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EMA/439369/2016
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Kyndrisa

International non-proprietary name: drisapersen

Procedure No. EMEA/H/C/003846/0000

Note

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

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List of abbreviations

6MWD	6-minute walking distance
ADA	Anti-drisapersen antibody
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AON	Antisense oligonucleotide
AP	Alkaline phosphatase
aPTT	activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
BLAST	Basic Local Alignment Search Tool
BMD	Becker Muscular Dystrophy
BMI	Body mass index
CI	Confidence interval
CSR	Clinical study report
DMD	Duchenne muscular dystrophy
DP	Drug product
DS	Drug substance
GSK2402968	Drisapersen
h51AON1	Drisapersen
h51AON23	Drisapersen
IL-6	Interleukin-6
IND	Investigational New Drug
ISE	Integrated Summary of Efficacy
ITT	Intent-to-Treat
MCID	Minimal clinically important differences
MCP-1	Monocyte Chemotactic Protein-1
NDA	New Drug Application
NH	Natural history
NSAA	North Star Ambulatory Assessment
PLB	Placebo
PP	Per protocol
PRO051	Drisapersen
QWBA	Quantitative Whole Body Autoradiography
SC	Subcutaneous
SD	Standard deviation
SE	Standard error
SMQ	Standardized MedDRA query

1. Recommendation

Based on the review of the data and the Applicant's response to the CHMP LoQ on quality, safety, efficacy and risk management plan, the CHMP considers that the application for Kyndrisa, an orphan medicinal product in the treatment of,

Duchenne muscular dystrophy (DMD) in ambulatory patients aged 5 years and older with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing (see sections 4.4 and 5.1)

is not approvable since major objections still remain, which preclude a recommendation for marketing authorisation at the present time.

Questions to be posed to additional experts

N/A

Inspection issues

GMP inspection(s)

A USA facility has been assigned to stability testing of the drug product. The inspection of the site has been deferred based on risk (to be conducted as a post-approval inspection) in agreement with the supervisory authority.

GCP inspection(s)

A routine inspection has been conducted without specific concerns (the main scope was to verify compliance with ICH GCP). The inspection focussed on the verification of selected efficacy and safety data reported in the Marketing Authorisation Application for a sample of patients to be determined by the inspectors (***study DMD114349***). Inspection sites were London Health Sciences Centre (No. 091313), Universitätsklinikum Essen (No. 091329), and Hacettepe Children`s Hospital in Ankara, Turkey (No. 091354). It should be referred to the detailed integrated inspection report (INS/GCP/2015/018) and respective appendices for the single study sites.

GCP inspection findings relevant for this application/ for the clinical assessment of data:

Clinical efficacy:

Initially, there were concerns as the data listings of at least two patients were incomplete with respect to baseline physiotherapeutic assessments and it was questioned whether this would have been influenced the study results. However, during the inspection process these concerns were resolved and it was considered that there is no impact on the interpretation of efficacy results.

Clinical safety:

Skin reactions:

The inspection of site 091354 revealed inadequate documentation and reporting of skin reactions (Finding MA5). No skin reactions have been reported from this site at the time of inspection. As this issue was not detected by the sponsor, e.g. during monitoring, it cannot be excluded that skin reactions were also not adequately reported by other sites, and/or in the feeder studies, which preceded the inspected extension trial.

As a response to this major finding, BioMarin committed “to review skin reaction data for all patients in DMD114349 and the feeder studies of DMD114117 and DMD114044 at this site by 31Jan2016”, and, “If appropriate, as a result of the review, [provide] additional supplemental data…”. Review was completed meanwhile and as a result, ISRs for subjects participating at this site (in studies DMD114349, and feeder studies DMD114117 and DMD114044) were presented:

In summary, six (6) mild injection site reactions (ISR) were identified **in 3/12 subjects** enrolled at this site. All six ISR events identified were reported as mild and reversible, including: rash (2 events), swelling (2), erythema (1) and hyperpigmentation (1). None of the patients withdrew from the study due to the event.

BioMarin commented on the clinical impact of ISR findings. ISRs were found at a high incidence in subjects treated with drisapersen depicting the adverse event with the highest frequency in clinical studies (affecting around 80% of subjects). Therefore, the additional 6 ISR events at this study site do not impact the safety profile in regard to the overall ISRs.

Summary relevant for clinical safety:

The Sponsor stated that there is no impact of the additional six ISRs on clinical safety reviewed at this study site. This is accepted. However, uncertainty remains on the documentation of (ISR) adverse events at this study site: the six documented ISRs reported by three subjects of a total of 12 subjects participating in study DMD114349 do not represent the high frequency of ISR data reported in the clinical study program (affecting around 80% of subjects). ISRs in 3 of 12 subjects would indicate that ISRs have occurred at this site in 25% of subjects, which is further to be questioned.

The Applicant is asked to reasonably clarify why overall frequency of ISRs at the turkey study site was only around 1/3 of the overall occurrence of ISRs in all studies up to 3.5 years (LoQ).

The Sponsor should additionally provide a listing of subjects included in any study at this study site together with overall frequency of the main adverse events of special interest (LoQ).

New active Substance status

Based on the review of the data and the Applicant’s response to the CHMP LoQ, the CHMP consider that the active substance drisapersen contained in the medicinal product *“Kyndrisa”*is to be qualified as a new active substance in itself.

2. Executive summary

2.1. Problem statement

Duchenne Muscular Dystrophy (DMD) is a rare, disabling, progressive and ultimately fatal X-linked genetic disorder caused by mutations in the gene for dystrophin, a cytoplasmic protein, which associates with other proteins to form the dystrophin-associated protein complex that connects the actin cytoskeleton with the extracellular matrix. Functional dystrophin is critical for the structural stability of myofibers in skeletal, diaphragm and cardiac muscle and is also of importance for smooth muscles.

DMD is caused by several types of mutations in the dystrophin gene such as deletions, duplications and point mutations, which produce a shift in the open reading frame of the dystrophin mRNA leading to

the absence of functional dystrophin protein. The disease primarily affects males with an incidence of 1 in 3600 – 6000 male newborns worldwide (Bushby *et al*., 2010). Initial signs of muscle weakness begin at the age of 2 and then progressively deteriorate, so that DMD patients become wheelchair-bound before the age of 12 and later die from respiratory failure and cardiomyopathy in their early twenties.

For a subpopulation of DMD patients aged 5 years and above, in which mutation created a nonsense stop codon in the dystrophin mRNA resulting in premature termination of translation and, hence, a truncated, non-functional protein, the API ataluren was granted central MA across the EU on 31st July 2014 (EMEA/H/C/2720, *"Translarna"*). Apart from ataluren, the current management of the disease focuses on prevention and management of complications. In addition, corticosteroids (e.g. prednisone or deflazacort) have been shown to temporarily reduce the decline in motor function in DMD patients.

2.2. About the product

Drisapersen (also termed PRO051, GSK2402968, h51AON1 and h51AON23 in the dossier) is a 20-mer 2'-O-methyl phosphorothioate antisense oligoribonucleotide (AON) that binds specifically to exon 51 of the human dystrophin pre-mRNA. As a consequence, exon 51 is excised with intron sequences during splicing (*"exon skipping"*), which restores the reading frame of the dystrophin transcript thereby allowing synthesis of an internally shortened but partially functional dystrophin protein. Deletions of one or more exons in the dystrophin gene are considered to represent more than 70 % of all mutations and are most frequently located in a *"hot spot region"* spanning exons 45-53 (Aartsma-Rus *et al*., 2006). This region corresponds to the central rod domain of dystrophin, where deletions in the number of the normally 24 spectrin-like repeats have been associated with a mild Becker Muscular Dystrophy(BMD) phenotype as long as the deleted region is not too large, the remaining repeats are properly positioned and the presence of the adjacent hinge regions to the actin-binding N-terminal domain and the cysteine-rich and C-terminal domains required for interaction with glycoproteins at the sarcolemma are maintained (van Deutekom and van Ommen, 2003). Skipping of exon 51 by drisapersen would be applicable to the largest group of 13 % of DMD patients with out-of-frame exon deletions in the *"hot spot"* region adjacent to exon 51, which encompass adjacent deletions in exons 45–50, 47–50, 48–50, 49–50, 50, 52, or 52–63 (Aartsma-Rus *et al*., 2009). Accordingly, drisapersen received orphan drug designation on 27th February 2009 for the treatment of DMD.

Drisapersen has been formulated as 200 mg/ml sterile, colourless to yellow sodium salt solution for once subcutaneous or intravenous injection. Following a loading dose of 6 mg/kg twice weekly for three weeks, it is proposed to continue the therapy with 6 mg/kg/week.

Three other oligonucleotides have been previously assessed as APIs in the EU: The 21-mer phosphorothioate AON fomivirsen (EMEA/H/C/244; *"Vitravene"*), that promotes RNase H-mediated degradation of its duplex with the mRNA of the immediate early transcriptional unit region 2 of the human cytomegalovirus (CMV), gained MA on 29th July 1999 for the intravitreal treatment of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS). However, the MA of fomivirsen was later voluntarily withdrawn by the MAH in 2002 for commercial reasons. Subsequently, the pegylated 28-mer oligonucleotide aptamer pegaptanib (EMEA/H/C/620, *"Macugen"*), which targets the Vascular Endothelial Growth Factor, was licensed on 31st January 2006 for intravitreal treatment of adults with neovascular age-related macular degeneration. Moreover, the MAA of the 20-mer phosphorothioate AON mipomersen (EMEA/H/C/2429, *"Kynamro"*), that promotes RNase H-mediated degradation of Apolipoprotein B-100 mRNA, was refused as subcutaneous treatment for inherited hypercholesterolaemia by the CHMP on 21th March 2013 for safety reasons. Thus, this MAA of drisapersen constitutes the first AON approach in Europe for restoration of protein synthesis by *"exon skipping"*.

2.3. The development programme/compliance with CHMP guidance/scientific advice

At present, the first EMA guideline in the field is being developed and is expected to be published as a final document by the end of the year (the latest draft available upon request).

The Applicant obtained national scientific advice by the competent authorities of the Netherlands and the United Kingdom with regard to non-clinical and clinical development on 15th May 2009, on 2nd June 2009 and on 27th March 2013, respectively.

In addition, the CHMP granted protocol assistance at different stages of pharmaceutical development:

- Follow-up protocol assistance on non-clinical and clinical development on 25th June 2009 (EMEA/CHMP/SAWP/357314/2009)
- Protocol assistance on quality and non-clinical aspects on 20th January 2011 (EMA/CHMP/SAWP/18788/2011) including clarification as a follow-up on 28th March 2011 (EMA/196773/2011)
- Follow-up protocol assistance on clinical development on 15th December 2011 (EMEA/CHMP/SAWP/945076/2011)
- Follow-up protocol assistance on quality issues on 15th December 2011 (EMEA/CHMP/SAWP/868016/2011) and on 17th January 2013 (EMA/CHMP/SAWP/3656/2013) including subsequent clarification on 8th March 2013

2.4. General comments on compliance with GMP, GLP, GCP

For EU manufacturing sites valid manufacturing authorisations/GMP certificates are included in module 1.

A USA facility has been assigned as site for stability testing of the drug product.

The inspection of this site has been deferred based on risk (to be conducted as a post-approval inspection) in agreement with the supervisory authority. FDA inspected the site in August 2015 with a positive outcome (inspection resulted in no cGMP deficiencies and no Form FDA 483, Inspectional Observations, was issued).

All pivotal safety pharmacology and toxicology studies of drisapersen were conducted in compliance with GLP.

For the clinical studies contained in this dossier compliance with Good Clinical Practice (GCP) regulations, the requirements of the Declaration of Helsinki and the ethical principles of Directive 2001/20/EC is claimed.

2.5. Type of application and other comments on the submitted dossier

- Legal basis

Art. 3(1) of Reg. (EC) 726/2004 and in accordance with the provisions of Art. 8(3) of Dir. 2001/83/EC.

- Conditional approval

N/A

- Approval under exceptional circumstances

N/A

- Accelerated procedure

A request for accelerated assessment pursuant to Article 14(9) of Reg. (EC) 726/2004 was submitted on 4thMay 2015. During the assessment the CHMP came to the following conclusion:

The potential benefits of drisapersen for Duchenne patients resulting from a mutation in the dystrophin gene correctable by exon 51 skipping are obvious. To date, therapy is limited to symptomatic treatment. The only approved disease modifying treatment option for Duchenne patients encompasses a subgroup of DMD patients with a nonsense mutation as the underlying genetic defect. Given the nature of the disease as life-threatening and chronic progressive, the unmet medical need clearly exists. Although drisapersen could be considered a significant therapeutic innovation, the claim that drisapersen is of major public health interest, as outlined in the Guideline on the Procedure for Accelerated Assessment (EMA/419127/05), is not considered justified based on the available evidence.

Regardless of the fact that the types of studies, intended to be submitted as part of the marketing authorization application, are expected to be sufficient for a proper B/R assessment, the strength of evidence on the expected beneficial effect on public health is undermined by the uncertainties created by the data from the failed phase III study. Due to this significant uncertainty hampering the strength of the evidence for the justification, the CHMP does not accept the request for accelerated assessment posed by the Applicant. This is notwithstanding to the outcome of the later evaluation of the marketing authorisation application. Based on the assessment of the request provided by the Applicant and considering the draft CHMP guideline on the procedure for accelerated assessment, the CHMP refused the granting of an accelerated assessment procedure pursuant to Article 14(9) of Reg. (EC) 726/2004for *"Kyndrisa"* (drisapersen).

- Biosimilarity

N/A

- 1 year data exclusivity

N/A

- Significance of paediatric studies

Pursuant to Art. 7of Reg. (EC) 1901/2006, the application included an EMA Decision P/0130/2015 on the agreement of a paediatric investigation plan (PIP). At the time of submission of the application, the PIP P/0130/ was not yet completed as some measures were deferred.

3. Scientific overview and discussion

Drisapersen is a 2'-O-methyl phosphorothioate antisense RNA consisting of 20 nucleotides with high specificity for exon 51 of the human dystrophin pre-mRNA. The methylation and phosphorothioate backbone increases the resistance of drisapersen to nuclease mediated degradation. Sequence alignment using the BLAST software (Basic Local Alignment Search Tool) of the US National Center for Biotechnology Information revealed no other complementary region in the human genome. Binding of drisapersen to exon 51 of the human dystrophin pre-mRNA results in excision of this exon with intron sequences during splicing. This restores the open reading frame of the dystrophin mRNA, so that a shortened dystrophin protein with partial function as found in BMD is produced.

3.1. Quality aspects

3.1.1. Introduction

The active substance of Kyndrisa is drisapersen as sodium salt. The drug product is a sterile, clear, colourless to yellow solution containing 188.7 mg/mL of drisapersen (corresponding to 200 mg/mL of drisapersen sodium). Kyndrisa is administered as a subcutaneous injection. The product is available in 0.9 mL fill volume and 0.6 mL fill volume. The product is filled into clear type I glass vials with fluoro resin polymer coated chlorobutyl rubber stopper sealed with aluminium overseals with a removable polypropylene flip-off cap. The vials are presented as a single vial pack or a 10 vial multipack.

3.1.2. Active Substance

General Information

Drisapersen sodium is a fully synthetic chemical entity, a 2'-O-methyl phosphorothioate oligoribonucleotide (5'-UCA AGG AAG AUG GCA UUU CU-3', molecular weight: 7395 Da). The structure of drisapersen sodium comprises twenty nucleotides connected via nineteen phosphorothioate linkages as the sodium salt. Due to the formation of the 19 phosphorothioate linkages the drug substance is a mixture of 2^{19} diastereoisomers. However, the stereochemistry cannot be controlled by the manufacturing process and the huge number of diasteroisomers is present in all therapeutic oligonucleotides containing phosphorothioate linkages.

Manufacture, characterisation and process controls

Full information on the drug substance is provided in the dossier. The ASMF or CEP procedures are not applicable.

The manufacturing process is described in sufficient detail in 3.2.S.2 and 3.2.S.4. Critical process parameters (CPP) and process parameters (PP) have been defined. The control strategy as proposed by the applicant consists of control of material attributes, control of the CPPs, in process controls and release testing.

A design space has been initially claimed for different unit operations and questions were raised concerning this approach. In the responses to the questions the applicant declared that no design space will be claimed anymore for this application.

The described quality target product profile (QTPP) is reasonable, although, somewhat reduced. All process parameters have been justified by data for up to 22 clinical and commercial drug substance batches. During process development an assessment of the criticality of the investigated parameters was done. Risk analyses (FEMA) are described in the dossier. The conclusions on the criticality are reasonable.

The starting materials are considered suitable for synthetic oligonucleotides. They are commercially available. The structure of these materials has been fully characterised and the relative stereochemistry at each position around the ribose ring is defined and controlled. The selection of the starting materials including the route of synthesis of the currently qualified suppliers has been described. It has been confirmed that the addition of an alternative vendor for the starting materials will be approved by variation. The specifications for the starting materials and all other materials are acceptable.

The structure and sequence of the drug substance has been adequately characterised by means of mass spectrometry. Some data and information relevant for the elucidation of structure have been

presented under S.1.3 (General Properties). This includes crystallinity, pH, melting point to a complementary 2'-OMe-PS-RNA strand, solubility, particle morphology, optical rotation, UV, FT-IR and ^{1}H, ^{13}C and ^{31}P NMR spectra. Data for elemental analysis, thermodynamic characterisation data (e.g. thermogravimetry and DSC) and on the three-dimensional structure have also been provided.

The information provided on potential and actual drug substance impurities in drisapersen sodium, prepared by the defined commercial manufacturing process is acceptable. Information relating to the origin and control of these impurities in drug substance manufacturing is also provided in S.2.6 manufacturing process development. The grouping of impurities is acceptable since the analytical methods are not able to separate this extremely high number of different structures.

Potential genotoxic impurities have been adequately addressed in the dossier. Information on process-related impurities and the depletion of all reagents used in the manufacturing process has been provided.

Specification

The specification comprises the quality attributes appearance, identity (molecular weight by ESI-MS, sequencing by ESI-MS, retention time by HPLC-UV), sodium content by flame AA spectrometry, impurity content by HPLC-UV, impurity content by HPLC-MS, purity by calculation, residual solvents by GC-FID, lead by ICP-MS, osmolality by freeze point depression, and pH by potentiometry. Bioburden and bacterial endotoxins are determined according to Ph. Eur.

The relevant CQAs have been included in the specification. A justification for each attribute and the respective acceptance criteria in the drug substance specification has been provided. Most of the proposed specification limits and acceptance criteria are acceptable and have been sufficiently tightened during the procedure. However, the applicant should commit to tighten the limits for the specified impurities in the drug substance specification when data for 5 additional drug substance batches with the commercial process are available. All other specifications limits have been adequately justified by historical batch analysis data. A justification for not including a biological activity test together with relevant data has been provided.

Descriptions of the analytical methods have been provided. These descriptions are acceptable. For the determination of impurities orthogonal methods are employed. The HPLC-UV method is used for the determination of n-1, n+1, n-2 and unspecified impurities. During validation of this method specificity resolution was investigated for drisapersen and 18 known potential impurities of synthesis and degradation. An orthogonal HPLC-MS method was developed to quantify these impurities and other process related impurities which were subsequently discovered. The combination of these two methods is adequate to control the impurity profile during routine analysis.

Validation data for analytical methods have been provided. It has been demonstrated that the methods are suitable for their intended use.

Sufficient information on reference standards and the container closure system has been provided.

Stability

Real time stability data for 9 batches have been provided in the dossier. These batches have been stored at -20°C and at refrigerated conditions. No significant changes or trends have been observed. All results are within the specifications. Data are available for up to 45 months. Stress test studies, forced degradation and photostability studies have been performed and it has been demonstrated that the analytical methods are stability indicating.

The proposed retest period of 36 months, being the storage conditions "Store in the freezer, -25ºC to -10ºC is acceptable.

Comparability exercise for Active Substance

N/A

3.1.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

Drisapersen sodium solution is a sterile, clear, colourless to yellow solution essentially free from visible particulates, containing 188.7 mg/mL of drisapersen (corresponding to 200 mg/mL of drisapersen sodium). The strength of the drug product was originally expressed as drisapersen sodium and not as drisapersen which is not in accordance with the Guideline on Summary of Product Characteristics (September 2009). The applicant has revised the Product Information to comply with the request to represent the strength of the drug product in terms of the active moiety (drisapersen).

Drisapersen sodium solution is administered as a subcutaneous injection. The product is available in 0.9 mL fill volume and 0.6 mL fill volume. The product is filled into clear type I glass vials with fluoro resin polymer coated chlorobutyl rubber stopper sealed with aluminium overseals with a removable polypropylene flip-off cap. The vials are presented as a single vial pack or a 10 vial multipack.

The links between drug substance CQAs and drug product CQAs are presented. They are obvious and need no further justification.

The method to detect the impurities in drisapersen sodium was only available after the pivotal clinical studies had been performed. The applicant analysed the drug substance batches used in the clinical studies, not the drug products, on these impurities. This is accepted as it is demonstrated that impurity contents in drug product batches produced after the clinical studies and the corresponding drug substance batches are not significantly different. The results show that the drug product specification limits with respect to the API content needs no revision compared to the limits of the specification applied during clinical studies.

Product development aimed at a stable and physiologically compatible drug product. A phosphate buffer was chosen as solvent for the drug substance. Data demonstrate stability and physiological compatibility (pH, osmolarity, clinical trials) of the chosen formulation. The formulation used in pivotal clinical studies is the same as that proposed for the commercial product.

Manufacturing process design and process performance qualification are described. Both, the previous manufacturer and the current manufacturer have contributed data for these lifecycle stages. Risk assessments followed by respective studies were performed to derive critical process parameters and in-process controls (IPCs) with respective proven acceptable ranges or set points.

No major process changes have been applied at the current manufacturing site. The differences comprise product contact material like tubings, mixing bags and filters as well as the container closure system where the suppliers are different and the stopper materials. The impact of the transfer and all changes has been investigated. The parameters identified as site dependent and high risk were investigated experimentally with the goal of establishing proven acceptable ranges for the site.

The bulk solution is aseptically prepared and sterile filtered. The original rationale for selection of sterile filtration instead of terminal sterilisation has not been sufficiently demonstrated. This was a major objection.

Further justification provided by the applicant is still not sufficient. The applicant justifies the choice of aseptic processing and sterile filtration with an increase of degradation products and an increase of the degradation rate corresponding to higher levels after initial heat exposure. The increase of the degradation rate is demonstrated on a sample which was subjected twice to terminal sterilised for 20 minutes at 122°C.

It is acknowledged that degradation products are heat dependent and that there could be an increase of the degradation rate based on the concentration after heat sterilisation. However, the impurity result after one cycle of 20 minutes at 122°C is only 0.3 % higher than with sterile filtration and the increase of the degradation rate was not investigated with these samples. Furthermore, only an overkill cycle (with 20 minutes instead of the standard 15 minutes) has been tried, results for a product specific cycle (F0≥ 8 minutes achieving an SAL of ≤ 10^{-6}) were not provided. It is not considered demonstrated that terminal sterilization is precluded. The major objection remains.

The pre-filtration bioburden has been limited to nmt 1 cfu/10 mL.

The validated filters are scale down models of the filters used for commercial manufacture of the drug product. Filter flush volume has been defined based on data.

The suitability of the container closure system has sufficiently been demonstrated based on drug product stability studies, glass delamination studies and studies on extractables/leachables from all components.

The evaluation on extractables/leachables also comprised the manufacturing equipment for the drug substance and drug product. No issue has been identified in this respect.

Manufacture of the product and process controls

Commercial batch size range is defined.

The unit operations with their associated process controls are described. The controls are a combination of manufacturing process parameters, in-process controls and quality attributes of input materials. The unit operations comprise buffer solution preparation, bulk solution preparation, filtration of the bulk solution, filling stoppering and capping, inspection, and packaging. All critical process parameters and in-process controls identified during development are included in section P.3.4.

The applicant claimed a continuous process verification approach and concluded that process validation data in section P.3.5 were not required. However, the applied process controls were the minimum to control the manufacturing process and were not sufficient for continuous process verification. As continuous process verification was not applicable at least three full scale were requested in accordance with the guideline on process validation. This was a major objection.

A continuous process verification approach is no longer claimed by the applicant. Section P.3.5 has been revised to include process validation data for three additional batches manufactured in 2015. The combination of the original Process Performance Qualification campaign (data are included in section P.2.3) and the batches filled in 2015 represent two lots at the maximum, minimum and intermediate batch volumes of the production scale. The bracketing approach to cover the production scale range and the fill volumes is accepted. Representative media fill results are included. However, the provided validation data is not complete to demonstrate that the manufacturing process consistently leads to

drug product of the desired quality. For two batches the pre-filtration bioburden is missing and for all batches manufactured in 2015 the release results are not provided.

Product specification

The drug product specification comprises all necessary quality attributes to control drisapersen sodium solution for injection. However, with respect to the limits there are some issues.

The limits proposed for assay at release and shelf-life are not acceptable. In accordance with the Annex I of the Directive 2001/83, as amended, and the provided data the assay limits at release and shelf life should be tightened to 95.0-105.0%. The shelf-life limit for one class of specified impurities is also not substantiated and should be tightened to the proposed limit for release.

An increase for these impurities has only been observed at accelerated storage conditions. The drug product will be stored at a refrigerator and therefore the proposed shelf-life specification for these Impurities has not been justified by stability data. The limit should be tightened to the proposed limit at release. The release limit is currently acceptable, however, the applicant should commit to revise this limit when data for 5 additional drug product batches with the commercial process are available. The applicant should also commit to revise the limit for a second specified impurity when data for 5 additional drug product batches with the commercial process are available.

Container Closure System

Descriptions of the container closure system are provided as requested. However, Ph. Eur. 3.2.9 is not applicable for fluoro resin polymer coated chlorobutyl rubber stoppers. A detailed specification should be provided.

Stability of the product

Stability study results have been provided for drug product manufactured at the current manufacturing site (primary stability batches stored for up to 12 months) and at the previous manufacturing site (supportive stability batches stored for up to 44 months). The applied manufacturing process is basically the same at both sites and the primary packaging is not identical but comparable. It is therefore in principle considered acceptable to utilize the stability data for the supportive batches as basis for the shelf-life proposal. However, the proposed shelf-life of 36 months based on data for the supporting stability batches is not acceptable. Though for 3 of the 7 supporting stability batches data after 44 months of storage at 2-8°C is provided, the testing after 44 months was not performed by the previous manufacturer, the original stability testing site, and partly different methods were applied. This precludes comparability with the data provided by the original testing site for 24 months and therefore only a shelf-life of 24 months for the drug products when stored in a refrigerator (2-8°C) can be accepted. The SmPC should be updated respectively.

The labelling statement to keep the vials in the outer carton, protected from light is in accordance with the results of photo stability testing.

3.1.4. Conclusions on the chemical, pharmaceutical and biological aspects

The application is not approvable as there are unresolved issues. One Major Objection remains concerning the choice of the sterilisation method of the drug product.

3.2. Non clinical aspects

3.2.1. Pharmacology

Primary pharmacodynamics *in vitro*

Drisapersen was selected from a set of 30 different 2'-O-methyl phosphorothioate AONs (length of 15 to 24 nucleotides), which had been designed to target exons 2, 29, 40-51 and 53 of the dystrophin pre-mRNA. The effective exon skipping effective activity of drisapersen was confirmed in primary human myoblasts and in myotubes from derived from different DMD patients carrying deletions of exons 50 or 52, exons 45-50, 48-50 or 49-50.

The novel transcript produced by exon skipping was always the major product, because out-of-frame transcripts are subject to nonsense-mediated mRNA decay. Hence, they are less stable and are not considered to increase the risk of side effects during therapeutic application.

Human myotube cultures also revealed that the metabolites generated by 3'-exonucleases from drisapersen, are generally capable to induce skipping of exon 51, but their efficiency gradually decreases with successive removal of nucleotides from the 3'-end. Accordingly, the metabolite that lacked 10 nucleotides from its 3'-end was completely inactive, which is generally attributed to the lower melting temperature of 3'-shortened AONs with the target pre-mRNA.

Primary pharmacodynamics *in vivo*

The sequence alignments of drisapersen and its potential degradants caused by either depurination of an adenosine nucleotide, or by removal of 1 to 5 nucleotides from their 3'-ends using the BLAST software and the human genomic and transcript database of the US National Center for Biotechnology Information only revealed the expected 100 % reverse complementarity with exon 51 of the human dystrophin pre-mRNA. No other reverse complementary region >80 % in the human genome was identified. For this reason, the risk of possible interaction of drisapersen or its degradants with other transcripts or sites in the human genome is regarded highly unlikely.

Among different species, the target sequences of drisapersen in the dystrophin pre-mRNA of humans, Cynomolgus and Rhesus monkeys are identical, whereas two mismatches exist in the mouse and rat sequence. This sequence specificity precluded primary pharmacodynamic studies in animals. To substitute for the lack of pharmacodynamic studies with drisapersen in animals, the restoration of dystrophin levels was demonstrated with the mouse-specific antisense oligonucleotide 23AON, which contains the same 2'-O-methyl phosphorothioate backbone like drisapersen in two mouse models of DMD: 1) in *mdx* mice that harbour a nonsense mutation in exon 23 of the mouse dystrophin gene and2) in the more severely affected *mdx/utrn*$^{+/-}$double mutant mice, which have a reduced lifespan of 2-3 months due to additional haploinsufficiency of the dystrophin homologue utrophin. With respect to the lack of more appropriate animal models of DMD, this approach is agreed, because it confirms the feasibility of exon skipping *in vivo*. As the phenotype of *mdx* mice is clearly less severe than that of DMD patients, the activity of any compound in *mdx* mice cannot directly predict its clinical efficacy. Consequently, the exon skipping activity of drisapersen in myotube cultures of healthy volunteers and DMD patients *in vitro* provides the only support of the clinical efficacy of drisapersen in DMD patients. Nevertheless, these pharmacodynamic studies indicate that effective AON doses need to be tightly balanced with AON levels in non-muscular tissues, which are subject to severe side effects (e.g. kidneys, liver).

