

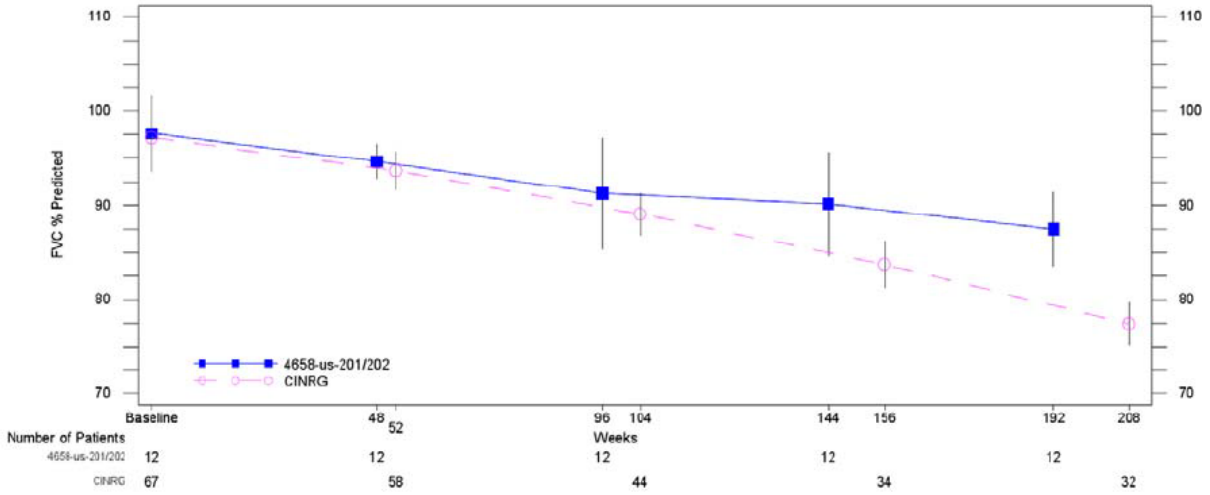
A new comparison was provided by the applicant with a control group identified from patients from the Cooperative International Neuromuscular Research Group (CINRG) database in order to sustain the effect of eteplirsen on pulmonary function over time (4 years of longitudinal data), The CINRG database contains data from 397 DMD patients across all ages; of which 198 patients were between the ages of 10 to <18 years and were treated with glucocorticoids.CINRG subsets of patients were selected according to age (7 to 13 years) and baseline FVC%p (84 to 124%), similar to those recruited in the eteplirsen clinical trial. Three subgroups were established:

- All CINRG (DMD patients excluding those amenable to exon 44 skipping treated with glucocorticoids) n= 75 (n=35 with data at year 4)
- Genotyped CINRG (all CINRG patients treated with glucocorticoids with genetic confirmation of DMD) n=67 (n=32 with data at year 4)
- Exon 51 CINRG (all CINRG patients treated with glucocorticoids with Exon 51 DMD) n=9 (n=2 with data at year 4)

Due to the limited number of subjects in Exon 51 cohort with available data at Year 4 Genotyped CINRG was chosen as the primary cohort for the analysis. FVC%p at baseline was 97.67% for eteplirsen patients vs 97.18% in Genotyped CINRG patients; Year 1: 94.67% vs 93.67%; Year 2: 91.25% vs 89.09%; Year 3: 90.17% vs 83.74%; Year 4: 87.50% vs 77.44%. During the first 2 years both trajectories are overlapped and separation is observed from Year 3. It is doubtful that at Week 192 (last time point for eteplirsen treated patients) a clear separation between both curves could be detected.

A similar pattern is observed for the global group (All CINRG group).

Figure 19 Temporal Pattern of FVC%p Decline – Study 201/202 vs. Baseline FVC%p and Age-Matched Genotyped CINRG Patients*



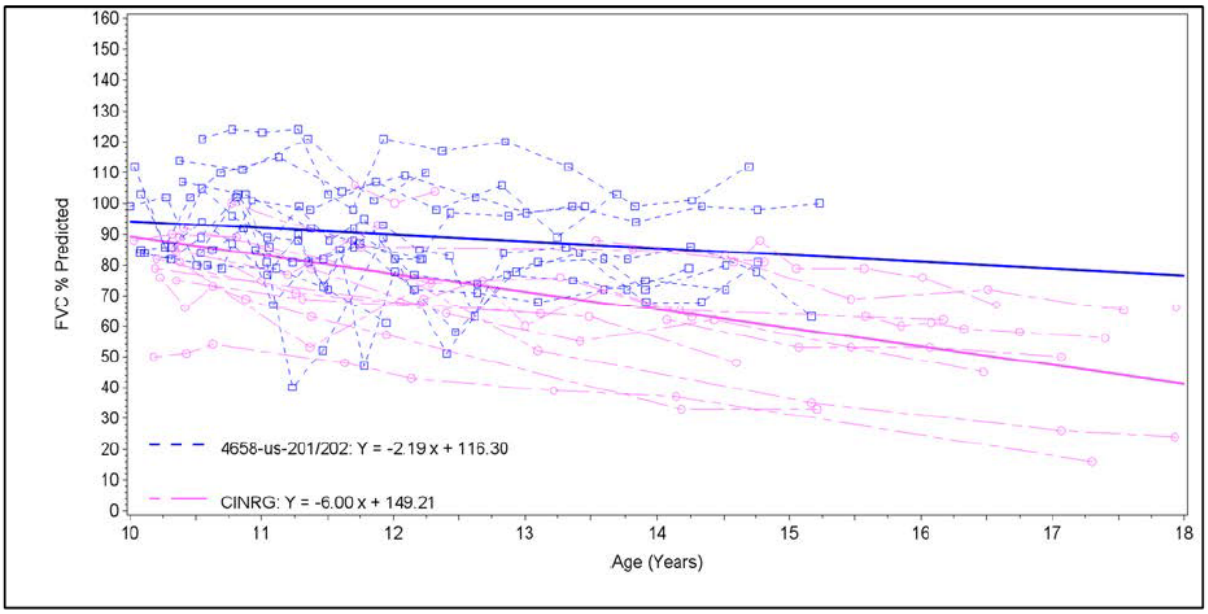
CINRG=Cooperative International Neuromuscular Research Group; FVC%p=forced vital capacity % predicted.
* Excluding milder exon 44 phenotype
Note: For Study 201/202, year is considered to be 48 weeks. For CINRG, year is 52 weeks, with each year windowed by ± 8 weeks from their baseline visit.
Source: SR-18-012 Figure IFVC.3.3.2.8

In addition to the limitations inherent to historical cohorts and the restricted number of subjects in the comparison (12 vs 32) the fact that the control group includes DMD patients other than exon 51 DMD patients makes groups not comparable. The modest observed differences raise concerns about its clinical impact, bearing in mind that a minimum of two-three years of treatment would be required to make evident an effect.

Exon 51 patients from eteplirsen patients and CINRG cohort were compared in the subgroup of patients aged 10 to 18 years (Study 201/202 n= 12 and CINRG cohort n =20 patients). A significantly reduced rate of FVC%p decline was observed in eteplirsen groups [Study 201/202 FVC%p annual change (%)= -2.19 (95% CI

-3.60,-0.79)] compared to CINRG database patients FVC%p annual change (%)= -6.00% (95% CI -6.80,-5.19) (p<0.001). Again, at individual level (Figure below) a high variability with remarkable overlapping between trajectories in both cohorts is observed.

Figure 20 Forced Vital Capacity % Predicted in Patients in the Exon 51 CINRG Subgroup or Enrolled in Study 201/202



Solid lines were estimated using an mixed model with repeated measures (MMRM) analysis.
Note: individual patient trajectories include multiple observations, which contribute to the reliability of the rate of FVC%p decline.
Source: SR-18-012 Figure IFVC 3.3.6

Comparative analysis of the safety and efficacy of eteplirsen-treated patients in study 301 and the external cohort of placebo-treated patients in study DMD114044 (drisapersen study).

As mentioned above, only 29 untreated patients were recruited in study 301. There was also a higher than expected dropout rate in this group (13 patients, 44.8%) that lead to only 7 patients reaching week 96 and only 4 among those with baseline 6MWT distance between 300 and 450 meters. In view of these limitations, the Applicant sought another control group amenable to exon 51 skipping treatment to evaluate the effects of eteplirsen treatment that came from the placebo arm of drisapersen Study 044 (n=61).

According to the Applicant, although there were differences in inclusion criteria between study 044 and study 301, there appeared to be enough overlap between the two studies to provide a placebo control group large enough for comparisons. The Applicant pre-specified the subset of placebo-treated patients from study 044 based on some inclusion criteria that were known to be prognostic for the 6MWT, including an age of 7 to 16 and a baseline 6MWT result of ≥300 meters. For the subset of patients who had a 6MWT distance of 300 to 450 meters at baseline, only 20 placebo patients were eligible. The primary analysis refers to the Efficacy Set although the Applicant is mainly referring to the subset of the Efficacy set in the CSR.

Analyses include comparison of change from baseline to week 48 in 6MWT, change in NSAA total score and other exploratory endpoints (i.e., time to 20% decline from Baseline in 6MWT through Week 48, time to LOA). Sensitivity and subgroup analyses were performed.

Additionally, safety and tolerability were summarized, including adverse events (AEs) occurring through week 48. FVC% predicted was not captured in study 044 and was therefore not specified as an endpoint in the SAP.

Results

As stated by the Applicant in the CSR, there was no statistically significant difference either in the mean change from baseline in 6MWT distance or change in NSAA total score between eteplirsen treated patients and placebo-treated patients at Week 48 either for the Efficacy set or the Subset. None of the patients either in the eteplirsen-treated or placebo-treated groups had loss of ambulation by week 48.

The Applicant stated that these results are consistent with those seen in studies 201/202 at week 48. However, it is difficult to draw any firm conclusions of the motor function efficacy results, especially as it is not evident that the dystrophin production that was observed after 24 and 48 weeks would not transfer into a clinical effect before one year of treatment. The results could also indicate a lack of clinical effect.

The Applicant has provided a number of additional comparisons with external controls for patients from Study 301:

a) Motor function: Interim results were compared with the Primary External Controls (those compared to Study 201/202 patients) over 2 years. Given the differences between cohorts on corticosteroids use (deflazacort use: Study 301 patients 28%; Primary EC 67%), the fact that only 35 of 60 patients from Study 301 fitted the efficacy set definition and the adjustments by age and walking distance at baseline, this additional analysis can be considered of little added value.

b) Pulmonary function: The selected external control was the Exon 51 CINRG cohort. This cohort included 20 patients who were between 10 to <18 years of age. The Study 301 Week 96 interim analysis provides results from 42 patients in the age group of 10 to <18 years. The annual FVC%p decline by age for eteplirsen-treated patients in Study 301 was significantly attenuated compared with patients in Exon 51 CINRG cohort (3.79% vs 6.00% annual decline, respectively).

Table 28 Comparison of Ambulatory Studies 201/202 and 301 to Matched CINRG Patient-Level CINRG Data (Matched for Glucocorticoid Status, Genotype [Exon 51-Amenable Mutations], and Age [10 to <18 years])

Parameter	CINRG (N=20)	Study 201/202 (N=12)	Study 301 (N=42)
# of observations	88	132	184
FVC%p annual change (%)	-6.00	-2.19	-3.79
SE	0.408	0.710	2.21
95% CI	-6.80,-5.19	-3.60,-0.79	-5.41, -2.16
Nominal p-value (comparison to CINRG)		<0.001	0.017

CI=confidence interval; CINRG=Cooperative International Neuromuscular Research Group; FVC%p=forced vital capacity % predicted; SE=standard error.
Source: SR-18-012 Tables IFVC 2.2.6.1 and 2.2.8.1

Supportive studies

Study AVI-4658-28

Dose-ranging study of AVI-4658 to induce dystrophin expression in selected Duchenne Muscular Dystrophy (DMD) patients

This was a phase 1b, open-label, multiple-dose, dose-ranging study designed to assess the safety, tolerability, pharmacokinetics (PK), and exploratory efficacy of eteplirsen in the treatment of boys with confirmed genotypic DMD who were amenable to treatment with exon 51 phosphorodiamidate morpholino oligomer (PMO). The study was conducted in two centres in UK.

The study was comprised of 3 study periods: Screening/Baseline (up to 12 weeks prior), Treatment (Weeks 1 through 12), and Follow-up (Weeks 14 through 26).

Eligible patients were allocated to 1 of 6 dose cohorts (of 2 to 4 patients per cohort) to receive eteplirsen administered intravenously (IV) over a 60-minute period once a week for 12 weeks. Weekly doses ranged from 0.5 to 20.0 mg/kg. Dose escalation to the next dosing cohort were to occur sequentially and only after review of the key safety data obtained from the previous cohort indicated that it was safe to proceed. Review of the data for dose escalation was conducted by an independent DSMB.

Study patients

Key Inclusion Criteria:

Male, between the ages of 5 and 15 years, with an out of frame deletion(s) that could be corrected by skipping exon 51 based on DNA sequencing data, and a muscle biopsy analysis showing <5% revertant fibers present were selected. Patients were required to walk independently for at least 25 meters, to have a forced vital capacity (FVC) ≥50% of predicted and receive the standard of care for DMD as recommended by the DMD care recommendations.

Patients were excluded if they have a DNA polymorphism within exon 51 that may have compromised PMO duplex formation, known antibodies to dystrophin or they lacked intact right and left biceps muscles or alternative arm muscle group. Other exclusion criteria were a calculated creatinine clearance <70% of predicted normal for age, aleft ventricular ejection fraction of <35% and/or fractional shortening <25% based on ECHO during Screening, a history of respiratory insufficiency, severe cognitive dysfunction, known immune deficiency or autoimmune disease, bleeding disorder or receipt of chronic anticoagulant treatment within 3 months of study entry or another clinically significant illness at time of study entry. Pharmacologic treatment, apart from corticosteroids, that might have affected muscle strength or function within 8 weeks of study entry was not permitted.

Efficacy endpoints

The study was primarily designed to assess the safety and PK of eteplirsen; therefore, no primary efficacy endpoint was defined. However, efficacy was assessed by evaluating dystrophin production and exon skipping in muscle biopsy tissue.

Muscle function using the 6MWT, Quantitative Muscle Testing (QMT), North Star Ambulatory Assessment (NSAA), and a StepWatch Activity Monitor (SAM) was also evaluated. The efficacy assessments were completed at Baseline (Week -1), Weeks 1, 6, and 12 (following study drug administration), and Weeks 18, 22, and 26/Early Termination visit.

No formal sample size calculations were performed for this Phase 1b dose escalation study. A minimum of 18 and a maximum of 24 patients were planned for this study.

Results

A total of 19 patients were enrolled and treated in this study; patients were enrolled sequentially into 1 of 6 dose cohorts. One patient withdrew from treatment after receiving 7 of the planned 12 doses due to an AE of cardiomyopathy. An additional patient was enrolled into the 4-mg/kg/wk dose cohort.

Overall, the mean age for the 19 patients was 8.7 years and ranged from 6 to 13 years. Mean weight and height across the 19 patients were 34.5 kg and 124.5 cm, respectively. Overall, the majority of patients were White (18 patients, 95%).

Approximately half (53%) of the 19 patients experienced an interruption in the infusion; interruptions were most commonly reported in the 2 highest dose groups (4 of 4 patients in the 10.0 mg/kg/wk group and 3 of 4 patients in the 20.0 mg/kg/wk group). Although infusions were interrupted for these patients, all patients completed the scheduled infusion.

With respect to the muscle function tests, in general, small changes from Baseline, both declines and improvements, were observed in all patients over the course of the study on each of the muscle function tests conducted.

Study 204

It was a Phase 2, multicenter, open-label study to explore the safety and tolerability of eteplirsen in patients with advanced Duchenne muscular dystrophy (DMD). In all, 24 patients between 7 to 21 years of age with advanced DMD and with confirmed genetic mutations amenable to exon 51 skipping were planned for enrolment. Patients were evaluated for eligibility, which included inability to walk ≥300 meters on the 6MWT during the screening period.

This study was performed in a different population than the one for which the indication is proposed so the value of the data to support the current application is limited.

During the treatment phase of this study (ie, Weeks 1 through 96), patients received 30 mg/kg of eteplirsen once weekly by intravenous (IV) infusion (over approximately 35 to 60 minutes).

The primary objective of this study was to evaluate the safety and tolerability of 30 mg/kg of eteplirsen in patients with advanced DMD. Safety was assessed throughout the collection of AEs, laboratory tests, ECGs, ECHOs, vital sign and physical examinations. The exploratory objectives were to evaluate the effect of eteplirsen on pulmonary function tests (PFTs) and other functional clinical measures; evaluations were completed approximately every 12 weeks over the first year and approximately every 24 weeks over the second year throughout the treatment phase of the study.

Patients were divided into 2 groups based on their ambulatory status at baseline (ambulatory and non-ambulatory). There was no control group in this study.

Results

In all, 24 patients were in the study, 17 were non-ambulant and 7 ambulant. There were obvious differences in the baseline characteristics related to the different stage of the disease.

According to the Applicant, there was a decline in FVC%p in the first year of eteplirsen therapy that was consistent with annual rates of ≥ 5% decline reported for untreated DMD patients in the literature (Khirani 2014; Mayer 2015) while the decline in the second year was less than the annual decline reported in the literature. In the study by Khirani et al predicted values showed a mean of 4.1±4.4% decline/year and in the study published by Mayer FVC and PEF showed a near linear decline of approximately 5% decline/year from ages 5 to 24. The interpretation of this data is hampered both by the small numbers and by the inherent variability of the test. In addition, there is no placebo arm for comparison. No sound conclusions can be drawn from this data.

The Applicant has provided an additional comparison with CINRG cohort for the age-based mixed model analyses was the Exon 51 CINRG cohort external control for patients from Study 301. For Study 204 in nonambulatory patients (n=20 aged from 10 to 18 years) annual decline was -3.66 (95% CI -5.00, -2.32) vs -6.00% (95% CI -6.80,-5.19) in the Exon 51 CINRG cohort.

Confirmatory study proposed in the context of the applied Conditional marketing authorization.

The Applicant applied for a Conditional marketing authorisation based on the data available at present, and their plans to perform a placebo-controlled study (study 302) as a post-approval, specific obligation for this application. The initially proposed Study 302 was meant to be a double-blind and randomised trial to confirm the clinically relevant benefit of eteplirsen in DMD patients amenable to exon 51 skipping. The study intended to recruit patients aged between 7 and 13 years of age who are stable to corticoids and with baseline 6MWT of 300-450 m. The study was planned to last 96 weeks with a sample size of 120 patients (2:1 randomization). The usual efficacy endpoints were to be used for assessment. The need of such study was strongly supported by the CHMP. However, the applicant was encouraged to re-think the length of the study, and the planned number of patients to be included, and present the efficacy data that the plans were based on. A revised design was then proposed in order to increase the feasibility of the study. The study was changed to recruit ambulatory and non-ambulatory patients aged 10 to 18 years on stable dose of oral corticosteroid and an FVC%p ≥ 50% to ≤ 80%. The primary endpoint was to be the measurement of FVC%p over 3 years (two years under placebo controlled and open label treatment with eteplirsen during the third year). Key secondary endpoints were to be evaluated in to corroborate the motor benefit of treatment with eteplirsen, and will include assessments of 6MWT results in ambulant patients and performance upper limb (PUL) in all patients. Approximately 150 ambulatory and non-ambulatory DMD patients, amenable to exon 51 skipping, were to be randomized in a 2:1 ratio of eteplirsen (30 mg/kg) to placebo. A minimum of 60 ambulatory patients with baseline 6MWT distance of 300 to 450 meters will be enrolled in order to provide 70% power for the evaluation of the 6MWT, a key secondary endpoint, a 2-sided alpha of 0.05.

In the latest stages of the assessment, the Applicant revised their proposal, by committing to perform a 96-week Randomized, Double-blind, Placebo-controlled Study in Ambulatory Patients with DMD Amenable to Exon 51 Skipping.

2.5.3. Discussion on clinical efficacy

The clinical programme for eteplirsen in the treatment of DMD muscular dystrophy amenable to be corrected by exon 51 skipping induced by eteplirsen consisted of 4 interventional clinical studies, a number of external control cohorts, and a review of literature describing the natural history of DMD.

Study 201 (randomized, double-blinded, placebo controlled Phase IIb study) and its open label extension Study 202 in a total of 12 DMD patients provide the main efficacy data. One proof –of-concept study (Study 33) and

a dose ranging study of eteplirsen 0.5 mg/kg up to 20 mg/kg IV over 12 weeks (Study 28) provide additional support.