Safety pharmacology

Drisapersen revealed no relevant inhibition of hERG currents *in vitro* and did not affect cardiovascular and respiratory function in monkeys at s.c. doses up to 12 mg/kg/week, which translated into C_{max} and AUC_{0-t} of 23.5 µg/ml and 492 µg·h/ml. In addition, drisapersen did not affect CNS and behavioural

parameters at s.c. doses up to 600 mg/kg in mice. The absence of relevant arrhythmogenic potential and effects on CNS function is known from other AONs and may be attributed to their large molecular size that most probably interferes with their interaction with the cardiac hERG channel and permeation of the blood-brain-barrier, respectively.

Pharmacodynamic drug interactions

Based on the specific mode-of-action of drisapersen, its molecular size and charge, no interaction with other drugs are foreseen. Indeed, the exon 51 skipping activity of drisapersen was not influenced by co-treatment with prednisolone in myogenic cell cultures from DMD patients *in vitro*. Similarly, the exon 23 skipping efficiency of 23AON in *mdx* mice was not altered by the concomitant administration of prednisolone *in vivo*. Thus, the possible co-medication of drisapersen with glucocorticoids in DMD patients is not expected to result in a pharmacodynamic interaction. Nevertheless, cautious use of anticoagulants is advisable during drisapersen therapy of DMD patients, because drisapersen prolongs aPTT and decreases platelet counts.

3.2.2. Pharmacokinetics

Absorption

The absorption of drisapersen was mainly investigated in toxicokinetic analyses of repeated-dose toxicity studies in mice, rats and monkeys. Following i.v. administration, the drisapersen plasma levels dose-proportionally increased in all species, but then rapidly declined below 1% of C_{max}. After repeated s.c. administration, drisapersen was also quickly absorbed in mice and rats with t_{max} around 0.25 h post dosing, whereas absorption was slightly delayed in monkeys with t_{max} between 3-5 h post dosing. Independent of the i.v., i.m. or s.c. routes of administration, the dose-proportionally increased plasma exposure rapidly declined in all species followed by very slow elimination phases. No differences between genders were noted in juvenile mice. After long-term repeated s.c. dosing, drisapersen slightly accumulated in plasma of mice (1.5- to 2.3-fold increased AUC) and to a lesser extent in monkeys. Drisapersen remained quantifiable in plasma until the end of the 39 weeks recovery periods of the two chronic toxicity studies in monkeys, which indicates extensive tissue distribution followed by continuous release of drisapersen from the s.c. injection depot into plasma. This coincides with a long half-life of 36.8 to 63.5 days in monkeys, which has been similarly reported for other AONs.

Distribution

Drisapersen showed very high plasma protein binding in mice, rats, monkeys and humans of 96.6 to >99.9 %.Quantitative Whole Body Autoradiography (QWBA) of i.v. administered ^{14}C-labelled drisapersen in CD-1 and *mdx* mice revealed comparably rapid and wide tissue distribution in both mouse strains with highest concentrations in kidneys, liver, spleen and lymphoid tissue, bone marrow, diaphragm, skin and rectum mucosa. In contrast, the lowest concentrations were determined in brain, spinal cord, seminal vesicle, testes and eye lens. The low levels in the CNS and testes are known from other AONs, because their molecular size and hydrophilicity interferes with the passage across blood-brain- and blood-testis-barriers. The radioactivity concentrations were slightly higher in skeletal muscle of *mdx* compared to CD-1 mice, which is in line with earlier results that had indicated limited distribution of 2'-O-methyl phosphorothioate AONs into healthy muscle tissue compared to dystrophic muscle.

Following repeated s.c. injections, the kidney concentrations of drisapersen increased dose-proportionally in mice and less than dose-proportionally in kidney cortex and liver of monkeys suggesting tissue saturation. In the chronic toxicity studies in both species, drisapersen remained detectable in the kidneys until the end of the respective recovery periods (20 and 35 weeks in mice, 39 weeks in monkeys).

Metabolism

The modification of AONs with a methylated phosphorothioate backbone improves stability against nuclease-mediated degradation. Accordingly, unchanged drisapersen was the major drug-related material in the chronic s.c. toxicity studies in mice and monkeys and following i.v. injection of ^{14}C-labelled drisapersen in the QWBA/mass balance study in CD-1 and *mdx* mice. The metabolic profiles in both mouse strains and in monkeys were generally comparable and were mainly characterised by decreasing amounts of metabolites with sequential exonucleolytic removal of nucleotides from the 3'-terminus (up to n-13). Minor metabolites revealed exonucleolytic cleavage from the 5'-terminus (up to n-12). The same metabolic pattern was determined in plasma samples of DMD patients. In samples of all species including humans, minor oxidative desulfurization was observed, which was attributed to the extraction procedure with Triton-X100.

Excretion

^{14}C-labelled drisapersen was similarly excreted in male CD-1 and *mdx* mice, predominantly by the renal route. Within 24 h, about 23 % and 12.4 % of the initially injected i.v. dose was eliminated by urine in CD-1 and *mdx* mice, respectively. Due to the extensive tissue distribution and accumulation of drisapersen, drug elimination slowly progressed thereafter and approximately 37.4 and 39.1 % of the dose were still determined in the carcasses of CD-1 and *mdx* mice at 28 days post dosing.

Pharmacokinetic drug interactions

Phosphorothioate AONs generally do not interact with CYP450 enzymes or drug transporters and drisapersen did neither induce, nor inhibit CYP450 enzymes in human hepatocytes (see Clinical AR for further information). Although phosphorothioate AONs like drisapersen are known to bind extensively to plasma proteins, no displacement of small molecule drugs has been described at therapeutically relevant concentrations. Thus, the risk for pharmacokinetic interactions of drisapersen is regarded low.

3.2.3. Toxicology

The toxicity of drisapersen was investigated after single and repeated administration in compliance with GLP. In view of the intended target population of male DMD patients and the proposed route of therapeutic administration, drisapersen was exclusively studied in male animals using s.c. injections for up 27 weeks in mice, up to 13 weeks in rats and up to 39 weeks in monkeys. In addition, i.v. injections were analysed in mice and monkeys, while i.v. and i.m. routes were tested in rats.

Pro-inflammatory effects

The toxicity of drisapersen is characterised by prominent pro-inflammatory effects that are related to its extensive and persistent distribution into various tissues and accounted for the majority of premature sacrifices or deaths in mice and monkeys. As early sign of inflammations, lymphocytic hyperplasia in draining lymph nodes around the injection site was apparent after single s.c. administration of drisapersen in mice. Following repeated s.c. administration in mice, rats and monkeys, lymphoid hyperplasia in spleen and lymph nodes and a reduced thymus were observed, which was accompanied by the release of pro-inflammatory biomarkers (MCP-1, IL-6) and reductions of the albumin/globulin ratio in all species. In addition, elevations of haptoglobin, CRP and fibrinogen were noted in monkeys. Increases of monocytes, macrophages and neutrophils were widely found across species indicating activation of the innate immune system. These inflammatory cells infiltrated multiple organs leading to accumulations of drisapersen as basophilic granules in proximal tubular epithelial cells of the kidney, centrilobular hepatocytes and Kupffer cells of the liver of mice and monkeys, which is known from other oligonucleotides. Basophilic granules were also detected in the pituitary gland and testicular Leydig cells of mice and in the adrenal and salivary glands of monkeys. In the lymph nodes and at the injection sites of all species, macrophages were found that had apparently taken up drisapersen by phagocytosis. Moreover, the complement system was activated in monkeys

(complement split factors C3a and Bb), but the concomitantly decreased total complement activity suggests functional impairment of the cascade. Activation of the complement system has been also reported for other phosphorothioate AONs in monkeys and, hence, appears to be a class-effect. However, long-term drisapersen therapy also decreased Complement factor C3 in clinical trials (study nos. DMD114876 and DMD114044; see clinical AR for further evaluation). Nevertheless, monkeys seem to be more susceptible for complement system activation than humans (see mechanistic considerations below).

The pro-inflammatory effects of drisapersen culminated in target organ toxicities in the kidneys, liver and at the injection sites of all species and in the vascular system of rats and monkeys.

Kidney toxicity

Drisapersen elicited glomerulopathies in the kidneys of mice, rats and monkeys with different cellular characteristics that increased in severity with dose and treatment duration and even progressed in the respective recovery periods. In mice, renal toxicities comprised changes in the basement membrane, hypertrophies of endothelial cells and lysosomal uptake of electron-dense material in podocytes, endothelial and mesangial cells. In addition, glomerular accumulation of hyaline matrix and amyloid leading to renal papillary necrosis or degeneration of proximal renal tubules were evident, which increased the mortality in mice. In rats, glomerulopathies with hyaline cast formation, renal tubular vacuolation and single cell necrosis in kidneys and adrenal glands were determined. In contrast, the glomerulopathy of monkeys was characterised by hypertrophied endothelial cells, lymphoid and mixed inflammatory cell aggregates within the interstitium and lysosomal inclusion bodies within proximal tubular epithelial cells, capillary endothelial cells, mesangial cells and podocytes. Within the glomeruli of monkeys, the deposition of complement split factor C3c was confirmed. These glomerulopathies coincide with those described for other phosphorothioate AONs. Signs of proteinuria were observed by hyaline casts in rats and by urinary excretion α1-microglobulin, KIM-1, β2-microglobulin, microalbumin and clusterin in monkeys. Accordingly, a warning for regular monitoring of nephrotic range proteinuria was proposed for section 4.4 of the SmPC and renal toxicity was named as an identified risk in the RMP.

Liver toxicity

The accumulation of drisapersen in the liver promoted increased metabolic activity as indicated by the enlarged organ in all animals and the concomitant elevations of ALT and AST and reduced levels of AP. In mice and monkeys, single hepatocellular necrosis was determined, whereas in monkeys, lympho-histiocytic infiltration and centrilobular vacuolation of the liver were additionally observed. Although these liver changes reversed in the respective recovery periods, a warning for regular testing of liver enzymes was suggested for section 4.4 of the SmPC and hepatotoxicity was named as an identified risk in the RMP.

Vascular inflammations and thromboembolic risk

Irreversible multi-organ perivascular inflammation with intimal and endocardial thickening were evident in drisapersen-treated monkeys, which have been previously described for other phosphorothioate AONs in monkeys and were attributed to activation of the complement system resulting in sustained decreases in plasma C3 concentrations and, hence, reduced clearance of immune complexes from the circulation. However, perivascular inflammations were also observed after i.v. drisapersen infusion into rats. Furthermore, three thromboembolic events were detected in monkeys after long-term s.c. drisapersen treatment over 26 and 39 weeks, respectively.

DMD is also associated with abnormal coagulation parameters, like fibrinogen degradation products, thrombin/anti-thrombin complex and prothrombin fragment and venous thromboembolism occurs frequently in DMD patients correlating with the decrease in muscle mass, prolonged immobility and ventilator use in these patients. It should be noted that the concomitant glucocorticoid medication in

the clinical drisapersen program presumably lowered the risk for vascular inflammations. Moreover, elucidation of the clinical risk for thromboembolic events is further complicated by the fact that healthy monkeys (and rats) were used in toxicity studies, which do not reflect the compromised status of DMD patients. For this reason, it is difficult to directly correlate the two thromboembolic SAEs in the clinical program of drisapersen with the thromboembolic events observed in two different chronic toxicity studies in monkeys. Although the Applicant included vasculitis and thromboembolism as important potential risks in the RMP, minimisation of the inherent hazard for thromboembolic events in DMD patients was considered still warranted and pursued with the clinical assessment.

Local tolerance

Local intolerabilities comprising scabs, erythema, subcutaneous oedema, haemorrhages and mixed infiltrates of mononuclear inflammatory cells in the subcutis and dermis including granulocytes and macrophages in association with myofiber degeneration/regeneration, fibroplasia/fibrosis, collagen degeneration and/or necrosis were noted at the s.c. injection sites in mice and monkeys. These injection site reactions increased in severity with prolonged dosing and only partially recovered in subsequent treatment-free periods. Local intolerance was also common in clinical trials and a warning for regular monitoring was therefore added to section 4.4 of the proposed SmPC and termed as an identified risk in the RMP.

Haematology

Drisapersen induced anaemia in all species. The increased erythrocyte sedimentation rate and the higher levels of neutrophils and monocytes were indicative of ongoing inflammations. In addition, reductions in thrombocytes as well as transient prolongations of aPTT and PT were determined in all species including DMD patients. These haematological alterations reversed in the long-term recovery periods in mice and monkeys. Mortality due to thrombocytopenia with haemorrhages in multiple organs were only observed in the 26 week chronic toxicity study in monkeys of Mauritius origin, but did not occur in the 39 week chronic toxicity study in monkeys of Chinese origin despite similar dosages. Nevertheless, a warning of thrombocytopenia was included in section 4.4 of the proposed SmPC and the concomitant clinical use of anticoagulants, thrombolytics or antiplatelet agents should be avoided. In addition, thrombocytopenia was named as an identified risk in the RMP.

Toxicokinetic

The exposure at the NOAELs for the observed target organ toxicities was either similar to or even below human therapeutic plasma levels at the recommended clinical s.c. dose of 6 mg/kg drisapersen. Hence, these non-clinical studies in animals just indicate the target organ toxicities of drisapersen, but do not provide reliable safety margins with regard to the proposed chronic treatment of drisapersen in DMD, which has been considered for the clinical safety assessment.

Genotoxic and carcinogenic potential

Drisapersen was negative in the standard battery of genotoxic tests, although these studies are not considered to provide meaningful data for oligonucleotides (see EMEA/CHMP/SWP/199726/2004). As drisapersen does not contain long homopurine stretches and is only 20 nucleotides in length, the potential for triplex formation with genomic DNA can be excluded. Thus, drisapersen is not considered to exert any relevant genotoxic potential.

The carcinogenic potential of drisapersen has not been elucidated yet, but short-term tolerability studies over 4 and 13 weeks in rats and over 4 weeks in wildtype CB6F1-nonTgrasH2 mice were conducted to aid in the selection of adequate dosages. With respect to the prominent toxicities observed in these studies, it is not regarded feasible to establish doses for life-time carcinogenicity studies. In view of the lack of a genotoxic potential and the severe nature of DMD with reduced life-expectancy, life-time bioassays for carcinogenicity in rodents are therefore not recommended.

Reproductive and developmental toxicity

As DMD almost exclusively affects male patients, the investigations of reproductive toxicity have been limited to potential influences of drisapersen on male fertility and general reproductive performance in mice, which is accepted. This study did not indicate adverse effects on male reproduction up to the highest s.c. dose of 300 mg/kg resulting in a more than 6-fold safety margin with regard to human therapeutic AUC.

The tolerability of drisapersen has so far only been investigated in two 4 week dose-range finding studies in juvenile male and female mice. In addition, repeated-dose toxicity studies were conducted in mice dosed from 4 weeks of age until adulthood, whereas monkeys were treated from 2 years of age. In line with the conclusions of the PDCO, the mice used in these studies are regarded equivalent to human development of a 5 year old child, while the monkeys corresponded approximately to the development of a 12 year old human child. Thus, further clinical development in DMD patients below 5 years of age will have to await prior completion of a pivotal juvenile toxicity study in mice, which has been considered for the proposed indication in section 4.1 of the SmPC.

3.2.4. Ecotoxicity / environmental risk assessment

As drisapersen is a chemically modified RNA, which will be subject to natural degradation pathways, the exemption from the ERA was agreed.

3.2.5. Discussion on non-clinical aspects

In view of the specificity of drisapersen for the human dystrophin sequence, the primary pharmacodynamic activity of drisapersen to induce skipping of exon 51 has only been demonstrated in myotube cultures of healthy volunteers and DMD patients *in vitro*. These investigations corroborate the feasibility of exon 51 skipping and indicate a gradually decreased activity of the 3'-metabolites of drisapersen, which are generated by successive exonucleolytic cleavage of nucleotides from the 3'-end. To substitute for the lack of pharmacodynamic *in vivo* studies with drisapersen, the exon skipping efficiency of the antisense oligonucleotide 23AON,which is specific for the mouse dystrophin pre-mRNA and contains the same 2'-O-methyl phosphorothioate backbone like drisapersen, was investigated in two mouse models of DMD, i.e. *mdx* and the more severely affected *mdx/utrn*$^{+/-}$double mutant mice. However, 23AON revealed limited exon skipping activity and restoration of dystrophin protein synthesis in these mice. Despite the structural similarity, the activity of 23AON in *mdx* mice can obviously not directly predict the clinical efficacy of drisapersen, because the phenotype of *mdx* mice is clearly less severe than that of DMD patients. Consequently, the exon skipping activity of drisapersen in myotube cultures of healthy volunteers and DMD patients *in vitro* provides the only support of the clinical efficacy of drisapersen in DMD patients.

Following s.c. administration, drisapersen was rapidly absorbed and distributed extensively into various tissues. The metabolic profile of drisapersen is consistent across species and mainly characterised by exonucleolytic removal of nucleotides from the 3'-terminus, whereas metabolites generated by 5'-exonucleolytic cleavage play a minor role. The elimination of drisapersen slowly progresses, predominantly by the renal route. These ADME properties and the absence of relevant interaction with CYP450 enzymes coincide with published experience with other phosphorothioate AONs that also do not suggest any interference with drug transporters or displacement of small molecule drugs from plasma protein binding.

Drisapersen elicited prominent pro-inflammatory effects in toxicity studies that are related to its extensive and persistent distribution into multiple tissues and culminated in target organ toxicities in the kidneys, liver, haematological parameters and at the injection sites of all species. The glomerulopathies in the kidneys of mice, rats and monkeys were associated with proteinuria and

showed different cellular characteristics, increased in severity with dose and treatment duration and even progressed in the treatment-free recovery periods. Other toxicities known for the class of AONs originating from drisapersen accumulation are the reversibly increased metabolic activity in the liver with single hepatocellular cell necrosis and the common local intolerability of drisapersen at the injection sites of all species including humans. The severity of local intolerance increased with the treatment duration and only partially recovered upon cessation of administrations. Moreover, haematological changes have been described for other AONs and were also observed in all species treated with drisapersen. These abnormalities included transient prolongations of aPTT and PT and reductions in platelets. Mortality due to thrombocytopenia with haemorrhages in various organs was only evident in the 26 week chronic toxicity study in monkeys, whereas the decrease in thrombocytes in the 39 week chronic toxicity study in this species did not lead to mortality despite similar dosages. Although these renal, liver and haematological toxicities of drisapersen are generally established for the class of AONs, they correlate with clinical adverse events. It was therefore deemed necessary that they are subject to additional monitoring during clinical treatment and result in restrictions of potential co-medications.

Drisapersen also evoked irreversible multi-organ perivascular inflammation with intimal and endocardial thickening in rats and monkeys. Inflammations of the vascular system have been previously described for other phosphorothioate AONs in monkeys and were attributed to activation of the complement system leading to sustained decreases in plasma C3 concentrations and reduced clearance of immune complexes from the circulation. Vasculitis appeared to culminate in three thromboembolic events in the two chronic toxicity studies in monkeys, which coincide with two clinical SAEs. However, DMD is generally associated with abnormal coagulation parameters and venous thromboembolism occurs frequently in DMD patients correlating with the muscle loss, prolonged immobility and ventilator use in these patients. Clarification of the clinical risk for thromboembolic events is complicated by the concomitant glucocorticoid medication in the clinical drisapersen program that presumably inhibited the occurrence of vasculitis. In addition, the healthy monkeys (and rats) used in toxicity studies do not reflect the compromised pathology of DMD. Therefore, the two clinical SAEs of thromboembolism cannot be directly correlated with the thromboembolic events in monkeys. Although the Applicant included vasculitis and thromboembolism as important potential risks in the RMP, minimisation of the inherent hazard for thromboembolic events in DMD patients was still required and pursued with the clinical assessment.

As the exposure of animals at the proposed NOAELs in toxicity studies was either similar or even below human therapeutic plasma levels, no reasonable safety margins with regard to the proposed chronic treatment of drisapersen in DMD were established.

Prominent toxicities in short-term tolerability studies in rats or after chronic treatment in mice question the feasibility to perform carcinogenicity investigations in these species at clinically meaningful dosages. With regard to the lack of a genotoxic potential of drisapersen and the severe nature of the proposed indication with reduced life-expectancy of the patient population, carcinogenicity studies in rodents were consequently regarded dispensable.

The investigation of potential effects of drisapersen on male fertility and general reproductive performance in mice did not reveal any cause for concern. In the light of the target population of male DMD patients, evaluations of influences on embryo-foetal or pre-/postnatal development were not deemed necessary. As the animals tested in repeated-dose toxicity studies covered the human development of a 5 year old child, the clinical use of drisapersen was limited to DMD patients aged 5 years or above.

3.2.6. Conclusion on non-clinical aspects

All non-clinical *"Other concerns"* have been either appropriately addressed by the Applicant or were further pursued with the clinical safety assessment.

3.3. Clinical aspects

The drisapersen clinical development programme is composed of nine clinical studies in 326 boys with DMD. Of the 326 subjects treated in the clinical development programme, 312 received at least one dose of drisapersen.

The cut-off date for inclusion of data in this submission is 31 August 2014. In September 2013 dosing was halted in all studies. No subjects received drisapersen from September 2013 up to the cut-off date.

At the clinical cut-off date for this submission, seven of the clinical studies were completed or terminated and had final clinical study reports (CSRs) available:

- Two single-dose Phase I/II studies (PRO051-01 and DMD114118)
- One open-label repeat-dose Phase I/II study (PRO051-02)
- Two Phase II placebo-controlled studies (DMD114117 and DMD114876)
- One pivotal Phase III placebo-controlled study (DMD114044)
- One long-term open-label extension study (DMD114349).

Subjects continue to be observed in two additional long-term extension studies:

- Study DMD114673 (long-term extension of PRO051-02) - an interim CSR was produced reporting data available up to Week 188 of the study and reporting the results of an intravenous sub-study undertaken in 7 subjects after Week 188.
- Study DMD115501 (long-term extension of DMD114876) - because very limited patient exposure had occurred prior to the halting of dosing by GSK, only information on serious adverse events (SAEs) is included in this submission.

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

Study No. Status	Study Design No. of centers / countries	Test product(s) Dosage regimen Route of administration	DMD Population	Duration of Treatment
Single-dose studies				
PRO051-01 Completed	Phase I/II Open-label, rising dose Single center	0.8 mg (4 x 0.2 mg) drisapersen Intramuscular	N=4 ambulant and non-ambulant subjects	Single dose
DMD114118 Completed	Phase I Randomized, placebo-controlled, rising dose 2 centers in 2 countries	3 mg/kg, 6 mg/kg, 9 mg/kg, or 12 mg/kg drisapersen SC, Dose-matched placebo No subjects received 12 mg/kg dose	N=20 non-ambulant subjects	Single dose
Short-term repeat-dose open label study				
PRO051-02 Completed	Phase I/II Open-label, rising dose 2 centers in 2 countries	0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg, or 6 mg/kg drisapersen SC once weekly	N=12 ambulant and non-ambulant subjects	5 weeks
Repeat-dose placebo-controlled studies in ambulant boys				
DMD114117 Completed	Phase II, pivotal Randomized, double-blind, parallel-group, placebo-controlled 13 centers in 9 countries	SC 6 mg/kg drisapersen twice weekly for 3 weeks (loading dose), then either: Continuous: SC 6 mg/kg/wk or Intermittent: alternating weeks of SC 6 mg/kg twice weekly and 6 mg/kg/wk for 6 weeks followed by 4 weeks off-dose period Dose–matched placebo	N=53 ambulant subjects; 6MWD≥ 75m, able to Rise from floor ≤7s	48 weeks
DMD114876 Completed	Phase II, pivotal Randomized, double-blind, parallel-group, placebo-controlled 13 centers in 1 country	3 mg/kg or 6 mg/kg drisapersen, SC once weekly Volume-matched placebo	N=51 ambulant subjects; 6MWD≥ 75m, able to Rise from floor ≤15s	24 weeks (followed by 24-week post-treatment period with no treatment)
DMD114044 Completed	Phase III, pivotal Randomized, double-blind, parallel-group, placebo-controlled 44 centers in 19 countries:	6 mg/kg drisapersen SC once weekly Dose-matched placebo	N=186 ambulant subjects; 6MWD≥ 75m	48 weeks
Long-term extension studies				
DMD114673 (Extension to PRO051-02) Ongoing	Phase I/II, pivotal Open-label uncontrolled extension of PRO051-02 – see above 2 centers in 2 countries:	6 mg/kg/wk drisapersen SC for 72 weeks. After an interval of 8 weeks (Weeks 73-80) off drug, subjects restarted an intermittent treatment regimen of 6 mg/kg/wk drisapersen for 8 weeks, followed by 4 weeks off treatment (12-week cycles) up to 188 weeks. An IV substudy was conducted following Week 188	N=12 ambulant and non-ambulant subjects at start of parent study PRO051-02	Planned: Until launch or termination of development. Actual: Dosing in the study was halted in September 2013, but was restarted after the data cut off for this submission.
DMD114349 Terminated	Phase III, pivotal Open-label extension of DMD114117 and DMD114044 58 centers in 24 countries	6 mg/kg/wk SC drisapersen Subjects with tolerability issues had the option to enter the intermittent arm of 6 mg/kg/wk for 8 weeks followed by 4 weeks off dose. Subjects who did not wish to receive drisapersen or who had to withdraw from both active arms during the study had the option to go into a natural history observation arm.	N=233 subjects, ambulant at start of parent study (DMD114044 or DMD114117)	Planned: Until launch or termination of development (minimum 104 weeks). Actual: Up to 101 weeks at time of termination of dosing in September 2013
DMD115501 Ongoing	Phase III Open-label extension of DMD114876 13 centers in 1 country	6 mg/kg/wk SC drisapersen Subjects with tolerability issues had the option to enter the intermittent arm of 6 mg/kg/wk for 8 weeks followed by 4 weeks off dose.	N=21 subjects, ambulant at start of parent study (DMD114876)	Planned: Until launch or termination of development Actual: Dosing in the study was halted in September 2013, but was restarted after the data cut off for this submission.

3.3.1. Pharmacokinetics

The pharmacokinetic profile of Drisapersen is typical for its substance class (antisense oligonucleotide with 2'O-methyl phosphorothioate backbone). The pK of drisapersen has not been fully characterised in humans (healthy volunteers or DMD patients). A number of assumptions are based on in vitro and non clinical studies and the knowledge of similar products (AONs), what is deemed acceptable.

Analytical Methods

Statement on GLP compliance and bio-analytical audits is given.

Concentrations of drisapersen were determined in 10% human plasma and dried blood spots using ELISA method. All assays were generally validated according to current FDA, EMA and ICH guidances for ligand binding assays.

The ELISA method in human plasma was validated by three different labs; Proxy Laboratories (Prosensa Therapeutics), Eurofins Medinet and Aptuit. A comparison of ELISA measurements from the three different labs showed that results obtained from all three labs were comparable (refer to 4169130001). In the cross validation experiment between Aptuit and Eurofins Medinet and between Prosensa Therapeutics and Eurofins Medinet, 95.8% and 70.8% of the samples, respectively, were within the 30% difference and therefore, the cross validation experiment was accepted.

The use of different methods is acceptable as there were no differences between laboratories in the cross validation. In the case of Pop-PK analyses performed with data from several studies, the validity of the pooling can be assumed.

In general, the pre-study validations of all the bioanalytical methods were consistent and demonstrated an adequate precision and accuracy (both intra- and inter-day) within the calibrated range. Some partial validations were necessary to confirm that minor changes in the methods did not affect the validity of the methods. A partial validation was preformed due to the samples were collected in Li-heparin while the analytical method uses KEDTA. No differences in the anticoagulant were observed.

Demonstration of stability of the analytes in one of the studies can be assumed for all the studies since the storage conditions were similar across studies. All plasma samples were analysed within the validated stability period (72 days at ambient temperature for the dried blood spots and 399 days and 365 days at -20 ºC and -80 ºC in human plasma, respectively).

The in-study validation shows an acceptable calibration standards and QC values met the acceptance criteria.

Some samples reanalysis were carried due to PK reason. For the PK re-assayed, the median values have been reported in the most cases. It could be acceptable take into account that it is not a BE study.

All results for ISR samples were within the 30% acceptance limits. %. The results confirm bio-analytical reproducibility in incurred plasma samples re-analysis.

Analytical Method for the Detection of Anti-Drug Antibody (ADA) by ELISA and western blot method was performed using a validated method. The ELISA results presented in this report demonstrate acceptable Non Specific Binding (NSB), Minimal Required Dilution (MRD), specificity, screening cut point, sensitivity, study drug interference, specificity confirmation cut point and reproducible measurements of anti-drisapersen antibodies in human heparin plasma. In addition, anti-drisapersen antibodies were found to be stable in human heparin plasma after 106 days stored at ≤ -70 ºC.