During the procedure, the Applicant has provided preliminary results from two other studies:

- Study 301 (PROMOVI), an open-label, multicentre study in 90 ambulant DMD patients amenable to be corrected by exon 51 skipping. A comparison is done with an external untreated group of DMD patients amenable to exon skipping of an exon other than exon 51.
- Study 204, a Phase 2, multicentre, open-label study to explore the safety and tolerability of eteplirsen in 24 patients with advanced Duchenne muscular dystrophy (DMD) with confirmed genetic mutations amenable to exon 51 skipping

In Study 201, a total of 12 boys were randomized to receive 50 or 30 mg/kg/wk eteplirsen or placebo (n= 4 patients/arm) for 24 weeks; placebo patients were then further randomized to 1 of 2 active groups for 4 weeks. Ambulatory patients with a mean age around 8.8 years (aged from 7 to 10 years) were recruited. About 67% of patients were older than 9 years. All patients were treated with corticosteroids. The majority of the patients were treated with deflazacort (67%) on a continuous regimen (92%). Subjects in eteplirsen groups walked at entry a mean distance of 375.6 metres and 394.5 metres in placebo group.

The aim of the treatment with eteplirsen is to produce a shorter, but functional dystrophin protein. It is considered that muscle quality is important for the therapeutic success, since dystrophin transcripts are only produced in muscle cells and not in the fibrotic and adipose tissue that replaces the muscle cells when the disease progresses. For this purpose inclusion of patients at early stages of the disease would be preferable. However, in young children ambulatory function may improve due to age-related growth and development, acting as a confounding factor. As the impact of a treatment on motor function can be more easily shown above the age of 7[2] when it is expected that ambulation annually declines, patients in the late ambulant phase (but still walking between 200-400 metres) were recruited. Given the muscle deterioration and the reduced options for regeneration existing at that stage it is unclear whether the included patients had sufficient ground for improvement.

The study was primarily aimed to show the effect of eteplirsen on dystrophin restoration. Efficacy endpoints were secondary endpoints.

The main efficacy measurement was the Six-Minute Walk Test (6MWT). The 6 minute walk test is currently being used as the primary outcome measure in most of the ongoing studies as it provides a global assessment of functional mobility, endurance, and ability to walk.[3] It is endorsed as it is recommended as primary outcome in several guidelines[4,5,6]. Several authors have described the natural course of the 6MWT in untreated patients[11,7,8]. The inter- and intra-personal variability, the influence of age, the learning effect and the fact that

[2] Mercuri E et al. Categorizing natural history trajectories of ambulatory function measured by the 6-minute walk distance in patients with Duchenne muscular dystrophy. Neuromuscular Disorders 26 (2016) 576-583.
[3] Pane M, Mazzone ES, Sivo S, Sormani MP, Messina S, et al. (2014) Long Term Natural History Data in Ambulant Boys with Duchenne Muscular Dystrophy: 36-Month Changes. PLoS ONE 9(10): e108205.
[4] Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy. EMA/CHMP/236981/2011, Corr. 11
[5] Duchenne Muscular Dystrophy and Related Dystrophinopathies: Developing Drugs for Treatment. Guidance for Industry. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
[6] Mercuri E et al. Towards harmonisation of outcome measures for DMD and SMA within TREAT-NMD. Neuromuscular Disorders 18 (2008) 894–903
[7] Mazzone ES, Pane M, Sormani MP, Scalise R, Berardinelli A, et al. (2013) 24 Month Longitudinal Data in Ambulant Boys with Duchenne Muscular Dystrophy. PLoS ONE 8(1): e52512
[8] Henricson E, Abresch R, Han JJ, Nicorici A, Goude Keller E, Elfring G, Reha A, Barth J, McDonald CM. Percent-Predicted 6-Minute Walk Distance in Duchenne Muscular Dystrophy to Account for Maturational Influences. PLOS Currents Muscular Dystrophy. 2012. doi: 10.1371/currents.

it is effort based and can be influenced by motivation may have an impact on the results at the time of the assessment.

In addition, the impact on global motor function tests, assessing activities other than ambulant capacity (North Star Ambulatory Assessment, timed 4-step test) as well as on complementary outcomes of muscular function (muscle strength, upper limb function) were also assessed in order to provide robustness to the claimed effect.

A total of 9 out of 12 patients experienced a decline in 6MWT after 24 weeks of treatment (4 subjects on eteplirsen 30 mg/kg, 2 subjects on eteplirsen 50 mg/kg and 3 on placebo). Two patients on eteplirsen 30 mg/kg (subject 009 and 010, twins aged 9 years, who were able to walk at recruitment 346 and 261 metres, respectively) showed a relevant decline, walking 296 and 218 metres less than the distance walked at entry of the study. These subjects lost the ambulation few weeks later, during the open label period.

In general a great variability was observed for most endpoints across patients and over time, where no separation is observed between placebo and any of the active groups.

After the initial placebo controlled phase of 24 weeks (Study 201) patients entered an open-label extension phase of up to 240 weeks (Study 202) in which all patients were treated with eteplirsen (30 or 50 mg/kg). All patients (n=12) completed the treatment period.

During the 4 years of treatment, patients experienced gradual decline in the measured functions. Patients showed a progressive deterioration of ambulation, even after excluding the two patients who lost early ambulation. No additional patients lost ambulation through the study. The Applicant has suggested a detrimental effect of the delayed onset group but the small number of patients included, the absence of a control (placebo) beyond 6 months and the variability between subjects prevent the CHMP from drawing sound conclusions. The results are not informative regarding the selection of the dose since no clear separation between curves by dose can be observed.

Similarly, those endpoints related to “burst activities” measured as Timed Function Tests (mean timed 4-step test score, 10-Meter Walk/Run Time and time to rise from a supine position) increased over time during the study. Results of muscle strength and pulmonary function are not easily interpretable. With only 12 patients in the study (two of them loosing ambulation early during the extension phase) and without a concurrent control it is difficult to conclude that they are reflecting a true change (slowing the progression) in the natural course of the condition.

In order to provide a control group for comparison for establishing the long-term clinical effectiveness of eteplirsen two natural history registries were initially selected. A total of twelve registries were identified worldwide and the Italian DMD Telethon registry and the Leuven Neuromuscular Research Center registry were finally chosen. The main criteria for selection were the availability of prospectively documented DMD natural history data and long-term 6MWT assessment (up to 3 years). The identification process of the external control group was intended to select a group of patients similar to the population included in the study so that it could reliably predict the course of the patients’ disease, in the case that they were not treated. Two external controls were finally selected: An external control group (n=12) amenable to exon 51 skipping was the primary comparator for the 4-year 6MWT data from Study 201/202 eteplirsen-treated patients. This was the Primary External Control Group. With the aim to have a larger population for comparison a 201/202 Secondary External Control Group (n=50) of patients amenable to any exon skipping was identified by the Applicant.

However, the post-hoc nature of the analysis and the fact that the cohorts were retrospectively identified within the untreated group of patients is of a serious concern (potential selection bias) and undermines the robustness of the data. As it is stated in ICH E10 “the control group is thus not derived from exactly the same population as

the treated population". Without randomization and blinding the comparability between groups is not properly established. In fact, in addition to the selection criteria (based on age, genotype and corticosteroid group) other factors, not considered in the matching, may have also influence in the course of the condition. This approach does not seem to alleviate the concerns derived from the Study 201/202.

With respect to the selected primary endpoint, the inter- and intra-subject variability and the fact that it is influenced by training and motivation, make 6MWT less suitable for external control group comparison. In general, the performance of tests used for assessing treatment effect may not be entirely superimposable to that standarized for both groups (test and control) in the clinical trial.

When eteplirsen and Primary External Control patients were compared, differences in relevant baseline characteristics were seen: eteplirsen patients started earlier glucocorticoid treatment (5.2 vs 6.5 years) and were more frequently on continuous regimen than control group (92% versus 82%); in addition, more patients on eteplirsen were able to rise from floor independently (92% versus 67%). Such differences can have an impact on walking distance and even more when the sample size is so small. Similar differences for ability to rise from floor were seen between the eteplirsen treated group and the Secondary External Control; the two other baseline characteristics (age at start of glucocorticoids, and glucocorticoid regimen) were not shown for the Secondary External Control. Moreover, although criteria for identification of external controls were predefined, the comparative analyses were post-hoc. There may be also unknown factors that can also have an impact on the course of the disease that may have not been considered by the Applicant.

In this indirect comparison both groups (eteplirsen treated patients and untreated external controls) experienced a decline in ambulation. A more pronounced deterioration was observed in the external control groups (both those amenable to exon 51 skipping and those amenable to any exon skipping) than in eteplirsen treated patients. After 4-year follow up patients in primary external cohort lost 330.3 metres (vs 166.9 metres in eteplirsen group). Separation between curves is apparent at Year 3. At individual level the variability between patients is evident and separation between groups is not so clear. According these results improvement in ambulation (even in this selected late ambulant population) could be seen after at least 2 years of weekly IV treatment.

With respect to the effect of eteplirsen on pulmonary function, the comparison of patients treated with eteplirsen with untreated patients from two other different DMD databases (United Dystrophinopathy Project cohort and Cooperative International Neuromuscular Research Group database) showed a slower annual decline of Forced Vital Capacity, percent predicted. In the age group of 7 to 15.5 years of age the annual decline FVC%p was 4.1% (95% CI; 1.9%, 6.3%) for the UDP control group compared with an annual decline of 2.3% (95% CI; 1.2%, 3.4%) in eteplirsen patients . In the subgroup of patients aged 10 to 18 years FVC%p annual change (%) declined 6.00% (95% CI -6.80,-5.19) in the CINRG database patients compared to annual decline of 2.19% (95% CI -3.60,-0.79)] in eteplirsen treated patients ($p<0.001$). In addition to the limitations inherent to historical cohorts, the substantial decrease in number of subjects in the external cohorts over time and the restricted number of subjects in the comparisons makes groups not comparable. At individual level a high variability with remarkable overlapping between trajectories in the cohorts is observed.

Although in principle one single pivotal study for the pursued indication could be acceptable, it was concluded that in this case Study 201/202 was not sufficient to provide the necessary evidence of efficacy. The main limitations come from the reduced number of patients by arm (which makes difficult the interpretability of the study) and the duration of the study under double-blind conditions (6 months). There is an increasing amount of evidence in DMD, suggesting that therapies aimed at restoring the expression of dystrophin may require longer duration of treatment to produce an evident clinical effect.

Even considering the rare nature of the condition and being aware that the number of patients available to be studied is significantly reduced, the CHMP was of the opinion that clinical development programme, aimed at supporting the efficacy and safety of the product was very limited, and therefore unsatisfactory to properly demonstrate the efficacy and safety of the product. Other products intended for treatment of this particular DMD population have been investigated in a significantly larger number of patients, proving that a more complete programme is feasible in real life conditions.

The clinical trials for eteplirsen were primarily designed to evaluate the effect of the product on dystrophin expression in target muscle. In addition, the effect of eteplirsen on clinical functions was also assessed. Given the critical role of the lack of dystrophin in this condition the demonstration of restoring the expression of a functional dystrophin represents a meaningful goal. However, according the current regulatory requirements (EMA guideline on DMD/BMD[9]), functional improvement (or at least delay of progression and deterioration) is considered the most relevant treatment goal for patients affected by DMD and BMD. In medicinal products intended to slow down the accumulation and the progression of disability, a sustained clinical effect on disability progression should be shown. Therefore, detection of dystrophin in muscle tissue can provide supportive information as proof of concept and support the validity of findings on the clinical endpoints.

Eteplirsen was effective in inducing skipping of exon 51. Results from dystrophin detection in muscle biopsies suggest a production of (truncated) dystrophin and, in addition, might indicate that eteplirsen-induced increase of truncated dystrophin is delayed up to several months from start of treatment. However, there are no satisfactory data, clearly establishing the effectiveness of the truncated dystrophin. The amount of protein produced seems very limited according to the Western blot results (0.44% of normal dystrophin at Week 48 [Study 301]; 0.93% at Week 180 [Study 201/202]). The minimum beneficial amount of dystrophin expression to be translated into a clinical benefit has yet to be established. In the absence of clinical data convincingly demonstrating a clinical effect, it cannot be concluded that the amount of dystrophin expressed with eteplirsen will translate into a clinical benefit to patients.

A confirmatory, prospectively powered trial appears necessary to complete the dossier for MAA. The Applicant is conducting three additional clinical studies: one Phase II study in young patients, one Phase II study in advanced patients (Study 204) and one Phase III study with the intended for marketing dose vs. untreated patients amenable to exon skipping different from Exon 51 following an open-label design (Study 301 PROMOVI). Although this strategy may result in producing additional data on efficacy, the methodological concerns as described above could still remain unaddressed by this approach.

During the procedure, the Applicant provided results of the interim report of study 301 that was performed after approximately 35 eteplirsen-treated patients with a baseline 6MWT between 300 and 450meters and 4 untreated subjects had completed their Week 96 study assessments. Comparisons at weeks 72 and 96 are difficult considering the low number of untreated patients available. At week 48, when almost all patients were still retained in the study, no clear beneficial effect was observed on eteplirsen treated patients (change from baseline at week: -37.3 meters and -47.5 meters for eteplirsen-treated and untreated group respectively). Whether this is expressing that an effect can only be detected after at least one year of treatment (as stated by the Applicant), or if it is reflecting the lack of beneficial effect of the treatment, cannot be concluded on at present. It is also unclear if considering the above mentioned limitations of the study (open nature, lack of reliable controls, etc.) the final results could dissipate all of the outstanding current concerns. Results for other relevant secondary endpoints (NSAA, loss of ambulation, FVC%p) all point in the same direction since no differences were seen between eteplirsen and untreated group. Concerning the final results from study 301

[9] Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy. EMA/CHMP/236981/2011, Corr. 11

(week 96) the lack of a placebo arm is a crucial drawback for the interpretation of the results considering the limitation of the external control groups.

Regarding Study 204, the small size of the study, the advanced stage of patients, the variability of the results and the lack of placebo control prevent from drawing valid conclusions for the pursued indication.

1) Positive benefit-risk balance: With the currently available data it is not possible to conclude that the benefit/risk ratio of eteplirsen is positive in DMD patients with mutations amenable to exon 51 skipping. Placebo comparative data beyond 24 weeks are not available and this comparison is only for 12 patients. The Applicant has provided additional comparative data but they have important limitations related to nature of the comparison groups. A variety of external controls coming from different studies and populations have been provided. This increases the uncertainties about the reliability of such comparisons rather than providing useful comparative data. The drawbacks of using external control groups are very well-known and have been previously discussed in this Assessment Report.

2) Provision of further comprehensive data: It is still doubtful that the Applicant can provide confirmation of a potential beneficial effect of eteplirsen in DMD (see above).

3) Unmet need: It is clear that there is an unmet need for medicinal products addressing mutations amenable to exon 51 skipping since currently there is no approved product with the same therapeutic indication as proposed for eteplirsen in the EU market.

4) Benefit of immediate availability: While it is clear that early availability on the market of a medicinal product for DMD patients with mutations amenable to exon 51 skipping would be relevant from the public health perspective results from the available clinical trials do not allow concluding that the benefit/risk of eteplirsen is positive, therefore the risk inherent in the fact that additional data are still required is not outweighed. Moreover, the availability of eteplirsen on the market would prevent from performing additional clinical trials that could address if eteplirsen or other products have a positive benefit/risk in DMD patients with mutations amenable to exon 51 skipping.

In conclusion, eteplirsen does not fulfil all of the criteria set out in Article 4(1) of Regulation (EC) No 507/2006, therefore the CHMP considers that the product does not fall under the scope of a conditional marketing authorisation.

2.5.4. Conclusions on the clinical efficacy

For this application clinical data were provided from a randomized, double-blinded, placebo controlled 24-week Phase IIb study (study 201) and its open label extension Study 202 in a total of 12 DMD patients. The original pivotal trial (study 201/202) provided a 24-week comparison of only 4 patients on eteplirsen exposed to the proposed dose of 30mg/kg/week versus placebo (n=4), and additionally 4 patients exposed to 50 mg/kg/week, in which no difference was observed in the 6MWD. Longer comparisons (up to 4 years) with 12 DMD patients on eteplirsen were performed versus two post-hoc defined, external and non-concurrent cohorts (Italian Telethon DMD Registry and Leuven Neuromuscular Reference Center Registry). Both groups (eteplirsen treated patients and untreated external controls) experienced a decline in ambulation. A more pronounced deterioration was observed in the external control groups (both the amenable to exon 51 skipping control group and the one including amenable to any exon skipping patients) than in eteplirsen treated patients. Separation between curves is apparent at Year 3 (Year 3 - 144 metres, p=0.0055; Year 4 - 161 metres, p=0.0007). At individual level the variability between patients is evident and separation between groups is not so clear. A trend favouring

eteplirsen treated patients was observed in loss of ambulation (2/12 in eteplirsen treated patients vs. 10/13 in external controls at Year 4), North Star Ambulatory Assessment and ability to rise from supine.

The main limitations in the dataset arise from the limited number of patients by arm (which hinder the interpretability of the study results), and from the duration of the placebo-controlled phase (6 months). The additional data provided from the open-label phase, a 4-year period of treatment, do not allow to convincingly conclude on a relevant effect of eteplirsen in this population. Without an appropriate concurrent control it is not possible to conclude that the results are reflecting a true and clinically meaningful change (slowing the progression) in the course of the condition. The comparison with external control cohort from natural history databases presents with methodological deficiencies, and its results can only be considered as exploratory or supportive. The Applicant has defined several external controls that have been used for different comparisons. The potential sources of bias, using this strategy, seriously affect the reliability of the subsets and comparisons, and the conclusions made thereof. This is even more relevant when the external controls are retrospectively selected. In general, this strategy increases the uncertainty about the results rather than providing reassuring comparisons.

At the time of the assessment, three additional clinical studies were being conducted, in order to provide additional data to support the application. The provided results from the interim analysis of one of them (study 301) and the performed comparisons with different controls, did not reveal significant differences in the clinical endpoints between eteplirsen treated and untreated patients. The limitation derived from the small numbers is acknowledged but by no means can reduce the uncertainties related to the results observed. Additional post-hoc comparisons with other external controls also have their limitations, as previously mentioned. A comparison versus a different external and non-concurrent cohort was provided (DMD patients on placebo from the pivotal trial of another medicinal product) for both the whole population and that restricted to those DMD patients walking between 300 and 450 m., and similar shortcomings regarding the comparison groups were identified there.

In terms of the pharmacodynamic proof of concept, a modest increase in dystrophin (truncated) production has been shown in some patients, while in a number of them no production was detected. As the minimum amount of truncated dystrophin expression that is needed to achieve a clinically relevant benefit remains unknown, the value of these data is mainly to serve as supportive for the proposed mechanism of action of the product.

The applicant requested a Conditional marketing authorisation based on their claim that the case fulfils all the requirements in the Regulation. However, the CHMP concluded that the medicinal product did not fulfil all of the criteria set out in Article 4(1) of the Regulation, as despite recognizing the unmet need in this condition, the current benefit-risk balance could not be considered positive based on the available, submitted data.

The CHMP considered that with the currently available data it is not possible to conclude that the benefit/risk ratio of eteplirsen is positive in DMD patients with mutations amenable to exon 51 skipping, since the efficacy of the above mentioned medicinal product is not sufficiently demonstrated.

2.6. Clinical safety

The current application is intended to support the use of eteplirsen for the treatment of Duchenne muscular dystrophy in adults, adolescents, and children aged 4 years and older who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping. DMD is a rare, serious, life-threatening, X-linked recessive

degenerative neuromuscular disease caused by mutations in the DMD gene. There is no approved treatment for this specific mutation.

Patient exposure

Eteplirsen application is based on 3 completed studies (studies 28, 33 and 201/201) and 3 that are currently ongoing (studies 203, 204 and 301) in which different dosages and methods of administration were used. The total number of DMD subjects exposed to any concentration of eteplirsen was 150 but only 116 received the proposed posology (30 mg/kg IV). Most of them (112) came from ongoing studies that do not include a placebo control arm. According to the Applicant, they have received eteplirsen at least for 12 weeks so far.

The only comparative data come from the pivotal study 201 in which 12 subjects were randomised to eteplirsen 30 mg/kg (n=4), 50 mg/kg (n=4) or placebo (n=4) for 24 weeks. This very limited dataset is a major limitation, even for this orphan condition, that seriously hinders the interpretation of the safety data.

At termination of study 201 patients entered the extension study 202 in which the 4 patients on placebo were randomised to eteplirsen 30 mg/kg or 50 mg/kg and received the correspondent dose for approximately 3 additional years. This small and non-comparative long-term dataset is another drawback of this application. Like for the short-term safety assessment drawing sound conclusions on long-term safety is impossible. . Therefore, the safety data presented in this application must be interpreted with extreme caution.

Adverse events

The Applicant has integrated all the safety data across studies population in order to perform a thorough safety assessment. Nevertheless, combining studies with different time exposure is likely to dilute the incidence of AEs making it difficult the evaluation of the safety profile of eteplirsen. Safety data for individual studies are also presented.

Individual studies

Study 201:

Patients originally randomised to placebo received at least 219 weeks of eteplirsen treatment, and patients randomized to the 30 or 50mg/kg arms had received at least 252 weeks of eteplirsen treatment. All patients treated with eteplirsen had AEs that were severe in 2 patients on eteplirsen 30 mg/kg.

From a qualitative point of view, similar AEs were reported for the placebo and 30 mg/kg dose groups. Some of them might be reflecting drug adverse events the target population of children enrolled in the trial (e.g., oropharyngeal pain or nasal congestion) rather than AEs, others seem to be related to the procedure (e.g., procedural pain or incision site pain) and others could be explained by the underlying disease (e.g., falls or pain in extremities). Hypokalaemia and dermatitis contact were also reported in 50% of patients. All these AEs, except contact dermatitis, were also reported in the placebo arm.

Since only 4 patients were treated with placebo, 4 with eteplirsen 30 mg/kg and 4 with 50 mg/kg a comparative safety exercise is not possible. Quantification of the safety profile is challenging as only AEs with higher incidence than 25% (but representing only 1 patient) can be identified while less frequent events will be missing, even those considered frequent with an incidence of 10%. To what extent the reported AEs are related to the drug or not is difficult to ascertain. No definite conclusions can be drawn from this very small dataset.

Study 202:

In the extension study 202 100% of patients had at least one TEAE and around 50% had at least one TEAE related to treatment. In patients treated with eteplirsen, around 100% of patients had at least 1 moderate or severe TEAE and around 30% has at least one serious TEAE.

The most common TEAEs were procedural pain (91.7%) followed by headache (75.0%), pain in extremity (75.0%), oropharyngeal pain (66.7%), arthralgia (66.7%), back pain (66.7%), contusion (58.3%), nasopharyngitis (58.3%), proteinuria (58.3%), cough (50.0%), vomiting (50.0%), upper respiratory tract infection (50.0%), hypokalemia (50.0%), arthropod bite (41.7%), balance disorder (41.7%), pyrexia (41.7%), excoriation (33.3%), prolonged aPTT (33.3%), increased C reactive protein (33.3%), muscle spasms (33.3%), musculoskeletal pain (33.3%), upper abdominal pain (33.3%), dyspepsia (33.3%), catheter site pain (33.3%), and infusion site extravasation (33.3%). The extension study 202 has the previously mentioned limitations: the small number of exposed patients and the lack of placebo control arm. Although drawing any sound conclusions on the long-term safety profile of eteplirsen appears difficult the number and variety of TEAEs suggest that the safety profile may worsens with the long-term administration of eteplirsen.

All TEAEs of study 201 were considered unrelated to the drug by the investigator, except for 1 case of nausea. All were assessed as mild or moderate except for 3 AEs occurring in 2 patients on 30 mg/kg (1 severe nasal congestion and 1 severe bone pain and severe loss of balance reported in 1 patient). There were no treatment-related or severe TEAEs reported during the last 4 weeks of Study 201 when all patients were receiving 30 or 50 mg/kg eteplirsen weekly.

In the remaining five clinical trials **(AVI-4658-28, AVI-4658-33, 4. 4659-203, 5. 4658-204 and 3. 4658-301)** eteplirsen was given at different doses and ways of administration (IM and IV). Three out of 5 studies were ongoing at the time of the MAA; in such studies patients were exposed to eteplirsen for at least 12 weeks, according to the Applicant.

In study **AVI-4658-28** (dosing from 0.5 to 20 mg/kg IV) the most common AEs were headache and upper respiratory tract infection (42%), back pain and rhinitis (37%), abdominal pain and fall (26%). All of them were mild to moderate. One event of cardiomyopathy was reported as severe. Most occurred after an extended time on drug.

In study **AVI-4658-33** (dosing 0.9 or 0.09 mg/kg IM) most of AEs were mild to moderate and the majority related to biopsy procedure (erythema, induration, pruritus, pain post biopsy).

Studies 4.4659-203, 5.4658-204 and 3.4658-301 in which patients received eteplirsen 30 mg/kg are currently ongoing and available data form part of “All patients database”.

<u>All patients receiving any dose of eteplirsen</u>

Around 50% of patients reported any AEs prior to study drug initiation. As mentioned for study 201 sme AEs may be reflecting the target population of children enrolled in the trial (e.g., upper respiratory tract infections or nasopharyngitis), be related with the disease itself (e.g., falls) or related to the method of administration (procedural pain). Vomiting and rash were reported in around 3% of patients. No patients on placebo reported any AEs except procedural pain; however, this comparison is difficult as only 4 patients were included in the placebo arm.

When looking at the TEAEs, around 87% of patients reported any TEAEs. The most common were cough (around 35%), vomiting (around 30%), back pain and headache (around 28%), nasopharyngitis (around 24%), upper respiratory tract infections, contusion and pain in extremity (around 23%). Most of these AEs were also

reported in patients on placebo. Again, comparison with placebo is difficult since only 4 subjects were included in this arm.

For the proposed dose (30mg/kg), 44% of patients reported any AE and around 16% of patients presented AE related to pain procedure (procedural pain and catheter site pain). Vomiting and rash were also reported in around 3% of patients.

Data provided for time to event onset suggest that most of TEAEs occurred more frequently over the first weeks of treatment. Given that most patients were exposed for short time (around 12 weeks) numbers of exposed patients at all time points (N=118) are not understood. Frequency of AEs over time changes may change if only the actual exposed patients are considered in each period of time. The Applicant should provide a revised table with the actual number of exposed patients and the AEs at all time points.

Overall treatment-related TEAEs were reported in 31% of patients. Cough (in around 35% of patients), vomiting (in around 30% of patients), back pain (in around 28% of patients) and headache (in around 28% of patients) were the most frequent TEAEs. The most common TEAEs related to treatment were headache (5.3%), vomiting (4%), nausea (2.7%) and diarrhoea (2.7%).

Eight TEAEs were considers serious (1 cardiomyopathy, 2 femur fractures, and 1 case of femoral neck fracture, myocarditis, nephrolithiasis, scoliosis, and tibia fracture). Only the cardiomyopathy event reported in study 28 was considered by the investigator to be possibly related to eteplirsen although further investigations concluded that this patient suffered of cardiomyopathy previously.

Adverse events of special interest

AESI focused on medical topics that were selected based on: potential safety-related findings observed in nonclinical toxicity studies of eteplirsen (renal function), safety-related findings that may be related to co-morbidities associated with the underlying DMD disease (falls, fractures, cardiac function), association with treatment with other RNA therapeutics (hepatic function, coagulopathy infusion site reactions and renal function), notably those with a phosphorothioate backbone, and/or general precautions (infusion related reactions, severe cutaneous reactions, hypersensitivity and leukopenia/neutropenia).

Hypersensitivity: Around 12% of all patients reported hypersensitivity events that in general were mild and considered as not related to treatment. Three patients had concurrent respiratory events (i.e., cough) and cutaneous events (i.e.,flushing or rash) that would suggest hypersensitivity. There were two mild cases that were considered related to treatment by the investigator, one with eteplirsen 30 mg/kg and another one with 50 mg/kg. The Applicant should discuss if hypersensitivity reactions should be included as potential risk in the RMP.

A case report of erythema describe reaction in a patient exposed to 50 mg/kg, the case report describe both positive dechallenge and positive rechallenge. The symptom is proposed to be included in section 4.8 of the SmPC, which is endorsed. Events of cough, rash and flushing are reported and described with limited time relation. AEs related to hypersensitivity are described and supported to be labelled in section 4.8 of the SmPC-(rash, erythema and flushing.) Further monitoring of hypersensitivity related ADRs should be considered.

Renal function: In non-clinical studies kidneys were identified as the main target organ. Proteinuria (defined as predefined abnormal change for protein in urine was the detection of 2+ or higher on dipstick assay) was reported in 13 patients (8.6%), 10 of them in patients on 30 mg/kg (all of them except one, in the ongoing studies). Five patients on 30 mg/kg had mild proteinuria that was considered possibly related to study drug by the investigator.

None of the events was serious, and all were reported as mild in intensity by the investigator. Renal toxicity has been proposed by the Applicant as a potential risk to be included in the RMP. This is supported.

Leukopenia and neutropenia: One patient treated with 50 mg/kg had 2 events of lymphocyte count decreased and 1 event of white blood cell count decreased that was considered as possibly related to study drug.

Infusion site reactions: 34 patients out of all patients receiving IV eteplirsen reported infusion site reactions: catheter site pain (9.1%), infusion site pain (6.3%) infusion site extravasation and peripheral swelling (3.5%) and application site rash, catheter site haemorrhage and infusion site rash (1.4%). Two of these events were considered definitely related to study drug although they completely recovered and continue with treatment. Three patients on 30mg/kg and one on 50 mg/kg interrupted treatment as a result of an infusion site reaction event although it was considered unrelated to treatment.

Severe Cutaneous Reactions: Six severe cutaneous reactions were reported in the whole population (3 conjunctivitis, 2 blisters, 1 dermatitis bullous and 1 drug eruption). According to the Applicant drug eruption (with the dose of 20 mg/kg) was possibly related to drug treatment and was solved without change of the dose.

Infusion-Related Reactions: 65 patients (43.3%) experienced infusion-related reactions. Vomiting and pyrexia were the most commonly reported events (28.7% and 14.0%, respectively). Most were mild and unrelated to study drug except in 8 patients in whom vomiting were moderate and possibly related to treatment (in 5 vomiting events patients were on eteplirsen 30 mg/kg). However, a consistent temporal relationship between the onset of vomiting and the timing of eteplirsen infusion has not been observed.

Five patients had moderate pyrexia possibly related to treatment. It seems that patients continued receiving treatment without recurrence of pyrexia, with the exception of one patient who experienced a subsequent mild, unrelated event of pyrexia.

Hepatic function: Two patients on eteplirsen 30 mg/kg in study 301 met the criteria for potential drug-induced hepatotoxicity, with GGT and hepatic enzymes increased.

- In one patient, GGT maintained stable over time while bilirrubin increased but decreased over time (3.42 µmol/L at baseline, 18.81 µmol/L at week 259 and 6.84 µmol/L at week 273) while transaminases increased and did not returned to normal values. The event was mild and not related to treatment but was not solved at cut-off date.

- The other patient experienced elevated GGT on Day 162. The total bilirubin value at the time was 3.42 µmol/L. The dose was not changed. This event was considered mild and possibly related to eteplirsen. The event was considered resolved on Day 246 when the patient's GGT returned to 28 U/L.

Given that antisense oligonucleotides have shown an effect on the liver, the Applicant should discuss if hepatotoxicity should be considered as a potential risk in the RMP.

Cardiac function: 23 patients (15.3%) experienced a cardiac-related TEAE. Most patients were on eteplirsen 30 mg/kg (15 patients). Six subjects had tachycardia, sinus tachycardia or arrhythmia events that were either moderate in severity or considered related to study drug by the investigator, 3 of them treated with eteplirsen 30 mg/kg. In two cases tachycardia was considered moderate and not related to treatment and in one case was mild and possibly related to treatment. Another 6 patients had cardiomyopathy, myocarditis, loss of consciousness or oedema peripheral that were considered either moderate or severe in intensity and/or related to study drug.

One patient from study 28 (receiving eteplirsen 4 mg/kg) had elevated troponin I levels at screening (0.07 and 0.08 µg/L; ULN of 0.04 µg/L). While receiving study drug, the patient had 3 reported mild sinus tachycardia

events. Retrospective review of echocardiograms obtained prior to study entry showed evidence for pre-existing cardiomyopathy.

In addition, the following AEs were reported in patients treated with 30 mg/kg of eteplirsen: one patient had moderate tachycardia and moderate cardiomyopathy (not related to eteplirsen); one patient had moderate worsening of bilateral pedal edema (not related to study drug); one patient experienced a moderate event of oedema peripheral (not related to eteplirsen); one patient experienced a serious, moderate event of loss of consciousness (not related), and one patient had severe myocarditis requiring hospitalization (study 301, not related to study drug). In the latter case the patient had not recovered at the time of the cut-off. During hospitalisation the patient had a cardiac MRI scan revealed a diffuse late gadolinuim enhacement. The patient also had normal right ventricular chamber size with normal systolic function and delayed myocardial enhancement. Cardiac catheterization did not show lesions. The patient was discharged from the hospital but needed to be re-admitted after having an unresolved chest pain episode that lasted approximately 2 hours accompanied by shortness of breath. Cardiac MRI was unchanged. The patient recovered and was discharged. The underlying disease was considered a reasonable etiology by the cardiologist but with an atypical presentation.

Three additional patients experienced mild and unrelated cardiac events. Two of them on 30 mg/kg of eteplirsen: one had myocardial fibrosis described as linear sub-epicardial late gadolinium enhancement in the inferolateral wall of the mid left ventricle without associated T2 hyperintensity consistent with myocardial fibrosis/scar. The investigator considered this mild and unrelated to study drug. Another one experienced a mild syncope (unrelated to eteplirsen).

Myoglobinuria: Three patients had mild and unrelated to drug myoglobinuria in patients receiving 0.9 mg/kg of eteplirsen. Myoglobinuria is a common finding in patients with damaged storage or use of energy by muscle cells.

Coagulopathy: Sixty one patients (40.7%) out of 150 had a TEAE indicative of a coagulation disorder. Most common events were contusions (34 patients, 22.7%), postprocedural contusions (4 patients, 2.7%), and incision site hemorrhage (3 patients, 2.0%).

There were 9 patients with moderate events of contusion or bruise that were considered unrelated to study drug by the investigator. There were 6 additional patients considered moderate and/or related to treatments (5 out of them on eteplirsen 30 mg/kg) reporting incision site hemorrhage (not related), thrombosis (2 related and 1 no related), and epistaxis (possibly related).

Others:

- Port-related events: Around 60% of all patients had study drug administered via central access port. Around 33% of these patients reported port-related events although mostly unrelated to treatment. In 5 patients treated with 30 mg/kg of eteplirsen such events were considered related to treatments by the investigator.

- Fractures: 27 patients (18%) reported fractures that were considered not related to treatment. Seven were moderate (1 compression fracture, 1 hand fracture, 1 foot fracture, 1 radius fracture and 1 lower limb fracture). Four patients reported severe fractures: 1 femoral neck fracture, 2 femur fracture and 1 tibia fracture.

- Falls were common in these patients and not unexpected in DMD population. However, in 2 patients fall were severe or considered related to treatment (one subject was on eteplirsen 30 mg/kg).

Serious adverse events and deaths

SAEs were not observed in the placebo arm (n=4). None was either reported in patients treated with 0.9 mg and 0.09 mg (n=7) and only vomiting was reported in those subjects on ≤20 mg/kg (n=19). No SAEs were reported during study 201. In study 202, 2 SAEs were reported in patients on eteplirsen 30 mg/kg ad 2 in those treated with 50 mg/kg. Bone fractures and worsening of scl¡oliosis were the reported SAEs in this study.

In the whole population 12 subject treated with any dose had SAEs, 9 of them in patients on eteplirsen 30 mg/kg although. Femoral neck fracture, femur fracture, foreign body, influenza, rhinovirus infection, scoliosis, loss of consciousness and nephrolitiasis were reported in the population treated with 30 mg/kg. In patients on eteplirsen 50 mg/kg femur and tibia fracture were the reported SAEs. All of them were considers unrelated to treatment by investigator. To what extent these events are related to the underlying disease or the age of patients rather than the study drug is unknown.

Three additional cases outside of treatment period were reported: oxygen saturation, ankle fracture and wound infection that were considered unrelated to treatment by investigator.

As previously mentioned, the present safety database is limited both in number of exposed patients and duration of exposure. Less common SAEs may have not been identified.

Laboratory findings

Several laboratory alterations were identified. Some observations like hypertransaminasemia may be attributed to muscle breakdown rather than to liver pathology although antisense oligonucleotdes have shown an effect on

the liver, and this should be discussed by the Applicant. Hyperglycaemia and hypokaliemia cases observed may be well explained by the use of corticosteroids in most on subjects included in the trials.

Hepatic function: One patient had concurrent ALT≥2x baseline and bilirrubin >2xULN. One additional patient had bilirrubin >1.5xULN although ALT did not raised from baseline. Since antisense oligonucleotides have adversely affect liver function tests, a potential higher risk in this population cannot be ruled out.

BUN, creatinine, cystatin C: In the clinical trials, BUN levels were within the normal range and remained like this at the time of the last observation. Among the slight increases, one patient on eteplirsen 30 mg/kg and 3 on 50 mg/kg had >1.5 x baseline and >ULN for BUN, observations that were not accompanied by alterations of creatinine or cystatin C.

Creatinine was in the low normal range or even decreased what could be explained by the decrease muscle mass. For cystatin C values were within normal range at baseline and at the end of the studies.

Glucose, potassium and CK: Some patients had slightly high levels of glucose and potassium that could be explained by the fact that most patients were taking corticosteroids. Some fluctuations were osberved for CK that could be attributable to the natural course of DMD.

Coagulation parameters: At baseline mean aPTT levels were within the normal range although with important variability, mainly in patients on eteplirsen 30 mg/kg. In these patients this variation was even more evident at the final observation point.

Haematological parameters: Six patients had decreases in haemoglobin level in more than 1 occasion but there were no concurrent events of bleeding or abnormalities in platelets or leukocytes.

Platelets reduced in 8 patients but they were isolated cases. For platelet values, 4 patients on eteplirsen 30 mg/kg and 1 on 50 mg/kg had a decrease of at least 100x109/L. According to the Applicant those patients did not experience any concurrent bleeding events.

There were small variations in neutrophil levels both in patients on eteplirsen 30 mg/kg and on 50 mg/kg although the minimum values were also below the lower normal range at baseline. For patients on 30 mg/kg this minimum value reduced a bit at the final observation while in patients on 50 mg/kg increased a bit. The interpretation of these changes is difficult also due to lack of a control placebo arm.

Safety in special populations

Safety has not been studied in hepatic insufficiency given that there is no indication that eteplirsen is metabolised by the liver. This is supported. No studies in patients with renal impairment have been performed either despite kidney was identified as a target organ in non-clinical studies. This should be considered as missing information in the RMP.

The lack of data in pregnant and lactating women is not considered relevant either as DMD is an X-linked genetic disorder causing disease only in boys.

Immunological events

It seems that antidystrophin antibodies were only assessed in study 28 in which detectable titres were not seen for any patients. Nevertheless, study 28 was a dose ranging study in which lower doses of eteplirsen were given (0.5 to 20 mg/kg weekly IV infusion) to only 19 patients.

Mean CD3, CD4 and CD8 lymhocyte counts decreased or remained stable from form baseline to 48 weeks suggesting lack of immunogenicity.

Immunogenicity has been proposed as missing information to be included in the RMP. This was supported.

Safety related to drug-drug interactions and other interactions

Given the low potential for DDI specific studies have not been performed.

Discontinuation due to AES

One patient discontinued due to proteinuria that was considered related to treatment. In addition, 7 patients missed or delayed infusions due to AEs. Three were serious (infuenza infection, femoral neck fracture and loss of consciousness) that were considered unrelated to treatment.

During the assessment the Applicant provided an update of the eteplirsen exposure (data as of 12 May 2017) with 167 patients exposed to eteplirsen, 124 for more than 48 weeks and 124 for more than 96 weeks. This means a total of 278 patient-years, as compared with the 150 patients treated for 164 patient-years in the original MAA submission.

Of the 167 patients as of the current cutoff date, 124 and 78 patients received at least 48 and 96 weeks of eteplirsen treatment, respectively. This includes 12 patients from Study 201/202 who have been treated for up to 5.5 years.

Overall, 97%of the patients in the Safety Population treated with the proposed posology had at least one TEAE, 11% were severe and 14% serious. No new TEAEs were identified in this updated population. Although a comparison with placebo is not possible (only 4 patients were on placebo for 24 weeks) most of them seem to reflect the target population of children enrolled in the study (e.g., upper respiratory infections or nasopharyngitis), related to the disease itself (e.g., falls) or the method of administration (procedural pain). Vomiting and nausea were also common although in most cases they were mild to moderate in severity. However, some events, such as hypersensitivity (including urticaria and nonspecific skin rashes), as well as erythema, flushing, and elevated temperature may be identified as ADRs.

A total of 22 patients experienced an SAE during treatment with eteplirsen. The most frequent serious TEAEs were "injury, poisoning and procedural complications" (5.9% of patients), "musculoskeletal and connective tissue" (3% of patients). However, none led to treatment discontinuation.