A limitation of the Western Blot assay is that the Western Blotting is performed using full-length dystrophin protein. In the unlikely event that an induced anti-dystrophin antibody would be only specific to the epitope unique to the truncated form of the protein resulting from the exon-skipping treatment, this antibody may not be detected with this assay. This method was used to qualitatively detect the emergence of dystrophin specific antibodies. No confirmatory assay could be developed as no purified dystrophin protein is available so any positive samples could not be confirmed.

Clinical pharmacology studies

All clinical studies have been performed in DMD subjects, none in healthy volunteers. Whereas early studies (PRO051-01, PRO051-02, DMD114118) included non-ambulant patients, the parmacokinetics of drisapersen has been mainly characterised in ambulant subjects. Although limited, no significant differences were observed between both populations.

Maximum plasma levels are generally reached between 2 to 4 hours after SC administration, after which the plasma levels decline during a rapid initial tissue (re)distribution phase, followed by a slower elimination phase. After 24 hours plasma concentrations have declined to 15% of Cmax or less, and after 1 week to 0.4% of Cmax or less. After repeated administration up to 48 weeks, some increases in AUCs are observed, indicating some accumulation, however not in all studies. Increases were less than 2-fold.

Trough concentrations after repeated dosing increase, indicative of prolonged and accumulating tissue exposure, until reaching steady-state. Dose proportionality was observed in exposures (AUC), especially after repeated dosing, whereas peak plasma levels (Cmax) did increase, but less than dose-proportional.

Table 1 Summary of pharmacokinetic parameters and tissue concentrations of drisapersen obtained during the clinical program

Study Ref No	Treatments (route, dose, regimen)	Parameter				Mean tissue concentration [c]		Study summary location
		Cmax (µg/mL) [a]		$AUC_{0\text{-}24h}$ (µg.hr/mL) [b]		(µg/g)	wk in study	
		Day 1	End of study	Day 1	End of study			
DMD114673 (acute phase)	SC, 0.5 mg/kg/wk for 5 weeks SC, 2 mg/kg/wk for 5 weeks SC, 4 mg/kg/wk for 5 weeks SC, 6 mg/kg/wk for 5 weeks	1.69 3.62 5.27 9.13	1.02 4.11 6.80 11.0	7.5 26.9 40.8 76.7	5.9 25.9 44.2 103	- - - 6.9	- - - 5	section 2.2.1.2.1
DMD114673[d] (extension phase)	SC, 6 mg/kg/wk SC, start of re-dosing	- 8.2	8.7 -	- 105	103 -	14.4 20.3	24 69	section 2.2.3.1.1
DMD114118	SC, 3 mg/kg single dose SC, 6 mg/kg single dose SC, 9 mg/kg single dose	4.99 8.14 8.94	- - -	44.6 87.8 97.8	- - -	- - -	- - -	section 2.2.2.1.1
DMD114117[e]	Continuous: SC, 6 mg/kg/wk or Intermittent: alternating 6 mg/kg biweekly and 6 mg/kg/wk for 6 weeks followed by 4 weeks off-dose period	- -	4.85 4.81	- -	45.5 52.5	11.0 9.8	24 24	section 2.2.2.2.1
DMD114876[f]	SC, 3 mg/kg for 24 weeks SC, 6 mg/kg for 24 weeks	3.05 5.73	2.84 6.24	23.0 46.7	30.2 57.3	2.7 10.8	24 24	section 2.2.2.3.1
DMD114044	SC, 6 mg/kg/wk for 48 weeks	-	-	-	-	4.1 5.2 9.3 16.6 14.0	8 12 24 36 48	section 2.2.2.4.1
DMD114349[g]	SC, 6 mg/kg/week up to 104 weeks divided into sub-groups of placebo in feeder study active treatment in feeder study	5.68 5.81	6.57 6.22	55.2 58.4	80.3 74.1			section 2.2.3.1.1

Note: All studies described in this section were performed using the sodium salt of drisapersen. Drisapersen sodium may be referred to as drisapersen, PRO051, GSK2402968, or h51AON23 in the study reports provided. PK parameters are reported in ng in the reports, however stated in µg in this summary document

Key:

a Geometric mean (CV%) ; b Median (range); c mean tissue concentrations provided in µg/g tissue; wk is the week in which the biopsy was obtained during the study; d up to 177 weeks; tissue concentrations reported are from visit 37 (week 24) and visit 81 (week 69) respectively, re-dosing commenced approximately one year after week 177; e no profile obtained at first dose, end of study is 29 week; subjects started with SC 6 mg/kg twice weekly for 3 weeks and thereafter continued with either the continuous or intermittent regimen; tissue concentrations determined in tibialis anterior muscle reported here (quadriceps excluded from mean); f tissue concentrations at week 24 reported in table; g Cmax and AUC reported are from subjects who had PK profiles on both occasions, end of study is week 48 profile, subjects were dosed up to 104 weeks

- = not determined/available; SC.=subcutaneous, wk = week

Although the median concentrations in muscle and plasma increased with increasing dose, there was no correlation between individual pre-dose plasma concentrations and drug concentrations in muscle.

Distribution of drisapersen was not studied extensively. No radiolabeled study was performed, this is deemed acceptable considering the target population. However reference is made to pre-clinical data.

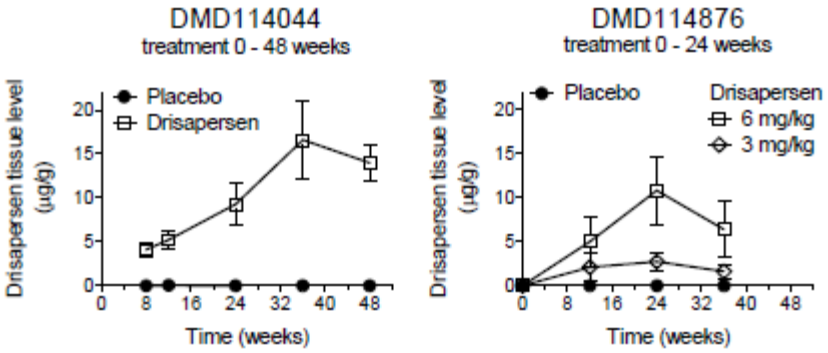
Distribution to the target i.e. muscle tissue was investigated in the muscle biopsies obtained from most subjects and this showed very variable tissue concentrations between subjects.

Muscle tissue concentrations increased after repeated dosing indicating accumulation. At 24-25 weeks, the average tissue concentration in the *tibialis anterior* muscle after repeated SC administrations of

6 mg/kg/wk is around 10 µg/g tissue and quite similar between the different studies, although the variation between subjects is quite high.

Tissue levels increased with increasing dose and after repeated dosing as seen between the 3 and 6 mg/kg dose groups in the DMD114876 study and between the different time points (12, 24 and 36 weeks) during the DMD114044 study and during the DMD114673 study (5, 24 and 69 weeks).

Figure 1 Drisapersen tissue concentrations (µg/g tissue) in muscle biopsies



Data from non-clinical studies and metabolite profiling in human suggest that the major drug-related component in plasma after repeated SC administrations was unchanged drisapersen. Metabolites were primarily formed by sequential cleavage of nucleotides from the 3' terminus of the molecule as was observed in the non-clinical species as well.

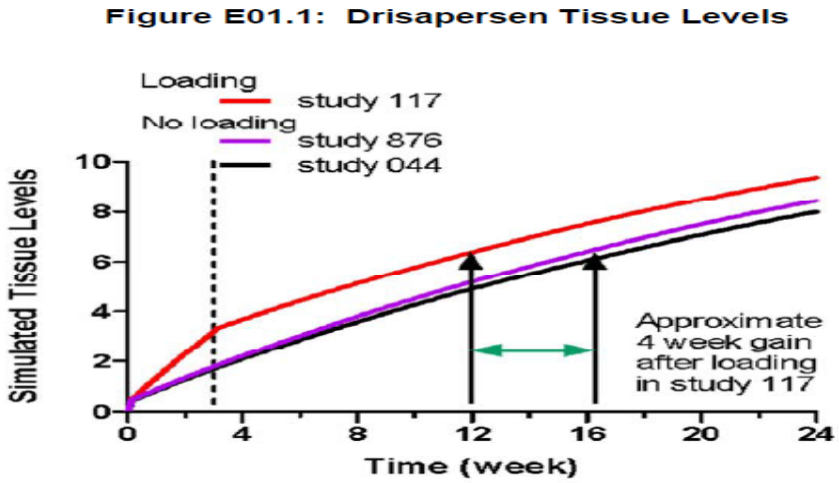
No studies have been performed specifically to evaluate excretion in humans. However, it is believed from radiolabeled data obtained in mice and from studies with similar compounds that initial excretion is rapid in the first 24 hours and mainly through the urinary route. Thereafter elimination is very slowly with most of the administered dose remaining in the tissues, as is evident from the long terminal half-life in both non-clinical species and human DMD subjects (decline of approximately 40% in biopsy homogenate tissue levels 12 weeks after drisapersen treatment is stopped).

Drisapersen has been shown not to be an inhibitor or inducer of CYP450 enzymes and is also not expected to be a substrate of CYP450 enzymes, as previously reported for other second generation AONs based on in vitro. No in vivo metabolic based drug-drug interactions have been reported for second generation AONs or first generation AONs no CYP450/drug transporter effects in humans, resulting from cytokine stimulation would be anticipated for the class of AONs. Hence, no clinical studies evaluating potential metabolic based drug-drug interactions of drisapersen with any concomitant medications have been conducted or deemed warranted.

Drisapersen is highly bound to plasma proteins bound (99.1% to >99.9%) over the concentration range 0.7 to 70 µg/mL apparently mainly to albumin, but also to many other plasma proteins however the potential pharmacokinetic interaction due to protein binding displacement is considered to be negligible (low plasmalevels of drisapersen, slow clearance, weak protein binding).

This tissue pharmacokinetic profile with a long tissue half-life in muscle in the range of 2-3 months supports a weekly dosing regimen. The pharmacokinetic results indicate that also a less frequent dosing or intermittent regimens could be applicable. In addition, the slow accumulation indicates that a loading dose regimen in the first weeks of treatment can be beneficial to reach significant drisapersen muscle levels more rapidly. Furthermore the pharmacodynamic results support the use of a loading dose, as only the study with a loading dose (DMD114117) showed a statistical significant increase of dystrophin compared to placebo. Simulated data indicated that desirable tissue level (>10µg/g) can be reached faster when a loading dose is applied.

Figure 2



The final drisapersen PK model developed in this population analysis can be described as a three compartmental model with first-order absorption. Elimination from the central compartment is described by a time-dependent process. Inter-individual variability is implemented on clearance, central volume and absorption rate. A constant coefficient of variation residual error model is used.

The following covariates are identified as influential on the PK of drisapersen:
Time: both clearance and peripheral volume 2 decrease as time progresses (by about 29 and 72%, respectively); The reason for these time-dependencies are unclear and could relate to disease progression, growing of the boys and/or treatment dependent changes to drug disposition or clearance processes.
Baseline body weight has an effect on absorption rate (-), clearance (+), central volume (+) and peripheral volume 2 (-).
Titer affects clearance (-), volume of second peripheral compartment (+) and inter-compartmental clearance between the central and second peripheral compartments (+).
The **number of injections in the thigh** and the **number of injections in the buttock** both decrease absorption rate.

The apparent volume of distribution is large and ranges between 1210 and 2650 L.

No formal comparative bioavailability or bioequivalence studies were conducted to compare drisapersen drug products as the formulation intended for marketing purposes (i.e., 200 mg/mL drisapersen sodium solution for SC administration) is the same as that was used in the pivotal clinical studies.

3.3.2. Pharmacodynamics

Mechanism of action

Antisense oligonucleotides (AONs) are small synthetic molecules that are chemically modified such that they are resistant to degradation by RNaseH, but instead induce specific exon skipping by blocking exon inclusion signals during pre-messenger ribonucleic acid (mRNA) splicing. Mutation-specific AONs of one or more exons in DMD patients allows restoration of the mutated open reading frame, introduction of novel dystrophin synthesis, and theoretical conversion of severe DMD into a milder BMD phenotype.

Drisapersen (GSK2402968 and PRO051) is a 20mer chemically-modified antisense oligonucleotide with a sequence designed to induce the skipping of exon 51 from the human dystrophin pre-messenger ribonucleic acid (mRNA) during the splicing process, restore the reading frame in mutations causing

truncation of translation, and thereby increase truncated dystrophin expression. The sequence of 5'-UCA AGG AAG AUG GCA UUU CU-3' is specific to human DMD exon 51.

Drisapersen results in exon 51 skipping

An increase in exon 51 skipped dystrophin product intensity after nested RT-PCR and capillary electrophoresis was observed in the 6 mg/kg/wk drisapersen treatment group compared to placebo at Week 48 in study DMD114044. This treatment effect is encouraging considering the large number of subjects in DMD114044 and that the increase was also observed at all time points after Week 12 and across the five main drisapersen specific deletion groups. Also in two other placebo controlled phase II studies increases in exon 51 skip were observed, albeit that the results were more prone to variability because of the lower number of subjects in these studies.

Table 2 Exon skipping across different studies

Study Treatment	Exon skip (a.u.)					
	Week 0			Week 25, 24 or 48[1]		
	Mean ± SE (SD)	Median	n	Mean ± SE (SD)	Median	n
DMD114117*						
Placebo	1.30 ± 0.24 (1.01)	1.1	17	0.73 ± 0.10 (0.41)	0.7	17
Weekly 6mg/kg	1.29 ± 0.57 (2.42)	0.7	18	1.30 ± 0.35 (1.50)	0.9	18
Intermittent 6mg/kg	1.72 ± 0.58 (2.38)	0.9	17	2.60 ± 1.29 (5.34)	0.8	17
DMD114876						
Placebo	2.01 ± 0.39 (1.52)	1.4	15	1.53 ± 0.31 (1.20)	1.4	15
Weekly 6mg/kg	2.69 ± 0.99 (4.09)	1.5	17	4.44 ± 0.86 (3.55)	3.8	17
Weekly 3mg/kg	2.41 ± 1.06 (4.38)	1.0	17	4.37 ± 1.65 (6.78)	3.0	17
DMD114044						
Placebo	-	-	-	1.45 ± 0.22 (1.68)	0.9	56
Weekly 6mg/kg	-	-	-	2.83 ± 0.48 (5.21)	1.6	118

Abbreviations: n = number, SD = standard deviation, a.u. arbitrary units
1: post-treatment biopsy at week 25 (DMD114117), week 24 (DMD114876) and week 48 (DMD114044) of treatment
* results (arbitrary units (a.u.)) of sample with highest tested template input was used for calculation.

Drisapersen results in dystrophin protein expression

Proof of concept for exon skipping in human subjects was obtained following local injections and systemic exposure of subjects to varying doses of drisapersen by increased dystrophin expression in a number of studies. Quantitation of these effects has improved through advancements in staining and in image analysis.

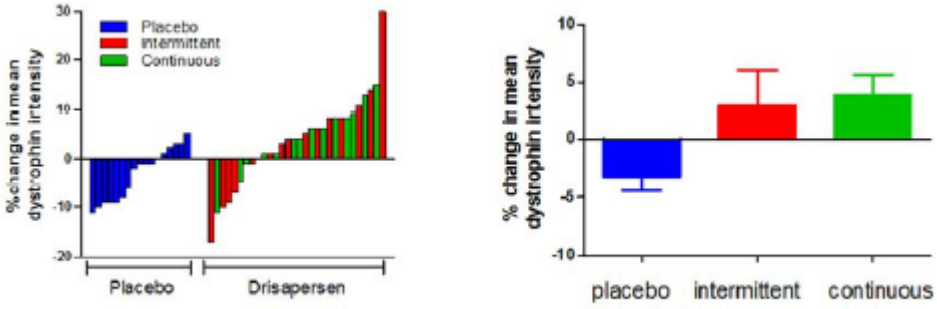
A dose-related effect of drisapersen on dystrophin expression was observed with the most prominent dystrophin signals observed in the two highest dose groups (4 and 6 mg/kg) in study PRO051-02. In addition, the persistence of the dystrophin intensity signal at 2 and 7 weeks after treatment suggested a prolonged drisapersen response. Potentially a combination of slow drug clearance and stability of dystrophin protein.

Treatment with 6 mg/kg/wk drisapersen resulted in an increase in membrane dystrophin protein expression from baseline of 3·9% ± 1.7% (n=15) in muscle biopsies at 25 weeks to a decrease of -3.1% ± 1.3 (n=17) in placebo treated subjects (P = 0.0026) in the placebo controlled phase II study DMD114117. Lower doses resulted in effects which were not statistical significant.

Combined results of immunofluorescence, Western blot analysis and exon skip RT-PCR analysis of pre- and post-treatment biopsies indicate that drisapersen is associated with an increase in dystrophin response in the majority (72 %) of active treated subjects compared with anincrease in dystrophin levels in one (6 %) subject in the placebo group.

The increase in membrane dystrophin protein intensity in DMD114117 indicated a relation with drisapersen drug levels in biopsies. For levels above 10 µg/g nearly all changes in dystrophin were positive, whereas mixed results were observed between 5-10 µg/g with more positive results for the continuous treatment arm compared to the intermittent. There was no straightforward linear relation between the change in dystrophin intensity with change in 6MWD for individual subjects, although more placebo subjects tended to decrease in dystrophin intensity and 6MWD from baseline compared to drisapersen treated subjects. This study was the only placebo controlled study with a significant increase of dystrophin and the only one using a loading dose regimen.

Figure 3 Drisapersen increased membrane dystrophin signal in immunofluorescence analysis in DMD114117 study



In DMD114876, no difference was observed in the change of dystrophin expression at week 24 for 6 mg/kg/wk drisapersen versus placebo. There may be a trend for an increase in dystrophin expression towards Week 36 compared to placebo, which could be the result of prolonged drisapersen muscle exposure even after stopping treatment due to a low tissue elimination rate. In this study, DMD114876, it may have taken longer to reach significant tissue levels of drisapersen exposure (>5 µ/g) because there was no loading dose regimen as applied in the DMD114117 study.

In DMD114044, no pre-treatment biopsy was taken and a pilot experiment performed.

Membrane dystrophin protein intensity appeared to increase over time in biopsies obtained between 8 and 48 weeks after 6mg/kg/wk drisapersen compared to placebo. Given the absence of a pre-treatment comparator and the inter-subject variability in relation to the potential effect and the high proportion of poor quality biopsies in this study (greatly reducing the number of evaluable biopsies in study DMD114044) no further immunofluorescence assessment was performed. Overall it can concluded that drisapersen leads to small increases of dystrophin what supports the postulated mechanism of action, however the predictability of dystrophin levels for clinical effects is considered as limited.

Drisapersen improves muscle pathology

In DMD subjects treated with 6mg/kg drisapersen, serum CK levels decreased compared with placebo treatment across the three placebo-controlled clinical studies, reaching statistical significance in DMD114044. These results are indicative of improvement of muscle fibre membrane integrity by drisapersen. Boys of younger age (5-7 years) with general less disease progression and more muscle mass appear to show a larger treatment effect in DMD114044.

In a sub-study of DMD114876, structural changes in six thigh muscles were assessed by MRI. The natural progression of disease with an increase in apparent fat fraction ranging from 2.7-5.2% in the placebo group (n=5) was reduced by approximately half in subjects receiving 6 mg/kg/wk drisapersen (range: 0.9-3.8%, n=6).

In addition, T2 weighted MRI signal, indicative of oedema and associated inflammation,

was decreased relative to baseline in the 6 mg/kg/wk drisapersen group in the evaluated thigh muscles (range: –0.07 – –0.23; n=14) compared to an increase in the placebo treated group (range: 0.07 – 0.14; n=10).

Secondary pharmacology

Immunogenicity

Plasma samples obtained for pharmacokinetic measurements in clinical study DMD114044, were analysed for anti-drug antibodies (ADA) presence. A total of 109 treated subjects and 50 placebo subjects were conclusively evaluated for ADAs. Overall, of the 109 evaluated treated subjects, 32 subjects (29.4%) had ADAs detected in several plasma samples obtained during the course of the study. Of the 107 treated subjects, 30 had a positive week 48 sample, and two subjects had a positive sample earlier in the study but no week 48 sample was available for these individuals. Only one subject tested positive at Week 8. In contrast, only 2% (one out of 50 subjects) of the placebo subjects were confirmed positive.

A statistical correlation analysis was performed to determine if any relation between the presence of these ADAs and the subjects PK, efficacy, and relevant safety parameters exists. The results of this analysis demonstrate that generally no relevant difference was observed.

3.3.3. Discussion on clinical pharmacology

A reduced clinical pharmacology study program has been conducted. No studies in human have been conducted in healthy subjects due to ethical reasons. This is considered acceptable. Most of the study populations were very small and PK data in the more extensive studies are partially generated only by sparse sampling. Moreover the comparison between the studies is complicated thorough different dosing regimen (continuous/intermittent, with/without loading dose).

Although most studies are limited to very small sample sizes and the results show high variability it can be concluded that the results of the pharmacokinetic profile in plasma and muscle tissue as well pharmacodynamic findings (dystrophin expression, exon skipping) support the proposed mechanism of action. These findings are in line with secondary parameter like significant decrease in serum CK levels and a positive trend in inhibition of structural changes (by MRI).

Drisapersen distributes to muscle and accumulates to steady state levels over a 6-9 month period. Drisapersen treatment resulted in dystrophin exon skipping and for doses above 6 mg/kg in an increase in dystrophin protein.

A sub-population of subjects (30-40%) treated with drisapersen developed ADAs against drisapersen, with a general onset of antibody generation between three to six months of treatment with 6 mg/kg drisapersen injected SC. The presence of ADA did not correlate to muscle concentration, so ADAs do not inhibit distribution of drisapersen to the target organ. A correlation was observed however between increased trough plasma concentration of drisapersen and the presence of ADAs. Further statistical correlation analyses were performed using actual ADA data for the one clinical study, or high trough concentrations as a surrogate for ADA presence for several clinical studies, and revealed no correlation with relevant safety and efficacy parameters.

Anti-dystrophin antibodies have also been detected in pre-treatment and placebo samples or only in sporadic samples. The results should be considered with caution as also placebo treated patients were found positive.

3.3.4. Conclusions on clinical pharmacology

The presented data allow a basic characterisation of the pharmacokinetic profile of drisapersen. Several conclusions are based on population PopPK analysis. The corresponding Pop PK model is considered acceptable.

3.3.5. Clinical efficacy

Dose-response studies and main clinical studies

Drisapersen has been developed for the treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing. An overview of the phase II and III clinical development is presented in the following tables.

Table 3 Summary of the placebo-controlled studies of drisapersen in Duchenne muscular dystrophy (DMD)

Study No. Status	Objective(s)	Study design No. of centres and location Study dates	Test product(s) Dosage regimen Route of administration	Study population	Planned duration of treatment	Number of subjects
DMD114117 Completed	Efficacy, tolerability, safety, PD and PK	Phase II Randomised, double-blind, parallel-group, placebo-controlled 13 centres in 9 countries: Australia, Belgium, France, Germany, Israel, Netherlands, Spain, Turkey, United Kingdom 01 Sep 2010 – 12 Sep 2012	Drisapersen solution for injection s.c. 6 mg/kg twice weekly for 3 weeks, then either: Continuous: 6 mg/kg/week or Intermittent: alternating weeks of 6 mg/kg twice weekly and 6 mg/kg/week for 6 weeks followed by 4 weeks off-dose period Dose–matched placebo	Subjects with DMD, ambulant boys	48 weeks	53 randomised/ 53 completed
DMD114876 Completed	Efficacy, tolerability. Safety, PD and PK	Phase II Randomised, double-blind, parallel-group, placebo-controlled 13 centres in 1 country: United States 26 Oct 2011 – 04 Nov 2013	Drisapersen solution for injection 3 mg/kg or 6 mg/kg drisapersen, s.c. once weekly Volume-matched placebo	Subjects with DMD, ambulant boys	24 weeks (followed by 24-week post-treatment period with no treatment)	51 randomised/ 51 completed
DMD114044 Completed	Efficacy, tolerability. Safety, PD and PK	Phase III Randomised, double-blind, parallel-group, placebo-controlled 44 centres in 19 countries: Argentina, Belgium, Brazil, Canada, Chile, Czech Republic, Denmark, France, Germany, Italy, Japan, Republic of Korea, Netherlands, Norway, Poland, Russian Federation, Spain, Taiwan, Turkey 02 Dec 2010 – 28 Jun 2013	Drisapersen solution for injection 6 mg/kg s.c. once weekly Placebo	Subjects with DMD, ambulant boys	48 weeks	186 randomised/ 181 completed

Table 4 Summary of the long-term extension studies of drisapersen in Duchenne muscular dystrophy (DMD)

Study No. Status	Objective(s)	Study design No. of centres and location Study dates	Test product(s) Dosage regimen Route of administration	Study population	Planned duration of treatment	Number of subjects
DMD114673 Ongoing (no treatment at cut-off date of 31 Aug 2014 for preparation of this summary)	Long-term safety, efficacy, and PK	Phase I/II Open-label extension of PRO051-02. DMD114673 commenced between 6 and 15 months after the completion of PRO051-02. 2 centres in 2 countries: Belgium, Sweden Ongoing (interim CSR for 30 Jul 2009 – 25 Jun 2013, including i.v. substudy)	Drisapersen solution for injection 6 mg/kg/wk s.c. for 72 weeks After an interval of 8 weeks (Weeks 73–80) off drug, subjects restarted an intermittent treatment regimen of 6 mg/kg/week for 8 weeks, followed by 4 weeks off treatment (= 12 weeks per cycle) up to 188 weeks. An i.v. substudy was conducted following Week 188: - At Weeks 189, 192, and 195, dosing with 0.5, 1.4, and 2.7 mg/kg, respectively via i.v. infusion over 4 hours - At Weeks 198 and 201, dosing with 2.7 mg/kg via i.v. infusion over 2 hours and 1 hour, respectively - At Weeks 190, 191, 193, 194, and 196, dosing with 6 mg/kg s.c. - At Weeks 197,199, and 200, no dosing with drisapersen - Intermittent s.c. schedule recommenced from Week 202 Subjects who did not participate in the i.v. substudy continued to receive the intermittent s.c. regimen after Week 188.	Subjects with DMD, ambulant and non-ambulant boys at start of parent study PRO051-02 The 7 subjects eligible for the i.v. substudy were those with peripheral venous access.	Until launch or termination of development. The main study up to and including Week 188 has been reported in an interim CSR dated 16 Jul 2014 along with the i.v. sub-study. Dosing in the study was halted in September 2013.	Main study: 12 entered / 0 completed (12 ongoing) i.v. substudy: 7 entered 7 completed
DMD114349 Terminated	Long-term safety, efficacy, and PK	Phase III Open-label extension of DMD114117 and DMD114044 58 centres in 24 countries: Argentina, Australia, Belgium, Brazil, Bulgaria, Canada, Chile, Czech Republic, Denmark, France, Germany, Hungary, Israel, Italy, Japan, Republic of Korea, Netherlands, Norway, Poland, Russian Federation, Spain, Taiwan, Turkey, United Kingdom 19 Sep 2011 – 17 Mar 2014 (study termination; no dosing after 20 Sep 2013)	Drisapersen solution for injection 6 mg/kg/week s.c. for minimum of 104 weeks Subjects with tolerability issues had the option to enter the intermittent arm of 6 mg/kg/week for 8 weeks followed by 4 weeks off dose. Subjects who did not wish to receive drisapersen or who had to withdraw from both active arms during the study had the option to go into a natural history observation arm.	Subjects with DMD, ambulant boys at start of parent study (DMD114044 or DMD114117)	Until launch or termination of development. Up to 710 days at time of termination	233 entered (228 on continuous arm, 4 on intermittent arm, 1 on natural history arm) 233 ongoing at time of study termination (205 on continuous arm, 11 on intermittent arm, 17 on natural history arm)

In the phase I/II studies, doses of drisapersen 0.5, 2, 4 or 6 mg/kg/week (study PRO051-02) and 6 mg/kg/week (study DMD114673), respectively have been evaluated. These studies are assessed in section 2. Based on the findings of phase I/II studies, it was concluded by the applicant, that the 6 mg/kg/week dosing regimen presents an appropriate safety and efficacy profile to be taken forward in the clinical programme.

The clinical programme for drisapersen consisted of three studies, i.e. study DMD114117, study DMD114876, both exploratory phase II studies, and one phase III study, study DMD114044, and two additional open-label extension studies. All studies were multicentre, randomised, double–blind, parallel-group, placebo-controlled studies conducted in ambulant boys with DMD resulting from a mutation correctable by exon 51 skipping induced by drisapersen.

Study DMD114117 and study DMD114876 had a rather similar study design. There was an initial screening period (2- to 4-week) followed by a double-blind treatment period. In one treatment group in each study, patients received a continuous regimen of once-weekly drisapersen 6 mg/kg. The main differences between the two phase II studies was the dosing with regard to different treatment arms and the existence/non-existence of a loading dose as well as the treatment duration:

In DMD114117, subjects received either placebo, continuous 6 mg/kg drisapersen, or intermittent 6 mg/kg drisapersen, randomized in a 1:1:1 ratio. All subjects also received a loading dose regimen of twice-weekly dosing with 6 mg/kg drisapersen (or matching placebo) for the first 3 weeks of treatment. Starting with Week 4, subjects then received either once-weekly continuous drisapersen or the intermittent regimen (or, for either group, a matched placebo regimen). In DMD114876, subjects received either 3 mg/kg drisapersen, 6 mg/kg drisapersen, or dose-matched placebo, randomized in a 1:1:1 ratio. In DMD114117, subjects were treated for 48 weeks (including the loading dose period). In DMD114876, subjects were treated for 24 weeks followed by a 24-week post-treatment period.