Cases of proteinuria were mild and transient as they were solved despite treatment with eteplirsen. One case of myocarditis was reported that recovered after hospitalisation without any dose change. Several cases of rash were identified. Although most were mild an transient there was a serious case of urticaria that was considered related to treatement and the Applicant proposes to include hypersensitivity as a new identified risk in the RMP.

This is considered acceptable. No cases of thrombocytopenia have been reported. Two patients had hepatic lab abnormalities without clinical correlation despite treatment with eteplirsen.

Postmarketing experience

Since eteplirsen is already authorised in the US the Applicant has safety data coming from the postmarketing experience. One hundred patients have started treatment with eteplirsen in the post-marketing phase and 29 additional subjects participanting in clinical trials continue to receive eteplirsen in the post-marketing setting as of 12 May 2017.

As of 12 May 2017, 5 postmarketing cases have been categorized as serious. With the exception of 1 serious case of pneumothorax in a 16-year-old patient with pulmonary blebs, the other 4 serious cases included cardiac and respiratory complications. They occurred in older DMD patients (24 to 28 years of age) at the end of their disease course at a time when cardiac and respiratory complications are the most frequent cause of death. The majority of postmarketing cases were similar in nature to those reported in the eteplirsen clinical trials, including events of flushing, pain in extremity, and pyrexia. Treatment with eteplirsen has been shown to be well tolerated with low rates of treatment-related SAEs or discontinuations due to AEs. A number of the commonly reported AEs are consistent with conditions that may arise in a population of pediatric patients with DMD.

The Applicant referred also to the serious AEs known to be reported with phosphorothioate oligomers (renal toxicity, thrombocytoprnia, inflammatory responses, coagulopathies, injection site reactions and hepatic toxicity. Only mild renal toxicity at high dose/exposure has been reported as well as mild and infrequent injection site reactions. For the time being data do not confirm (but do not rule out either) that phosphorothioate oligomers and phoshorodiamedate morpholino oligomers have the same serious safety profile due to the still low number of patients exposed so far.

In summary, the Applicant's conclusion that events related to hypersensitivity reactions (including e.g. urticarial, rash, erythema, and flushing) have been identified as ADRs can be agreed. Accordingly, and as requested previously, it is proposed that section 4.8 , of the SmPC be revised, removing AEs that most likely reflect the disease condition and/or patient population and for which a possible causal relationship to eteplirsen seems difficult to establish until more data become available. In this regard, it is noted that the Applicant is planning to conduct an additional placebo-controlled study that may further establish the safety profile of eteplirsen.

2.6.1. Discussion on clinical safety

Safety data from 7 completed or ongoing clinical studies are included to support the indication for eteplirsen. Clinical safety results from the eteplirsen development program are presented for the pivotal studies 201/202, Study 28, Study 33 and as pooled data across the studies.

The total number of DMD subjects exposed to any concentration of eteplirsen was 150 but only 116 received the proposed posology (30 mg/kg IV). Most of them (112) come from ongoing studies that did not include a placebo control arm and have received eteplirsen at least for 12 weeks. The lack of a controlled arm and the different time exposure to eteplirsen in these studies are the main uncertainties in the safety assessment of this set of patients.

The only comparative data come from the pivotal study 201 in which 12 subjects were randomised to eteplirsen 30 mg/kg (n=4), 50 mg/kg (n=4) or placebo (n=4) for 24 weeks. This very limited dataset is a major limitation, even for this orphan condition, that seriously hinders the interpretation of the safety data.

At termination of study 201 patients entered the extension study 202. Patients originally randomised to placebo received at least 219 weeks of eteplirsen treatment, and patients randomized to the 30 or 50mg/kg arms had received at least 252 weeks of eteplirsen treatment. This small and non-comparative long-term dataset is another drawback of this application. Like for the short-term safety assessment drawing sound conclusions on long-term safety is very difficult. Therefore, the safety data presented in this application have to be interpreted with a lot of caution.

The analysis of safety is based on AEs, laboratory findings and vital signs. There are severe adverse events reported, however there are no events evaluated to be serious adverse reactions.

Study 201

From a qualitative point of view, similar AEs were reported for the placebo and 30 mg/kg dose groups. Some of them might be reflecting drug adverse events the target population of children enrolled in the trial (e.g., oropharyngeal pain or nasal congestion) rather than AEs, others seem to be related to the procedure (e.g., procedural pain or incision pain site) and others could be explained by the underlying disease (e.g., falls or pain in extremities).

All AEs were reported in 25% of patients (representing 1 event) except hypokalaemia and dermatitis contact that were reported in 50% of patients (2 events). All these AEs, except contact dermatitis, were also reported in the placebo arm. Since only 4 patients were treated with placebo, 4 with eteplirsen 30 mg/kg and 4 with 50 mg/kg a comparative safety exercise is hardly possible. Quantification of the safety profile is challenging as only AEs with higher incidence than 25% (but representing only 1 patient) can be identified while less frequent events will be missing. No firm conclusions canbe drawn from this very small dataset.

Extension study 202 (from study 201)

All patients had at least one TEAE and around 50% had at least one TEAE related to treatment. Around 100% of patients had at least 1 moderate or severe TEAE and around 30% has at least one serious TEAE. The extension study 202 has the previously mentioned limitations: the small number of exposed patients and the lack of placebo control arm. Although drawing any sound conclusions on the long-term safety profile of eteplirsen appears difficult the number and variety of TEAEs might suggest that the safety profile may worsen with the long-term administration of eteplirsen.

Patients treated with any dose of eteplirsen

The Applicant integrated all the safety data across studies population in order to perform a thorough safety assessment. Nevertheless, combining studies with different exposure duration is likely to dilute the incidence of AEs making it difficult the evaluation of the safety profile of eteplirsen.

Overall treatment-related TEAEs were reported in 31% of patients. Cough (in around 35% of patients), vomiting (in around 30% of patients), back pain (in around 28% of patients) and headache (in around 28% of patients) were the most frequent TEAEs. The most common TEAEs related to treatment were headache (5.3%), vomiting (4%), nausea (2.7%) and diarrhoea (2.7%).

Eight TEAEs were considers serious (1 cardiomyopathy, 2 femur fractures, and 1 case each of femoral neck fracture, myocarditis, nephrolithiasis, scoliosis, and tibia fracture). Only the cardiomyopathy event reported in study 28 was considered by the investigator to be possibly related to eteplirsen although further investigations concluded that this patient suffered of cardiomyopathy previously.

Among the *adverse events of special interest* the following ones deserve to be commented on:

Hypersensitivity: Around 12% of all treated patients reported hypersensitivity events that in general were mild and considered as not related to treatment. Three patients had concurrent respiratory events (i.e., cough) and cutaneous events (i.e.,flushing or rash) that would suggest hypersensitivity. There were two mild cases that were considered related to treatment by the investigator, one with eteplirsen 30 mg/kg and another one with 50 mg/kg.

Renal function: In non-clinical studies kidneys were identified as the main target organ. Proteinuria was reported in 13 patients (8.6%), 10 of them in patients on 30 mg/kg (all of them except one, in the ongoing studies).

Hepatic function: Two patients on eteplirsen 30 mg/kg in study 301 met the criteria for potential drug-induced hepatotoxicity, with GGT and hepatic enzymes increased. The events were mild and considered not related to treatment but one of them in which bilirrubin and transaminases increased was not solved at cut-off date. Given that antisense oligonucleotides have shown to have an effect on the liver, hepatotoxicity should be monitored as AE of special interest.

Cardiac function: One patient had severe myocarditis requiring hospitalization (study 301, not related to study drug) that was not recovered at the cut-off date. Additional information is available clarifying that during hospitalisation the patient had a cardiac MRI scan that revealed a diffuse late gadolinuim enhancement. The patient also had normal right ventricular chamber size with normal systolic function and delayed myocardial enhancement. Cardiac catheterization did not show any lesions. The patient was discharged from the hospital but needed to be re-admitted after having an unresolved chest pain episode that lasted approximately 2 hours accompanied by shortness of breath. Cardiac MRI was unchanged. The patient recovered and was discharged. The underlying disease was considered a reasonable aetiology by the cardiologist but with an atypical presentation.

SAEs were not observed in the placebo arm (n=4). None was either reported in patients treated with 0.9 mg and 0.09 mg (n=7) and only vomiting was reported in those subjects on ≤20 mg/kg (n=19). No SAEs were reported during study 201. In study 202, 2 SAEs were reported in patients on eteplirsen 30 mg/kg ad 2 in those treated with 50 mg/kg. Bone fractures and worsening of scoliosis were the reported SAEs in this study.

In the whole population 12 subject treated with any dose had SAEs, 9 of them in patients on eteplirsen 30 mg/kg although. Femoral neck fracture, femur fracture, foreign body, influenza, rhinovirus infection, scoliosis, loss of consciousness and nephrolitiasis were reported in the population treated with 30 mg/kg. In patients on eteplirsen 50 mg/kg femur and tibia fracture were the reported SAEs. All of them were considers unrelated to treatment by investigator. To what extent these events are related to the underlying disease or the age of patients rather than the study drug is unknown.

As previously mentioned, the present safety database is limited both in number of exposed patients and duration of exposure. Less common SAEs may have not been identified.

Several *laboratory findings* were identified. Some observations like hypertransaminasemia may be attributed to muscle breakdown rather than to liver pathology, although hepatotoxicity could also be expected in patients treated with antisense oligonucleotides. Hyperglycaemia and hypokaliemia cases observed may be well

explained by the use of corticosteroids in most on subjects included in the trials. Proteinuria and hepatotoxicity events have already been mentioned.

Safety has not been studied in hepatic insufficiency given that there is no indication that eteplirsen is metabolised by the liver. This is supported. No studies in patients with renal impairment have been performed either despite kidney was identified as a target organ in non-clinical studies.

The lack of data in pregnant and lactating women is not considered relevant either as DMD is an X-linked genetic disorder causing disease only in boys.

Antidystrophin *antibodies* were only assessed in study 28 in which detectable titres were not seen for any patients. Nevertheless, study 28 was a dose ranging study in which lower doses of eteplirsen were given (0.5 to 20 mg/kg weekly IV infusion) to only 19 patients.

Although particularly serious concerns have not been identified so far, the safety profile of eteplirsen that cannot be considered characterized. The limitation of the database size does not allow identifying frequent AEs (≥10%), and the lack of a comparator placebo arm makes it impossible to distinguish between AEs related to the disease or the age of the population and those related to the drug. Moreover, data from previously assessed oligonucelotides show a worrisome safety profile affecting several organs and systems. Based on the scarce available data it cannot be rule out that eteplirsen has a similar effect. The ongoing clinical studies will not provide sufficient information to solve this uncertainty. Safety data for a sufficient number of patients compared to placebo would be needed to conclude on the safety profile of eteplirsen.

2.6.2. Conclusions on the clinical safety

The assessment of the safety profile of eteplirsen is hampered by the limitations safety database. The only comparative data comes from study 201 (n=12) in which 4 subjects were randomized to 30 mg/kg, 4 to 50 mg/kg and 4 to placebo for 24 weeks. This is a major limitation for the interpretation of safety data, even for this orphan condition. There are available data from the extension study 202 in which only 6 patients were treated with eteplirsen, 30 mg/kg and 6 with eteplirsen 50 mg/kg for approximately 3 additional years. This small and non-comparative dataset makes the assessment of the long-term safety hardly possible. Therefore, safety data have to be interpreted with caution, precluding any firm conclusions on the safety profile of the product.

All patients (100%) included in the pivotal trial and around 50% of all patients treated with any dose of eteplirsen reported AEs, mainly hypokaliemia, dermatitis contact, oropharyngeal pain, procedural pain, vomiting, balance disorder and cough. Comparison with untreated patients is challenging as only 4 patients were on placebo for 24 weeks.

For the long-term safety assessment, safety data are available only for a small set of patients (6 on eteplirsen 30 mg/kg and 6 on 50 mg/kg). Nevertheless, the variety and number of AEs suggest that the safety profile of eteplirsen may worsen with the long-term administration of the drug. The lack of a placebo controlled arm prevent from drawing any conclusions. One myocarditis event with an atypical presentation was identified in one patient on eteplirsen 30 mg/kg.

Regarding the laboratory findings the main concern is proteinuria that has already been observed in non-clinical studies. Other findings already seen for other antisense oligonucleotides, like elevation of transaminases have also been identified.

In conclusion, the safety profile of eteplirsen has not been thoroughly characterized. The limitation of the database size does not allow identifying frequent AEs (≥10%) and the lack of a comparator placebo arm makes it impossible to distinguish between AEs related to the disease or the age of the population and those related to the drug. Moreover, the known serious safety profile of other antisense oligonucleotides adds further concern. The ongoing clinical studies will not provide sufficient information to solve this uncertainty. Safety data on a sufficient number of patients compared to placebo would be needed to conclude on the safety profile of eteplirsen.

2.7. Risk Management Plan

The CHMP and the PRAC, having considered the data submitted, are of the opinion that, due to the concerns identified with this application, the RMP for eteplirsen is not acceptable at this stage.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

Not applicable.

2.9. New Active Substance

The applicant compared the structure of eteplirsen with active substances contained in authorised medicinal products in the European Union and declared that it is not a salt, ester, ether, isomer, mixture of isomers, complex or derivative of any of them.

The CHMP, based on the available data, considers eteplirsen to be a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union. However, in light of the negative recommendation, the new active substance status is not applicable at this stage.

2.10. Product information

In light of the negative recommendation, a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.10.2. Additional monitoring

Not applicable.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Exondys is indicated for the treatment of Duchenne muscular dystrophy (DMD) in adults, adolescents, and children aged 4 years and older who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping.

DMD is a rare, serious, life-threatening, X-linked recessive degenerative neuromuscular disease caused by mutations in the DMD gene. Approximately 13% of all DMD patients have mutations amenable to therapies that skip exon 51, corresponding to approximately 1,500 to 2,300 patients in the EU who would potentially benefit from exon 51 skipping therapy.

Dystrophin has a structural role as a cytoskeletal stabilisation protein protecting muscle fibres against contraction-induced damage, but also a signalling role including mechano-transduction of forces and localisation of signalling proteins. Lack of dystrophin results, through mechanisms not precisely understood, in degeneration of muscle fibres, attracting inflammatory cells and ultimately replacement by fibrotic tissue and adipose tissue.

The progression of muscle degeneration in DMD is well documented, showing a proximal-to distal progression of muscle weakness leading to progressive functional decline with eventual loss of ambulation, loss of upper limb function, trunk, and neck function, severely affecting patient quality of life, as well as that of caregivers and families. Complications from this loss of ambulation have a major cascading effect, including scoliosis. There is also an increased risk of cardiomyopathy with DMD, which usually manifests after 10 years of age as dilated cardiomyopathy with reduced left ventricular ejection fraction (LVEF). Many DMD patients require ventilation assistance by their late teens and die of respiratory or cardiac failure in their 20s or 30s.

3.1.2. Available therapies and unmet medical need

There are no approved treatments to cure or stop the ultimately fatal progression of DMD. As a result, supportive care (e.g., physiotherapy) and glucocorticoids are currently the primary means to help improve the quality of life of affected boys. Even with the introduction in the 1990s of assisted ventilation in the later stages of the disease, the mean age of survival (for those ventilated patients who do not develop early and severe cardiomyopathy) is still only 24 years.

Despite improvements in the standard of care, including steroids and other supportive care, these measures do not address the underlying absence of dystrophin. For treatment of DMD patients with exon 51 deletion mutations, there are no approved specific treatments for this subset of DMD patients in the European Union. Translarna™ (ataluren) has received conditional marketing authorization in the EU but in DMD patients with nonsense mutations. Therefore, an unmet medical need remains for DMD patients with exon 51 deletion.

The Applicant was initially seeking approval for Exondys (containing eteplirsen) for the treatment of Duchenne muscular dystrophy (DMD) in adults, adolescents, and children aged 4 years and older who have a confirmed

mutation of the DMD gene that is amenable to exon 51 skipping. The current proposed indication is restricted to ambulatory patients older than 4 years.

Whole exon deletions that disrupt the messenger ribonucleic acid (mRNA) reading frame, also referred to as "out-of-frame deletions", are the primary cause of DMD. Out-of-frame mutations prevent translation of functional dystrophin protein downstream of the mutation, creating an unstable protein lacking a C-terminal dystroglycan-binding domain.

A potential therapeutic approach to the treatment of DMD is suggested by Becker muscular dystrophy (BMD), a milder dystrophinopathy with "in-frame" mutations that do not disrupt the reading frame and result in the production of internally shortened, functional dystrophin protein. The clinical literature also demonstrates that the presence of only low or exceptionally trace levels of "in-frame" dystrophin could result in a milder disease course. The ability to convert an out-of-frame mutation to an in-frame mutation would consequently hypothetically preserve the mRNA reading frame and produce an internally shortened, functional, dystrophin protein. Eteplirsen was designed to accomplish this.

This submission is based on the demonstration of restoring dystrophin expression in muscle biopsies and the results from the early phase of the clinical development of the product.

3.1.3. Main clinical studies

This application for eteplirsen is supported by efficacy data derived from 4 interventional clinical studies, a external control cohort, and a review of literature describing the natural history of DMD.

1. Study AVI-4658-33 (Study 33), a proof-of-concept clinical trial performed in the United Kingdom (UK) in which boys with DMD due to dystrophin mutations amenable to exon 51 skipping were given a single dose of eteplirsen by intramuscular (IM) injection (0.09- or 0.9-mg dose levels).
2. Study AVI-4658-28 (Study 28), a dose ranging study of eteplirsen IV 0.5 mg/kg/wk (n=4), 1.0 mg/kg/wk (n=2), 2.0 mg/kg/wk (n=2), 4.0 mg/kg/wk (n=3), 10.0 mg/kg/wk (n=4), and 20.0 mg/kg/wk (n=4) over 12 weeks to induce dystrophin expression in DMD patients. The safety of escalating doses of eteplirsen as well as the PK and efficacy of eteplirsen were also evaluated.
3. Study 4658-us-201 (Study 201), a randomized, double-blind, placebo-controlled, multiple-dose efficacy, safety, tolerability, and PK study of eteplirsen 30 and 50 mg/kg IV administered over 28 weeks in the treatment of ambulant subjects with DMD. Study 201 was a randomized, single-center, double-blind, placebo-controlled, multiple-dose study. A total of 12 patients were randomized to receive 50 (n=4) or 30 mg/kg/wk (n=4) eteplirsen or placebo (n=4); after 24 weeks, placebo patients were further randomized to 1 of 2 eteplirsen groups for 4 additional weeks.
4. Study 4658-us-202 (Study 202), an open-label, multiple-dose, efficacy, safety, and tolerability extension study of eteplirsen 30 and 50 mg/kg IV in subjects with DMD who participated in Study 201. Patients continued to receive treatment with once-weekly IV eteplirsen (30 or 50 mg/kg), at the same dose they were receiving at their completion of Study 201 until Study Week 240.

Given the relatively short duration of 24 weeks for the placebo-controlled portion of Study 201/202, the applicant identified external observational registries with longitudinal clinical outcome data to make it possible to compare some of the clinical outcomes results from studies 201/202 to the data from the control groups

obtained from the registers. The external control groups were recruited from two registries in Europe. The studies 201/202 were conducted at one site in US.

Three additional clinical studies provided supportive data:

• Study 4658-301 (Study 301 [PROMOVI]) is a confirmatory 96-week, open-label Phase 3 study of eteplirsen (30 mg/kg by weekly IV infusion) in ambulatory patients (N = 120) with DMD, ages 7 to 16 years old.

• Study 4658-203 (Study 203) is a 96-week, open-label Phase 2 study to evaluate safety and pharmacokinetics of eteplirsen (30 mg/kg by weekly IV infusion) in younger patients (N = 40) with DMD, aged 4 to 6 years old.

• Study 4658-204 (Study 204) is a 96-week, open-label Phase 2 study primarily to evaluate safety of eteplirsen (30 mg/kg by weekly IV infusion) in patients (N = 24) with advanced stage DMD (including non-ambulatory patients), ages 7 to 21 years old.

3.2. Favourable effects

The pharmacodynamic effect of eteplirsen was confirmed as in the study 201 exon skipping was observed in all eteplirsen exposed patients. An increase of dystrophin-positive fibers in the eteplirsen group exposed for the longest time-period 24 weeks could indicate a production of a (truncated) dystrophine and, in addition, indicate that eteplirsen-induced increases of truncated dystrophin is delayed up to several months from start of treatment as no increase was measured after 12 weeks. Consistent with its effects on the percentage of dystrophin-positive fibers, treatment with eteplirsen for 24 weeks appeared to increase the mean total amount of dystrophin protein in muscle tissue homogenates (as measured by Western blot using MANDYS106).

In the study 202 a positive RT-PCR response was observed for all 12 patients at Week 48 (including the placebo-to-eteplirsen group who had started eteplirsen treatment on Week 25) indicating the induction of skipping of exon 51. Treatment with eteplirsen increased the mean percentage of dystrophin positive fibers from baseline to week 48 for both the placebo-to-eteplirsen group and the all eteplirsen group.

In the added analysis of Week 180 biopsy samples, all 11 evaluated patient samples displayed a positive RT-PCR response all 11 evaluated patient samples indicating the induction of skipping of exon 51. The mean percent dystrophin positive fibers and the dystrophin fiber intensity were significantly higher for eteplirsen treated patients relative to normal tissue controls in the Week 180 biopsy samples.

A mean dystrophin protein level of 0.93% of normal in eteplirsen-treated patients during 180 weeks compared to 0.08% in untreated controls (p = 0.007) was shown.

Indirect comparison with external control cohort from two European DMD registries showed a positive effect on clinical endpoints. The 2 patient groups (i.e. 30 and 50 mg/kg groups combined, and external controls) had similar disease progression trajectories through Year 1. Differences in 6MWT in favour of eteplirsen were observed by Year 2 (Δ 62 metres, p=0.1550) until the end of the study (Year 3 Δ 144 metres, p=0.0055; Year 4 Δ 161 metres, p=0.0007). A positive trend was observed in loss of ambulation (2/12 in eteplirsen treated patients vs. 10/13 in external controls at Year 4) North Star Ambulatory Assessment and ability to rise from supine. The decrease in FVC% predicted in the eteplirsen-treated group shows approximately half of the expected decrease in FVC% predicted that has been observed in some natural history studies of DMD.

3.3. Uncertainties and limitations about favourable effects

Despite the relatively high percentage of dystrophin positive fibers reported, in the originally planned analysis patients treated with eteplirsen showed levels of dystrophin 0.9% by western blot compared with normal controls. As a reference, dystophin levels around 30% of normal dystrophin levels in western blots have been reported in near-asymptomatic Becker patients[10]. Also, dystrophinopathy patients of intermediate clinical severity have been associated with dystrophin levels of between 10 and 25% of normal levels while in-frame deletions in BMD patients with severe DMD phenotype have been associated with less than 10% dystrophin[11].

Even considering that a minimum dystrophin level would be required for improving the phenotype, other factors such as the muscle deterioration, fibre loss, or the time required after dystrophin expression to be functional may also have a role. It is expected that the late ambulant patients included in the Study 201/202 present a basal impairment of muscle hystology and motor function with limited ground for improvement.

The current application relies on data from one short term placebo controlled study (Study 201) and its open label extension (Study 212) with 12 patients; 6 patients on the recommended 30 mg/kg/week dose. One single pivotal study for the pursued indication could be acceptable but in this case several concerns have been raised. Main limitations come from the reduced number of patients by arm (which may make difficult the interpretability of the study) and the duration of the placebo controlled phase (6 months). Previous clinical developments on Duchenne have shown that therapies restoring the expression of dystrophin may require long (undetermined) duration of treatment to make evident an effect although this has not been demonstrated yet. The study was primarily aimed to show the effect of eteplirsen on dystrophin restoration. Efficacy endpoints were considered as secondary endpoints.

In the study 201 no overall improvements were measured in clinical efficacy endpoints in the 30 mg/kg/wk or 50 mg/kg/wk eteplirsen group compared to the placebo group after 24 weeks. However, it was noted that patients in the 30 mg/kg/wk eteplirsen groups showed a much more extensive mean declines in 6MWT and some of the other clinical test compared to the other groups. This decline was directly attributable to two (of the four) patients who showed rapid disease progression during the first few weeks of the study. In addition, also the 50 mg/kg/wk had a worse outcome than the placebo group in some clinical tests at week 24.

In the study 202, overall the clinical functional efficacy tests indicated a worsening during the approximatively 4 years long study period for both the placebo-to-eteplirsen group and the all eteplirsen group.

No dose response studies were performed. Two doses (30 mg/kg/week and 50 mg/kg/week) were selected for Studies 201/202 based on the preclinical data and the results from Study 28 (tolerability and PD response). Distribution to the target i.e. muscle tissue has not been investigated in the muscle biopsies so that tissue concentrations achieved in subjects treated with eteplirsen is unknown. It can be relevant since it is believed that the efficacy of AONs depends partly on the amount of AON that reaches its target, i.e. the muscle fibre [12].When eteplirsen was tested versus placebo, the overlapping images between placebo and the two doses, and the wide inter-subject variability do not allow to support one dose over the other one. Similar conclusions can be drawn from the long-term administration (Study 202). As the two doses were pooled in the indirect comparison with external controls in order to increase the total number of patients the potential differences

[10] van Putten M, Hulsker M, Nadarajah VD, van Heiningen SH, van Huizen E, van Iterson M, Admiraal P, Messemaker T, den Dunnen JT, 't Hoen PA, Aartsma-Rus A. The effects of low levels of dystrophin on mouse muscle function and pathology. PLoS One. 2012;7(2):e31937.

[11] Lu Q, Cirak S and Partridge T. What Can We Learn From Clinical Trials of Exon Skipping for DMD? Molecular Therapy—Nucleic Acids (2014) 3, e152

[12] Ingrid E. C. Verhaart and Annemieke Aartsma-Rus (2012). AON-Mediated Exon Skipping for Duchenne Muscular Dystrophy, Neuromuscular Disorders, Dr. Ashraf Zaher (Ed.), InTech, DOI: 10.5772/33938. Available from: http://www.intechopen.com/books/neuromuscular-disorders/aon-mediated-exon-skipping-for-duchenne-muscular-dystrophy

between doses in the long-term effect cannot be evident. At request the Applicant have explained that given that neither production of dystrophin nor clinical data were better with the highest dose 30 mg/kg was conservatively chosen as the most appropriate for chronic use.

During the 4-year open label treatment patients experienced gradual decline in functional measures. Although a detrimental effect on ambulation has been suggested by the Applicant as a proof of efficacy in those patients originally treated with placebo (delayed onset group), the small number of patients and the variability between subjects do not allow concluding on this effect.

The comparison of eteplirsen treated patients (regardless the dose received) with external controls covers only part of the concerns derived from the clinical studies submitted. Even though the patients from the registries match the patients in the studies 201/202 in a number of baseline characteristics, the differences in inclusion criteria between the study population and the registries population, the fact that some patients were evaluated in the context of a clinical study and some in a registry where training and coaching may be crucial for test performance, contribute to the uncertainties of any measured differences between the two populations. Still resulting in a limited efficacy data (eteplirsen treated patients n=12; external controls n = 13), this comparison was defined and conducted after data collection (post hoc). Both groups (eteplirsen treated patients and untreated external controls) experienced a decline in ambulation. A more pronounced deterioration was observed in the external control groups (both those amenable to exon 51 skipping and those amenable to any exon skipping) than in eteplirsen treated patients. After 4-year follow up patients in primary external cohort lost 330.3 metres (vs 166.9 metres in eteplirsen group). Separation between curves is apparent at Year 3. At individual level the variability between patients is evident and separation between groups is not so clear. Improvement in ambulation (even in this selected late ambulant population) could only be detected after patients were treated at least 2 years.

Even if the decrease in FVC% predicted favoured the eteplirsen-treated group compared to what has been observed in some natural history studies (but not all) a none-biased conclusion regarding any effect of eteplirsen on FVC% predicted would have to be confirmed in a placebo-controlled study.

During the procedure results from of the interim report of study 301 has been reported. It includes 35 eteplirsen-treated patients with a baseline 6MWT between 300 and 450meters and 4 untreated subjects (from untreated control arm of DMD patients amenable to exon skipping of an exon other than exon 51) after having completed their week 96 study assessments. No clear beneficial effect has been observed at Week 48; comparison beyond this point is difficult considering the low number of untreated control patients available. Results from comparisons with other post-hoc defined external controls do not provide further reassurance.

3.4. Unfavourable effects

One hundred per cent of patients included in the pivotal trial 201 and around 50% of all patients treated with any dose of eteplirsen reported adverse events. The main AEs were procedural pain, hypokaliemia, oropharyngeal pain, vomiting, balance disorder, dermatitis contact and cough.

For the long-term safety assessment (extension study 202), the variety and number of AEs suggest that the safety profile of eteplirsen may worsen with the long-term administration of the drug.

Proteinuria has been observed in non-clinical studies and also in the clinical trials and it is considered an important potential risk.

3.5. Uncertainties and limitations about unfavourable effects

The assessment of the safety profile of eteplirsen is hampered by the limitations safety database. The only comparative short-term safety data comes from study 201 (n=12) in which 4 subjects were randomized to 30 mg/kg, 4 to 50 mg/kg and 4 to placebo for 24 weeks. This is a major limitation for the interpretation of safety data, even for this orphan condition, that seriously hinders the interpretation of the safety data.

There are available data from the extension study 202 in which only 6 patients were treated with eteplirsen, 30 mg/kg and 6 with eteplirsen 50 mg/kg for approximately 3 additional years. This small and non-comparative dataset makes the assessment of the long-term safety hardly possible. Safety data have to be interpreted with extreme caution.

A serious event of urticaria that was considered moderate in severity and related to eteplirsen has been reported. Accordingly, hypersensitivity has been included in the safety specification as an important identified risk. Other findings already seen for other antisense oligonucleotides, like elevation of transaminases also need to be addressed.

3.6. Benefit-risk assessment and discussion

3.6.1. Importance of favourable and unfavourable effects

The main basis of the Marketing Authorization Application (MAA) for eteplirsen is the efficacy analysis of the clinical outcomes of the pooled dataset of 12 patients who received eteplirsen from Studies 201/202 compared to the primary external control cohort amenable to exon 51 skipping derived from the DMD registries, supplemented by the data from the pharmacodynamic (biological) results.

The pharmacodynamic (biological) results for dystrophin expression are viewed as supportive of the proof of principle. It is currently uncertain how predictive of sustained functional improvement the detected dystrophin level could be, and what levels may be required for a meaningful clinical improvement in Duchenne patients to be registered. However, it is important to note that the dystrophin produced is an internally shortened protein and the clinical effect of the truncated dystrophin is still not fully known. At present, as it has been reflected in the EMA Guideline for DMD, the CHMP is of the opinion that a convincing demonstration of sustained clinical improvement (or at least delay of progression and deterioration) is necessary for a medicinal product to be licensed in this condition, and the presented data in this application fail to fulfill these requirements.

When patients received treatment with eteplirsen 30 or 50 mg/kg/week for 24 weeks no relevant differences were observed in ambulation, timed function test, NSAA, muscle strength, pulmonary function or quality of life with respect to placebo. A great variability was observed for most endpoints across patients and over time. When patients extended the treatment up to 240 weeks they showed a gradual decline in measured functions. A detrimental effect of the delayed onset of eteplirsen group originally assigned to placebo has been suggested but the small number of patients included, the duration of exposure to placebo limited to 24 weeks and the variability between subjects prevent from drawing up sound conclusions, and subsequently prevents establishing a positive B/R balance.

When external non-concurrent cohorts of untreated patients were used as control, eteplirsen patients appeared to perform better, although a clear benefit was not evident until the third year of treatment. This together with

the methodological deficiencies derived from a post hoc analysis and indirect comparison create serious doubts about the robustness of the results or the time until a potential benefit could be demonstrated.

The presented results from the on-going phase III study 301 could not alleviate the main concerns, regarding this application. Even if the external untreated control arm is concurrently and appropriately recruited, the low number of patients who remain in the study after 1 year, preclude any sound conclusions from being made after analysis of the data. Although in principle one single pivotal study for the pursued indication could be an acceptable regulatory approach, it is considered that the available data in this case are insufficient to provide sufficient evidence of efficacy. A confirmatory trial appears to be necessary to complete the dossier for MAA.

The safety profile of eteplirsen has not been thoroughly characterized. The limitation of the database size does not allow for the identification of frequent AEs (≥10%), and the lack of a comparator placebo arm makes it impossible to distinguish between AEs related to the disease or the age of the population and those related to the drug. The ongoing clinical studies were not considered sufficient to supplement the information and therefore they were not able to resolve this uncertainty. Safety data on a sufficient number of patients compared to placebo would be needed to conclude on the safety profile of eteplirsen.

3.6.2. Balance of benefits and risks

The Applicant is seeking approval for eteplirsen for the Treatment of Duchenne muscular dystrophy (DMD) in ambulatory patients aged 4 years and older who have a confirmed mutation of the *DMD* gene that is amenable to exon 51 skipping.

The available data on eteplirsen have shown that there is a very modest increase of dystrophin expression in DMD patients treated with the product, when compared to the pre-treatment biopsies and a low expression compared to controls. The clinical relevance of this low amount of dystrophin is unknown and insufficient to establish the clinical benefit of the product at this stage Convincing demonstration of sustained functional effects in DMD patients is necessary to support the claims for efficacy of the medicinal product. In addition, due to the limited number of patient exposed to eteplirsen the safety profile has not been characterized.

The CHMP considered that with the currently available data it is not possible to conclude that the benefit/risk ratio of eteplirsen is positive in DMD patients with mutations amenable to exon 51 skipping, since:

- Efficacy of eteplirsen has not been demonstrated. There are no comparative data with patients on placebo beyond 24 weeks, and the available data for patients on treatment are derived from only a limited number of patients (n=12). There was no difference in 6MWD between eteplirsen and placebo during this 24 week treatment period.
- The provided additional comparative data from a variety of external controls, derived from different studies and populations, suffer from important limitations related to the nature of the methodology used (non-concurrent, retrospectively selected, post-hoc defined). This increases the uncertainty about the reliability of such comparisons rather than providing confirmatory data for efficacy.
- It is unknown whether expression of the observed very low amount of truncated dystrophin after treatment with eteplirsen can translate into any clinical benefit to patients. Although the evidence of truncated dystrophin production may support the mechanism of action of the product, convincing demonstration of sustained functional effect is necessary to support the claim for efficacy of the medicinal product in the intended indication.

- Due to the limited number of patients exposed to eteplirsen, the safety profile has not been thoroughly characterised.

The CHMP is of the opinion that pursuant to Article 12 of Regulation (EC) No 726/2004, efficacy and safety of the above mentioned medicinal product are not properly or sufficiently demonstrated. Therefore, the CHMP has recommended the refusal of the granting of the conditional marketing authorisation for Exondys.

3.6.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

The Applicant is proposing a conditional approval for eteplirsen and several designs for post-approval specific obligations have been proposed by the Applicant during the procedure. The latest proposal referred to a confirmatory Study 4658-302 that will be a 96-week randomized, double-blind, placebo-controlled study in ambulatory patients with DMD amenable to Exon 51 Skipping. A total of 120 patients aged 7-13 years are planned to be enrolled.

It is agreed that patients with DMD mutations amenable to exon 51 skipping represent a population where an unmet medical need exists, and that patients would benefit from the immediate availability on the market of a product with a positive benefit risk balance in that population. However, as stated above, the currently available data does not allow to conclude that such balance is positive in the referred population, the first condition to be fulfilled in a request of CMA.

Although the Applicant considers that the study is feasible in the post-authorisation phase, previous experience in similar circumstances goes in the opposite direction. There are reasonable doubts that this post-authorisation study versus placebo is feasible once the product is on the European market.

3.7. Conclusions

The overall B/R ratio of Exondys is considered negative in the proposed indication.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy for Exondys in the treatment of Duchenne muscular dystrophy (DMD) in ambulatory patients aged 4 years and older who have a confirmed mutation of the DMD gene that is amenable to exon 51 skipping, the CHMP considers by consensus that the efficacy and safety of the above mentioned medicinal product is not sufficiently demonstrated, and, therefore recommends the refusal of the granting of the conditional marketing authorisation for the above mentioned medicinal product.

The CHMP considers that:

- Efficacy of eteplirsen has not been demonstrated. There are no comparative data with patients on placebo beyond 24 weeks, and the available data for patients on treatment are derived from only a

limited number of patients (n=12). There was no difference in 6MWD between eteplirsen and placebo during this 24 week treatment period.

- The provided additional comparative data from a variety of external controls, derived from different studies and populations, suffer from important limitations related to the nature of the methodology used (non-concurrent, retrospectively selected, post-hoc defined). This increases the uncertainty about the reliability of such comparisons rather than providing confirmatory data for efficacy.
- It is unknown whether expression of the observed very low amount of truncated dystrophin after treatment with eteplirsen can translate into any clinical benefit to patients. Although the evidence of truncated dystrophin production may support the mechanism of action of the product, convincing demonstration of sustained functional effect is necessary to support the claim for efficacy of the medicinal product in the intended indication.
- Due to the limited number of patients exposed to eteplirsen the safety profile has not been thoroughly characterised.

The CHMP is of the opinion that pursuant to Article 12 of Regulation (EC) No 726/2004, efficacy and safety of the above mentioned medicinal product is not properly or sufficiently demonstrated. Therefore, the CHMP has recommended the refusal of the granting of the conditional marketing authorisation for Exondys.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system and risk management plan cannot be agreed at this stage.

Furthermore, the CHMP, in light of the negative recommendation, was of the opinion that it is not appropriate to conclude on the new active substance status and similarity at this time.

5. Re-examination of the CHMP opinion of 31 May 2018

Following the CHMP conclusion that Exondys was not approvable based on the fact that efficacy and safety of the medicinal product was not properly or sufficiently demonstrated, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

Detailed grounds for re-examination submitted by the applicant

Following a request from the applicant at the time of the re-examination, the CHMP convened a Scientific Advisory Group (SAG) inviting the experts to provide their views on the CHMP grounds for refusal, taking into account the applicant's response.

The applicant presented in writing and at an oral explanation the following arguments:

Ground #1

"Efficacy of eteplirsen has not been demonstrated. There are no comparative data with patients on placebo beyond 24 weeks, and the available data for patients on treatment are derived from only a limited number of patients (n=12). There was no difference in 6-minute walk distance between eteplirsen and placebo during this 24 week treatment period."

Summary of the Applicant`s Response

The Applicant re-emphasized that the comparative efficacy data in this submission are multi-year evaluations (up to 4 years) in comparison with appropriate external controls. Concurrent placebo data are available for the first 24 weeks of Study 201 and comparative data with external control patients are available for periods of 2 to 4 years.

The efficacy data in this submission are based on 96 eteplirsen-treated patients; 72 of which are in a similar phase of ambulatory decline and 74 of these patients are in a linear phase of pulmonary decline. Through the application of key prognostic factors these groups of eteplirsen-treated patients and their corresponding external controls have been identified. The ***"limited number of patients (n=12)"*** refers to the original MAA submission. Since then comparative analyses for an additional 84 patients have been submitted in response to the Day 180 LoOIs.

The Applicant agreed that in retrospect the placebo-controlled study period of 24 weeks in Study 201/202 was too short to detect a treatment benefit for 6MWD. When Study 201/202 was designed, it was not yet appreciated that dystrophin accumulates gradually over time with significant levels of dystrophin first apparent at Year 1 and increasing through 3.5 years. Therefore, longer study periods were needed to understand the clinical benefit for eteplirsen. This is consistent with the CHMP Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy.

The Applicant re-emphasized the body of clinical evidence demonstrating benefit related to ambulatory (6MWT, NSAA, and loss of ambulation) and pulmonary (FVC%p) function from 96 eteplirsen-treated patients in Studies 201/202, 301 and 204. The value of the primary ambulatory endpoint of 6MWT was confirmed in the CHMP Scientific Advice and Protocol Assistance dated December 2014 and December 2015, respectively. Furthermore, the 6MWT test is presented alongside other ambulatory endpoints (ie, NSAA, loss of ambulation) and pulmonary analyses providing a totality of evidence for benefit of eteplirsen including the timing of benefit and clinical relevance to patients.

> Studies 201/202 and 301 enrolled 72 patients in homogeneous phase of ambulatory decline and the endpoints assessing ambulatory function include 6MWT, NSAA, and loss of ambulation, which demonstrated that the benefit of eteplirsen becomes clinically evident after Year 1 and is sustained through Year 4, consistent with the gradual increase of dystrophin
>
> Studies 201/202, 204, and 301 included 74 patients in linear phase of pulmonary decline and the FVC%p endpoint demonstrates that the benefit of eteplirsen on pulmonary function is evident by Year 2 and is sustained through Year 4

Slowing the rate of respiratory decline is vital to the lives of DMD patients. Treatment with eteplirsen has been shown to preserve lung function over multi-year studies which would potentially provide a cumulative benefit extending the time to FVC%p decreases to other clinically relevant pulmonary thresholds such as nocturnal ventilation (FVC%p < 50%).