Patients enrolled in study DMD114117 were required to be able to rise from the floor in ≤7 seconds without aids/orthoses, whereas an ability to rise from the floor in ≤15 seconds was required in DMD114876 (changed from ≤7 seconds by protocol amendment 3). However, only two patients had a rise from the floor time >7 seconds - ≤15 seconds at screening in study DMD114876. Therefore, these small baseline differences are not expected to significantly influence the treatment outcome.

Included patients were Duchenne patients, who are earlier in the disease process. Key inclusion criteria encompassed Duchenne patients with a genetic defect believed to be correctable by drisapersen and that have been treated with corticosteroids for at least 6 months with a stable dose for at least 3 months immediately prior to screening. Included patients were at least 5 years of age, able to walk at least 75 meters in the 6 minute walking distance (6MWD) test and be able to rise from the floor in ≤7 seconds.

Primary endpoint for the phase II studies was the change from baseline at week 24/25 in the 6 minute walking distance (6MWD) test for the different active treatment arms compared to the combined placebo group of each study. During the 6MWD, subjects were asked to walk, at their own preferred speed, up and down a fixed distance until they were told to stop after 6 minutes. The subjects were warned of the time and were told that they could stop earlier if they felt unable to continue. The total distance walked within 6 minutes (or until the subject stopped in case of early termination of the test), the 6MWD, was recorded in meters, as well as any falls.

A wide battery of secondary endpoints was included: Timed function tests (times and grading): change from baseline in rise from floor time, change from baseline in 10 m walk/run time, change from baseline in 4-stair climb; change from baseline in muscle strength total score; change from baseline in the North Star Ambulatory Assessment (NSAA) total score; frequency of accidental falls (during 6MWD); time to loss of ambulation; change from baseline in serum CK concentrations; change from baseline in pulmonary function parameters; Dystrophin expression (muscle biopsies); Clinician Global Impression of Improvement (CGI-I).

The mean age of Duchenne patients for both studies was about 7-8 years. Mean age of diagnosis was about 4 years. All included patients were diagnosed by genetic testing. Overall the treatment arms for both studies were balanced with regard to concomitant medication and glucocorticosteroid usage.

Study DMD114117 (phase II study): A total of 53 patients were randomized in this study. Demographic characteristics were relatively balanced across treatment groups. About 60% of subjects were on a continuous regimen of glucocorticosteroids (combined placebo 61%, continuous drisapersen 67%, and intermittent drisapersen 53%). The time since first symptoms, diagnosis and first

corticosteroid use in the intermittent drisapersen treatment group were slightly longer compared to the other two treatment groups. However, this aspect is consistent with the slightly older mean age of patients in the intermittent treatment group (mean age: combined placebo 6.9 years, continuous 6 mg/kg drisapersen 7.2 years, intermittent 6 mg/kg drisapersen 7.7 years; age >7 years: combined placebo 27.8%, continuous 6 mg/kg drisapersen 38.9%, intermittent drisapersen 58.8%). Mean baseline values for the 6MWD test were slightly higher in the continuous group (427.61 m) compared to the placebo (403.18 m) and the intermittent (394.57 m) treatment group.

Study DMD114876 (phase II study): A total of 51 patients were randomized in this study. Demographic and baseline characteristics were relatively balanced across treatment groups, with the drisapersen 6 mg/kg/week group having slightly lower mean age (combined placebo 8.0 years, 3 mg/kg/week 7.8 years, 6 mg/kg/week 7.6 years) than placebo. The proportion of subjects > age 7 was 9/16 (56%) in the placebo group, 9/17 (53%) in the 3 mg/kg/week group, and 8/18 (44%) in the 6 mg/kg/week group. The time since diagnosis was similar across treatment groups. More than 90% of subjects were on a continuous regimen of glucocorticosteroids (combined placebo 94%, 3 mg/kg/week 88%, 6 mg/kg/week 100%). The time since first symptoms was longest in the drisapersen 3 mg/kg group, while the time since first corticosteroid use was longest in the placebo group and shortest in the drisapersen 6 mg/kg group. Mean baseline values for the 6MWD test were shortest in the drisapersen 6 mg/kg group (396.18 m) and similar in the drisapersen 3 mg/kg and placebo treatment groups, 415.21 m and 416.41 m, respectively.

Study DMD114044 (phase III study): Based on an open-label extension study (Study DMD114673) that was ongoing and in which patients received drisapersen 6mg/kg/week for at least 3 months the drisapersen dose 6 mg/kg was selected for this phase III study. Subjects received either drisapersen 6 mg/kg or placebo (2:1 ratio) given s.c. once weekly for 48 weeks.

In principle, key inclusion and exclusion criteria for study DMD114044 were similar to those used for the two phase II studies, study DMD114117 and study DMD114876, with the exception that in study DMD114044 no definite time was required to be able to rise from the floor (mean baseline RFF time in the placebo group: 13.41 s, in the drisapersen 6 mg/kg/week group: 12.34 s). This lead to the inclusion of a population of broader disease severity only bounded by the ability to walk a minimum of 75 metres in the 6MWD.

Primary endpoint was the change from baseline in muscle function using the 6MWD test assessed at week 48. Most of the selected secondary endpoints were similar to those used in the phase II studies.

A total of 186 patients were randomized in this study. Demographic characteristics were rather similar across treatment groups with the exception, that mean weight was higher in the drisapersen 6 mg/kg/week group (30.1 kg) than in the placebo group (26.9 kg). Age was similar for both treatment groups (mean age: placebo 8.0 years, drisapersen 6 mg/kg/week 8.3 years). The time since first symptoms, diagnosis, and first corticosteroid use were longer in the drisapersen 6 mg/kg/week group than in the placebo group (mean time since first symptoms: 71.8 versus 66.7 months; mean time since diagnosis: 58.0 versus 54.2 months; mean time since first steroid taken: 35.6 versus 29.1 months). Overall concomitant medication and glucocorticosteroid usage was similar across treatment groups. The majority of subjects in both treatment groups received a continuous regimen of glucocorticosteroids (placebo: 85%; drisapersen 6 mg/kg/week: 86%). Mean baseline values for the 6MWD test were slightly lower in the drisapersen 6 mg/kg/week group (337.46 m) than in the placebo group (348.00 m). Overall, in reference to baseline characteristics, the included patient population was compared to those of the two phase II studies more heterogeneous, older (min: 5, max 16 years of age) and not able to walk at baseline as far as patients did in the phase II studies.

Summary of main efficacy results

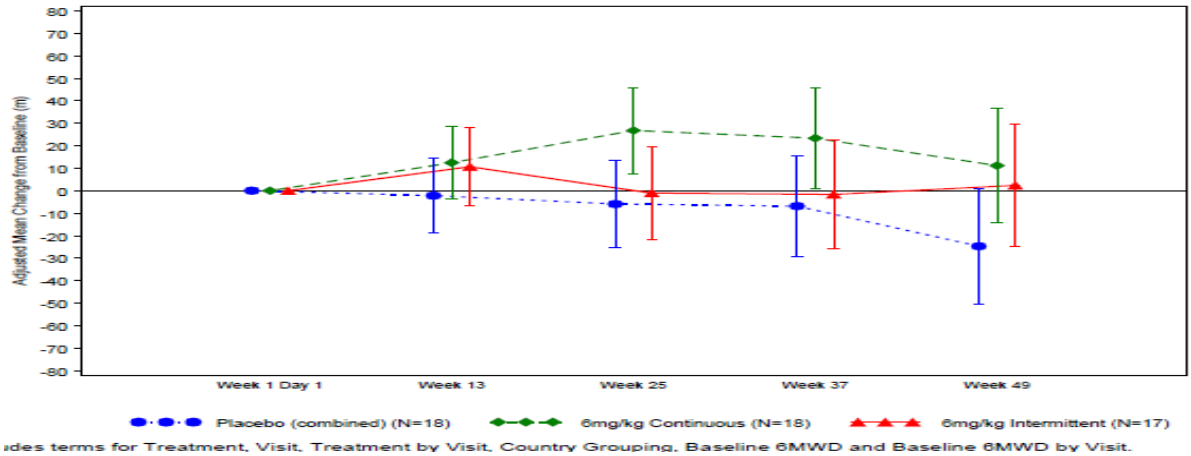
<u>Study DMD114117:</u> Primary endpoint: Change from Baseline in the 6MWD at Week 25

The primary efficacy analysis was conducted when all subjects had completed 24 weeks of dosing (Week 25). All subjects were to remain on study until the final efficacy evaluation after 48 weeks of dosing (Week 49). The ITT population was the primary population for efficacy parameters. The PP population was analyzed as a sensitivity analysis.

Table 5 Change from baseline in 6MWD (m) at Week 25 and Week 49 (ITT population)

	Placebo (combined) (N=18)	6mg/kg Drisapersen Continuous (N=18)	6mg/kg Drisapersen Intermittent (N=17)
Baseline			
n	18	18	17
Mean (SD)	403.18 (45.131)	427.61 (70.045)	394.57 (66.952)
Week25			
n	16	16	15
Adjusted mean change (SE)	-3.6(9.73)	31.5 (9.75)	-0.1(10.34)
Adjusted mean difference vs.placebo		35.09	3.51
95% CI		(7.59, 62.60)	(-24.34,31.35)
p-value		0.014	0.801
Week49			
n	17	18	15
Adjusted mean change (SE)	-24.7 (12.75)	11.2 (12.64)	2.4 (13.63)
Adjusted mean difference vs.placebo		35.84	27.08
95% CI		(-0.11,71.78)	(-9.83,63.99)
p-value		0.051	0.147

Figure 4 Adjusted mean change from baseline (95% CI) in 6MWD (m) (ITT population)



ıdes terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit.

In the primary analysis of change from baseline in 6MWD (m) at Week 25, a statistically significant difference was demonstrated for the drisapersen 6 mg/kg/week continuous regimen when compared

against the combined placebo group (p = 0.014) representing a mean difference of 35.09 meters on the 6MWT. A 30 m change in the 6MWT was earlier accepted for Duchenne clinical programmes as a clinically relevant effect. No statistically significant difference was shown for the drisapersen 6 mg/kg/intermittent treatment regimen when compared against placebo (p = 0.801). The intermittent regimen group was almost not distinguishable from placebo (3.51 meters). However, in the context of efficacy assessment, it generally should be considered that the study was planned as an exploratory study and not designed to have sufficient power to show a statistical difference of a clinically important effect size.

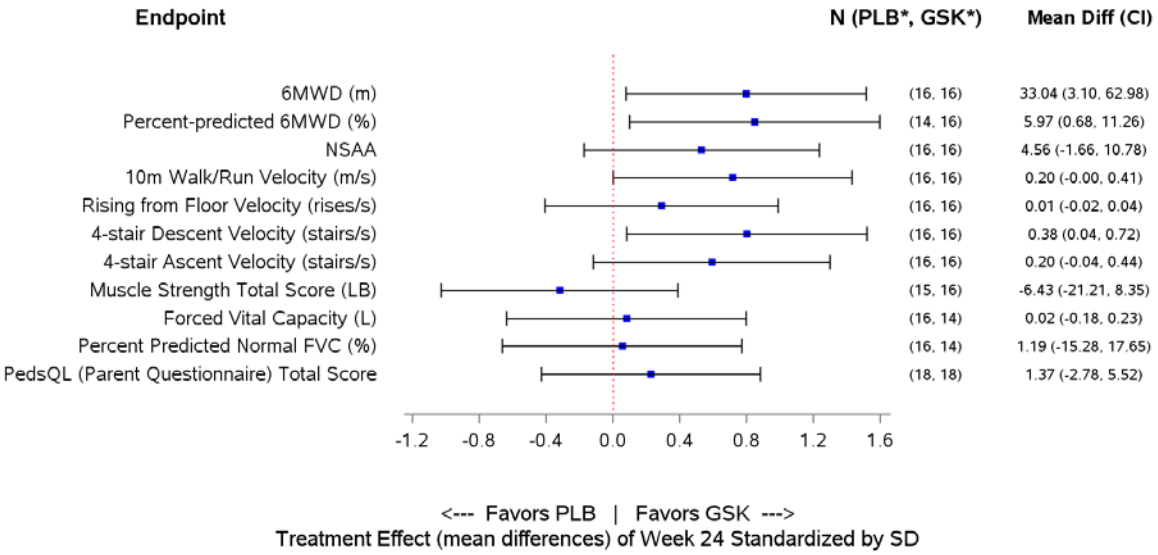
In the analysis of change from baseline in 6MWD (m) at Week 49, also positive results were shown for the continuous regimen when compared against the combined placebo groups (p = 0.051, not adjusted for multiplicity of measurement time points). The continuous regimen group had a mean of 35.84 meters treatment difference on the 6MWT when compared to placebo at week 49. This treatment difference in the 6MWD over placebo was of similar magnitude to that received at Week 25, around 35 meters. The continuous group showed an increase above baseline which persisted throughout the 48 weeks. It showed some decline towards baseline after the initial increase in 6MWD up to Week 25. The intermittent regimen group had a mean of 27.08 meters treatment difference on the 6MWT when compared to placebo at week 49.

Favourable trends (compared with placebo) were observed in the timed function tests (rise from floor, 10 m walk/run, and 4-stair climb/descent) at Week 25 and Week 49 for the continuous group and at Week 49 for the intermittent group. However, none of these treatment differences reached statistical significance. There were directionally favourable changes compared with placebo for the NSAA and CK at Week 25 and Week 49 for the continuous group and at Week 49 for intermittent groups. Little change was seen in total muscle strength, compared to slight improvements with placebo, and changes in pulmonary function measures were small and variable in both treatment groups.

No subjects in any treatment group lost ambulation during the study. Few subjects across treatment groups had an accidental fall during the 6MWD assessment performed in the study and there were no treatment-related trends.

The forest plots below present efficacy outcome for all endpoints for the drisapersen 6 mg/kg/week continuous group compared against placebo at week 24 and at 48. These endpoints use different scales and units of measurement, so in order to display the efficacy of these endpoints together, the estimates are standardized by the applicant to a common scale of measurement by dividing the estimate and confidence interval bounds for each endpoint by the standard deviation of that estimate.

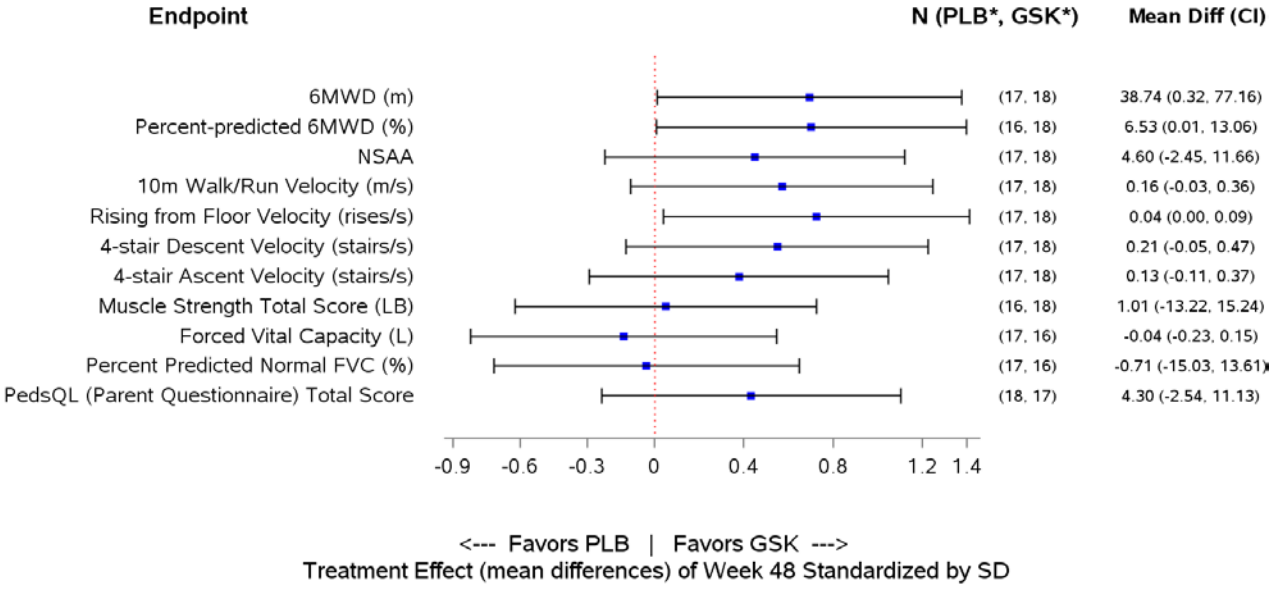
Figure 5 Treatment Effect of Drisapersen 6 mg/kg/week on Efficacy Endpoints at Week 24



*PLB includes subjects from Placebo group of Study 114117; GSK includes subjects from 6 mg/kg/week Drisapersen of Study 114117.

Source: /ace/acedev/drisapersen/dmd/isse201503a/progstat/stats_g_117_6mg_w24_w48_forest.sas

Figure 6 Treatment Effect of Drisapersen 6 mg/kg/week on Efficacy Endpoints at Week 48

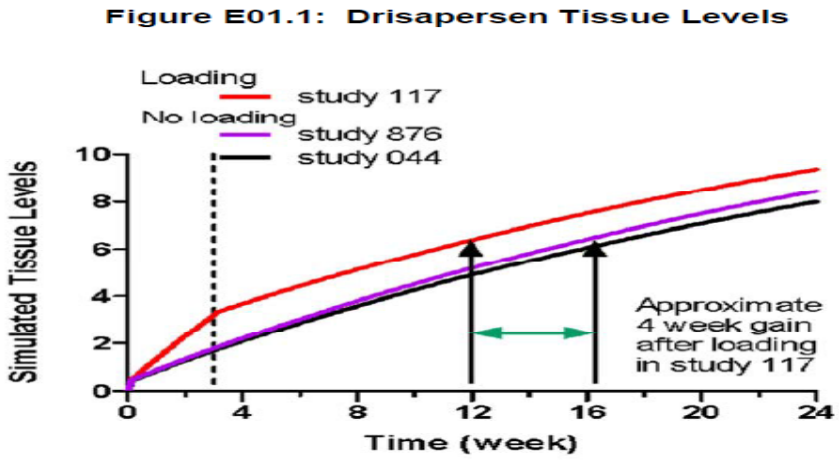


Source: ISE Figure 22

To substantiate the inclusion of a loading dose further, the applicant discussed within the answer to the day 120 LOQ the long tissue-half-life of drisapersen of approximately 3 months. The applicant postulated that higher drisapersen tissue concentrations as provided in this study caused by twice weekly administrations over the first 3 weeks of treatment (with three additional drisapersen administrations) compared to those dosing regimens of studies DMD114876 and DMD114044 were associated with a greater clinical benefit on the primary endpoint.

To further justify the influence of a loading dose on the treatment effect at week 24 in the sense that the clinical outcome depends on tissue levels that are reached most early, the applicant performed a tissue exposure model. Simulated tissue levels were demonstrated over the first 24 weeks of treatment across the different studies. The figure below shows that comparable tissue levels where reached approximately 4 weeks earlier in case of the use of a loading dose.

Figure 7



As results from study DMD114117 demonstrated that patients with drisapersen tissue levels above 10 µg/g showed the highest increase in dystrophin protein expression compared to placebo, the applicant further assessed the relationship between the clinical outcome, based on the efficacy endpoint at week 48, and muscle biopsy tissue concentrations at week 24. This analysis showed that the 6MWD in fact improved best at tissue concentrations above 10 µg/g. However, although for all studies the best results were achieved for the tissue levels above 10 µg/g, it also has to be taken into account that the change in 6MWD at week 48 according to this analysis was higher in study DMD11476 compared to study DMD11417 although patients in study DMD11476 were treated with drisapersen only for 24 weeks.

Although the provided analyses support the fact that the positive results of study DMD114117 are caused by the initial use of a loading dose the applicant`s answer based on the presented models does not fully resolve all doubts. Some uncertainties also exist in reference to the positive results observed at week 24 in study DMD114876 although no loading dose has been used. Although the included study population in study DMD114117 and DMD114876 was comparable in reference to baseline characteristics, results obtained under placebo treatment were not that similar.

The best evidence to assess the influence of a loading dose would have been derived from a study that uses the same treatment regimen in two treatment arms and that in addition includes a loading dose in one of these arms. However, in reference to the treatment administration in general, the three additional drisapersen administrations during the first three weeks of treatment are not considered relevant for the long-term treatment effect of this chronic disease. Whether the positive results of study DMD114117 in fact were caused by the use of a loading dose cannot totally be resolved from the information provided. Therefore, the posology recommendation of using a loading dose is not entirely substantiated.

Whereas the efficacy of the loading dose (vs a standard posology) is not demonstrated, safety data from Phase II Study DMD114117 suggest a worse safety profile. When continuous versus intermittent administration (in which drisapersen 6 mg/kg was twice weekly administered for several weeks

although in an intermittent schedule) higher incidence of injection site reaction was observed in the intermittent arm than in continuous arm . Although limited, and until more evidence on the safety and efficacy is available, there are reasonable doubts on the pertinence of the recommended loading dose.

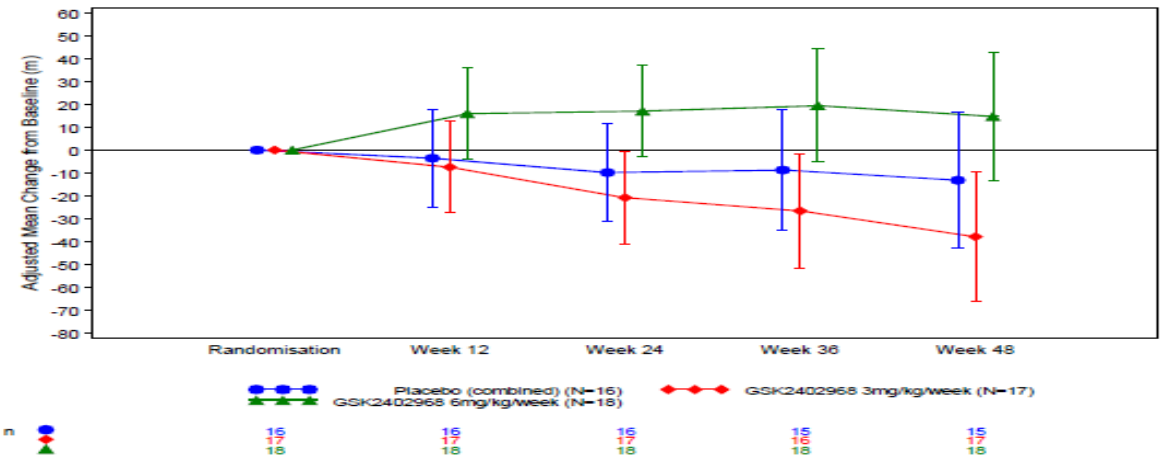
Study DMD114876: Primary endpoint: Change from Baseline in the 6MWD at Week 24

Table 6 change from baseline in 6MWD (m) at Week 24 and Week 48 (ITT population)

	Placebo (combined) (N=16)	Drisapersen 3 mg/kg/week (N=17)	Drisapersen 6 mg/kg/week (N=18)
Baseline			
n	16	17	18
Mean (SD)	416.41 (56.988)	415.21 (58.049)	396.18 (60.662)
Week 24 primary analysis			
n	16	17	18
Adjusted mean change (SE)	-10.98 (10.666)	-19.93 (9.964)	16.12 (9.941)
Adjusted mean difference vs. placebo		-8.946	27.099
95% CI		(-39.122, 21.229)	(-2.210, 56.408)
p-value		0.554	0.069
Week 48 (end of post treatment period)			
n	15	17	18
Adjusted mean change (SE)	-13.17 (14.843)	-37.92 (14.059)	14.69 (13.891)
Adjusted mean difference vs. placebo		-24.750	27.866
95% CI		(-66.371, 16.871)	(-13.043, 68.775)
p-value		0.238	0.177

Source: DMD114876 CSR, Week 24 Analysis Table 2.2, Week 48 Analysis Table 2.3
Note: Subjects received study treatment for 24 weeks, followed by a 24-week post-treatment period where subjects did not receive drisapersen (shaded area).

Figure 8 Adjusted mean change from baseline (95% CI) in 6MWD (m) (MMRM analysis, ITT population)



Source: DMD114876 CSR, Figure 2.1

Subjects received study treatment for 24 weeks, followed by a 24-week post-treatment period where subjects did not receive drisapersen.The figure presents data from the MMRM analysis including all data up to and including Week 48. The results for Weeks 12 and 24 are therefore slightly different to those obtained from the primary analysis, due to the influence of additional data

In the primary analysis of change from baseline in 6MWD at Week 24, the treatment difference over the combined placebo group (27 metres) observed for the drisapersen 6 mg/kg/week group was not

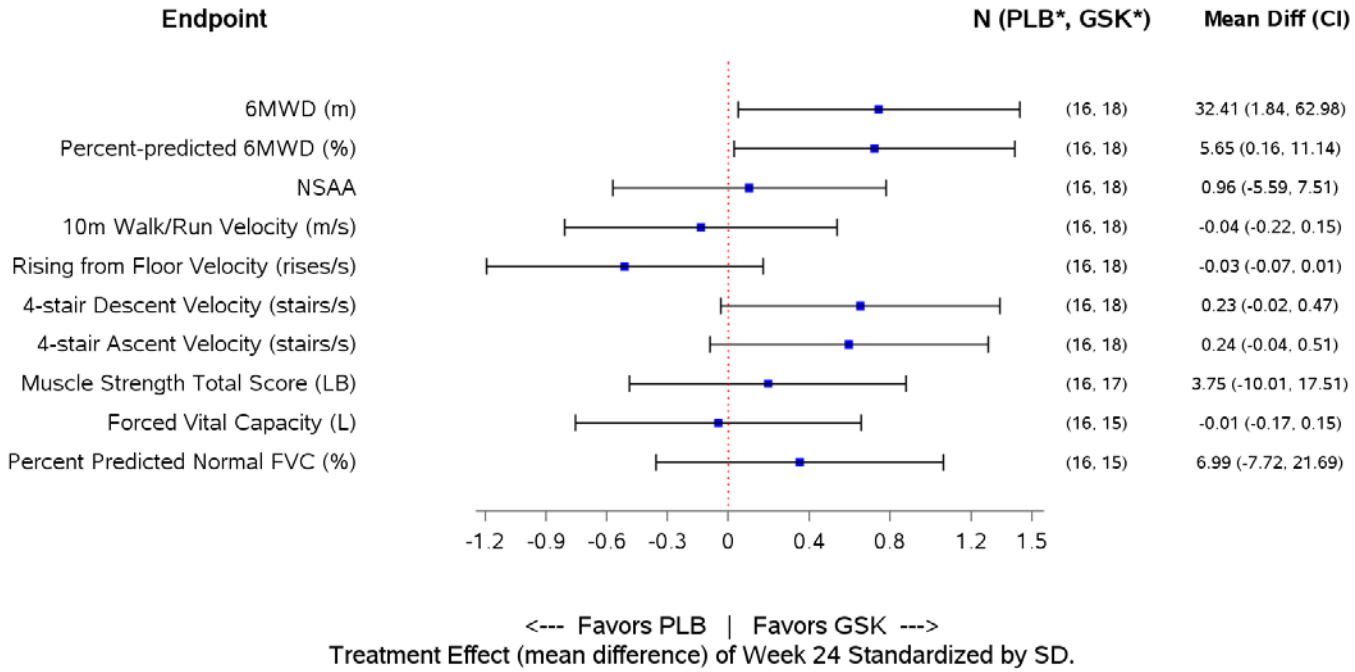
statistically significant (p=0.069). Since a hierarchical testing approach was used, conclusions regarding the statistical significance of the 3 mg/kg group cannot be made since the 6 mg/kg group did not reach statistical significance. A mean difference (27.10 m) over placebo was observed for the drisapersen 6 mg/kg group. The drisapersen 3 mg/kg group had a greater deterioration (8.9 m) at week 24 when compared against placebo. Generally, in the context of efficacy assessment, it should be noted that the study was exploratory in nature and not designed to have sufficient power to show a statistically significant difference.

An MMRM analysis was also conducted for the 6MWD including all data at Week 48, after subjects had been off of treatment with drisapersen for 24 weeks. The model was analogous to that performed for the primary analysis at Week 24.

At Week 24, the differences from placebo in secondary endpoints (with the exception of CK) showed variable and insignificant changes for both drisapersen groups. For most of the endpoints, subjects in the drisapersen 6 mg/kg/week dosing group showed a more positive response than subjects receiving placebo with the exception for the timed function tests 10m walk/run and rising from floor and pulmonary function.

Data on creatine kinase (CK) showed a decrease compared with placebo at Week 24 for both drisapersen groups, with a greater treatment difference in the drisapersen 6 mg/kg group than the drisapersen 3 mg/kg group. At Week 48, the difference between the drisapersen 3 mg/kg group and placebo had increased in favour of drisapersen, but within the drisapersen 6 mg/kg group, CK values had increased post-treatment, leading to little difference compared to placebo at Week 48.