CHMP position

The Applicant summarized the current efficacy data regarding use of eteplirsen in treatment of Duchenne muscular dystrophy with confirmed mutation of the DMD gene that is amenable to exon 51 skipping.

The main limitations are:

Ground for refusal #1 relates to the extremely limited double blind, placebo controlled component of the pivotal efficacy dataset. The clinical study 201 was a phase 2 study, with primary endpoint evaluating the change from baseline in the percentage of dystrophin-positive fibers as measured in muscle biopsy tissue using immunohistochemistry (IHC) at Week 12 for the 50 mg/kg/wk eteplirsen and matching placebo groups (Groups 1 and 3a) and at Week 24 for the 30 mg/kg/wk eteplirsen and matching placebo groups (Groups 2 and 3b). It is considered as pivotal study for demonstration of efficacy in the proposed indication by the Applicant. However, it seems that the study was designed primarily to evaluate mechanism of action of dystrophin increase in the muscle cells. There are several concerns which create doubts about suitability of this study as the main confirmatory body of evidence. These concerns arise from the nature of the study design. Despite the fact that the occurence of disease is rare, the overall number of patients in the study is very low (only 12 subjects). Above that the proposed dose of 30 mg/kg of eteplirsen was administered in only 4 patients during the first 24 weeks. This is the only period in which the placebo arm was included in the study, so a higher number of patients will be desirable to provide clear interpretability of the study results. In this context the limited number of subjects represents a crucial fault of the study design.

Furthermore, after 24 weeks, no clinically relevant and statistically significant differences between placebo and treatment arms in favour to eteplirsen were demonstrated, in ambulation , timed function tests, North Star Ambulatory Assessment, muscle strength, pulmonary function or quality of life. This could be understandable as it can be assumed that the effect of eteplirsen will be demostrated after longer period of use. The results from the extended treatment up to 240 weeks (study 202) suggested this possibility, however the small number of patients who used the proposed dose 30 mg/kg and absence of the placebo arm does not allow for a clear conclusion regarding the clinical efficacy of eteplirsen.

The Applicant argues that efficacy data have been derived from a total of 96 eteplirsen treated patients; however, the vast majority of these were not studied in a randomised controlled setting and were not blinded to their treatment which presents a risk of bias. This is particularly significant, when the primary efficacy variable was the 6MWT that requires a voluntary effort and therefore could be the subject of motivational influence. Although the Applicant describes the use of "*Scripted encouragement from the testing staff at regular intervals to provide a standardized level of motivation*" when executing the 6MWT, this will only serve to reduce variability but does not override the risk of bias due to knowledge of receiving an investigational drug, particularly in a disease where there is a very high unmet need, and therefore patients, carers and investigators will be particularly susceptible to motivational influence. The additional "burst activity" endpoints also require voluntary effort and again carry similar risks of bias in an open label study setting.

The design of the study 301 seems to be more appropriate in the sense of the number of enrolled patients and length of treatment period with respect to the placebo comparison. However due to high number of droup outs in the untreated control arm, the comparative analyses could not be provided.

As no robust and evaluable long-term control group was included in the studies 201/202 and 301, the Applicant decided to perform comparison of efficacy results with the External Controls.

Based upon results of studies 201/202 and 301 the Applicant concluded, that results of 6MWT, NSAA, and loss of ambulation in 72 patients in homogeneous phase of ambulatory decline demonstrated that the benefit of eteplirsen becomes clinically evident after Year 1 and is sustained through Year 4, consistent with the gradual increase of dystrophin compared to external control groups.

According to the Applicant, by Year 3, the treatment benefit for 6MWT more than doubled with a significant 147 meter-difference, which is in parallel with dystrophin increases observed through Year 3. The treatment

difference at Year 4 (165 meters) could be argued to show the sustained and cumulative benefit of eteplirsen treatment that corresponds to the sustained production of dystrophin compared to external control groups.

The Applicant further concluded that Studies 201/202, 204, and 301 included 74 patients in linear phase of pulmonary decline, and that the FVC%p endpoint demonstrates that the benefit of eteplirsen on pulmonary function is evident by Year 2 and is sustained through Year 4.

However, it is necessary to emphasise the fact that the control group for comparison of pulmonary function includes DMD patients other than with Exon 51 skip-amenable patients and there was significant loss of control group subjects over 4 years. In addition, there was a high variability in individual trajectories of pulmonary results which further precludes drawing a sound conclusion.

The greatest benefit for FVC% was visible in results from study 201/202 when compared with external control groups. However, due to small number of patients, the possibility that these differences were achieved by chance cannot be excluded.

Pulmonary function data from eteplirsen treated patients in study 201/202 were compared with retrospectively defined external cohorts derived from the CINRG natural history dataset, in which pulmonary function was evaluated. Two external cohorts were defined in the CINRG dataset, for comparison with N=12 eteplirsen treated patients in study 202: i) genetically confirmed DMD patients (glucocorticoid treated “genotyped” CINRG control; N=67), data from whom were compared with eteplirsen treated patients over a period of 4 years; and ii) exon 51 skip amenable CINRG patients treated with glucocorticoids; N=20, in which annual decline in %p FVC was compared with eteplirsen treated patients. Pulmonary function data from eteplirsen treated patients were obtained between the ages of 10 and <18 years in order to coincide with the phase of “linear” pulmonary decline.

The patients from the Exon 51 CINRG cohort demonstrated a 6.00% annual decline in %pFVC by year of age. In contrast, eteplirsen-treated boys experienced a decline of 2.19% annually. This difference was nominally significant ($p<0.001$).

By year 4 of eteplirsen treatment, there was an absolute percentage point difference of 10.1% in %pFVC between the eteplirsen treated patients in study 202 and the genotyped CINRG cohort, suggesting an apparent slowing of pulmonary decline in eteplirsen treated patients. The reliability of this interpretation is however questioned, for the reasons detailed below.

Aside from the general concerns in relation to bias arising from the use of external controls, there are specific concerns in relation to the assumption of a linear trajectory of pulmonary decline in DMD patients above the age of 10 years. The Applicant’s response refers to the most recent review published by Maher (2017, US Neurology; 13(1):35-41) in which a trend is described for patients to enter the stage of pulmonary decline from the age of 10. However, Maher also makes it clear that the threshold of meaningful pulmonary decline for %pFVC is 80%, with values above this considered to be within the normal range. Moreover, at values above 80 %pFVC, the trajectory of decline is sensitive to glucocorticoid treatment, with potential delay in reaching the 80% threshold of established respiratory decline of up to 3 years, with glucocorticoids. Below the threshold of 80%, the slope of decline appears to be unaffected by glucocorticoid treatment. Therefore, although in the DMD population as a whole there is an overall trend to decline in pulmonary function after the age of 10 years, this does not become clinically meaningful until %pFVC has fallen below the threshold of 80% and above this, glucocorticoids can substantively delay the point when this is reached.

Individual plots for %pFVC decline, presented in the response to the Grounds for Refusal #1, suggest baseline imbalance between eteplirsen treated patients in study 201/202 and the Exon 51 CINRG cohort. Meaningful

baseline imbalance in %pFVC is confirmed in the summary of baseline characteristics presented in Figure 13 in the response to the Grounds for Refusal #2: median %pFVC in eteplirsen treated patients at the baseline for this analysis (age of ≥10yrs) was 92.00% which is substantively above the threshold for established respiratory decline. Moreover, the range of 84.00 – 121.00% indicates all patients were above this threshold. Whereas, in the Exon 51 CINRG cohort the median %pFVC was 81.00% which is on the cusp of clinically meaningful respiratory decline and the lower end of the %pFVC range was 50.0%, indicative of established pulmonary function decline that would be sufficient to require nocturnal assisted ventilation. The baseline characteristics therefore clearly demonstrate meaningful differences between the two populations. Eteplirsen treated patients, given they were above the 80% threshold, would also be sensitive to glucocorticoid-mediated delay in reaching the 80% threshold of meaningful decline; whereas the Exon 51CINRG patients who were in established respiratory decline would have been unaffected by concomitant glucocorticoids. This would bias in favour of the eteplirsen group. The individual patient also data indicate substantial variability in the trajectories of decline which may have been in part due to concomitant glucocorticoids.

While it is agreed that on a population level, there is a trend to linear pulmonary decline in DMD above the age of 10 years, this can be substantially delayed by administration of glucocorticoids in patients with %pFVC above the meaningful threshold of decline which is considered to be 80%.

The level of pulmonary function in the study 201/202 patients is consistent with their ambulant status and as also explained in Maher et al, meaningful respiratory decline generally coincides with loss of ambulant status. Therefore, it is challenging to investigate both outcomes in the same population.

Therefore, the eteplirsen treated patients are not in a clinically meaningful stage of respiratory decline, and the clinical relevance of any apparent slowing of decline in %pFVC is unclear. Moreover, the apparent slowing of decline in eteplirsen patients compared with Exon 51 CINRG cohort patients could be explained by concomitant glucocorticoid treatment. Although both groups of patients were receiving glucocorticoids, eteplirsen patients would be sensitive to glucocorticoid-mediated modulation of decline in %pFVC whereas not all CINRG cohort patients would be, due to the differences in pulmonary function at baseline.

The pulmonary function data are therefore not considered to provide reassurance of meaningful efficacy benefit.

The effect of eteplirsen in comparison with data from the external controls (apart from comparison with placebo arm from study with drisapersen) has been observed, however considering the shortcomings regarding the chosen registries (for further details, please see the assessment of Ground 2), it is difficult to draw any firm conclusion.

Absence of long-term control arm makes it impossible to establish the true clinical efficacy of eteplirsen.

Ground for refusal #1 is considered unresolved.

Ground #2

"The provided additional comparative data from a variety of external controls, derived from different studies and populations, suffer from important limitations related to the nature of the methodology used (non-concurrent, retrospectively selected, post hoc defined). This increases the uncertainty about the reliability of such comparisons rather than providing confirmatory data for efficacy."

Summary of the Applicant`s Response

In their response to this issue the Applicant has tried to re-establish the rationale for the variety of external controls used to evaluate the clinical efficacy of eteplirsen in slowing the ambulatory and the pulmonary progression of disease and tried to show that they have been chosen according to a robust process that reduced bias. Different sources of control patients were needed for different endpoints; the Italian Telethon and Leuven NMRC registries, provided the ambulatory endpoints and a different registry, the Cooperative International Neuromuscular Research Group (CINRG) database, provided the comparative pulmonary data. Different populations were needed as the external controls for ambulatory outcomes had to be from a slightly younger age group in ambulatory decline (≥ 7 years of age) compared with the external controls needed to evaluate the pulmonary outcomes during the linear phase of FVC%p decline (10 to < 18 years). Finally, in the analysis of pulmonary outcomes, 2 distinct populations were needed for the 2 analytical approaches for evaluation of FVC%p (annual change by year of age and the mean change over time).

The process for identifying the DMD registries considered all available sources:

- Twelve global DMD registries were identified, 3 of which had longitudinal, patient-level data that allowed for the collection of genotype, glucocorticoid status, age and clinical outcomes; all 3 registries were used as a source for the external controls, minimizing the potential for selection bias from choice of registry.

- Identification of individual patients from those registries was conducted prior to comparative analyses and based on 2 sets of well -established criteria, prognostic for ambulatory and pulmonary decline. The clear rationale for the identification criteria and utilization of all identified patients, minimizes the potential for bias from the retrospective identification.

It was also argued that the use of multiple comparators contributes to the certainty of the beneficial findings for eteplirsen by providing context for the study results observed with eteplirsen. In their opinion eteplirsen has demonstrated favourable results across ambulatory and pulmonary endpoints against optimal comparators and in each case, the comparison of the eteplirsen data with additional comparators has provided sensitivity analyses which strengthen the evidence for eteplirsen efficacy.

- Study 201/202 demonstrated a compelling reduction in the risk for loss of ambulation for eteplirsen boys compared with the Primary External Control. Further, comparisons of eteplirsen loss of ambulation compared with data from the CINRG database have reinforced the favorable findings for eteplirsen. The eteplirsen boys are still walking at older ages. Therefore, the CINRG comparison provides a sensitivity analysis, supporting the evidence that eteplirsen reduces the risk for loss of ambulation.

- Studies 201/202, 301, and 204 demonstrated a statistically significant slowing in the FVC%p annual change for eteplirsen compared with the Exon 51 CINRG control. Further comparisons to the larger Genotyped CINRG control group (N=148 prognostically comparable with Exon 51 CINRG) consistently demonstrated a significant slowing for eteplirsen patients across all 3 studies. The Genotyped CINRG comparison provides a sensitivity analysis, supporting the evidence that eteplirsen is able to slow pulmonary decline.

Once external control groups were identified, evaluation of baseline demographics confirmed that the external control groups generally resembled eteplirsen patients for key baseline prognostic factors and other characteristics. Other baseline characteristics were further evaluated through sensitivity analyses of the 6MWT and FVC%p, which have indicated minimal influence on the overall results and remain favourable for eteplirsen-treated patients.

CHMP position

The Applicant tried to further substantiate the decision for the use of external controls to demonstrate efficacy of eteplirsen and the criteria upon which these external controls were selected.

Although it is agreed, that in registries useful information was captured, not all data were recorded, and serious shortcomings were identified. Nevertheless, the CHMP recognized that there have been substantial efforts in the DMD field to produce additional data in trying to establish a better use of concurrent controls in the drug development. While these efforts were supported and stimulated by the Committee, it was highlighted that the position expressed in this procedure was related to the specific way such controls were used, and particularly to the very low numbers, hence it cannot be ruled out that effects observed in a few patients may have a significant influence on the final outcome.

The most important issue is that the external control groups were selected post hoc, when results of study 201 were already evaluated. The results of this study did not demonstrate any beneficial effect of eteplirsen and although it is agreed with the Applicant that 24 week seems to be a too short period, to reveal a potential treatment effect, no other placebo-controlled study was submitted.

When an external non-concurrent cohort of untreated patients was used as control, eteplirsen patients appeared to perform better, although a clear benefit was not evident until the third year of treatment.

As noted in the International Conference on Harmonization (ICH) E10 Guideline, blinding and randomization, used to decrease bias in randomized controlled trials, are not utilized in externally-controlled trials, which is a critical limitation of externally controlled trials. Despite the Applicant's extensive arguments, there are several dissimilarities between groups which could influence the study results. In addition, in patients' baseline characteristics there are several other factors important for disease progression and without the uniform methodology of patients' inclusion and exclusion criteria, which is used in placebo control studies, these factors are not possible to be captured and standardized. The influence over the study results, thus, cannot be excluded.

Primary external control for ambulatory analyses were the patients from the Italian Telethon and Leuven NMRC registries.

The patients from these registries had slightly older age of glucocorticoid initiation and less proportion of continuous (daily) glucocorticoid treatment regimen compared to patients treated with eteplirsen in study 201/202. The difference in corticosteroid use was also noted in study 301.

For pulmonary assessment, another external control group was identified - Exon 51 CINRG Identification. Due to small number of patients, also patients with other mutations, not amenable with exon 51 skipping, were used as a control arm for external comparison.

The pulmonary function data add a significant further concern of bias arising from the comparison with external controls. As discussed previously, there was evidence of clinically meaningful baseline imbalance between eteplirsen treated patients in study 201/202 and the Exon 51 CINRG cohort, with the eteplirsen group entirely above the 80% (%pFVC) threshold for established respiratory decline, a stage when the patients would be sensitive to the modulatory effect of glucocorticoids on pulmonary function; whereas, the Exon 51 CINRG cohort were at a more advanced stage of respiratory decline, with a median %pFVC of 81.00% below which patients would not be sensitive to the ameliorating influence of glucocorticoids on respiratory function. The two groups were therefore not at a comparable stage of respiratory decline.

It is not agreed, as claimed by the Applicant, that comparable behaviour of placebo and external control groups is confirmed by the study Mercuri 2017. Although the external controls were selected from the same registries as primary external control for ambulatory decline assessment, different active treatments than eteplirsen were evaluated in the Mercuri study. As a consequence, different inclusion and exclusion criteria, as well as different length of the treatment necessary for demonstration of efficacy, prevents reliable comparison with eteplirsen. Moreover, the conclusion from the study was that knowledge of untreated status (in the external controls) did not negatively impact 6MWT performance compared with patients who were receiving placebo as a blinded treatment. The more relevant question, however, is whether certain knowledge of receiving an investigational drug provides positive reinforcement to 6MWT performance where not only the patient but the investigator could be influenced. Indeed, as discussed previously, the Applicant describes the use of "Scripted encouragement from the testing staff at regular intervals to provide a standardized level of mo`tivation" when executing the 6MWT, which may reduce variability but highlights the importance of potential influence from the testing staff. The same concerns would apply to the outcomes dependent on "burst activities" as well as spirometry to obtain pulmonary function data which is also subject to voluntary effort. It is questionable therefore whether any of the key efficacy outcomes were suited to investigation in an open label setting, where the comparison was with external controls.

The Applicant stated that other characteristics at the time of first FVC%p assessment, such as specific age, type of glucocorticoid or ambulatory status, would not be anticipated to impact on the rate of FVC%p decline. In addition, the patients were identified regardless of ambulatory status. Since ambulatory status is key for further performance of the subjects and is strongly associated with age of the patients and glucocorticoid use, it is not agreed that this factor has no influence on study results.

The Applicant performed several post-hoc analysis – as such – they should be taken as supportive only. Sensitivity analyses in Study 201/202 are performed for variables 6MWT and FVC%p where treatment effect is adjusted for other important characteristics. Especially, results from sensitivity analyses for 6MWT based on ANCOVA model with high number of predictor variables (up to 4) should be interpreted cautiously. As there are small number of patients both in eteplirsen group (N=12) and in primary external control group (N=12), parameter estimates from ANCOVA model can be unreliable. The same holds for sensitivity analysis of FVC%p which includes height as additional predictor variable.

Sensitivity analyses in Study 301 for treatment difference in 6MWT and NSAA, respectively, are based on ANCOVA model with treatment effect adjusted for used type of corticosteroid, age and 6MWT. If primary external group is considered, then there is similar problem with results as in study 201/202 but these are more acceptable as eteplirsen group includes more patients (N=60). Other external control groups (placebo based on Study 044, untreated control group) seem more acceptable in sensitivity analyses due to higher number of patients (N=20).

However, sensitivity analyses are performed post-hoc, so they have rather an exploratory than confirmatory character regarding the effect of treatment difference.

Demonstration of efficacy solely on studies with external control is substantially affected by the possible dissimilarity of patients' population.

In summary, in addition to the methodological concerns arising from the use of non-concurrent external controls that were retrospectively defined, there is evidence of a potential bias in favour of eteplirsen for the key efficacy outcomes including ambulatory and pulmonary function outcomes.

The Applicant's response fails to provide reassurance in relation to uncertainties arising from the use of external controls in the pivotal efficacy analysis of eteplirsen.

Ground for refusal #2 is considered unresolved.

Ground #3

"It is unknown whether expression of the observed very low amount of truncated dystrophin after treatment with eteplirsen can translate into any clinical benefit to patients. Although the evidence of truncated dystrophin production may support the mechanism of action of the product, convincing demonstration of sustained functional effect is necessary to support the claim for efficacy of the medicinal product in the intended indication."

Summary of the Applicant`s Response

The Applicant argues that the entirety of pharmacologic data demonstrates definitive production of dystrophin by eteplirsen (including exon skipping, significant dystrophin levels, and the correct subcellular location of the truncated dystrophin) which not only confirms mechanism of action, but also demonstrates a sustained pharmacodynamic effect that translates into a sustained functional effect.

The mechanism of action of eteplirsen has been consistently confirmed through preclinical data and exon skipping in 100% of evaluated patients. It is also supported at the subcellular level preclinically and clinically through IHC which demonstrates correct localization of dystrophin within muscle fibers. Finally the sustained production of dystrophin over a course of 1 to 3.5 years has been demonstrated. Eteplirsen is the first therapeutic agent to demonstrate sustained dystrophin production over a multi-year course, based on validated Western Blot methods. Muscle biopsies from patients in Study 301 (N=12) show a 2.8-fold increase in dystrophin over baseline at Year 1 (48 weeks) and biopsies from patients in Study 201/202 (N=11) show there is an 11.6-fold increase over untreated controls at Year 3.5 (180 weeks). This sustained pharmacodynamic effect has translated to a sustained clinical benefit for the eteplirsen treated boys who received eteplirsen for over 4 years in Study 201/202. The timing of dystrophin production with significantly increased levels of dystrophin at Week 48 (Study 301) correlates with clinically evident ambulatory benefit on the 6MWT that emerges after Year 1. Further increase in significant dystrophin production at Week 180 (Study 201/202) correlates with widening of the clinically evident benefit with significant slowing in the rates of ambulatory and pulmonary decline observed by Years 3 and 4.