Figure 9 Treatment Effect of Drisapersen 6 mg/kg/week on Efficacy Endpoints at Week 24



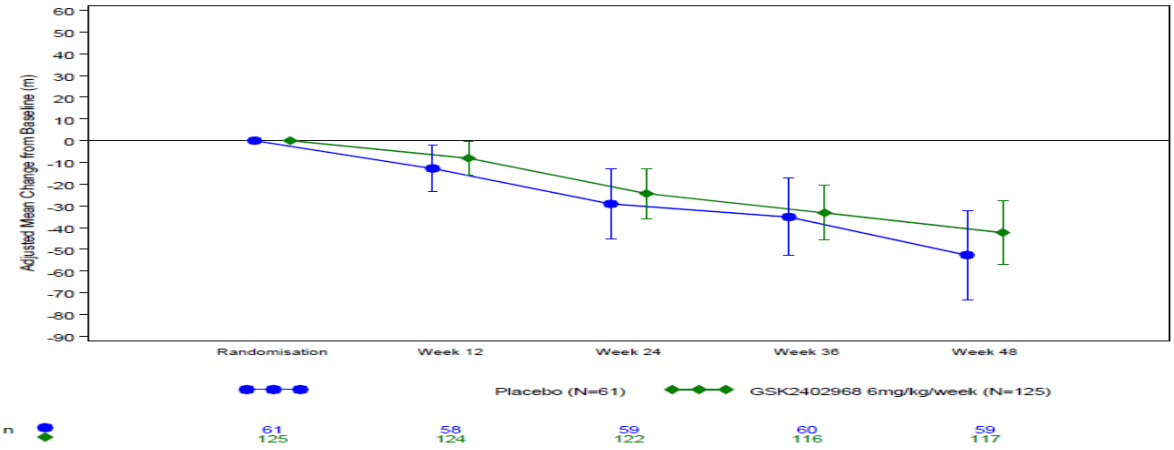
<u>Study DMD114044:</u> Primary endpoint: Change from Baseline in the 6MWD at Week 48

Table 7 Change from baseline in 6MWD (m) at Week 24 and Week 48 (ITT population)

	Placebo (N=61)	Drisapersen 6 mg/kg/week (N=125)
Baseline n Mean (SD)	 61 348.00 (92.153)	 125 337.46 (95.594)
Week 24 n Adjusted mean change (SE) Adjusted mean difference vs. placebo 95% CI p-value	 59 -29.11 (8.267)	 122 -24.34 (5.815) 4.767 (-14.896, 24.431) 0.633
Week 48 n Adjusted mean change (SE) Adjusted mean difference vs. placebo 95% CI p-value	 59 -52.65 (10.423)	 117 -42.32 (7.378) 10.334 (-14.645, 35.312) 0.415

Source: DMD114044 CSR, Table 2.2 and Table 2.3
Notes:
A positive difference compared to placebo represents benefit over placebo.
Model includes terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit.

Figure 10 Adjusted mean change from baseline (95% CI) in 6MWD (m) – (ITT Population)



l includes terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit.
Source: DMD114044 CSR, Figure 2.1
Notes:
A positive change from baseline indicates improvement. Model includes terms for Treatment, Visit, Treatment by Visit, Country Grouping, Baseline 6MWD and Baseline 6MWD by Visit

The primary analysis of change from baseline in 6MWD (m) at Week 48 failed to show statistical significance when the drisapersen 6 mg/kg/week treatment group was compared against placebo (p=0.415). The 10.3 m treatment difference over placebo observed for the drisapersen treatment group is considered to be not clinically relevant. Mean decreases from baseline in 6MWD were observed for both the placebo and the drisapersen 6 mg/kg/week treatment group, indicating a decline in ambulatory function over 48 weeks.

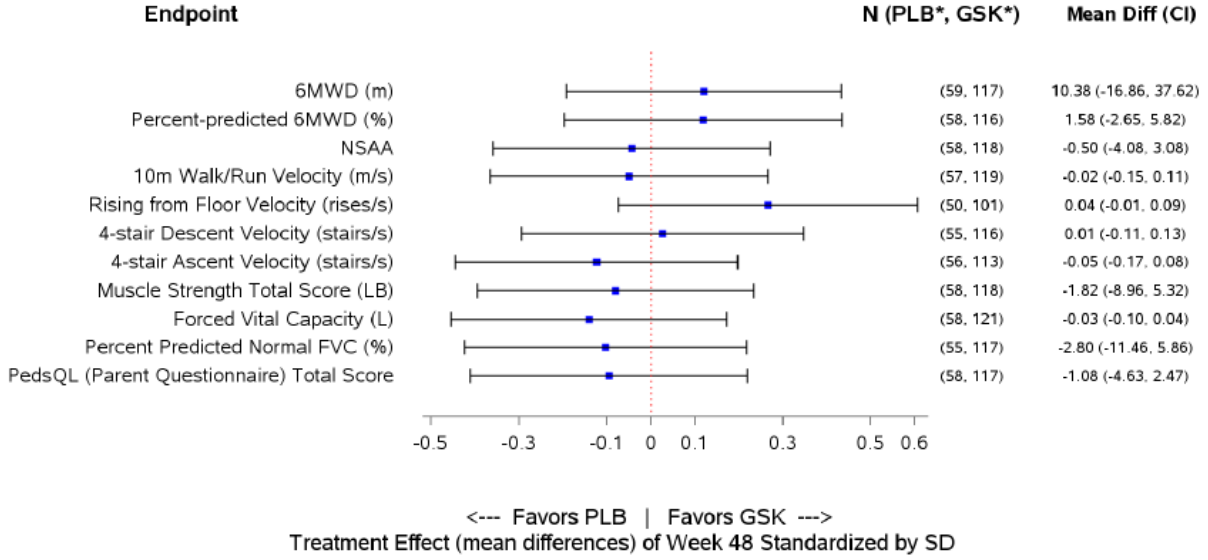
In the analysis of time to persistent 10% decrease in 6MWD, 26 (43%) subjects in the placebo group and 45 (36%) subjects in the drisapersen group had a persistent decrease in 6MWD. However, the results for the drisapersen group were not statistically significant compared with placebo (p=0.368).

For the primary endpoint, the applicant provided further selected subgroup analyses post-hoc with different age ranges (≤ 7 years and >7 years) and baseline 6MWD (> 330 m and ≤ 330 m). It seemed that for the combined subgroup ≤ 7 years and baseline 6 MWD about ≤ 330, the most promising numerical differences on the 6MWD in comparison to placebo were achieved. A greater treatment difference in change from baseline in 6MWD over placebo was observed for the drisapersen treatment group at Week 48 in subjects ≤7 years (21.5 metres) compared with subjects >7 years (6.9 metres). A greater treatment difference for the drisapersen group compared with placebo for the change from baseline in 6MWD at Week 48 was observed in the ≤330 m subgroup (18.4 m) than in the >330 m subgroup (7.4 m), however neither treatment differences were considered clinically meaningful.

The applicant explained that although nowadays age and baseline 6MWD are considered relevant prognostic factors for the clinical outcome, patients in the provided clinical study program performed several years ago were not stratified to ensure balance across all treatment groups with respect to age and baseline walk. The subgroup of patients with baseline 6MWD >330 meters consisted for the placebo group of patients tended to be younger: 66% (25/38) of placebo subjects were ≤7 years old, as compared to 49% (33/67) in the drisapersen group. In the applicant`s view this might have confounded the estimates for the ≤330 meters and the >330 meters subgroups. However, it also has to be considered that the robustness of the results is considerably influenced by the low number of patients in some subgroups used for a stratified analysis.

There were no statistically significant differences between drisapersen and placebo on the majority of secondary endpoints. A statistically significant decrease in CK (p<0.001) was observed at Week 48 for the drisapersen group compared with placebo. A total of 1 (2%) subject in the placebo group and 12 (10%) subjects in the drisapersen group were considered responders (much improved or very much improved) on the CGI-I at Week 48. There were no significant treatment differences between drisapersen and placebo for the PedsQL Neuromuscular Module, HUI health outcomes assessments and activities of daily living. A total of 6 (10%) subjects in the placebo group and 15 (12%) subjects in the drisapersen group lost ambulation during the study. There were no statistically significant or clinically meaningful differences in the change from baseline in linearized NSAA total score at Week 48 for the drisapersen group compared with placebo. There were no statistically significant or clinically meaningful differences in the change from baseline in muscle strength at Week 48 for the drisapersen group compared with placebo.

Figure 11 Treatment Effect of Drisapersen 6 mg/kg/week on Efficacy Endpoints at 48 Weeks



Source: ISE Figure 23

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 8 Summary of efficacy for trial DMD114117

Title: A phase II, double blind, exploratory, parallel-group, placebo-controlled clinical study to assess two dosing regimens of GSK2402968 for efficacy, safety, tolerability and pharmacokinetics in ambulant subjects with Duchenne muscular dystrophy			
Study identifier	DMD114117		
Design	Multicenter, international, randomized (2:1), double-blind, exploratory, placebo-controlled, parallel-group For the primary analysis the 2 matched placebo groups were combined		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		48 weeks, including the loading dose regimen 3 weeks 104 weeks
Hypothesis	Superiority		
Treatments groups	Placebo		once-weekly
	6 mg/kg/ drisapersen		once-weekly
	Intermittent regimen; 6 mg/kg drisapersen		twice weekly on 1st, 3rd and 5th weeks, once weekly on 2nd, 4th and 6th weeks, and no active drug on 7th to 10th week of each 10 week cycle
Endpoints and definitions	Primary endpoint	6MWD	change from baseline at Week 25 in the 6MWD

	Main secondary endpoints (found relevant by CHMP)	TFT	Change from baseline in: Rise from floor time 10 m walk/run time 4-stair climb
		NSAA	
		Muscle strength	
		Frequency of accidental falls	
		CGI-I	
		CK	Change from baseline in serum CK concentrations
		PD endpoint	Muscle dystrophin expression
Database lock			

Results and Analysis

Analysis description	**Primary Analysis**The observed case (OC) data from the ITT population was analysed using a mixed model for repeated measures (MMRM). The model included treatment, visit, treatment by visit interaction, country/country grouping, baseline 6MWD and baseline 6MWD-by-visit as fixed effects and used an unstructured covariance matrix. The primary analysis was planned with comparisons to the combined the placebo groups. In the event that differences were observed, additional analyses considered the placebo groups separately.			
Analysis population and time point description	Intent to treat, time point week 25			
Descriptive statistics and estimate variability	Treatment group	Placebo	6 mg/kg/ drisapersen continuous	6 mg/kg/ drisapersen intermittent
	Number of subject	18	18	17
	Baseline	403.18	427.61	394.57
	Change observed in 6MWD Adjusted Mean	-3.6	31.5	-0.1

Effect estimate per comparison	The model estimated differences in adjusted mean differences vs. placebo	Comparison groups	6 mg/kg/ drisapersen continuous vs combined Placebo
		Difference (meters)	35.09
		95%CI	7.59, 62.60
		P-value	0.014
	The model estimated differences in adjusted mean differences vs. placebo	Comparison groups	6 mg/kg/ drisapersen intermittent vs combined Placebo
		Difference (meters)	3.51
		95%CI	-24.34, 31.35
		P-value	0.801

Table 9 Summary of efficacy for trial DMD114876

Title: An exploratory study to assess two doses of GSK2402968 in the Treatment of Ambulant boys with Duchenne Muscular Dystrophy			
Study identifier	DMD114876		
Design	Multicenter, national, randomized (2:2:1:1), double-blind, exploratory, placebo-controlled, parallel-group For the primary analysis the 2 volume-matched placebo groups were combined		
	Duration of main phase: Duration of post-treatment:		24 weeks 24 weeks
Hypothesis	Superiority		
Treatments groups	Placebo (drisapersen 3 mg/kg volume matched, drisapersen 6 mg/kg volume-matched)		once-weekly
	3 mg/kg/ drisapersen		once-weekly
	6 mg/kg/ drisapersen		once-weekly
Endpoints and definitions	Primary endpoint	6MWD	change from baseline at Week 25 in the 6MWD
	Main secondary endpoints (found relevant by CHMP)	TFT	Change from baseline in: Rise from floor time 10 m walk/run time 4-stair climb
		NSAA	
		Muscle strength	
		Frequency of accidental falls	
		CGI-I	
		CK	Change from baseline in serum CK concentrations
		PD endpoint	Muscle dystrophin expression
Database lock			
Results and Analysis			

Analysis description	**Primary Analysis**Change from baseline in the 6MWD was analysed for the observed cases (OC) dataset using a mixed model for repeated measures (MMRM). The MMRM model for change from baseline in 6MWD included fixed categorical terms for treatment, visit, treatment by visit interaction, center grouping, and continuous fixed covariates of baseline 6MWD and baseline 6MWD-by-visit. Primary inferences regarding treatment differences for the changes from baseline in the 6MWD was derived from the MMRM models at Week 24. As additional supportive information, treatment differences for Week 12 were also estimated using the MMRM models. The primary analysis was planned with comparisons to the combined the placebo groups. In the event that differences were observed, additional analyses considered the placebo groups separately.			
Analysis population and time point description	Intent to treat, time point week 24			
Descriptive statistics and estimate variability	Treatment group	Placebo	3 mg/kg/ drisapersen	6 mg/kg/ drisapersen
	Number of subject	16	17	18
	Baseline	416.41	415.21	396.18
	Change observed in 6MWD Adjusted Mean	-10.98	-19.93	16.12
Effect estimate per comparison	The model estimated differences in adjusted mean differences vs. placebo	Comparison groups	3 mg/kg/ drisapersen vs combined Placebo	
		Difference (meters)	-8.946	
		95%CI	-39.122, 21.229	
		P-value	0.554	
	The model estimated differences in adjusted mean differences vs. placebo	Comparison groups	6 mg/kg/ drisapersen vs combined Placebo	
		Difference (meters)	27.099	
		95%CI	-2.210, 56.408	
		P-value	0.069	

Table 10 Summary of efficacy for trial DMD114044

Title: A phase III, randomized, double blind, placebo-controlled clinical study to assess the efficacy and safety of GSK2402968 in subjects with Duchenne muscular dystrophy		
Study identifier	DMD114044	
Design	Multicenter, international, randomized (2:1), double-blind, placebo-controlled, parallel-group	
	Duration of main phase: Duration of Extension phase:	48 weeks, including the loading dose regimen 104
Hypothesis	Superiority	
Treatments groups	Placebo	once-weekly
	6 mg/kg/ drisapersen	once-weekly

Endpoints and definitions	Primary endpoint	6MWD	change from baseline at Week 48 in the 6MWD
	Main secondary endpoints (found relevant by CHMP)	TFT	Change from baseline in: Rise from floor time 10 m walk/run time 4-stair climb
		NSAA	
		Muscle strength	
		Frequency of accidental falls	
		CGI-I	
		CK	Change from baseline in serum CK concentrations
		PD endpoint	Muscle dystrophin expression
Database lock			

Results and Analysis

Analysis description	**Primary Analysis**: Change from baseline in the 6MWD was analysed for the OC dataset using a mixed model for repeated measures (MMRM) with restricted maximum likelihood estimation and an unstructured covariance matrix. The MMRM model for change from baseline in 6MWD included fixed categorical terms for treatment, visit, treatment by visit interaction, country grouping, and continuous fixed covariates of baseline 6MWD and baseline 6MWD by visit.			
Analysis population and time point description	Intent to treat, time point week 48			
Descriptive statistics and estimate variability	Treatment group	Placebo	6 mg/kg/ drisapersen	
	Number of subject	61	125	
	Baseline	348.00	337.46	
	Change observed in 6MWD Adjusted Mean	-52.65	-42.32	

Effect estimate per comparison	The model estimated differences in adjusted mean differences vs. placebo	Comparison groups	6 mg/kg/ drisapersen vs Placebo
		Difference (meters)	10.334
		95%CI	-14.645, 35.312
		P-value	0.415

Figure 12 DMD114117: Individual profile plots of 6MWD (m) versus age by treatment (ITT population)

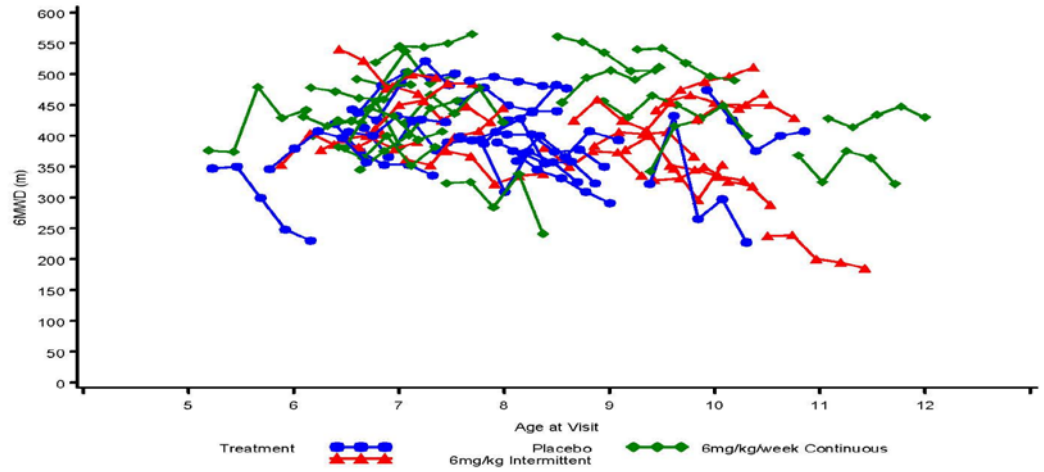


Figure 13 DMD114876: Individual profile plots of 6MWD (m) versus age by treatment (ITT population)

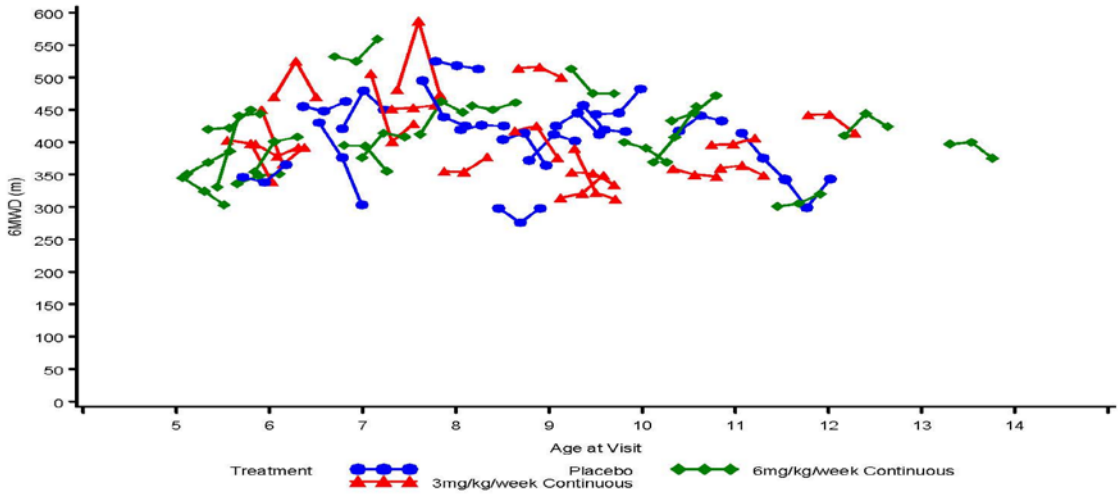
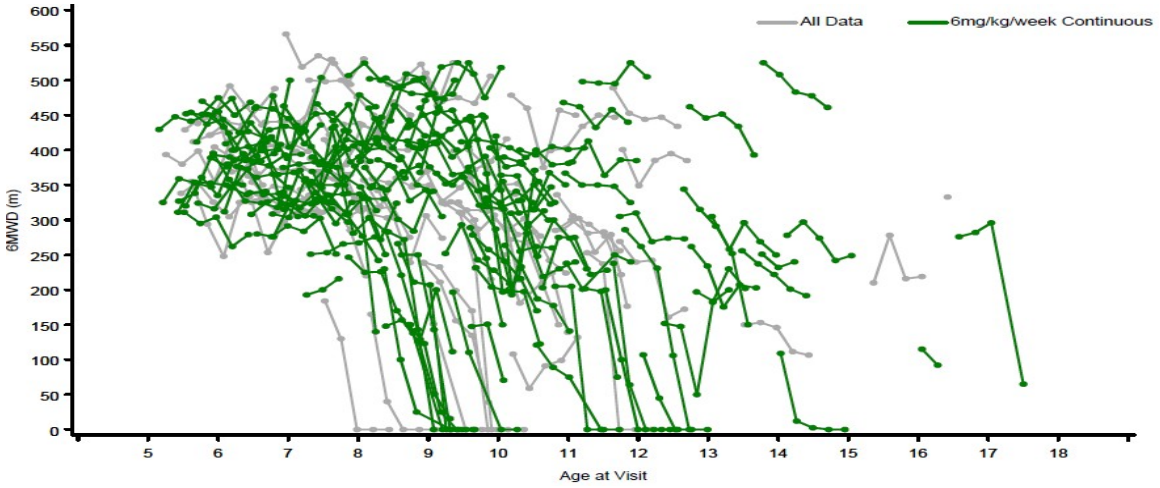


Figure 14 DMD114044: Individual profile plots of 6MWD (m) versus age by treatment (ITT population)



The figures above show the individual profile plots of the 6MWD versus age by treatment groups for the three clinical studies. In the phase 3 study, study DMD114044, patients decline much more in the 6MWD compared to those in the phase II studies, study DMD114117 and DMD114876, also under 6mg/kg/week drisapersen continuous treatment. This is considered indicative for a patient population included in study DMD114044 with more progressive disease.

Clinical studies in special populations

Since the target patient population in Duchenne is predominantly a paediatric population, no elderly patients were included. This is in accordance with the short life expectancy for this patient population.

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

The data from studies DMD114117, DMD114876 and DMD114044 were combined, and summaries and analyses of the change from baseline in efficacy endpoints were provided to further investigate the efficacy of drisapersen administered for up to 1 year. The focus of these analyses was primarily set on the '48-week ambulant, placebo-controlled studies' grouping (studies DMD114117 and DMD114044).

In addition, data from studies DMD114117 and DMD114876 were combined for the analyses of the change from baseline in efficacy endpoints at the 24-week time-point.

For all summaries and analyses, only the 6 mg/kg/week dosing regimen for drisapersen was included in addition to placebo as no other treatment regimen was common between the studies, and the 6 mg/kg/week dosing regimen is the dose for which the applicant claimed approval.

Table 11 Change from baseline in 6MWD (m) - Integrated analyses (ITT population)

	Ambulant, placebo-controlled studies (DMD114044 / 117 / 876)		48-week ambulant, placebo-controlled studies (DMD114044 / 117)		Phase II placebo-controlled studies (DMD114117 / 876)	
	Placebo (N=95)	Drisapersen 6 mg/kg/week (N=161)	Placebo (N=79)	Drisapersen 6 mg/kg/week (N=143)	Placebo (N=34)	Drisapersen 6 mg/kg/week (N=36)
Baseline, n	95	161	79	143	34	36
Mean (SD)	369.98 (84.844)	354.11 (94.962)	360.57 (86.711)	348.81 (97.302)	409.41 (50.699)	411.89 (66.517)
Week 12, n	92	160	76	142	34	36
Adjusted mean change (SE)	−6.92 (4.57)	1.28 (4.09)	−8.80 (5.11)	−3.22 (4.47)	−4.95 (5.93)	12.78 (5.76)
Mean difference vs. placebo	–	8.20	–	5.57	–	17.73
95% CI	–	(−2.19, 18.59)	–	(−5.88, 17.03)	–	(1.23, 34.22)
p-value	–	0.1215	–	0.3385	–	0.0356
Week 24, n	93	158	77	140	34	36
Adjusted mean change (SE)	−21.22 (6.43)	−9.53 (5.37)	−22.98 (7.22)	−15.64 (5.86)	−11.20 (7.10)	19.92 (6.90)
Mean difference vs. placebo	–	11.68	–	7.33	–	31.11
95% CI	–	(−3.62, 26.99)	–	(−9.63, 24.30)	–	(11.34, 50.88)
p-value	–	0.1339	–	0.3950	–	0.0025
Week 36, n	–	–	78	134	–	–
Adjusted mean change (SE)	–	–	−27.72 (7.93)	−23.44 (6.38)	–	–
Mean difference vs. placebo	–	–		4.27	–	–
95% CI	–	–		(−14.55, 23.10)	–	–
p-value	–	–		0.6551	–	–
Week 48, n	–	–	77	135	–	–
Adjusted mean change (SE)	–	–	−45.32 (9.03)	−32.71 (7.16)	–	–
Mean difference vs. placebo	–	–		12.62	–	–
95% CI	–	–		(−9.02, 34.26)	–	–
p-value	–	–		0.2517	–	–

Source: ISE Table 10.52, Table 10.53, Table 10.54, Table 10.76, Table 10.77.1, Table 10.78

Notes:

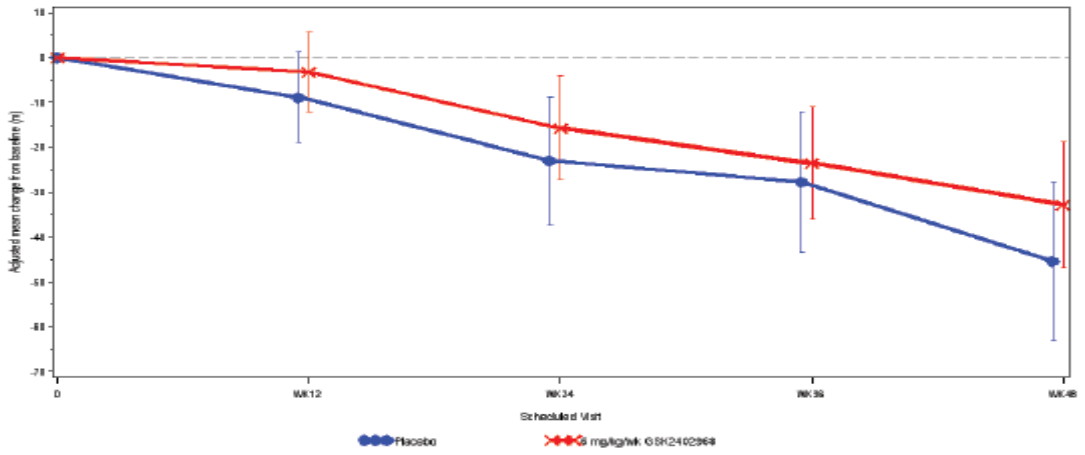
Differences in adjusted least square means are shown (drisapersen 6 mg/kg/week minus placebo). A positive difference compared to placebo represents benefit over placebo.

n = Number of subjects with evaluable data.

MMRM model includes terms for treatment, visit, treatment by visit interaction, study, baseline 6MWD, and baseline 6MWD by visit interaction.

Subjects in DMD114876 received study treatment for 24 weeks, followed by a 24-week post-treatment period where subjects did not receive drisapersen (shaded area).

Figure 15 Adjusted mean change from baseline (95% CI) in 6MWD (m) - MMRM analysis / 48-week ambulant, placebo-controlled studies (ITT population)



Model includes terms for treatment, visit, treatment by visit interaction, study, bs baseline 6MWD by visit interaction.

Source: ISE Figure 10.13.2a

The applicant further provided a large number of analyses performed across the clinical studies. However, due to the heterogeneous patient population included across the studies, evidence of these pooled analyses is rather limited.

Supportive studies

Long-term extension studies

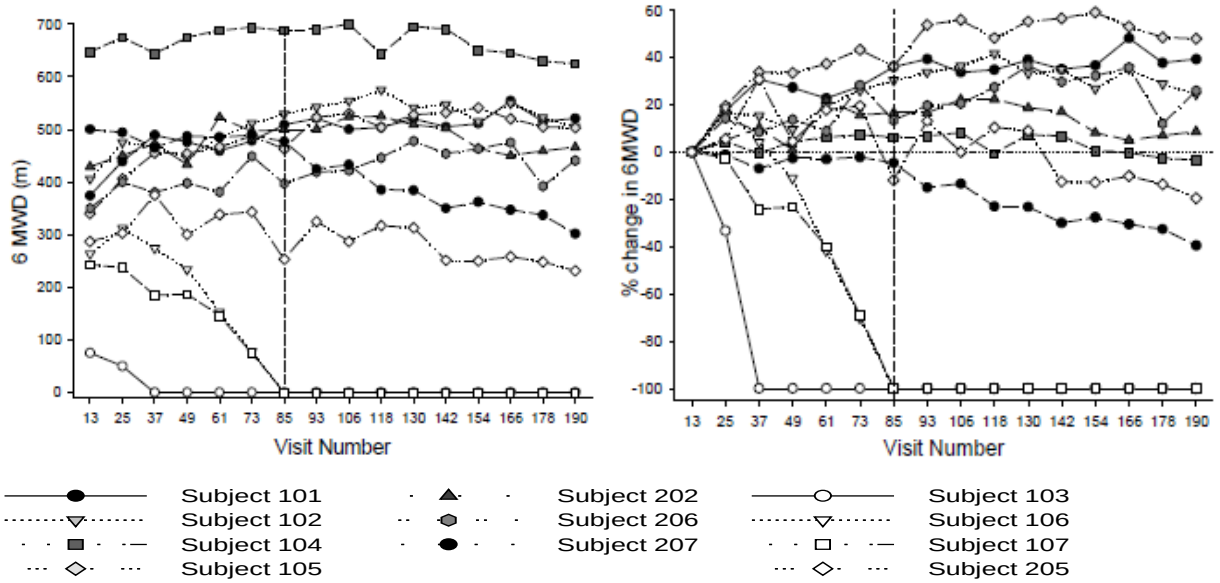
Study DMD114673

Study DMD114673 was the long-term extension phase of PRO051-02, a Phase I/II, open-label, escalating-dose, pilot study to assess the effect, safety, tolerability, and pharmacokinetics of multiple s.c. doses of drisapersen (0.5, 2.0, 4.0, 6.0 mg/kg once weekly for 5 weeks) in male subjects with DMD. Participants in this study were any subjects who completed study PRO051-02 and for whom the subject and/or his parents, the investigator, and sponsor all agreed that drisapersen administration appeared to improve the subject's clinical status.

Between PRO051-02 and DMD114673 (6-15 months), subjects received no drisapersen treatment. All subjects who entered DMD114673 were to be treated with 6 mg/kg s.c. injections of drisapersen once weekly.

The protocol was amended due to emerging safety and pharmacokinetic data, whereby all subjects had a 8-week washout period from Weeks 73 to 80 (Visits 86 to 93) inclusive. Subjects restarted drisapersen treatment at Week 81 (Visit 94) and received an intermittent 6 mg/kg/week dosing regimen (8 weeks of treatment followed by 4 weeks off treatment; 12 weeks per cycle). A subsequent amendment introduced the option to return to a continuous weekly dosing regimen, following discussion with the investigator and medical monitor, if a perceived continuous decline in efficacy was observed and where safety and tolerability was acceptable. Any change from intermittent to continuous weekly dosing was to be implemented at the start of a 12-week cycle.

Figure 16 Absolute and percent change from baseline (Visit 13) in 6MWD (m)



Source: DMD114673 CSR, Listing 16.2.29.1, Listing 16.2.49.

Notes:

'Stable' subjects have filled symbols; 'Decline' subjects have open symbols. Vertical line denotes start of intermittent dosing regimen at Visit 85.

Overall, there appeared to be a general improvement in the distance walked in 6 minutes within 12 weeks of dosing in DMD114673 which was maintained up to Visit 142 (Week 129). Some reductions

were observed subsequently up to Visit 190 (Week 177, when the last efficacy assessments were conducted).

In the 11 subjects who were able to attempt the 6MWD test at least one time during the study, the median change from Visit 13 to Visit 190 (Week 177) was –22 metres (mean change: –29.1 metres). In the 10 subjects who were able to complete the 6MWD at Visit 13, the median change in 6MWD from Visit 13 to Visit 190 (Week 177) was 7.5 metres (mean change: –24.5 metres).

Five of the 10 subjects who could complete the 6MWD at baseline in DMD114673 (Visit 13) could still walk further (range: 37 to 163 m) at Visit 190, with 2 subjects still being able to walk over 140 m further at Visit 190 than they could at baseline (Visit 13). Six of the 11 subjects who could complete the 6MWD at the beginning of PRO051-02 (Visit 1) could still walk further (range: 53 to 158 m) at Visit 190. Although the data are limited, the introduction of an intermittent dosing regimen following Week 72 (Visit 85) did not appear to adversely affect efficacy parameters. However, open-label studies should generally be interpreted with caution. Nevertheless, the presented data may be indicative of a maintained effect of drisapersen.

Study DMD114349 (Phase III study, long-term extension)

Study DMD114349 (phase III long term extension) was a Phase III, multicentre, open-label, uncontrolled extension study in male subjects with DMD who had completed the double-blind treatment phase in DMD114117 or DMD114044. In addition, subjects who had to withdraw from those studies due to safety or tolerability issues may have been able to enrol after Principal Investigator (PI) consultation with the Medical Monitor. This study was aimed to evaluate long-term safety, tolerability, and efficacy. The study was terminated on 17 March 2014. No dosing occurred after 20 September 2013.

Subjects were assigned to 1 of 2 active treatment groups, either a continuous dosing arm (6 mg/kg/week for a minimum 104 weeks) or an intermittent dosing arm (6 mg/kg/week for 8 weeks followed by 4 weeks of no dosing for a minimum 104 weeks). At any point during the study, subjects could discontinue active treatment and move to the natural history observation arm for the duration of the study or until early withdrawal.

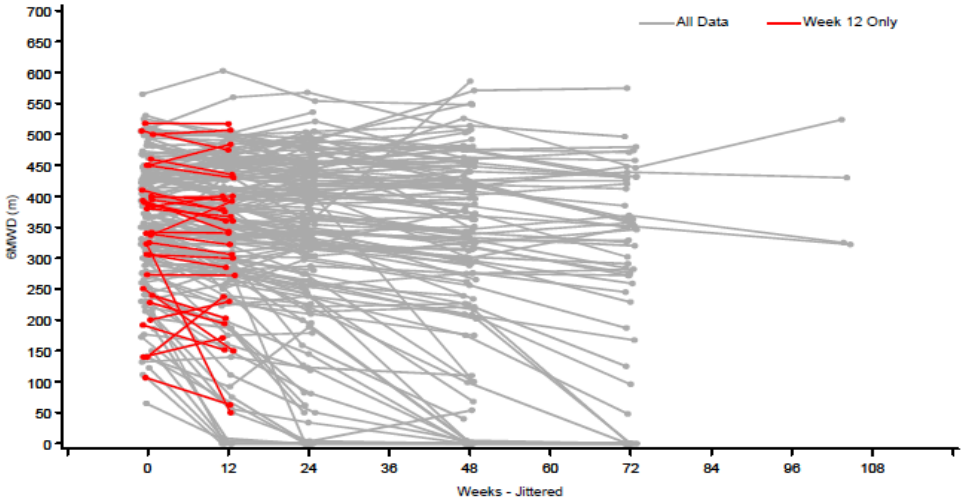
The long-term efficacy and safety population included 239 subjects: 233 subjects were enrolled in the long-term extension study (53 subjects from DMD114117 and 180 subjects from DMD114044) and 6 subjects who participated in study DMD114044 but did not enter study DMD114349.

Prior to entry into study DMD114349, 79 of the 239 subjects were treated with placebo, 143 were treated with drisapersen 6 mg/kg/week, and 17 were treated with intermittent drisapersen 6 mg/kg.

The primary efficacy endpoint for this study was the difference between baseline and Week 104 in 6MWD for subjects on the continuous drisapersen treatment group for the Modified Ambulant ITT population. However, at the time of the early termination of this study only 4 subjects in the continuous drisapersen group and 1 subject in the natural history arm had efficacy data at Week 104. Therefore, the efficacy results focus on data up through Week 72.

Primary Efficacy Analysis: Mean Change from Baseline in the 6MWD

Figure 17 Profile Plots of 6MWD by Time and Continuation Pattern



Week 0 = Derived baseline.
Derived baseline = data from feeder study within 3 months of the baseline assessment in DMD114349 or data collected at the baseline assessment in DMD114349.
tad66240: /arenv/arprod/gsk2402968/dmd114349/final/drivers/f_profile_6mwd.sas 22APR2014 14:09

Table 12 Summary Statistics for Baseline and Change from Baseline in 6MWD (m) (Modified Ambulant ITT Population)

Visit	6 mg/kg Drisapersen Continuous (N=210)	6 mg/kg Drisapersen Intermittent (N=10)	Natural History (N=15)
Derived Baseline			
n	198	6	11
Mean (SD)	359.73 (98.974)	352.95 (105.912)	349.78 (87.065)
Median Min,	372.00	359.00	341.00
Max	65.0, 565.0	221.7, 473.0	138.0, 450.0
Week 12			
n	185	1	0
Mean (SD)	-18.03 (49.827)	-1.00	NA
Median Min,	-15.50	-1.00	NA
Max	-273.0, 103.0	-1.0, -1.0	NA
Week 24			
n	155	1	2
Mean (SD)	-30.89 (65.050)	-6.00	-99.00 (55.154)
Median	-21.50	-6.00	-99.00
Min, Max	-345.0,92.0	-6.0,-6.0	-138.0, -60.0
Week 48			
n	120	1	8
Mean (SD)	-58.99 (91.532)	-21.00	-85.86 (102.234)
Median Min,	-38.50	-21.00	-67.65
Max	-350.0, 200.0	-21.0, -21.0	-280.6, 29.0
Week 72			

n	56	3	2
Mean (SD)	-90.81 (99.732)	-88.57 (115.366)	-41.00 (137.179)
Median	-66.50	-26.00	-41.00
Min, Max	-353.0, 81.0	-221.7, -18.0	-138.0, 56.0
Week 104			
n	4	0	1
Mean (SD)	-20.25 (76.142)	NA	-429.00
Median Min,	-33.00	NA	-429.00
Max	-97.0, 82.0	NA	-429.0, -429.0
Follow-up12Weeks Postdose			
n	101	5	1
Mean (SD)	-54.47 (77.248)	-47.00 (35.602)	9.00
Median Min,	-33.50	-26.00	9.00
Max	-307.0, 96.3	-89.0, -13.0	9.0, 9.0

Source: Table 2.2
Abbreviations: ITT=Intent-to-Treat; Max=maximum; Min=minimum; 6MWD=6-minute walking distance; NA=not applicable; SD=standard deviation
Notes: (1) Derived baseline = data from feeder study within 3 months of the baseline assessment in DMD114349 or data collected at the baseline assessment in DMD114349. (2) Only subjects with post-baseline data for a particular treatment group have data included in the derived baseline for that treatment group.

Table 13 Summary of Repeated Measures Analysis of Change from Baseline in 6MWD (m) by Visit – 6 mg/kg Drisapersen Continuous (Modified Ambulant ITT Population)

Visit [a]	N	n	Adjusted Mean (SE)	Difference vs. Baseline [b]	95% CI for Difference	P-value for Difference
Derived Baseline	210	188	357.82 (7.454)			
Week 12	210	187	339.57 (9.149)	-18.25	(-25.42,-11.08)	<0.001
Week 24	210	157	325.85 (10.294)	-31.97	(-41.79,-22.14)	<0.001
Week 48	210	120	297.05 (12.465)	-60.77	(-76.18,-45.37)	<0.001
Week 72	210	56	265.54 (14.588)	-92.28	(-113.31,-71.26)	<0.001

Source: Table 2.3
Abbreviations: CI=confidence interval; ITT=Intent-to-Treat; 6MWD=6-minute walking distance; n=number of subjects with evaluable data; SE=standard error
Notes: (1) A positive difference compared to baseline represents benefit over baseline. (2) Model includes terms for visit and country grouping. (3) Derived baseline = data from feeder study within 3 months of the baseline assessment in DMD114349 or data collected at the baseline assessment in DMD114349. (4) Only subjects with post-baseline data on drisapersen continuous are included in the analysis.

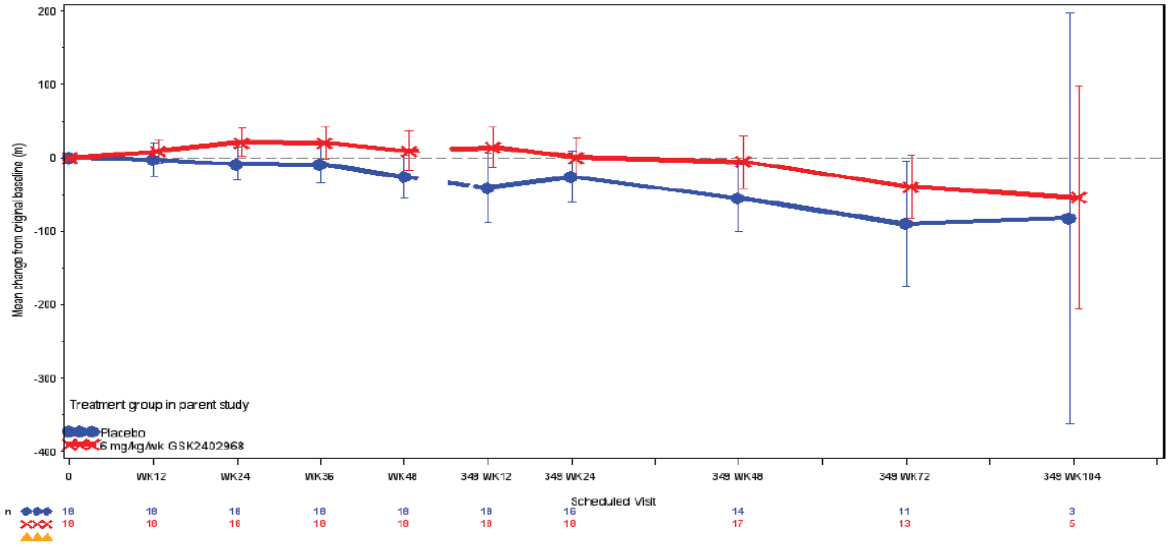
a. Due to the small number of subjects reaching Week 104, only data from visits up to and including Week 72 were included in the analysis.
b. Difference in adjusted least squares means are shown (visit vs baseline).

Statistically significant mean decreases from baseline in 6MWD (m) were observed for the drisapersen 6m/kg drisapersen continuous treatment group over 72 weeks. However, open label studies should generally be interpreted with caution.

Additional post-hoc analyses of the DMD114349 data set by feeder study were conducted.

Figure 18

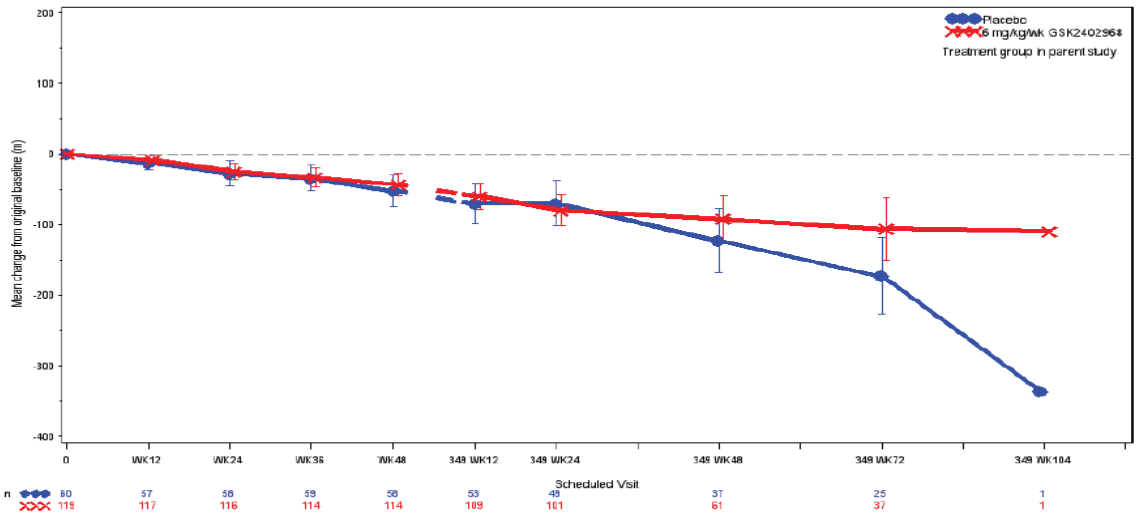
Figure 15 Mean change from original baseline in 6MWD (m) for subjects from parent study DMD114117 (long-term efficacy and safety, ITT)



Source: ISE Figure 10.1.1

Figure 19

Figure 16 Mean change from original baseline in 6MWD (m) for subjects from parent study DMD114044 (long-term efficacy and safety, ITT)



Source: ISE Figure 10.1.2

Results from secondary endpoints were consistent with the primary analysis and showed a general decline in function over 72 weeks of this long-term extension study (e.g., muscle strength, timed muscle function tests [rise from floor, 10-meter walk/run, 4-stair climb (ascent/descent)], NSAA total score). Serum CK, a potential marker of muscle cell integrity, showed a decline for the continuous drisapersen treatment group over 72 weeks.

Within the answer to the day 120 LoQ the applicant provided a subgroup analysis for the primary endpoint with different age ranges (≤7 years and >7 years) and baseline 6MWD (>330 m and ≤330

m) for those patients from study DMD114044 who were included in study DMD114349. Patients who received placebo in the feeder study DMD114044 and switched to drisapersen at week 48 were compared to those patients who continued on active treatment. Overall, the results from this subgroup analysis exercise exhibit a considerable variability, specifically for results beyond week 24. This is expected, as numbers in the subgroups are low and the extension study was stopped early at an index date with the phase III program.

When results of study DMD114349 at week 48 were compared to those at week 48 from study DMD114044, the same subgroups, e.g. those with younger and less affected patients, provided the most promising numerical differences in comparison to placebo. This finding supports the applicants conclusion to start treatment with drisapersen in younger patients and/or earlier in the disease course, e.g. below the age of 7 years. However, as these findings rely on small numbers and are derived from a post-hoc analysis of an open label study which was stopped early, results should be interpreted with caution.

3.3.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The clinical programme to support the efficacy of drisapersen in the treatment of Duchenne muscular dystrophy amenable to be corrected by exon skipping induced by drisapersen consisted of three pivotal studies, i.e. study DMD114117 and study DMD114876, both exploratory phase II studies, one phase III confirmatory study (DMD114044) and two open-label extension studies (DMD114673 and DMD114349). Given the rare nature of the condition the clinical development aimed to support the efficacy and safety of the product appears reasonable.

In general, the studies are in line with the current EMA recommendations (Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy; EMA/CHMP/236981/2011).

In the phase I/II studies, doses of drisapersen 0.5, 2, 4 or 6 mg/kg/week (study PRO051-02) and 6 mg/kg/week (study DMD114673), respectively have been evaluated. Based on these findings, it was concluded by the applicant, that the 6 mg/kg/week dosing regimen presents an appropriate safety and efficacy profile to be taken forward in the clinical programme. Two Phase II studies (DMD114118, n=51; and DMD114117, n= 53) were conducted in order to explore dosing recommendation.

All pivotal studies were multicentre, randomised, double–blind, parallel-group, placebo-controlled studies conducted in ambulant boys with DMD resulting from a mutation correctable by exon 51 skipping induced by drisapersen.

Study DMD114117 and study DMD114876 had a rather similar study design. There was an initial screening period (2- to 4-week) followed by a double-blind treatment period. In one treatment group in each study, patients received a continuous regimen of once-weekly drisapersen 6 mg/kg. The main differences between the two phase II studies was the dosing with regard to different treatment arms and the existence/non-existence of a loading dose as well as the treatment duration:

In DMD114117, subjects received either placebo, continuous 6 mg/kg drisapersen, or intermittent 6 mg/kg drisapersen. All subjects also received a loading dose regimen of twice-weekly dosing with 6 mg/kg drisapersen (or matching placebo) for the first 3 weeks of treatment. Starting with Week 4, subjects then received either once-weekly continuous drisapersen or the intermittent regimen (or, for either group, a matched placebo regimen). In DMD114876, subjects received either 3 mg/kg drisapersen, 6 mg/kg drisapersen, or dose-matched placebo. In DMD114117, subjects were treated for

48 weeks (including the loading dose period). In DMD114876, subjects were treated for 24 weeks followed by a 24-week post-treatment period.

An ability to rise from the floor in ≤7 seconds (without aids/orthoses) was required in DMD114117, defining this population as an early ambulant DMD population, whereas an ability to rise from the floor in ≤15 seconds was required in DMD114876 (changed from ≤7 seconds by protocol amendment 3). However, only two patients had a rise from the floor time >7 seconds - ≤15 seconds at screening in study DMD114876. Therefore, these small baseline differences are not expected to significantly influence the treatment outcome.

Included patients were ambulant Duchenne patients, who were earlier in the disease process. Key inclusion criteria encompassed Duchenne patients with a genetic defect believed to be correctable by drisapersen and that have been treated with corticosteroids for at least 6 months with a stable dose for at least 3 months immediately prior to screening. Included patients were at least 5 years of age, able to walk at least 75 meters in the 6 minute walking distance (6MWD) test and be able to rise from the floor in ≤7 seconds.

The mean age of Duchenne patients for both studies was about 7-8 years. Mean age of diagnosis was about 4 years. All included patients were diagnosed by genetic testing. Overall the treatment arms for both studies were balanced with regard to concomitant medication and glucocorticoid usage.

Study DMD114117: A total of 53 patients were randomized in this study. Demographic characteristics were relatively balanced across treatment groups. The time since first symptoms, diagnosis and first corticosteroid use in the intermittent drisapersen treatment group were slightly longer compared to the other two treatment groups. However, this aspect is consistent with the slightly older mean age of patients in the intermittent treatment group. Mean baseline values for the 6MWD test were slightly higher in the continuous group (427.61 m) compared to the placebo (403.18 m) and the intermittent (394.57 m) treatment group.

Study 114876: A total of 51 patients were randomized in this study. Demographic characteristics were relatively balanced across treatment groups. The time since diagnosis was similar across treatment groups. The time since first symptoms was longest in the drisapersen 3 mg/kg group, while the time since first corticosteroid use was longest in the placebo group and shortest in the drisapersen 6 mg/kg group. Most patients were on a continuous regimen of glucocorticosteroids. Mean baseline values for the 6MWD test were shortest in the drisapersen 6 mg/kg group (396.18 m) and similar in the drisapersen 3 mg/kg and placebo treatment groups, 415.21 m and 416.41 m, respectively.

Proof of efficacy was based on the walking ability as a major parameter of muscular function. Primary endpoint was the change from baseline at week 24/25 in the 6 minute walking distance (6MWD) test for the different active treatment arms compared to the combined placebo group of each study. Although the six-minute walking test was originally developed to evaluate the functional capacity, monitor the efficacy of therapies and establish the prognosis of patients with cardiorespiratory diseases this test has been broadly used in clinical settings[1], including DMD condition. In ambulant DMD boys it provides a global assessment of functional mobility, endurance, and ability to walk[2]. Factors such as age, disease heterogeneity, steroid regimen, learning effect, may influence the inter- and intra-subject variability[3] in the results and make it difficult in the interpretation of the results. Of importance, any

[1]Zuniga V. Reference Equations for the 6-Minute Walk Test in Healthy Individuals. Arq. Bras. Cardiol 2011; 96:128-138.
[2] Mazzone ES et al. 24 Month Longitudinal Data in Ambulant Boys with Duchenne Muscular Dystrophy. PLoS ONE 8(1): e52512
[3] 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 Feb 2 [last modified: 2012 Mar 26].

improvement in the walked distance should be representative of a clinically relevant benefit for the intended population.

A wide battery of secondary endpoints was included: Timed function tests (times and grading): change from baseline in rise from floor time, change from baseline in 10 m walk/run time, change from baseline in 4-stair climb; change from baseline in muscle strength total score; change from baseline in the North Star Ambulatory Assessment (NSAA) total score; frequency of accidental falls (during 6MWD); time to loss of ambulation; change from baseline in serum CK concentrations; change from baseline in pulmonary function parameters; Dystrophin expression (muscle biopsies); Clinician Global Impression of Improvement (CGI-I).

Study DMD114044 is the confirmatory trial to support the efficacy of drisapersen in the treatment of patients with Duchenne muscular dystrophy caused by a mutation amenable to be corrected by exon skipping induced by drisapersen. Subjects received either drisapersen 6 mg/kg or placebo (2:1 ratio) given s.c. once weekly for 48 weeks.

In principle, key inclusion and exclusion criteria for study DMD114044 were similar to those used for the two phase II studies, study DMD114117 and study DMD114876, with the exception that in study DMD114044 no definite time was indicated to be able to rise from the floor. This lead to the inclusion of a population of broader disease severity only bounded by the ability to walk a minimum of 75 metres in the 6MWD.

Primary endpoint was the change from baseline in muscle function using the 6MWD test assessed at week 48. One year duration of treatment is considered a reasonable time period to detect changes in the trajectory of decline in the placebo arm and likely, the required time to substantiate the efficacy of the product in the intended indication. Most of the selected secondary endpoints were similar to those used in the phase II studies.

According to the Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy (EMEA/CHMP/236981/2011) a therapy aiming at restoring the expression of dystrophin (such as drisapersen) may be expected to translate into a clinical improvement in motor function as the most relevant outcome measure in patients with DMD. The relevance of the preservation of independent ambulation for ambulant patients is acknowledged. The 6MW test has been chosen as the primary outcome measure in several DMD clinical trials and normative data are available. Therefore, the primary endpoint selected for this confirmatory trial is considered appropriate. A mean change from baseline between drug and placebo of 30 meters was assumed as clinically relevant. This difference has been recently reported [4] and considered as a predictive factor of disease progression [5]. In any case, a positive impact on global motor function tests, assessing activities other than ambulant capacity (North Star Ambulatory Assessment, timed function tests) as well as on complementary outcomes of muscular function (muscle strength and mobility) would provide robustness to the claimed effect.

The perception of the patients (and parents/caregivers) is part of the relevant assessment of the efficacy of the product. It provides reassurance to the clinical relevance of the changes observed on the quantitative outcomes.

A total of 186 patients were randomized in this study (placebo n = 61, drisapersen 6 mg/kg n = 125). Most of them (97%) completed the study. Demographic and baseline characteristics were rather

[4] the 6-Minute Walk Test and other clinical endpoints in Duchenne muscular dystrophy: reliability, concurrent Validity, and minimal clinically important differences from a multicenter study. McDonald CM et al. Muscle Nerve 48: 357–368, 2013

[5] EPAR Traslarna (EMA/369266/2014)

similar across treatment groups with the exception, that mean weight was higher in the drisapersen 6 mg/kg/week group (30.1 kg) than in the placebo group (26.9 kg).

Most of the patients received corticosteroids on a continuous basis (placebo: 85%; drisapersen 6 mg/kg/week: 86%). Mean baseline values for the 6MWD test were slightly lower in the drisapersen 6 mg/kg/week group (337.46 m) than in the placebo group (348.00 m). Overall, in reference to baseline characteristics, the included patient population compared to those of the two phase II studies was more heterogeneous, older (min: 5, max 16 years of age) and not able to walk at baseline as far as patients did in the phase II studies. Mean baseline RFF time was in the placebo group: 13.41 s, and 12.34 s in the drisapersen 6 mg/kg/week group.

Two long-term extension studies have been conducted. Study DMD114673 (extension phase of Phase I/II PRO051) included only 11 subjects that were followed around 3.5 years. Drisapersen administration was interrupted during a 8 week-period and some patients received the product also on an intermittent regimen basis.

Study DMD114349 (extension phase of DMD114117 and DMD114044): A Phase III, multicenter, open-label, uncontrolled extension study in male subjects with DMD who had completed the double-blind treatment phase in DMD114117 or DMD114044. In addition, subjects who had to withdraw from those studies due to safety or tolerability issues may have been able to be enrolled after Principal Investigator (PI) consultation with the Medical Monitor. Subjects were assigned to 1 of 2 active treatment groups, either a continuous dosing arm (6 mg/kg/week for a minimum 104 weeks) or an intermittent dosing arm (6 mg/kg/week for 8 weeks followed by 4 weeks of no dosing for a minimum 104 weeks). At any point during the study, subjects could discontinue active treatment and move to the natural history observation arm for the duration of the study or until Early Withdrawal. Study DMD114349 was terminated early because results of Study DMD114044 showed the lack of efficacy of drisapersen. Although patients were treated weekly up to 104 weeks, the number of patients providing measurements is especially small between Week 104 and Week 130, precluding the ability to draw definitive conclusions about the maintenance of efficacy over this time interval.

Efficacy data and additional analyses

Study DMD114117:

In the primary analysis of change from baseline in 6MWD (m) at Week 25, a statistically significant difference was demonstrated for the drisapersen 6 mg/kg/week continuous regimen when compared against the combined placebo group ($p = 0.014$) representing a mean difference of 35.09 meters on the 6MWT. No statistical significant difference was shown for the drisapersen 6 mg/kg/intermittent treatment regimen when compared against placebo ($p = 0.801$). The intermittent regimen group was almost not distinguishable from placebo (3.51 meters). However, in the context of efficacy assessment, it should be considered that the study was planned as an exploratory study and not designed to have sufficient power to show a statistical difference of a clinically important effect size.

In the analysis of change from baseline in 6MWD (m) at Week 49, also positive results were shown for the continuous regimen when compared against the combined placebo groups ($p = 0.051$, not adjusted for multiplicity of measurement time points). The continuous regimen group had a mean of 35.84 meters treatment difference on the 6MWT when compared to placebo at week 49. This treatment difference in the 6MWD over placebo was of similar magnitude to that at Week 25, around 35 meters. The continuous group showed an increase above baseline which persisted throughout the 48 weeks. It showed some decline towards baseline after the initial increase in 6MWD up to Week 25. The intermittent regimen group had a mean of 27.08 meters treatment difference on the 6MWT when compared to placebo at week 49.

For most of the secondary endpoints, there were directionally favourable changes for the continuous group compared with placebo (timed function tests (rise from floor, 10 m walk/run, and 4-stair climb/descent), NSAA and CK). Little change was seen in total muscle strength, compared to slight improvements with placebo at week 48, and changes in pulmonary function measures were small and variable in both treatment groups.

Study DMD114876:

In the primary analysis of change from baseline in 6MWD (m) at Week 24, no statistically significant difference was demonstrated for the drisapersen 6 mg/kg group when compared against the combined placebo group (p=0.069). Since a hierarchical testing approach was used, conclusions regarding the statistical significance of the 3 mg/kg group cannot be made since the 6 mg/kg group did not reach statistical significance. A mean difference (27.10 m) over placebo was observed for the drisapersen 6 mg/kg group. The drisapersen 3 mg/kg group had a greater deterioration (8.9 m) at week 24 when compared against placebo. Generally, in the context of efficacy assessment, it should be noted that the study was exploratory in nature and not designed to have sufficient power to show a statistically significant difference.