The Applicant re-emphasizes the dystrophin levels observed with eteplirsen provide clinical benefit. It is clear that the presence of some dystrophin results in disease amelioration. This is supported by multiple studies in dystrophic mouse models showing preservation of muscle force, as well as improvement of survival. Furthermore, evidence reported in the literature supports the hypothesis that low levels of dystrophin are indeed beneficial, as indicated by some exon 44 amenable patients who express low levels of dystrophin resulting from naturally-occurring exon skipping and have a milder phenotype (Wang 2018).

In addition, the levels of dystrophin produced by eteplirsen compare favourably with those produced by ataluren, a treatment for DMD that has received a positive CHMP opinion. Previous dystrophin expression levels resulting from treatment with ataluren are substantially lower than those observed with eteplirsen (Finkel 2013). Based on a quantitative IHC analysis of ataluren patients for 28 days, a mean change in dystrophin fiber intensity from pre-treatment to posttreatment of 11.0% in dystrophin expression was observed ($p = 0.008$, paired t-test). Using a comparable calculation, eteplirsen at Week 180 of Study 201/202 had a mean change in dystrophin fiber intensity from pre-treatment to posttreatment of 22.6% in dystrophin expression was observed ($p<0.001$).

Importantly, the timing of dystrophin production as measured by the validated Western blot method in 23 patients from 2 clinical studies shows significantly increased levels of dystrophin at Week 48 (Study 301) correlating with clinically evident benefit that emerges after Year 1. The continued and significant increase in dystrophin production at Week 180 (Study 201/202) correlates with widening of clinically benefit evidence by the significantly slowing in the rates of ambulatory and pulmonary decline observed by Years 3 and 4.

CHMP position

The Applicant's argumentation was related to the levels of dystrophin after treatment with eteplirsen based upon results of levels of dystrophin analysis from studies 201/202 and 301.

The proposed mechanism of action is the production of an internally shortened dystrophin protein.

Although the results of dystrophin levels obtained from patients in studies 201/202 and 301 indicate that after eteplirsen administration there are indeed elevated levels of dystrophin protein in muscle tissue, clinical consequence of these findings still has not been sufficiently demonstrated by the Applicant. It is acknowledged that a greater accumulation of dystrophin could be expected for a much longer period of eteplirsen administration than the period which has been observed in the submitted clinical studies. Nonetheless, the levels of dystrophin protein are still very low, much lower than are values of healthy boys and men, and substantively below the levels in milder forms of muscular dystrophy (Becker).

The CHMP considers that it is not possible to replace the evidence of clinical efficacy with discussion regarding a potential correlation between amount of dystrophin and expected clinical effect. Any such demonstrated correlation could be supportive, but not the sole evidence of clinical efficacy, needed to support the marketing authorization. Furthermore, the minimum effect of dystrophin expression that translates into the clinical effect is unknown yet and no clear and definitive threshold, above which a certain level of clinical benefit can be expected, can be defined. There is no expert consensus either, about the necessary levels of dystrophin that can predict a clinical benefit of the treatment.

In addition, during the clinical development, various methods of assessment of dystrophin levels were used. This could lead to a difference in results' interpretation.

Dystrophin production was evaluated by Western blot analysis and immunohistochemistry in muscle tissue obtained from patients in the pivotal Studies 201/202 following eteplirsen treatment of increasing durations. Immunofluorescent staining demonstrated an increase in the mean percentage of dystrophin-positive fibres, from a baseline of 18.19% to 41.14% in the 4 patients in study 201 treated with eteplirsen 30 mg/kg for 24 weeks. However, 6MWT performance in two of these patients declined more rapidly than placebo treated patients for reasons that are unknown. Moreover, the increase in dystrophin fibre positivity was observed with one anti-dystrophin antibody only whereas other anti-dystrophin antibodies did not demonstrate an increase. This highlights the susceptibility of antigenic domains to techniques used in immunohistochemistry such as fixation and permeation methods. The method is useful for qualitative evaluation but is very limited in its ability to provide quantitative information.

Quantification of dystrophin protein by densitometric analysis of Western blots in study 201/202 patients demonstrated a cumulative increase in dystrophin expression with increasing duration of eteplirsen treatment. The Applicant highlights that there is an 11.6-fold increase over untreated controls at Year 3.5 (180 weeks). The more relevant comparison however is with normal levels of dystrophin expression in healthy individuals. It is acknowledged that in some forms of muscular dystrophy, dystrophin expression levels 10-20% of normal levels is associated with milder clinical manifestation and therefore subnormal dystrophin expression levels may still have potential for clinical benefit.

However, in study 201/202 patients, even after more than 3 years of eteplirsen treatment, dystrophin protein levels in muscle tissue are still less than 1% of normal (0.93%) which is extremely low.

The Applicant does not discuss the possible reasons for this very low level of expression, except to make reference to the recognised poor uptake by target tissues of other oligonucleotide therapeutics. The Applicant has not investigated target tissue uptake of eteplirsen thus far but intends to do so, which is clearly important.

Given that the reason for the very low levels of dystrophin expression is unknown, it would have been helpful if more consideration had been paid to this in order to inform future studies. Although poor uptake of the oligonucleotide may be a contributory factor, there is no evidence at present for this and there could be additional reasons for the low protein levels. The demonstration of exon 51 splicing by RT-PCR provides evidence that the targeted oligonucleotide is exerting its intended mechanism of action on the target pre-mRNA. However, it is unclear whether the efficiency of this has been studied for example by comparing relative levels of exon 51 skipped and non-exon 51 skipped mature mRNA in eteplirsen treated muscle.

What is also relevant is the apparent susceptibility of misfolded dystrophin protein to enzymatic degradation in the cell, which the Applicant surmises is the reason for the failure to detect exon 51 skipped dystrophin protein in healthy non-human primates exposed to eteplirsen. This can be understood given that exon 51 skipping will disrupt, rather than restore, translational reading frame in wild-type dystrophin that is therefore likely to have more severely misfolded/truncated dystrophin, compared with exon 51 skipped in DMD patients with exon 51 skip amenable mutations. In the latter, reading frame is restored and the result is an internally truncated protein. Nonetheless, even an internal truncation may be sufficient to disrupt protein conformation to cause some degree of protein instability and degradation. It is possible that the low protein levels are at least in part due to intracellular degradation. This could have been investigated for example by exposure of muscle cells cultured from DMD patients treated with eteplirsen (as was done in the proof of concept investigation of splicing) to a proteasome inhibitor, to inhibit intracellular degradation and/or to a pharmacological chaperone to correct protein folding. If either of these approaches were to increase protein levels, this would suggest that the low dystrophin levels are at least in part due to post-translational reasons where there may be some hope of rectification.

In the meantime there continues to be of significant concern that the extremely low levels of dystrophin expression are unlikely to achieve meaningful clinical efficacy benefit and the clinical data have failed to provide reassurance in this regard.

Although the Applicant discussed the possible mechanism of action of eteplirsen by gradual accumulation of dystrophin based upon data from nonclinical evaluation as well as cellular level function, it has not been sufficiently demonstrated that eteplirsen administration leads to such amount of dystrophin levels which is able to restore missing function of dystrophin in Duchenne disease necessary to alleviate the disease progression.

Regardless demonstration of correlation between amount of dystrophin and clinical effect, it cannot substitute for the need to provide the appropriate clinical study with sufficiently robust control group of patients in order to clearly show the clinical effect. Therefore the main issue, the lack of appropriate confirmatory clinical trial, still persists as appropriate confirmatory results have not been submitted at the moment.

Ground for refusal #3 is considered unresolved.

Ground #4 (re-examination of the safety profile)

"Due to the limited number of patients exposed to eteplirsen the safety profile has not been thoroughly characterized".

Summary of the Applicant`s Response

The Applicant considers that the current size of the safety database and approach to evaluation, including a comparison to external placebo control data, have sufficiently characterized the safety profile of eteplirsen, given the rarity of the disease, for a conditional approval.

- As of 27 October 2017, 171 DMD patients have been exposed to eteplirsen in clinical trials, including 142 patients who have been treated with ≥30 mg/kg. Exposure for clinical trial safety data at ≥30 mg/kg is 309 patient-years. The exposure of 142 patients at ≥30 mg/kg in clinical trials would provide 95% probability to detect any AE occurring in 2.1% of patients.

- No significant safety concerns have been identified in clinical or post-marketing data to date. Infusion-related reactions, most of which are mild to moderate are important identified risks with eteplirsen; however, they were generally manageable with few events leading to interruption or discontinuation of eteplirsen.

- The severe risks associated with other antisense oligonucleotides, such as hepatotoxicity, renal toxicity, infusion-site reactions, severe cutaneous reactions, and thrombocytopenia/coagulation disorders have been monitored and have not been identified as risks with eteplirsen to date.

A brief summary of clinical and post-marketing safety data through 27 October 2017 is provided below.

Exposure

As of 27 October 2017, 171 DMD patients have been exposed to eteplirsen in clinical trials, including 142 patients who have been treated with ≥30 mg/kg. Exposure for clinical trial safety data at ≥30 mg/kg is 309 patient-years. These data allow for identification of AEs occurring in ~ 2 to 3% of patients.
The estimated EU prevalence of exon 51 skip-amenable mutations is 3360. Therefore, there are approximately 1680 males with DMD who are amenable to exon 51 skipping. The number of patients treated with eteplirsen in clinical trials at ≥30 mg/kg (N=142) represents approximately 8.5% relative to this EU DMD population.

Adverse Events

Cases with Fatal Outcomes
There were no AEs from clinical trials with a fatal outcome. There were 5 postmarketing cases with fatal outcomes reported through 27 October 2017. All were in older patients (21 to 28 years of age) who, consistent with the literature, succumbed to the respiratory and cardiac sequelae of DMD in their third decade of life. No new safety signals have been detected. Cases with a fatal outcome are monitored closely in routine pharmacovigilance activities.

Review of Risks

As part of pharmacovigilance activities, the Applicant has reviewed identified and potential risks of eteplirsen, in addition to risks associated with other antisense oligonucleotides using specified search strategies (Table 1). Below are overviews of these risks.

Table 29 Review of Risks

Rationale for Review	Risk
Important identified risk in Risk Management Plan	Infusion related reactions
Important potential risk in Risk Management Plan	Renal toxicity
Risks associated with other antisense oligonucleotides	Hepatotoxicity
	Infusion site reactions
	Severe cutaneous reactions
	Coagulopathy
Routine pharmacovigilance	Hypersensitivity

- Important identified risk
  - Infusion-related reactions (IRRs): Review of both clinical and postmarketing data reveal that IRRs occur with eteplirsen treatment. The most frequent IRRs were rash (including urticaria and erythema), headache, vomiting, nausea, abdominal pain/discomfort, and pyrexia. Several of the reactions occur with the first infusion, indicating that a type 1 hypersensitivity reaction is not likely. Most were mild to moderate and resolved without treatment except slowing or discontinuation of the infusion. All clinical trial patients and many postmarketing patients continued with eteplirsen without recurrence of these events. Of note, review of the clinical data revealed that nearly half of the events categorized as IRRs did not have an exact start time, thus it was not possible to determine if these occurred prior to the infusion versus during or after the infusion. Queries to obtain these data, as well as improvement to the electronic Case Report Form for capturing the timing of the events going forward are being implemented; a reanalysis will be performed once additional data are collected.
- Important potential risk
  - Renal toxicity: Due to nonclinical findings, renal toxicity is classified as an important potential risk. Analysis of AEs retrieved by the search criteria identified no significant safety risk with eteplirsen. Evaluation of biochemical laboratory parameters including blood urea nitrogen, creatinine, and serum cystatin C identified no pattern of drug effect. There were no postmarketing cases identified by the search strategy.
- Risks associated with other antisense oligonucleotides
  - Hepatotoxicity: No significant risk of hepatotoxicity has been identified in the eteplirsen clinical or postmarketing safety database to date, which is consistent with the nonclinical data for eteplirsen. Hepatotoxicity associated with other antisense oligonucleotides was seen in both nonclinical and clinical data.
  - Infusion site reactions: No significant risk of infusion site reactions has been identified in the eteplirsen clinical or postmarketing safety data to date. Review of the data revealed that infusion site reaction events were non-serious and mild in severity. They appear to occur at a similar rate for patients receiving infusions via a port and those receiving infusions peripherally. Given the clinical exposure as well as the postmarketing exposure, the rate of reported infusion site reactions to date is in line with what would be expected for administration of an intravenous product.

- Severe cutaneous reactions: No significant risk of severe cutaneous reactions has been identified with eteplirsen to date. There were few events identified by the search strategy, including non-serious and mild events of blister and skin erosion, and an association with eteplirsen is not likely. No cases were identified in the postmarketing data.

- Coagulopathy: No significant risk of coagulopathy, including thrombocytopenia, has been identified in the eteplirsen clinical or postmarketing safety database to date. The most common events identified by the search strategy were contusions and epistaxis, both of which are common in children. Thrombocytopenia and decreased platelets manifested by coagulopathy, seen with different antisense oligonucleotides, has not been identified as a risk with eteplirsen to date.

- Routine pharmacovigilance

  - Hypersensitivity: Review of the clinical data revealed few events that occurred during the infusion however, because of missing event start times, ultimate conclusions cannot be made. Conservatively including events with missing start times, the events that were reported on days of infusions were primarily reported as rash and urticaria, which is consistent with IRRs seen with eteplirsen treatment. The remaining events are generally evenly distributed over the 7 days following an infusion. Postmarketing data indicates that cases of hypersensitivity were consistent with the IRR cases. The Applicant agrees to add hypersensitivity to the Risk Management Plan.

Comparison to external placebo controlled data

Safety data from all enrolled patients in Study 301 (n=79; including patients not in ambulatory decline) were compared with 48weeks of safety data from patients randomized to the placebo arm in a drisapersen study (Study DMD114044; referred to as Study 044; n=61). Notwithstanding the challenges of cross study comparisons, these data provide an opportunity to assess eteplirsen safety data in the context of a placebo group of a randomized, blinded, controlled clinical study in patients with DMD amenable to exon 51 skipping. Study 044 was determined to be valid for a safety comparison based on inclusion and exclusion criteria for the studies and baseline demographics.

Similar rates of AEs were observed across the 2 groups during 48 weeks of follow-up; 92% of the eteplirsen-treated patients and 95% of the placebo patients experienced AEs. Serious AEs were infrequent, occurring in 5% and 8% of eteplirsen-treated and placebo patients, respectively. No patterns or trends were noted in comparison of serious AEs in eteplirsen-treated patients to those reported in placebo patients. The majority of all AEs across both groups were mild in severity. Patients with severe events were generally balanced across the 2 groups (4% and 3%, respectively). All serious and severe events were considered unrelated to study drug and none occurred in >1 patient in either group.

The review of events occurring more frequently in eteplirsen-treated patients is supportive of the adverse drug reactions (ADRs). Headache, vomiting, back pain, nausea and oropharyngeal pain all occurred more frequently in eteplirsen-treated patients and are considered ADRs with eteplirsen.

Summary

The Applicant has demonstrated that the evolving safety data, with > 300 patient-years from clinical trials and > 113 patient-years from postmarketing exposure through 27 October 2017 represents a considerable portion of the patient population of this rare disease and allows for the identification of AEs with an incidence of a few percent. It is important to note that 106 patients have received ≥ 30 mg/kg of eteplirsen for a period of 96 weeks or more. Patients in Study 201/202 have received ≥ 30 mg/kg of eteplirsen for ≥ 5 years.
No significant safety issues have been identified to date in the clinical or postmarketing data. IRRs were generally mild to moderate and resolved without treatment except slowing or discontinuation of the infusion.
The eteplirsen safety data have also been monitored for the severe risks associated with other antisense oligonucleotides, such as hepatotoxicity, nephrotoxicity, infusion site reactions, severe cutaneous reactions and thrombocytopenia/coagulation disorders. No signals have been identified.
Comparison with external placebo control data from a relevant drisapersen study does not identify any additional safety issues.
The size and evaluation of the safety database is considered by the Applicant to be adequate for a CMA, given the rarity of the disease. The Applicant will continue to expand the safety database through a prospective confirmatory trial, a 5-year registry and other planned studies. The Applicant agrees to add hypersensitivity to the Risk Management Plan. The Applicant will continue to closely monitor clinical trial and postmarketing data to evaluate risks and identify any new risks or changes to the risk-benefit profile and submit analyses in the Periodic Safety Update Reports.

CHMP position

At the present time, the available safety data for eteplirsen include 171 patients with DMD, of which 106 patients received the products at or above the proposed 30 mg/kg dose for over 96 weeks. The safety database constitutes of 7 clinical studies including Study 201 with extension part 202 (n= 12), which was the only completed, double blind, placebo-controlled, long-term trial. The other two finalized Phase 1 studies are dose-ranging study 28 and single-dose study 33. The ongoing studies are Phase 3 study 301, Phase 2 study 203 and study 204 with intended participation of 184 patients, which were originally designed to be 96-week. The post-marketing data from 182 newly exposed patients from US were provided. In addition, two Phase 1 studies 101 and 103 were completed, which evaluated single dose administration to non-DMD study subjects but were not included in the integrated analysis of safety.

The clinical safety analysis of eteplirsen is based on clinical trials providing very limited data; the enrolled patients were exposed to different doses, methods of administration and exposure duration. Another crucial shortage of clinical development is lack of the control groups in ongoing studies, except for post hoc addition of placebo arm from Phase 3 BioMarin sponsored study with drisapersen to study 301 (and 201/202), which is considered to be questionable.

It has to be noted, that the safety profile of eteplirsen is mainly based on Study 201/202, where only 4 patients out of 12 were treated with the proposed posology 30 mg/kg for 240 weeks. These limited comparative data do not allow to obtain sufficient view on short or even long-term safety profile of eteplirsen. Other eteplirsen studies 301, 203 and 204 have not been finished yet (available results are part of integrated analysis), but due to their limited design, their role in clinical safety assessment needs to be further considered.

Due to limited dataset the incidence and severity of adverse events and laboratory findings cannot be entirely determined. In addition, the misinterpretation of results and incomplete identification of all treatment-related AEs cannot be excluded in such a small sample of patients. Moreover, the influence on children development and possible accumulation of eteplirsen should be specified. Paediatric patients with DMD are specific population

suffering from cardiac, pulmonary and musculoskeletal complications and establishment of side effect origin is problematic in absence of control arm.

Although the Applicant stated that results from clinical trials or post-marketing did not reveal significant safety risks accompanying therapy with eteplirsen, the submitted safety database needs to be supplied with relevant long-term controlled data confirming the safety of use of this medicinal product in paediatric population.

From CHMP point of view, the safety database is considered to have serious limitations that preclude a sufficient characterisation of the safety profile of eteplirsen. Hence in this context of the efficacy not being sufficiently demonstrated, this remains an issue. Despite that, the CHMP agreed that the safety of the product as an important component of the B/R ratio, has to always be considered in the totality of the data, and it was agreed that in the event of sufficient efficacy data being presented, the remaining safety concerns will have to be re-discussed in that context.

Ground for refusal #4 is considered unresolved.

Report from the SAG

SAG Neurology answers

1. Efficacy of eteplirsen has not been demonstrated. There are no comparative data with patients on placebo beyond 24 weeks, and the available data for patients on treatment are derived from only a limited number of patients (n=12). There was no difference in 6MWD between eteplirsen and placebo during this 24 week treatment period.

2. The provided additional comparative data from a variety of external controls, derived from different studies and populations, suffer from important limitations related to the nature of the methodology used (non-concurrent, retrospectively selected, post-hoc defined). This increases the uncertainty about the reliability of such comparisons rather than providing confirmatory data for efficacy.

- SAG experts endorsed the position of the Rapporteurs that there is the clear need for comparative data with PBO in this condition.
- The issue of what constitutes an acceptable PBO controlled duration was discussed in detail.

  The patient representative commenced the discussion commenting that a 96 week PBO controlled period is perceived as unethical and unacceptable in most cases. Based on the available data for similar performance during the first year between the Concurrent controls and PBO patients in trials, a 48 week PBO-controlled period was proposed by the representative, to be combined with the use of concurrent controlled data to establish a picture of the expected efficacy.
- The SAG experts recognised the difficulties, but nevertheless the majority of them supported a two-year PBO-controlled, double-blind period as a minimum requirement (with one suggesting a 3 year duration). This period was suggested to be followed up by an open-label extension of at least another two years of follow up. The ratio of patients on PBO vs ones on active can also be adjusted to minimise PBO exposure, provided the size of the PBO group is sufficient for a proper statistical evaluation to be performed. The discussion was then continued with the question if it would be an option to use a combination of data generated in the PBO phase with the available data from concurrent controls, in order to supplement the full picture on efficacy. An alternative that was discussed was that it would be possible to have a pre-defined interim analysis for superiority after a2 year PBO-controlled, DB phase, which combined

with the use of the concurrent control data can provide the basis for conclusions on the efficacy of the product. In the case of negative analysis, or borderline trend for significance, this analysis could provide grounds to discuss a one year PBO-controlled extension.