For the analyses of secondary endpoints, no relevant differences from placebo were observed when other complementary outcomes of muscular function were measured: NSAA, timed function test, muscle strength.

The reduced size of these two trials and the variability shown by the results prevent from achieving sound conclusions. Of note, these studies were primarily aimed to provide PK data and not based on statistical considerations for efficacy. Results offer some hints on the effect of drisapersen and the selection of the dose and regimen of administration to be used in the confirmatory trial.

Study DMD114044:

The primary analysis of change from baseline in 6MWD (m) at Week 48 failed to show statistical significance when the drisapersen 6 mg/kg/week treatment group was compared against placebo (p=0.415). The 10.3 m treatment difference over placebo observed for the drisapersen treatment group is considered far from the minimum distance defined as clinically relevant. Mean decreases from baseline in 6MWD were observed for both the placebo and the drisapersen 6 mg/kg/week treatment group, indicating a decline in ambulatory function over 48 weeks.

For the primary endpoint, the applicant provided further selected subgroup analyses post-hoc with different age ranges (≤ 7 years and >7 years) and baseline 6MWD (> 330 m and ≤ 330 m). From the analyses provided it seemed that for the combined subgroup ≤ 7 years and baseline 6 MWD about ≤ 330, the most promising numerical differences on the 6MWD in comparison to placebo were achieved. A greater treatment difference in change from baseline in 6MWD over placebo was observed for the drisapersen treatment group at Week 48 in subjects ≤7 years (21.5 metres) compared with subjects >7 years (6.9 metres). A greater treatment difference for the drisapersen group compared with placebo for the change from baseline in 6MWD at Week 48 was observed in the ≤330 m subgroup (18.4 m) than in the >330 m subgroup (7.4 m), however neither treatment differences were considered clinically meaningful.

The applicant explained that patients in the clinical study program for drisapersen were not stratified to ensure balance across all treatment groups with respect to age and baseline walk. The subgroup of patients with baseline 6MWD >330 meters consisted for the placebo group of patients that tended to be younger: 66% (25/38) of placebo subjects were ≤7 years old, as compared to 49% (33/67) in the drisapersen group. In the applicant`s view this might have confounded the estimates for the ≤330 meters and the >330 meters subgroups. However, it also has to be considered that the robustness of

the results is considerably influenced by the low number of patients in some subgroups resulting from stratification.

There were no statistically significant differences between drisapersen and placebo on the majority of secondary endpoints. A statistically significant decrease in CK ($p<0.001$), a marker of muscle cell membrane integrity, was observed at Week 48 for the drisapersen group compared with placebo. A total of 1 (2%) subject in the placebo group and 12 (10%) subjects in the drisapersen group were considered responders (much improved or very much improved) on the CGI-I at Week 48. There were no significant treatment differences between drisapersen and placebo for the PedsQL Neuromuscular Module, HUI health outcomes assessments and activities of daily living. A total of 6 (10%) subjects in the placebo group and 15 (12%) subjects in the drisapersen group lost ambulation during the study.

To further substantiate the efficacy of Kyndrisa for the treatment of Duchenne muscular dystrophy, the applicant focused in his answer to the day 120 LOQ on the differences between the studies, e.g. the study design and the more heterogeneous study population that has been included in the failed phase III study. Thus, older and more advanced patients have been included in this study, e.g. the rise from floor criterion (in the phase II studies patients had to be able to rise from floor in less than 7 seconds) was removed as inclusion criterion.

To further illustrate that the phase III study failed its primary endpoint due to the broader patient population included rather than due to lack of efficacy, the applicant presented cumulative distribution function plots for each study. These figures demonstrate differences in the number of patients who increased their walking distance in the 6MWD test in each study. While 72% of the drisapersen treated patients compared to 44% and 56% of the placebo treated patients in study DMD114117 and DMD114876, respectively experienced an increase in 6MWD from baseline, only 37% of the active treated patients compared to 24% of placebo treated patients improved in their walking distance in the phase III study. These data support the conclusion that study DMD114044 included a patient population with more advanced disease compared to those included in the two phase II studies.

In a clarification meeting on the Day 120 questions the importance of identifying a subset of patients that would show a consistent beneficial effect in all three placebo controlled studies was highlighted. Following this discussion, the applicant provided two additional subgroup analyses:

For these, the middle 50% of subjects with regard to either baseline 6MWD or baseline RFF of the pooled population across the 3 placebo-controlled studies were selected for an analysis at week 48 for the different studies. Intention of the analyses based on the middle 50% of patients was to remove the 25% most severely affected and the 25% least severely affected patients as patients with strong muscle mass are unlikely to show decline over a 1 year trial while those with poor muscle mass are unlikely to show a relevant treatment effect as their ambulatory capacity and RFF are on an inexorable downward path over 1 year. In this context it has to be considered that study DMD114876 only lasted 24 weeks and that at week 48 patients already had stopped treatment for 24 weeks. In reference to the selected subgroups patients had either a baseline 6MWD between 313 to 419 meters or a baseline RFF between 4.2 and 13.3 seconds.

Results for the middle 50% of subjects with regard to baseline 6MWD (313 to 419 meters) analysis showed a treatment benefit of about 72.8, 37.1 and 19.9 meters for studies DMD114117, DMD114876 and DMD114044 meters, respectively. The effect of about 20 meters seen in an effect-maximized subpopulation in study DMD114044 is rather small and not clinically relevant. It substantially differs from those effects seen for the phase II trials and therefore the demonstration of consistency of effects cannot be regarded definite.

Also the data from the middle 50% of the patient population based on baseline rise from floor time analysis showed treatment differences of 31.4, 35.6 and 18.1 meters in study DMD114117,

DMD114876 and DMD114044, respectively. Again the treatment difference of about 18 meters in study DMD114044 is rather small and not comparable to those seen in the phase II studies.

Table 14 MO1.2: Treatment Effect at Week 48 in 6MWD by Study and Pooled in Subjects (Baseline 6MWD Middle 50%a)

	Study				
	DMD114117 (N=8/13)	DMD114876 (N=12/7)	DMD114044 (N=56/32)	Pooled (P2 PCTs) (N=20/20)	Pooled (3 PCTs) (N=76/52)
Treatment difference (Drisa-plbo)	72.8	37.1	19.9	55.6	31.3
95% CI	(12.4, 133.1)	(-5.0, 79.2)	(-8.8, 48.7)	(19.8, 91.3)	(9.3, 53.2)

Abbreviations: 6MWD = six-minute walking distance, CI= confidence interval, Drisa = Drisapersen, N=number, P2 = phase II, PCT = placebo-controlled trials, plbo = placebo.

Middle 50% based on baseline 6MWD between 313 and 419 meters

Table 15 MO1.3: Treatment Effect at Week 48 in 6MWD by Study and Pooled in Subjects (Baseline RFF Middle 50%a)

	Study				
	DMD114117 (N=10/12)	DMD114876 (N=11/6)	DMD114044 (N=58/28)	Pooled (P2 PCTs) (N=21/18)	Pooled (3 PCTs) (N=79/46)
Treatment difference (Drisa-plbo)	31.4	35.6	18.1	36.6	25.8
95% CI	(-22.3, 85.1)	(-3.1, 74.3)	(-9.2, 45.6)	(4.2, 69.0)	(5.1, 46.6)

Abbreviations: CI= confidence interval, Drisa = Drisapersen, N=number, P2 = phase II, PCT = placebo-controlled trials, plbo = placebo, RFF = rise from floor.

Middle 50% based on baseline RFF between 4.2 and 13.3 seconds

The applicant provided one 6MWD based subgroup analysis as sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters, which suggests that the effect estimates could considerably vary with different cut-off values. It showed a treatment effect ranging from 67.8 - 27.8 meters across the different studies, with a markedly higher estimate in study DMD114044 and a lower estimate in study DMD114876 compared to those analyses with a baseline window from 313 m to 419 m. In study DMD114044 the difference was 27.8 meters, representing an almost clinical relevant difference in reference to the recently as clinically relevant accepted effect of 30 meters.

Table 16 MO1.4: Treatment Effect at Week 48 in 6MWD by Study and Pooled in Subjects with 300<=baseline 6MWD<=400

	Study				
	DMD114117 (N=8/9)	DMD114876 (N=11/3)	DMD114044 (N=52/31)	Pooled (P2 PCTs) (N=19/12)	Pooled (3 PCTs) (N=71/43)
Treatment difference (Drisa-plbo)	67.8	29.6	27.8	58.6	35.7
95% CI	(-0.2, 135.9)	(-46.7, 105.9)	(-7.5, 63.1)	(12.5, 104.7)	(7.6, 63.8)
P value	0.051	0.411	0.121	0.015	0.013

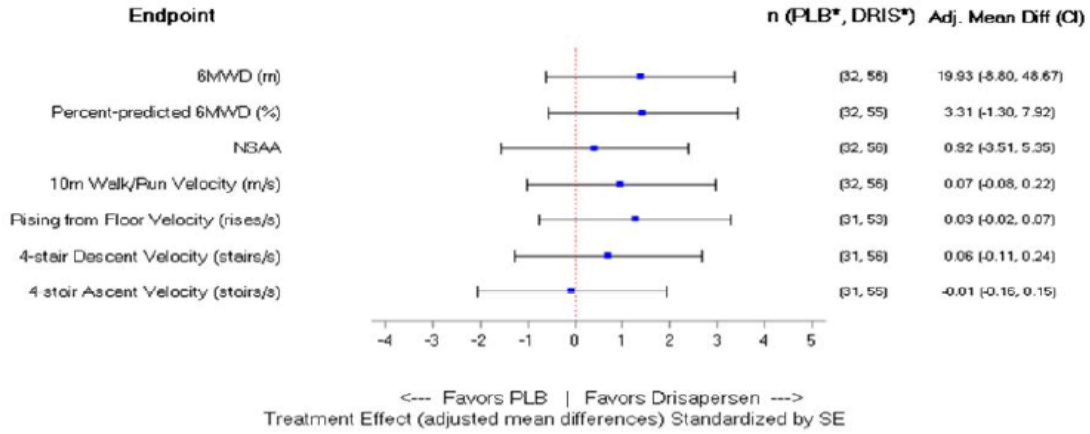
Abbreviations: CI= confidence interval, Drisa = Drisapersen, N=number, P2 = phase II, PCT = placebo-controlled trials, plbo = placebo.

For the above described subgroup analyses based on baseline quartiles the number of patients in the middle 50% groups for the three studies is limited and results could depend on the choice of the cut-off values. From the analyses provided it can be concluded that the large variability of the observed effect makes the results problematic to interpret: as for Study DMD114044 when the baseline 6MWD cut off point is moved from 313-419 metres (middle 50%) to 300-400 meters, the treatment effect versus placebo changes from 19.9 metres (95% CI -8.8, 48.7) to 27.8 (95% CI -7.5, 63.1). The confidence intervals are even wider for Phase II trials estimations. These results appear highly dependent on the selected cut-off point of 6MWD and are thus far from being robust. Therefore further sensitivity analyses with the same window width (of 100 m) and cut-off values below and above 300 m and 400 m, respectively are deemed necessary to assess how sensitive the results are with respect to the choice of the cut-off.

The applicant also provided a subgroup analysis based on the middle 50% of patients according to baseline 6MWD for several secondary endpoints, e.g. the ambulatory timed function tests and the NSAA at week 48. It is agreed that these endpoints are considered sensitive to change in the included ambulant patient population and also over the treatment duration of 48 weeks. As in study DMD114876 timed function tests of rise from floor, 10 m walk/run and 4-stair climb/descent, and the NSAA only were performed during the 24 week treatment period study DMD114876 was excluded from analysis.

The forest plots at week 48 based on the middle 50% of subjects according to baseline 6MWD showed for these secondary endpoints in both studies comparable results in favor of drisapersen. However, to provide support for robustness of the effects received, sensitivity analyses based on different baseline 6MWD cut-off values as requested for the primary endpoint should also be performed for the secondary endpoints.

Figure 20 MO1.8: Forest Plot of Adjusted Mean Difference from MMRM Analysis at Week 48 Ambulatory Function Endpoints; DMD114044 (Baseline 6MWD Middle 50%a)



Middle 50% based on baseline 6MWD between 313 and 419 meters

Abbreviation: 6MWD = six minute walking distance, Adj= Adjusted, CI = Confidence Intervals, Diff = Difference, Drisa = Drisapersen, m = meter, n = number, NSAA = North Star Ambulatory Assessment, PLB = Placebo, SE = Standard Errors

The applicant presented also data for quality of life scores, functional outcomes and global clinical impression for the three studies. However, given the different tests that were chosen across the studies, no adequate comparison can be derived for the clinical global impression from the information provided.

Study DMD114673 (extension phase of Phase I/II PRO051):

Some effect appears to be observed as stabilisation of walking performance (in principle 6 out of 12 subjects treated). No positive effect was observed in muscle strength, which declined over time in all subjects. As this extension study is not placebo-controlled, the findings need to be interpreted with caution.

DMD114349 (phase III long term extension):

A progressive decline in ambulation was observed along the study, both in patients previously treated with drisapersen or placebo. At week 72 the 6MWD showed an average overall decline of -92.28 metres. The mean change was -88.79 metres in subjects who had received drisapersen in the previous study, as compared to -141.78 metres in subjects who switched to drisapersen in the extension study after receiving placebo in the former study. Secondary endpoints were consistent with the pattern shown by the primary endpoint. However, open label studies should generally be interpreted with caution.

Within the answer to the day 120 LOQ the applicant provided a subgroup analysis for the primary endpoint with different age ranges (≤7 years and >7 years) and baseline 6MWD (>330 m and ≤330 m) for those patients from study DMD114044 who were included into the open –label long term extension study DMD114349 as requested. Patients who received placebo in the feeder study DMD114044 and switched to drisapersen at week 48 were compared to those patients who continued active treatment. Overall, the results from this subgroup analysis exercise exhibits a considerable variability, specifically for results beyond week 24. This is expected, as numbers in the subgroups are low and the extension study was stopped early at an index date with the phase III program. When

results of study DMD114349 at week 48 were compared to those at week 48 from study DMD114044, the same subgroups, e.g. those with younger and less affected patients, provided the most promising numerical differences in comparison to placebo. This finding supports the applicants conclusion to start treatment with drisapersen in younger patients and/or earlier in the disease course, e.g. below the age of 7 years. However, as these findings rely on small numbers and are derived from a post-hoc analysis of an open label study which was stopped early, results should be interpreted with caution.

Anti-drisapersen antibodies have been evaluated in study DMD114044. At week 48, 29.4% of the drisapersen treated patients tested positive for anti-drisapersen antibodies. The same analyses were performed for samples from patients participating in studies DMD114117, DMD114876 and DMD114349. Analyses stratified by the presence or absence of anti-AON antibodies did not show any differences with respect to efficacy. Consequently, there seems to be no apparent relationship between immune response (patients with ADA) and efficacy of drisapersen.

Additional expert consultation

N/A

Assessment of paediatric data on clinical efficacy

The target patient population in Duchenne muscular dystrophy is predominantly a paediatric population.

3.3.7. Conclusions on clinical efficacy

Two exploratory phase II studies were performed of which one, study DMD114117, provided statistically significant results for the pre-defined primary endpoint, change from baseline at week 25 in the 6 minute walking distance (6MWD) test. The drisapersen 6m/kg/week continuous regimen group in this study had a mean difference of 35.09 meters on the 6MWT when compared to placebo. The intermittent regimen group was almost not distinguishable from placebo (3.51 meters). A 30 m change in the 6MWT was earlier accepted for Duchenne clinical programmes as a clinically relevant effect. Generally, the patients included in the phase II studies were DMD patients with regard to their baseline characteristics of earlier disease process.

Only study DMD110117 included a loading dose at the beginning. Whether results of this single positive study were caused by the initial loading dose or a chance finding was discussed by the applicant within the answer to the day 120 LOQ. Due to the long tissue-half-life of drisapersen of approximately 3 months it was discussed that higher drisapersen tissue concentrations in study DMD114117 were associated with a greater clinical benefit on the primary endpoint. The applicant therefore performed a tissue exposure model showing that comparable tissue levels where reached approximately 4 weeks earlier in case of the use of a loading dose.

As study DMD114117 showed that patients with drisapersen tissue levels above 10 µg/g showed the highest increase in dystrophin protein expression compared to placebo, the applicant further assessed the relationship between the clinical outcome, based on the efficacy endpoint at week 48 and muscle biopsy tissue concentrations at week 24. The provided analysis showed that the 6MWD in fact improved best at tissue concentrations above 10 µg/g.

Although the provided analyses support the assumption that the positive results received in study DMD114117 are caused by the initial use of a loading dose the applicant`s answer based on the presented models does not fully resolve all doubts.

Some uncertainties also exist in reference to the results received at week 24 in study DMD114876. Although the included study population in study DMD114117 and DMD114876 was comparable in reference to baseline characteristics results received under placebo treatment were not that similar.

The main concern still relates to the unconvincing evidence of efficacy. Although the phase II study DMD114117 showed statistical significance and clinical relevance for its primary endpoint, the distance from baseline to week 24 in the 6MWD, and also the phase II study DMD114876 provided results that pointed into this direction it should be considered that these studies were planned as exploratory studies and not designed to have sufficient power to show a statistical difference of a clinically important effect size. The pivotal study failed for the primary endpoint assessed at Week 48 (p=0.415). The 10.3 m treatment difference over placebo in the 6MWD is considered far from the minimum distance defined as clinically relevant. As the phase III study included a population of broader disease severity and in more progressed disease compared to those populations of the two phase II studies, the applicant now provided further subgroup analyses and one sensitivity analysis to determine a subset of patients that would show a consistent treatment effect in all three placebo controlled studies.

These additional analyses in subgroups defined by “rise from floor time“ and “6MWD“ are considered useful and reasonable to assess the consistency between the Phase II and Phase III data for the primary endpoint. It is acknowledged that the subgroup definitions are based on subgroup ranges of single baseline variables rather than a combination of criteria. It is also noted that these analyses are post-hoc subgroup analyses and interpretation of the results has to be made with caution.

In principle, the definition of a subgroup more sensitive to changes in the primary endpoint for comparisons across studies is considered acceptable, given the difficult setting for studies in Duchenne patients with a wide range of baseline capability as assessed by either baseline 6MWD or rise from floor time as shown by the differences in inclusion criteria between the studies. Current scientific knowledge supports the view that a range of baseline values from about 300 m to 400 m defines a population with more sensitivity for changes in the 6MWD in Duchenne patients. McDonald et al (McDonald CM et al, The 6-minute walk test and other endpoints in Duchenne muscular dystrophy: Longitudinal natural history observations over 48 weeks from a multicentre study, Muscle Nerve, September 2013, 48:343-356) described a baseline 6MWD of <350 meters to be associated with greater functional decline. Also Pane M et al (Pane M et al, Long Term Natural History Data in Ambulant Boys with Duchenne Muscular Dystrophy: 36-Month Changes, PLOs One October 2014, Vol 9,e108205 1-69) states that according to their examinations changes in the 6MWD were significantly different according to baseline age and the baseline 6MWT values (below and above 350m).

However, the provided data showed that results cannot be regarded as consistent concerning the size of the effect for the primary endpoint across the three studies. While the sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters demonstrated a treatment difference of about 27.8 meters that should be considered as almost clinical relevant, the treatment effect in the subgroup analyses for the middle 50% of patients with respect to baseline 6MWD and RFF was away from what was considered clinically relevant in the past.

As the results based on the middle 50% of subjects according to baseline 6MWD were different from those of the sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters, the two analyses suggest that results could be sensibly dependent on the choice of the cut-off value. Thus, a set of sensitivity analyses for different cut-off values is requested to evaluate how results are influenced by the cut-off value. In fact, from the analyses provided it can be concluded that the large variability of the observed effect makes the results problematic to interpret: as for Study DMD114044 when the baseline 6MWD cut-off point is moved from 313-419 metres (middle 50%) to 300-400 meters, the treatment effect versus placebo changes from 19.9 metres (95% CI -8.8, 48.7) to 27.8 (95% CI -7.5, 63.1). The

confidence intervals are even wider for Phase II trials estimations. These results appear highly dependent on the selected cut-off point of 6MWD and are thus far from being robust.

The lack of consistent favourable results on complementary meaningful clinical outcomes such as timed-function tests, the North Star Ambulatory Assessment (NSAA) total score, muscle strength or activities of daily living do not support the weak effect observed in the primary endpoint.

The applicant now provided also a subgroup analysis based on the middle 50% of patients according to baseline 6MWD for several secondary endpoints, e.g. the ambulatory timed function tests and the NSAA at week 48. It is agreed that these endpoints are considered sensitive to change in the included ambulant patient population and also over the treatment duration of 48 weeks. As in study DMD114876 timed function tests of rise from floor, 10 m walk/run and 4-stair climb/descent, and the NSAA only were performed during the 24 week treatment period study DMD114876 was excluded from analysis.

The forest plots at week 48 based on the middle 50% of subjects according to baseline 6MWD showed for these secondary endpoints in both studies comparable results in favor of drisapersen. However, to provide support for robustness of the effects received, sensitivity analyses based on different baseline 6MWD cut-off values as requested for the primary endpoint should also be performed for the secondary endpoints.

3.3.8. Clinical safety

The clinical program of drisapersen in DMD includes nine clinical phase I to III studies. Clinical safety mainly focuses on the results of six of these nine clinical studies (repeat-dose studies). These studies comprise one Phase I/II open-label study (*PRO051-02*), two Phase II placebo-controlled studies (*DMD114117 and DMD114876*), one phase III placebo-controlled study (DMD114044), one completed long - term open-label extension study (DMD114349), and one ongoing long - term open-label extension study (DMD114673). Studies contributing to clinical safety were completed at time of submission except for two long-term extension studies (DMD114673 and DMD115501) with an updated cut-off date for clinical safety data on October 19th 2015. Study DMD115501 has not been integrated in the grouping of studies due to differences in study design and study objectives. The main body of the integrated safety data base is considered comprehensive for a rare disease like Duchenne muscular dystrophy (DMD).

Presentation of data was made by grouping these studies in:

- *"all multiple dose studies"* (PRO051-02, DMD114673, DMD114117, DMD114876, DMD114044, DMD114349; including all study periods),
- "ambulant placebo-controlled studies" (DMD114117, DMD114876, DMD114044; *one – year placebo - controlled safety data of drisapersen*),
- 48-week ambulant placebo-controlled studies (DMD114117, DMD114044) and
- studies contributing to long-term safety of drisapersen (DMD114117, DMD114044, DMD114349) referred to as "*Study DMD114349 and parent studies".*

Single dose studies are considered to be supportive in nature.

Safety parameters were recorded in the majority of the clinical studies except for renal ultrasound, which was exclusively conducted in study DMD114876. In addition, laboratory parameters are considered adequate and cover the most important safety issues with drisapersen taking into account the signals from the preclinical data and the mechanistic features of the drug.

Patient exposure

Safety data of drisapersen derived from a total of 326 subjects with DMD of which 312 subjects received at least one dose of drisapersen. 302 of 326 subjects in the clinical program were included in the *all repeat-dose studies*. The remaining subjects were included in single-dose studies (Table S1).

Table S1: Number of subjects treated in each study (cut off: 31st August 2014)

Study	Number of treated subjects	Number of subjects receiving at least one dose of drisapersen[b]
Repeat-dose studies		
DMD114044	186	125
DMD114117	53	35
DMD114876	51	35
PRO051-02 / DMD114673[a]	12	12
DMD114349	(233 [c])	78 not previously treated with drisapersen in DMD114117 or DMD114044 (232 in total [c])
Single-dose studies		
PRO051-01	4	4
DMD114118	20	15
Other		
Ongoing study DMD115501	(21[d])	8 not previously treated with drisapersen in DMD114876 (21 in total [d])
Total	326 [c,d]	312 [e]

Source: DMD114044 CSR, Section 5.1; DMD114117 CSR, Section 5.1; DMD114876 CSR, Section 5.1; PRO051-02 CSR, Section 10.1; DMD114673 CSR, Section 5.1; DMD114349 CSR, Section 5.1 and ISS Table 9.2; PRO051-01 CSR, Section 10.1; DMD114118 CSR, Section 4.1.

a All subjects who participated in PRO051-02 entered DMD114673.
b Subjects may have received more than one dosing level in any study.
c Subjects in DMD114349 were previously treated in either DMD114044 or DMD114117. These subjects are only counted once in totals in this table. One subject entered the natural history arm of DMD114349 and did not receive any treatment with drisapersen in this study.
d Subjects in DMD115501 were previously treated in DMD114876. These subjects are only counted once in totals in this table.
e Includes subjects treated with drisapersen in DMD 114044, DMD114117, DMD114876, PRO051-02/DMD114673, and those subjects treated for the first time with drisapersen in the long-term extension studies DMD114349 and DMD115501.

Patient exposure in all repeat-dose studies

285 subjects were exposed to drisapersen in the all repeat-dose studies with 267 subjects receiving the 6 mg/kg/wk dose. Completion rates were high with 266 patients on drisapersen (93.3%) completing week 24 and 214 patients on drisapersen (75.1%) completing week 48. Only 16 subjects discontinued study prematurely (11 patients reported a withdrawal of consent).

A total of 271 (95.1%) subjects were treated with drisapersen (all regimens) for at least 24 weeks and 223 (78.2%) subjects were treated for at least 48 weeks. A total of 122 (42.8%) subjects were treated for at least 96 weeks and the maximum exposure was at least 192 weeks (3 [1.1%] subjects). 64 subjects (67.4%) received placebo for 48 weeks.

The number of subjects treated with drisapersen all regimens included in the repeat-dose studies accounts for 490.1 subject-years of exposure (the extent of exposure for drisapersen 6 mg/kg/wk was 432.7 subject-years).

Most of the subjects were treated in Europe and North America, followed by the rest of the world.

Concerning demographic and study population characteristics (age, ethnicity, race, region), the all repeat-dose studies group was well balanced with respect to placebo and drisapersen 6 mg/kg/wk. The

drisapersen 6 mg/kg intermittent (8 doses in a 12-week cycle) group was older than other treatment groups with a mean age of 9.3 years and 20.8% of subjects were ≤7 years.

Patient exposure in ambulant placebo-controlled studies

290 patients were included of which 195 received drisapersen (161 received drisapersen 6 mg/kg/wk) and 95 received placebo. Completion rates were high with 160 patients on drisapersen 6 mg/kg/wk (99.4%) completing week 24 and 139 patients on drisapersen 6 mg/kg/wk (86.3%) completing week 48. Only 4 subjects discontinued study prematurely (due to adverse events and withdrawal of consent). Exposure for at least 48 weeks was slightly higher for placebo (67.4%) compared to drisapersen 6 mg/kg/wk (59%) (confirmed in the 48-week ambulant placebo-controlled studies), which was requested to be further clarified.

Concerning demographic and study population characteristics, the ambulant placebo-controlled studies group was generally similar to that of the all repeat-dose studies.

Patient exposure in DMD114349 and parent studies (**long-term safety**)

The majority of subjects assigned to placebo in the parent studies continued on drisapersen 6 mg/kg/wk. Only few subjects on drisapersen 6 mg/kg/wk and on drisapersen 6 mg/kg/wk intermittent switched to other treatment options throughout the extension study (natural history arm, continuous drisapersen, intermittent drisapersen or no treatment). Most of the subjects withdrew from study DMD114349 due to study closed/terminated (n=221). The most common reason for withdrawal from the study was 'consent withdrawn' (11 subjects). Since studies in this pool comprised a subset of those from the all repeat-dose studies, cumulative exposure was slightly lower: A total of 238 subjects were treated with drisapersen (all regimens) for at least 1 week, 234 (98.3%) for at least 24 weeks and 211 (88.7%) subjects were treated for at least 48 weeks. Almost half of the subjects still had an exposure of more than 96 weeks (46.2%) and 20 subjects (8.4%) from the drisapersen all regimen groups had an exposure of 144 weeks.

Baseline demographics of subjects included in the different groupings were similar: Approximately three-fourths of patients on placebo and drisapersen regimens were White/of Caucasian origin. The most common exon mutations were DMD 45-50, DMD 48-50 and DMD 49-50 deletions. The majority of subjects had no pre-existing cardiomyopathy.

Concomitant medication use mainly comprised glucocorticoids generally administered to all subjects either continuously or intermittently with more subjects being continuously treated. The mean age at which corticosteroids were initiated was similar for all treatment groups, ranging from 5.0 to 5.7 years.

To conclude, DMD is a disease with life-long prevalence and therefore long-term safety data need to be considered. The numbers of patients exposed for more than 6 months is slightly lower than proposed by ICH E1 (regarding the all repeat-dose studies group a total of 271 (95.1%) subjects were treated with drisapersen (all regimens) for at least 24 weeks). Exposure data for at least one year were in accordance with ICH E1 (n=223 subjects with drisapersen exposure for at least 48 weeks of which n=219 subjects have been assigned to the drisapersen 6 mg/kg/wk group). The maximum exposure to drisapersen was ≥192 weeks (4 years) for 3 subjects.

Adverse events

Adverse events emerging with drisapersen were analysed within each of the two groupings ("all repeat-dose studies" and "ambulant placebo-controlled studies"). Analyses were also provided for maximum intensity of on-treatment AEs, adverse event causality and time to occurrence of adverse events.

The incidence of adverse events of subjects from all repeat-dose studies was in a range of 94.7 – 100% for drisapersen and placebo groups. Reporting of severe AEs and drug-related AEs was higher for drisapersen compared to placebo (drisapersen 6 mg/kg/wk: 16.5%; placebo: 4.2% and drisapersen 6 mg/kg/wk: 92.5%; placebo: 48.4%).