- The SAG experts acknowledged the efforts by the community to collect and increase the reliability of concurrent controls data, but they agreed that at present these data cannot fully replace the need for PBO controlled data. The concurrent controls data can certainly be used in trial designs to increase trial feasibility and reduce the exposure to PBO, and to alleviating in part the concerns expressed by parents and patients.

3. It is unknown whether expression of the observed very low amount of truncated dystrophin after treatment with eteplirsen can translate into any clinical benefit to patients. Although the evidence of truncated dystrophin production may support the mechanism of action of the product, convincing demonstration of sustained functional effect is necessary to support the claim for efficacy of the medicinal product in the intended indication.

- The SAG experts agreed that the current data on the observed dystrophin production, can only be seen as supportive of the mechanism of action of the product, and that data on a functional effect are necessary to support the claim for efficacy of the medicinal product in the intended indication. The patient representatives supported the notion that more dystrophin is bound to be more beneficial than no dystrophin, and that in the case of patients with exon 45 deletion, it was shown that this leads to a different phenotype in the affected boys.
- The reliability of the dystrophin quantification methodology was discussed, and it was acknowledged that there are efforts in the DMD community to standardize the collection of these data. The SAG experts discussed the point that any potential correlation between the observed dystrophin levels and objective functional effects could help to support the expected clinical efficacy claim.

4. Due to the limited number of patients exposed to eteplirsen the safety profile has not been thoroughly characterised.

- The SAG experts agreed that the safety profile has not been thoroughly characterised and more data are needed. Nevertheless it was made clear that the limited safety data should be interpreted in the context of the presence of insufficient efficacy data, contributing to the uncertainties about the Benefit-Risk balance of the product.
- Patients' representatives expressed the view that the patients would be comfortable with potential risks, provided that efficacy has been shown.

Overall conclusion on grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant, and considered the views of the Scientific Advisory Group, and concluded that after re-examination the grounds for refusal of the original procedure still remain.

6. Benefit-risk balance following re-examination

Therapeutic Context

Disease or condition

Duchenne muscular dystrophy (DMD) is a rare, serious, disabling and fatal X-linked recessive degenerative neuromuscular disease caused by mutations in DMD gene. Product of this gene is the protein dystrophin.

Dystrophin has a structural role as a cytoskeletal stabilisation protein protecting muscle fibres against contraction-induced damage, but also a signalling role including mechano-transduction of forces and localisation of signalling proteins.

Affected boys develop symptoms around 5 years of age with slower functional gains compared with normal boys.

The predictable path of ambulatory decline begins around 7 years of age and with the current standard of care, most patients will experience loss of ambulation between 11 and 13 years of age. The prevalence of DMD in the European Union (EU) is estimated to be approximately 15000 cases. The most common cause of DMD is deletion mutations of 1 or more DMD exons. The remaining DMD cases are accounted for by nonsense mutations (amenable to therapy with ataluren, approximately 13%) or other types of mutations. A very small number, approximately 13%, of all patients with DMD have mutations amenable to exon 51 skipping (1950 boys in the EU). Given the well-described natural course of DMD, disease progression can be quantified by ambulatory and pulmonary measures.

Available therapies and unmet medical need

There are no authorised curative treatments for DMD. Supportive care (e.g., physiotherapy and ventilation assistance at latter stage) and glucocorticoids are currently the primary means to help improve the quality of life of affected boys. According to Cochrane review of 05 May 2016 glucocorticoids improve muscle strength and function for up to six months and strength up to two years (evidence on function at two years is limited). Data from other study types suggest that corticosteroids produce better function over a five-year period in many patients. Overall, long-term benefit remains unclear, and has to be weighed against long-term side effects. Aside from glucocorticoids, none of these interventions have been shown to impact loss of ambulation. Even with the introduction in the 1990s of assisted ventilation in the later stages of the disease, the mean age of survival (for those ventilated patients who do not develop early and severe cardiomyopathy) is still only 24 years.

Despite improvements in the standard of care, including steroids and other supportive care, these measures do not address the underlying absence of dystrophin. For the treatment of DMD patients with exon 51 deletion mutations, there are no approved specific treatments for this subset of DMD patients in the European Union (eteplirsen was approved in the USA in 2016 under accelerated approval based on an increase in dystrophin in skeletal muscle observed in some patients).

Other exon skipping therapy, ataluren, has received conditional marketing authorization in the EU but in DMD patients with nonsense mutations. Therefore, an unmet medical need remains for DMD patients with exon 51 deletion.

Main clinical studies

The MAA for eteplirsen included efficacy data derived from 4 interventional clinical studies (201, 202, 28 and 33, see Table 2 below), external control cohorts, and a review of literature describing the natural history of DMD and preliminary results from two other studies (Study 301 and 204).

Study 301 is a confirmatory 96-week, open-label Phase 3 study (30 mg/kg by weekly IV infusion) in ambulatory patients (N = 120) with DMD, ages 7 to 16 years old compared an untreated control group of patients with DMD amenable to skipping of any exon, with the exception of exon 51.

Study 204 is a 96-week, open-label Phase 2 study primarily to evaluate safety of eteplirsen (30 mg/kg by weekly IV infusion) in patients (N = 24) with advanced stage DMD (including non-ambulatory patients), ages 7 to 21 years old.

	Study number			
	Pivotal		**Supportive**	
	Study 201	**Study 202**	**Study 28**	**Study 33**
Study design	Randomized, double blind, placebo-controlled, multiple-dose, singlecenter, (US) study	Multicenter (US), open-label, multiple-dose, extension study	Dose-ranging study, Open-label, multipledose, (UK)	Proof of concept, Single-blind, placebo-controlled, single-dose, investigator-sponsored, (UK)
Dosing regimen	Eteplirsen 30 or 50 mg/kg/week, or placebo (weekly IV infusion) Weeks 1-24, then eteplirsen 30 or 50 mg/kg Weeks 25-28	Eteplirsen 30 or 50 mg/kg/week (weekly IV infusion)	Eteplirsen 0.5, 1.0, 2.0, 4.0, 10.0, or 20.0 mg/kg/week (weekly IV infusion)	Eteplirsen 0.09 or 0.9 mg IM in the EDB of 1 foot and placebo (IM) in the EDB of the opposite foot
Clinical Endpoints	**Primary**: 6MWT, LOA, NSAA, rise time and PFTs	**Primary**: Change from BL in 6MWT through Week 240 (combined), LOA, NSAA, rise time and PFTs	**Primary**: Safety and tolerability	**Primary**: Safety
Age at entry (yrs)	7 - 13	7 - 13	5 - 15	10 - 17

Favourable effects

The primary pharmacodynamic endpoint of Pivotal Study 201/202 was the percent dystrophin positive fibers assessed in muscle biopsies obtained pre- and post-treatment using immunohistochemical (IHC) detection with the MANDYS106 antibody. This assessment was conducted at several time points: Week 12, 24, 48, and 180. Dystrophin fiber intensity was used to verify de novo production of dystrophin at the correct sarcolemmal location of the muscle fiber, and dystrophin quantification was determined by both dystrophin fiber intensity and Western blot. Treatment with 30 mg/kg eteplirsen (N = 4) for 24 weeks increased the mean percentage of dystrophin-positive fibres from a baseline of 18.19% to 41.14%

The primary functional efficacy endpoint was the change from baseline in distance walked on the 6MWT. Supportive functional efficacy endpoints included the loss of ambulation, and the North Star Ambulatory Assessment (NSAA), and the ability to rise independently from a supine position to standing (derived from a NSAA subscore). Analysis of these variables was conducted on a post-hoc basis by comparing data from the 12 eteplirsen-treated patients in Study 201/202 to the data of a highly comparable untreated external control cohort derived from the Italian Telethon DMD Registry database and the Leuven Neuromuscular Reference Center (LNMRC) database.

Eteplirsen-treated patients showed a slower rate of decline in ambulation, endurance, and muscle function, as measured by the 6-minute walk test (6MWT), compared to the untreated primary and secondary external controls. Fewer eteplirsen-treated patients lost ambulation compared to untreated, external control cohorts. Two of the 12 (16.7%) eteplirsen-treated patients lost ambulation by Year 4 compared to 10 of the 13 (76.9%) primary external control patients. Eteplirsen-treated patients had a slower annual rate of decline (2.8%) in respiratory muscle function, as measured by forced vital capacity (FVC)% predicted compared to rates of ≥5% for untreated DMD patients described in the literature.

Uncertainties and limitations about favourable effects

The clinical study 201 is considered as pivotal study for demonstration of efficacy in the proposed indication by the Applicant. However, there are several concerns which create doubts about suitability of this trial as the main confirmatory study. These concerns arise from the nature of the study design. Despite the fact that DMD is a rare disease, the overall number of patients in the study is very low (only 12 subjects). The proposed dose 30 mg/kg of eteplirsen was administered in only 4 patients during the first 24 weeks. This is the only period in which a placebo arm was included in the study, and a higher number of patients will be desirable to provide clear interpretability of the study results. In this context the limited number of subjects represents a crucial shortcoming of the study design.
After 24 weeks no statistical difference between placebo and treatment arms in favour to eteplirsen have been seen in ambulation, timed function test, North Star Ambulatory Assessment, muscle strength, pulmonary function or quality of life. Based on this finding, the Applicant made the assumption that the effect of eteplirsen will be demonstrated after longer period of use. The results from the extended treatment up to 240 weeks (study 202) suggested this possibility, however the small number of patients who used the proposed dose 30 mg/kg and absence of the placebo arm do not allow for a clear conclusion regarding clinical efficacy of eteplirsen.

The design of the study 301 seems to be more appropriate in the sense of the number of enrolled patients and length of treatment period with respect to the placebo comparison. However due to high number of drop outs in the untreated control arm, no comparative analyses can be made.

As no robust and evaluable long-term control group was included in the studies 201,202 and 301, the Applicant decided to perform comparison of efficacy results with the External Controls.

When an external non-concurrent cohort of untreated patients was used as control, patients treated with eteplirsen demonstrated some clinical efficacy although a clear benefit was not evident until the third year of treatment. Nonetheless, the most important issue related to these results is that external control groups were selected post hoc and despite the Applicant's extensive arguments, there are several dissimilarities between groups which could influence the study results.

In that regards, the Applicant presented results of 6MWT, NSAA, and loss of ambulation in 72 patients in homogeneous phase of ambulatory decline. The benefit of eteplirsen becomes clinically evident after Year 1 and is sustained through Year 4, consistent with the gradual increase of dystrophin compared to external control groups. Results FVC%p from 74 patients in linear phase of pulmonary decline demonstrates that the benefit of eteplirsen on pulmonary function is evident by Year 2 and is sustained through Year 4. Despite the fact that the effect of eteplirsen in comparison with data from the external controls (apart from comparison with placebo arm from study with drisapersen) has been observed, considering the shortcomings regarding the chosen registries, it is difficult to draw any firm conclusion. Absence of long-term control arm makes it impossible to establish clinical effectiveness of eteplirsen.
The Applicant further performed several sensitivity analyses, which were also performed post-hoc, so they have rather an exploratory than confirmatory character regarding significant effect of treatment difference.

In addition, although the results of dystrophin levels obtained from patients in studies 201/202 and 301 indicate that after eteplirsen administration there are elevated levels of dystrophin protein in muscle tissue, clinical consequence of these findings still has not been sufficiently demonstrated by the Applicant. It is acknowledged that the greater accumulation of dystrophin is expected for the much longer period of eteplirsen administration than period which has been observed in the submitted clinical studies. Nonetheless, the levels of dystrophin protein are still very low, much lower than are values of healthy man and substantively below levels in milder forms of muscular dystrophy.
The minimum effect of dystrophin expression that translates into the clinical effect is unknown and neither literature references nor other data can provide clear and definitive threshold above which the clinical benefit can be expected. There is no consensus about levels of dystrophin necessary to predict clinical benefit of the treatment. In addition, during the clinical development various methods of assessment of dystrophin levels were used. This could also lead to difference in results interpretation.

Although the Applicant discussed the possible mechanism of action of eteplirsen by gradual accumulation of dystrophin based upon data from nonclinical evaluation as well as cellular level function, it has not been sufficiently demonstrated that eteplirsen administration leads to such amount of dystrophin levels which is able to restore missing function of dystrophin in Duchenne disease necessary to alleviate the disease progression. Regardless a potential demonstration of a correlation between amount of dystrophin and clinical effect, the appropriate clinical study with sufficient robust control group of patients should be submitted and the clinical effect should be clearly shown.

In conclusion, the main issue, the lack of an appropriate confirmatory clinical trial, still persists and appropriate confirmatory results have not been submitted.

Unfavourable effects

Around 50% of all patients receiving any dose of eteplirsen reported any AEs prior to study drug initiation. The main AEs were procedural pain, contact dermatitis, hypokalemia, falls, nasal congestion, back pain, headache, pyrexia and vomiting.

For the long-term safety assessment (extension study 202), the variety and number of AEs suggest that the safety profile of eteplirsen may worsen with the long-term administration of the drug.

Due to nonclinical findings, renal toxicity is classified as an important potential risk. Analysis of AEs retrieved by the search criteria identified no significant safety risk with eteplirsen. Evaluation of biochemical laboratory parameters including blood urea nitrogen, creatinine, and serum cystatin C identified no pattern of drug effect. There were no postmarketing cases identified by the search strategy.

Uncertainties and limitations about unfavourable effects

The clinical safety analysis of eteplirsen is based on clinical trials providing very limited data; the enrolled patients were exposed to different doses, methods of administration and exposure duration.

Another crucial shortage of clinical development is lack of the control groups in ongoing studies, except for post hoc addition of placebo arm from Phase 3 BioMarin sponsored study with drisapersen to study 301 (and 201/202), which is considered to be questionable.

It has to be noted, that the safety profile of eteplirsen is mainly based on Study 201/202, where only 4 patients out of 12 were treated with the proposed posology 30 mg/kg for 240 weeks. These limited comparative data do not allow to obtain sufficient view on short or even long-term safety profile of eteplirsen. Other eteplirsen studies 301, 203 and 204 have not been finished yet (available results are part of integrated analysis), but due to their limited design, their role in clinical safety assessment needs to be further considered.

Due to limited dataset the incidence and severity of adverse events and laboratory findings cannot be entirely determined.

Effects Table

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects: Please see foot note.						
Unfavourable Effects						
Hypokalemia		% of pts.	Eteplirsen 30 mg/kg: 50	Placebo: 50	Small safety database (eteplisen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201
Dermatitis contact		% of pts.	Eteplirsen 30 mg/kg: 50	Placebo: 0	Small safety database (eteplisen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201
Procedural pain		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplisen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201

Effect	Short Description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Falls		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplirsen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201
Nasal congestion		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplirsen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201
Back pain		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplirsen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201
Headache		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplirsen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201
Pyrexia		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplirsen n=4; Placebo n=4)	Study 201
Vomiting		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplirsen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201
Haematoma		% of pts.	Eteplirsen 30 mg/kg: 25	Placebo: 25	Small safety database (eteplirsen n=4; Placebo n=4). Only AEs with an incidence higher than 25% can be identified.	Study 201

Abbreviations: AE … adverse event

Notes: Clinical studies were primarily aimed to show the effect of eteplirsen on dystrophin. Efficacy results were considered exploratory. Data from limited number of patients included (n=12) questions the interpretability the results. Similarly, comparison with external controls has been conducted posthoc; so it has rather exploratory than confirmatory character regarding significant effect of treatment difference. Due to the lack of robustness of the results they are not included in the Effect table.

Benefit-risk assessment and discussion

Importance of favourable and unfavourable effects

An effect of eteplirsen in comparison to data from the external controls has been observed, however considering the shortcomings regarding the chosen methodology, it is difficult to draw any firm conclusions about its reliability. The absence of long-term control arm makes it impossible to establish clinical effectiveness of eteplirsen.

The most important issue is that the external control groups were selected post hoc, when results of study 201 were already evaluated. The results of the pivotal study did not demonstrate a clear beneficial effect of eteplirsen. Since ambulatory status is key for further performance of the subjects and is strongly associated with age of the patients and glucocorticoid use, it is not agreed that this factor has no influence on study results. Also sensitivity analyses were performed post-hoc, so they have rather an exploratory than confirmatory character regarding significant effect of treatment difference.

For pulmonary assessment, another external control group was identified - Exon 51 CINRG Identification. Due to small number of patients, also patients with other mutations were used as placebo control arm for external comparison. The demonstration of efficacy solely on studies with external control is substantially affected of the possible dissimilarity of patients' population. The results from the on-going phase III study 301 cannot address the main uncertainties.

The limited comparative data do not allow to obtain sufficient view on short or even long-term safety profile of eteplirsen. Other eteplirsen studies 301, 203 and 204 have not been finished yet (available results are part of integrated analysis), but due to their limited design, their role in clinical safety assessment needs to be further considered. Due to limited dataset the incidence and severity of adverse events and laboratory findings cannot be entirely determined.

Balance of benefits and risks

Favourable effects observed cannot overweigh the effect of the observed limitations (missing data beyond 24 weeks, limitations of submitted clinical trials, post-hoc analysis, selection of controls, unclear clinical outcome of the truncated dystrophin production) and the limited dataset available for the analysis of safety.

On the basis of the assessment of the responses and the grounds for re-examination submitted by the Applicant in response to the grounds for refusal of the marketing authorisation, the CHMP concluded that serious concerns remain with regard to the demonstration of therapeutic efficacy and clinical benefit. Additionally, the safety dataset has serious limitations that prevent an adequate evaluation; and while evidence of dystrophin expression in muscle tissue from eteplirsen treated DMD patients is considered useful as a proof of principle, the levels of expressed protein are too low to be considered clinically meaningful at this stage. Moreover, the reasons for the very low expression levels are not understood.

Additional considerations on the benefit-risk balance

The Applicant proposed a conditional marketing authorisation for eteplirsen. It is agreed that there is an unmet medical need, however, the currently available data and their limitations cannot lead to a conclusion that the benefit-risk in the target population is positive.

The Clinical Trial 302 (a double-blind randomised trial to confirm a clinically relevant benefit of eteplirsen in DMD patients amenable to exon 51 skipping) has been proposed by the Applicant. There are concerns regarding the possibility of recruiting sufficient number of patients older than 10 years with this ability to positively conclude on any results observed. Although the Applicant considers that the study 302 is feasible in the post-marketing phase, however there are reasonable doubts that this study will remain feasible should the product be authorised on the European market.

In view of the above, the CHMP maintains that the product does not fulfil the requirements of a conditional marketing authorisation.

Risk Management Plan

The CHMP and PRAC, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application, the risk management plan cannot be agreed at this stage.

7. Recommendations following re-examination

Based on the arguments of the applicant and all the supporting data on quality, safety and efficacy, the CHMP re-examined its initial opinion and in its final opinion concluded by consensus that the benefit-risk for Exondys remains negative in the applied indication.

The CHMP considers that:

- Efficacy of eteplirsen remains not demonstrated. There are no comparative data with patients on placebo beyond 24 weeks, and the available data for patients on treatment are derived from only a limited number of patients (n=12). There was no difference in 6MWD between eteplirsen and placebo during this 24 week treatment period.
- The provided additional comparative data from a variety of external controls, derived from different studies and populations, suffer from important limitations related to the nature of the methodology used (non-concurrent, retrospectively selected, post-hoc defined). This increases the uncertainty about the reliability of such comparisons rather than providing confirmatory data for efficacy.
- It remains unknown whether expression of the observed very low amount of truncated dystrophin after treatment with eteplirsen can translate into any clinical benefit to patients. Although the evidence of truncated dystrophin production may support the mechanism of action of the product, convincing demonstration of sustained functional effect is necessary to support the claim for efficacy of the medicinal product in the intended indication.
- Due to the limited number of patients exposed to eteplirsen the safety profile remains not thoroughly characterised.

The CHMP is of the opinion that pursuant to Article 12 of Regulation (EC) No 726/2004, efficacy and safety of the above mentioned medicinal product is not properly or sufficiently demonstrated.

Therefore, the CHMP has recommended the refusal of the granting of the conditional marketing authorisation for Exondys.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system and risk management plan cannot be agreed at this stage.

Furthermore, the CHMP, in light of the negative recommendation, was of the opinion that it is not appropriate to conclude on the new active substance status and similarity at this time.