Drug-related SAEs and on-treatment AEs resulting in investigational product discontinuation or study withdrawal were reported with drisapersen only.

Differences in AE incidences between drisapersen (6 mg/kg/wk group is depicted) and placebo could be seen on the level of the following system organ classes (differences of ≥ 10% except for blood and lymphatic system disorders) with drisapersen compared to placebo for *general disorders and administration site conditions* (46.3% placebo vs 86.1% drisapersen), *investigations* (29.5% placebo vs 56.9% drisapersen), *renal and urinary disorders* (28.4% placebo vs 50.6% drisapersen), *nervous system disorders* (27.4% placebo vs 39.7% drisapersen), and *blood and lymphatic system disorders* (1.1% placebo vs 9% drisapersen). In general, results for drisapersen 6 mg/kg intermittent and drisapersen all regimens were very similar to those seen for drisapersen 6 mg/kg/wk.

The most commonly reported on-treatment AEs in subjects treated with drisapersen 6 mg/kg/wk were injection site erythema (drisapersen 6 mg/kg/wk 53.2% vs placebo 8.4%), injection site discolouration (drisapersen 6 mg/kg/wk 47.2% vs placebo 5.3%), proteinuria (drisapersen 6 mg/kg/wk 43.1% vs placebo 16.8%), nasopharyngitis (drisapersen 6 mg/kg/wk 36% vs placebo 32.6%), headache (drisapersen 6 mg/kg/wk 35.6% vs placebo 21.1%), vomiting (drisapersen 6 mg/kg/wk 32.6% vs placebo 24.2%), and pyrexia (drisapersen 6 mg/kg/wk 32.6% vs placebo 23.2%). With the exception of nasopharyngitis, all were reported in a substantially higher percentage of subjects treated with drisapersen than with placebo.

Exposure differences between placebo and drisapersen limit the definite conclusion and therefore the additional analyses to account for exposure-adjusted incidence rates were provided. AEs from the all repeat-dose studies grouping for which **exposure-adjusted incidence rates were higher with drisapersen 6 mg/kg/wk compared to placebo** (in at least 5% of subjects) were injection site related AEs, renal abnormalities (proteinuria, haematuria, albuminuria, protein urine present, red blood cells urine positive, cystatin C increased, urine protein/creatinine ratio increased, protein urine), thrombocytopenia, complement factor C3 decreased, glutamate dehydrogenase increased, arthralgia, gastroenteritis, and abdominal pain upper.

The main findings from the ambulant placebo-controlled studies were very similar to those of the repeat-dose studies.

Differences in AE incidences between drisapersen (6 mg/kg/wk group is depicted) and placebo could be seen on the level of the following system organ classes (differences of ≥ 10%) with drisapersen compared to placebo for *general disorders and administration site conditions* (46.3% placebo vs 85.7% drisapersen), *investigations* (29.5% placebo vs 47.2% drisapersen), and *renal and urinary disorders* (27.4% placebo vs 41.0% drisapersen). The incidence of on-treatment AEs with drisapersen 6 mg/kg/wk compared to placebo is highest for *injection site erythema, discolouration, reaction, pain, induration, swelling, atrophy, urticaria, gastroenteritis, diarrhoea, protein urine present, Cystatin C increased, urine protein/creatinine ratio increased, protein urine, proteinuria, haematuria, headache and arthralgia*.

Adverse events reported from *all repeat-dose studies grouping* are very similar to those reported from the ambulant placebo-controlled studies group, giving an overall consistent picture of controlled and controlled/uncontrolled studies.

Analysis of maximum intensity of on-treatment AEs (*all repeat-dose studies*) revealed most of the AEs to be mild and moderate in intensity similar for drisapersen and placebo except for severe AEs, which

were reported with a higher incidence for drisapersen 6 mg/kg/wk compared to placebo (16.1% vs. 4.2%). Thrombocytopenia was the AE reported to be severe in intensity with the highest incidence (2.6% of drisapersen 6 mg/kg/wk treated subjects versus 0% for placebo-treated subjects).

Regarding adverse event causality analysis in the *all repeat-dose studies*, on-treatment treatment-related AEs were found nearly double as high with drisapersen 6 mg/kg/wk compared to placebo (92.5% vs. 48.4%) and were also adverse events of special interest (AESI) in a majority of cases.

The time to occurrence of the most frequent AEs (≥2% in any treatment group) was investigated using data from the *ambulant placebo-controlled studies* DMD114117, DMD114876 and DMD114044 and data from the DMD114349 and parent studies (long-term safety) grouping. On-treatment AEs that occurred at a higher incidence with drisapersen 6 mg/kg/wk compared to placebo and early in treatment were injection site-related AEs like injection site erythema and injection site discolouration (32.3% and 11.2% within the first week to 4 weeks of treatment). In contrast, AEs from the renal and urinary disorders SOC and investigations SOC (including protein urine present, red blood cells urine, Cystatin C increased) developed most of all later during treatment within the first 24 weeks.

From the DMD114349 and parent (long-term safety) experience it could be concluded that occurrence of most of the AEs was highest within the first 12 months of treatment with substantial increases already starting from the first six months on except for injection site erythema and injection site discoloration (from the first month on).

Adverse drug reactions were defined on statistical grounds (as AE with an incidence of ≥5% and double the placebo rate; see Table S2), on known pharmacology, class effects, biological plausibility, reversibility upon drug withdrawal and rarity of the event in the DMD population.

Table S2: Adverse drug reactions with ≥5% incidence in drisapersen 6 mg/kg/wk arm and at least twice the placebo rate (ambulant placebo-controlled studies, updated at Day 120 due to grouping of preferred terms of haematuria and proteinuria)

System organ class **PT/combined PT**	**Placebo N=95** **n(%)**	**Drisapersen 6mg/kg/wk N=161** **n(%)**
General disorders and administration site conditions "Injection site reactions"	14(14.7)	121(75.2)
Investigations		
Cystatin C increased	4(4.2)	17(10.6)
Protein urine	0	8(5.0)
Urine protein/creatinine ratio increased	4(4.2)	14(8.7)
Musculoskeletal and connective tissue disorders		
Arthralgia	2(2.1)	10(6.2)
Renal and urinary disorders		
Haematuria	10(10.5)	26(16.1)

In addition, the grouped term “injection site reactions” was dissolved and the following ADRs were found: *injection site erythema, injection site discolouration, injection site pain, injection site pruritus, injection site reaction, injection site induration, injection site swelling, injection site atrophy, injection site urticaria. Proteinuria* was also evaluated to be an ADR (updated incidences 43.5% drisapersen vs. 23.2% placebo after recalculation of preferred terms).

The *all repeat-dose studies* additionally revealed ADRs of increased GLDH and increased GGT, as well as alopecia (although with an incidence of <5%).

For the evaluation of long-term safety of drisapersen (DMD114349 and parent studies), which is most of all related to the open-label experience without relying on a placebo group, criteria for ADRs were an incidence of ≥10% and the factors described above. Additionally to the aforementioned ADRs, *injection site haematoma, injection site bruising, protein urine present, red blood cells urine positive, and red blood cells urine* were found.

Thrombocytopenia was also reported as an ADR based on the class effect of this AE.

Adverse events of special interest (AESI)

Table S3 depicts the incidences of AESI in the all repeat-dose studies group, which is very similar (except for thrombocytopenia) for the ambulant placebo-controlled studies group.

Table S3: Overview of on-treatment adverse events of special interest (all repeat-dose studies)

Adverse event preferred term	Placebo N=95 n (%)	Drisapersen 3mg/kg/wk N=17 n (%)	Drisapersen 6mg/kg/wk N=267 n (%)	Drisapersen 6mg/kg intermittent[a] N=38 n (%)	Drisapersen all regimens[b] N=285 n (%)
Injection site reaction	21 (22.1)	11 (64.7)	210 (78.7)	31 (81.6)	224 (78.6)
Renal abnormality	32 (33.7)	2 (11.8)	191 (71.5)	29 (76.3)	194 (68.1)
Inflammation events	26 (27.4)	1 (5.9)	102 (38.2)	14 (36.8)	111 (38.9)
Coagulation abnormality	14 (14.7)	0	32 (12.0)	1 (2.6)	33 (11.6)
Hepatic abnormality	2 (2.1)	0	28 (10.5)	7 (18.4)	31 (10.9)
Thrombocytopaenia	0	0	19 (7.1)	2 (5.3)	20 (7.0)

Source: ISS Table 11.63

Injection site reactions

On-treatment ISRs were reported more frequently with drisapersen compared to placebo in the *all repeat-dose studies* (78.7% in the drisapersen 6 mg/kg/wk groups vs. 22.1% in the placebo groups). 2 of 267 subjects (0.7%) from the drisapersen 6 mg/kg/wk group reported SAEs (*severe injection site oedema*).

The most commonly reported injection site reaction events (>10% of subjects) were *injection site erythema and injection site discolouration*, reported in 52.1% and 47.2% of subjects, respectively in the drisapersen 6 mg/kg /wk group, followed by *injection site induration* (26.9%), *IS pain* (19.5%), *IS pruritus* (16.9%), *IS reaction* (18.4%), *IS atrophy* (12%), *IS bruising* (13.1%), *IS haematoma* (12%) and *injection site swelling* (10.1%). The most common PTs of ISRs in *the ambulant placebo-controlled studies* were injection site erythema (51%), discolouration (which describes both, hyperpigmentation and less commonly hypopigmentation, 35%), pain (16%), reaction (16%), pruritus (15%), bruising (12%) and induration (11%) (vs. incidence of 1 to 8% in the placebo group). Injection site necrosis was not reported in any study. These ISRs were also rated **ADRs** of drisapersen.

The effect of rotating injection sites was not systematically studied. It was stated that there are 8 weeks between injections in the same injection site when rotating. CHMP already commented on injection site rotation and was concerned that rotation would not reduce ISRs and tumorigenic changes could be possible from these chronic inflammation sites. Upon request, the Applicant provided additional analyses to address the impact of injection site rotation in studies DMD114673 and DMD114349 (both open-label long-term studies). Analyses comprise an interval of 96 weeks or 96 doses. Most subjects in study 114673 received abdominal injections within the first 96 weeks/doses. In contrast, rotation of injection sites (abdomen, thigh, buttock, arm) was favoured from the beginning in DMD114349 (including feeder studies).As a result, the overall incidence of ISRs in study DMD114673 was higher for the pre-defined time period (100%, no injection site rotation) compared to those in study DMD114349 (90%, rotation applied). The range of reported ISR terms was broader on DMD114349, probably due to the higher number of subjects included. The most significant result was the higher incidence of *injection site induration* (including skin hardening through descriptions of sclerosis) in subjects in DMD114673 (100%) compared to DMD114349 (36.3%). These comparative data suggested that rotation of injection sites might be beneficial for at least a subset of ISRs (e.g. injection site induration).

The occurrence of *Injection site recall reactions* was asked for by CHMP. Recall injection site reactions were not specifically evaluated except for study DMD115501, which is still ongoing and for which "memory responses" of ISRs were systematically prompted.

As a result, nearly 1/3 of subjects included in the study reported an ISR memory response, which could be either a hyperpigmentation or a more severe form of ISR (atrophy, rash, and erythema). In addition, one subject from study DMD114673, an ulcer has been reported to occur at an injection site at which no injection was administered for 8 months. The mechanism of recall injection site reactions is poorly understood. However, experience with etanercept showed that recall injection site reactions respond to antihistamines and therefore a hypersensitivity reaction is assumed (Rajakulendran et al. 2004). Further information was requested to be provided on treating memory responses.

Injection site oedema was reported as SAE in two subjects leading to study withdrawal in one subject.

Severe injection site AEs (n=16) were reported by 10 subjects: two SAEs of injection site oedema, injection site atrophy, injection site discolouration, injection site induration, injection site pain, injection site inflammation, injection site erythema, injection site warmth, injection site nodule.

Injection site erythema was commonly reported to occur early in treatment (within the first month of treatment). Injection site discolouration, injection site pruritus, IS reaction, IS bruising and IS haematoma were more frequently reported within the first six months of treatment. Injection site induration, injection site atrophy and injection site swelling were not commonly seen during the first month of treatment and were more commonly reported after longer treatment duration (6 to 24 months).

The outcome of ISRs after study termination was "not resolved" for 739 of the 3477 events with drisapersen 6 mg/kg/wk (21.4%; *all repeat-dose studies*). The following ISRs were most likely not to be resolved: lipodystrophy acquired, injection site atrophy, injection site induration, hyperaemia and injection site nodule. Mean duration of resolved ISR was 57.9 days. Data for ambulant placebo-controlled studies were similar. Each of the subjects in study DMD114673 (n=12) had at least one ISR that has been reported as "not resolved".

98 subjects in the open-label extension study **DMD114349** were found with significant ISRs. 50% of these subjects had one or more persistent injection site reactions of induration; approximately 20% of the 98 subjects had persistent lipoatrophy, atrophy or lipodystrophy; 2 had injections site reactions which were described as sclerosis and 2 as fibrosis; and 2 individuals had persistent injection site

reaction oedema. One individual had a report of a persistent injection reaction nodule. All of the 12 subjects in study **DMD114673** had persistent injection site induration (100%, see above). Seven of these 12 subjects also had ongoing injection site discolouration; 3 of the 12 had unresolved injection site atrophy and a similar proportion had unresolved injection site erythema. One subject was reported with microcalcification. Over the course of study DMD114673, all subjects had various injection site related AEs getting more frequent with longer treatment periods. ISRs of subcutaneous nodules, injection site atrophy and IS ulcer were reported during later time periods (from week 80 on). IS ulcers reported in study DMD114673 progressed with bacterial infections.

CHMP was concerned on the severity and long-term implications of ISRs seen in the clinical program. The Applicant provided additional information on ongoing open-label long-term extension studies DMD114673 and DMD115501, including ISR grading system, medical photography, and dermatological evaluation. A significant amount of ISRs worsened with chronic exposure to drisapersen even in periods without treatment supporting the idea of an immunological cause. Medical photographs were taken from moderate to severe ISRs of 8 subjects from study DMD114673 and from 2 subjects from study DMD115501. ISRs (based on visual assessment) could be summarized as massive, large-scaled and multiform inflammations of the skin, including nodules, formation of granuloma, sclerosis, skin ulcer (partly superinfected), scarred structures (hypotroph or hypertroph), and residual tissue deficiencies (“hole in the skin”). The size of the measured ISRs was extensive with worsening of some of the ISRs in drug-free intervals predictive for broad inflammatory and also immunological processes.

Unfortunately, dermatological assessment was foreseen in the aforementioned studies, but no expert evaluation was presented for the events with medical photographs. In addition, firm conclusion on the type of ISR and etiology of ISRs would have been facilitated with histopathological evaluations, which have been implemented at least in study protocols for the ongoing extension studies. It was hence concluded, that the impact of ISRs on the long-term safety profile of drisapersen is still insufficiently characterized

Renal abnormalities

The kidney is a target organ for drisapersen with drug accumulating in the proximal tubule. More subjects on drisapersen 6 mg/kg/wk reported renal abnormality AEs compared to subjects on placebo in the *all repeat-dose studies* (71.5% vs. 33.7%).

Four (1.4%) subjects treated with drisapersen experienced renal abnormalities that were reported as SAEs, 3 in the drisapersen 6 mg/kg/wk group (moderate glomerulonephritis, severe proteinuria, moderate renal impairment) and 1 (severe haematuria) in the drisapersen 6 mg/kg intermittent group. Two SAEs (glomerulonephritis and proteinuria) led to the withdrawal of the subjects. These were also the only 2 subjects who reported nephrotic levels of urinary protein (defined as 3.5 g/24 hours or >40 mg/m2/hour) or urinary protein levels >1 g/24 hours. The severe proteinuria and severe haematuria recovered. The case of glomerulonephritis was not recovered at the end of the study, but subsequent follow-up indicated that the subject was recovering.

The most common on - treatment renal abnormalities AEs were (drisapersen 6 mg/kg/wk vs. placebo) proteinuria (43.1% vs. 16.8%), protein urine present (15.7% vs. 6.3%), haematuria (16.1% vs. 5.3%), cystatin C increased (12.4% vs. 4.2%), red blood cells urine positive (13.5% vs. 4.2%), and urine protein/creatinine ratio increased (11.2% vs. 4.2%). Other renal abnormality AEs occurred in less than 5% of subjects on drisapersen 6 mg/kg/wk, including albuminuria (3.4%), protein urine (4.9%), and alpha-1-microglobulin urine increased (4.1%).

Data from *ambulant placebo-controlled studies* were generally similar to those of the all repeat-dose studies (98 (60.9%) subjects treated with drisapersen 6 mg/kg/wk compared with 32 (33.7%) subjects treated with placebo reported renal abnormalities).

Urinalysis and laboratory parameters were evaluated:

There were **mean and median increases** in **urine protein** for drisapersen 6 mg/kg/wk: mean change from baseline was 47.1 mg/L at Week 12, 69.9 mg/L at Week 24, 72.0 mg/L at Week 36, and 63.9 mg/L at Week 48 compared with mean changes of 3.4, 4.6, 8.4, and 5.6 mg/L, respectively for placebo. Changes in **urine α1-microglobulin** were significantly greater for drisapersen 6 mg/kg/wk than for placebo. Highest changes from baseline were found around week 36. Changes from baseline at week 24/week 48 were 19.28 mg/L and 23.34 mg/L (placebo: 0.21 and 0.15 mg/L). Shifts from normal to high for drisapersen 6 mg/kg/wk were found in up to 81% of subjects (Week 36)!

Red blood cells and white blood cells behaved contrarily in the drisapersen and placebo group regarding their overall absence: drisapersen 6 mg/kg/wk group absence of red (white) blood cells from baseline to week 48 decreased from 94.4% (87.0%) to 88.2% (75%). In the placebo group, the portion of subjects with no red or white blood cells in urine increased over time.

Coarse granular casts, fine granular casts, RBC casts, WBC casts and waxy casts were neither found in placebo-treated subjects nor in the drisapersen-treated subjects. Hyaline casts were found in single subjects in both groups.

Results from the open-label extension study DMD114673 confirmed that positive urinalysis results were more often retrieved with the progress of the studies. Further analyses show that drug-free periods (washout in weeks 73 to 80, 8 weeks treatment phase, 4 weeks off-drug) were in favour of some of the parameters to decrease, e.g. urine protein values and alpha-1-microglobulin. However, these data should be carefully interpreted since only 12 subjects participated in this study.

Further clarification in regard to monitoring recommendations for renal parameters was presented:

A re-calculation of proteinuria and haematuria for drisapersen 6 mg/kg/wk and placebo has been undertaken for the ambulant placebo - controlled studies to combine similar preferred terms on proteinuria and haematuria. Proteinuria was recalculated to be present in 43.5% of drisapersen 6 mg/kg/wk treated subjects vs. in 23.2% of placebo-treated subjects (haematuria: 16.1% drisapersen vs. 10.5% placebo). The laboratory findings of random protein in urine called for a highly conservative proceeding in the clinical studies. Drisapersen was interrupted and 24-h urine measurement was performed. Most of the findings from random protein could not be confirmed in the 24-h urine and treatment was resumed (only 14% of subjects from the all repeat - dose studies with random protein have been confirmed with proteinuria in the 24-h urine samples). Most of the protein findings were found resolved after treatment interruption. However, the collection of 24-hour urine samples is fraught with error, and the collection often has to be repeated (Loghman-Adham M. Evaluating proteinuria in children, Am Fam Physician. 1998). The Applicant was requested to additionally indicate how collection of 24h urine has been checked in the studies to confirm that collection was complete. This was assumed to affect feasibility of monitoring in the SmPC.

Baseline mean **cystatin C values** were similar for the two groups (ambulant placebo-controlled studies). At Week 24 and Week 48, mean changes were higher for drisapersen (0.11 and 0.16 mg/L, respectively) than for placebo (0.05 and 0.04 mg/L, respectively). For drisapersen 6 mg/kg/wk, the percentage of subjects with shifts from normal to high increased during treatment and was 17.3% at Week 12, 31.0% at Week 24, and 40.4% at Week 48. The corresponding values for placebo were 8.0%, 6.4%, and 5.3%, respectively. The gradual increase of serum cystatin C was confirmed in the open-label extension study DMD114349 (up to week 40).

Mean changes for **creatinine** were 0.33 µmol/L at Week 12, 1.03 µmol/L at Week 24, and 1.90 µmol/L at Week 48 for drisapersen 6 mg/kg/wk. For placebo, the mean changes were 0.72, -0.20, and -0.25 µmol/L, respectively. At Week 48, the percentage of subjects with shifts to abnormal values was higher for drisapersen than for placebo. In the drisapersen 6 mg/kg/wk group, 34.1% had no shift, 61.5%

had a shift to Grade 1, and 4.4% had a shift to Grade 2. For placebo, the percentages were 55.8%, 42.9% and 1.3%, respectively. Low numbers of subjects in both groups had shifts to Grade 3 or 4. Similarly, there were larger mean increases in **BUN** with drisapersen 6 mg/kg/wk throughout the 1 year study period. All subjects had BUN values within normal limits.

Four SAEs of renal abnormalities were documented, two of which led to study withdrawal (*SAE of membranous glomerulonephritis, SAE of proteinuria*). Proteinuria of the subject with membranous glomerulonephritis was significant (up to 9g/L) and renal biopsy supported urinalysis results. No anti-drug antibody status was available from this subject at Week 48 (anti-drug antibody status at week 24 was negative). Membranous glomerulonephritis was found clearly related to drisapersen treatment setting the patient at risk for drug-induced alteration of the immune system. The condition emerged later during treatment, therefore it could not be ruled out that drisapersen causes more significant (renal) safety problems later during treatment.

The second SAE “severe proteinuria” also peaked in high urine protein concentration (up to 11g/24h). However, a renal biopsy was not taken and hence renal damage could not be verified. One SAE of renal impairment showed up with high BUN and creatinine as well as haematuria. For this SAE alternative causes may be discussed (dehydration subsequent to virus infection). Last but not least, (macroscopic) haematuria as a SAE was reported (subject also had proteinuria). Two additional severe AEs were reported: protein urine present (self-limiting) and red blood cells urine positive/ severe protein urine present.

Most of the renal abnormalities were resolved by the end of the studies (94% of drisapersen – related AEs). The mean duration of resolved renal abnormality events was 40.1 days (maximum duration 1002 days) *versus 19.2 days in the placebo group*.

To conclude, two different findings were thought to be taken into account for judging the safety profile of drisapersen in regard to renal damage probably caused by this drug. **Tubular interaction** seemed to be the prevailing mechanism of renal findings in the clinical program (drisapersen inhibits tubular reabsorption of alpha-1 microglobulin and albumin via competition for reabsorption) and no signs of tubular damage were noted neither in the preclinical program (no cell necrosis) nor in the clinical program (blood pressure elevation and electrolyte disturbances). The second possible mechanism was thought to imply an **immune-mediated alteration** during later stages of drisapersen treatment causing nephrotic range proteinuria (including membranous glomerulonephritis). Further predictive parameters defining the type of renal interaction were lacking (IgG urine, erythrocyte morphology). More data were requested to be systematically collected in ongoing long-term studies and post-marketing to address this uncertainty

Monitoring proteinuria in clinical routine was proposed in line with the study protocols. Urine protein were recommended to be measured by dipstick at baseline and every two weeks with quantitative measurement (24 –h urine) being initiated with more than trace amounts of urine protein to confirm proteinuria. Even though dip stick controls are considered easily to conduct, 24-h urine sampling in a substantial number of subjects may become necessary being cumbersome and fraught with error. Hence, feasibility of this subsequent risk minimisation measure is not indisputably shown and should be further addressed by the Applicant.

Inflammation events

Pro-inflammatory effects were found in animal species and are a known class effect of phosphorothioate oligonucleotides (including activation of the alternative complement pathway).

102 of 267 subjects (38.2%) on drisapersen 6 mg/kg/wk and 26 of 95 subjects (27.4%) receiving placebo reported inflammation related AEs in the *all repeat-dose studies*. One SAE of pyrexia was

reported for a subject on drisapersen 6 mg/kg/wk and no patient was withdrawn due to an inflammation AE.

The most common on - treatment inflammation AEs were (drisapersen 6 mg/kg/wk vs. placebo) pyrexia (29.6% vs. 22.1%), complement factor C3 decreased (6.4 % vs. 0%), and C-reactive protein increased (3.4% vs. 0%). In regard to the incidences in the *ambulant placebo-controlled studies*, inflammation events were similarly distributed between drisapersen and placebo-treated subjects. No safety issue could be detected in the one year studies.

Biomarkers for inflammation events in the *ambulant placebo-controlled studies* were:

Mean baseline values of **hsCRP** were not similar for placebo and drisapersen 6 mg/kg/wk (0.51 mg/L and 0.82 mg/L). A mean increase was first noted in drisapersen 6 mg/kg/wk treated subjects compared to placebo **from Week 48** (0.83 mg/L and 0.48 mg/L). At week 48, more subjects on drisapersen 6 mg/kg/wk had a shift from normal to high hsCRP compared to placebo (10.4% vs. 3.8%). Intermittent treatment seemed to have no beneficial effects on hsCRP values over time. Mean changes of **Complement C3** from baseline were -0.047 g/L at Week 12, -0.075 g/L at Week 24, and -0.085 g/L at Week 48 (8% decrease from baseline to Week 48), respectively, for drisapersen. For placebo, the mean changes were 0.041, 0.004, and -0.025 g/L, respectively. At week 48, more subjects on drisapersen 6 mg/kg/wk had a shift from normal to low complement C3 compared to placebo (11.7% vs. 1.3%). Data from study DMD114349 up to week 104 indicate slight decreased but mainly stable values: in regard to study DMD114349 baseline complement C3 values, the mean decrease up to week 104 was 2.4% and regarding the original baseline complement C3 values of the ambulant placebo-controlled studies, there was a mean decrease of 6% in total. For study DMD 114349 it could be ascertained that no further decrease in complement C3 concentration over longer treatment periods took place.

To further substantiate complement activation, the Applicant was asked to present data on complement split products measured in two phase I/II studies (DMD114118 and PRO051-02). Drisapersen was administered as a single dose in study DMD114118 and over a period of five weeks in study PRO051-02. There seemed to be no strong evidence from the two small phase I/II studies that drisapersen triggers formation of complement split products. A mean concentration of split product C3a was found to be above the upper range of normal already at screening in study DMD114118, which needed further discussion. The small number of subjects included in these two studies and treated with single dose or short – term drisapersen was not considered representative to reassure the absence of complement activation in a larger data set or with longer drisapersen treatment. Complement split product measurement was also implemented in ongoing open-label long-term study DMD114673. Any available data were requested to be presented to the competent authority for further clarification. Further monitoring was deemed necessary to be implemented in any ongoing study with drisapersen.

Differences between placebo and drisapersen in **MCP-1** were observed as early as from week 24 on. The mean increase for placebo was 18.21 ng/L compared with an increase of 162.80 ng/L with drisapersen and at Week 48 there was a mean decrease of -87.83 ng/L for placebo compared with an increase of 305.08 ng/L for drisapersen. At Week 48, there were 10 (8.1%) subjects in the drisapersen 6 mg/kg/wk group with a shift from normal to high for MCP-1 compared with 1 (1.5%) subject in the placebo group. Differences between placebo and drisapersen for **Fibrinogen** were observed as early as from week 48 on. The mean change for placebo was 0.041 g/L compared with a mean change of 0.289 g/L with drisapersen. Shift analysis was not indicative of a specific pattern.

Differences in haptoglobin (binding of free haemoglobin to prevent toxic renal damage) between placebo and drisapersen were observed as early as from week 48 on. The mean change for placebo was - 0.005 g/L compared with a mean change of 0.101 g/L with drisapersen. Shift analysis was not indicative of a specific pattern. **Immunglobulin IgG:** mean baseline values were slightly higher for

placebo vs. drisapersen-treated subjects. Differences were observed as early as from week 12. The mean change for placebo at week 12 (week 24/ week 48) was - 0.010 g/L (- 0.170/ - 0.198) compared with a mean change at week 12 (week 24/ week 48) of 0.451 g/L (0.878/ 1.786) with drisapersen 6 mg/kg/wk.

Box plot analyses from long-term study grouping including DMD114349 and parent studies revealed higher hsCRP values with intermittent compared to continuous drisapersen treatment.

Most of the inflammation AEs were found recovered or resolved by the end of study treatment. The only AEs not resolved were 4 events of complement factor C3 decreased and one AE of blood fibrinogen increased, which may be indicative of a longer effect on immunological functioning. For the recovered inflammation events the mean duration was 19.4 days.

As a conclusion, the long-term clinical relevance of increases of single inflammatory biomarkers and decreases of complement factors could not be judged and remains to be further evaluated within ongoing studies and post-marketing.

Coagulation abnormalities

Nonclinical experience with phosphorothioate oligonucleotides showed various haematological effects including a transient, dose-proportional prolongation in aPTT (Henry et al., 2007; Kwoh, 2007).

14 of 95 subjects on placebo (14.7%) and 32 of 267 subjects on drisapersen 6 mg/kg/wk (12%) reported AEs of coagulation abnormalities in the *all repeat-dose studies*. SAEs only occurred within the drisapersen 6 mg/kg/wk group (0.7%; 2 subjects). Severe coagulation abnormalities were reported in two subjects. A relation to drug was found in 9.5% of subjects on placebo and in 8.2% of subjects on drisapersen 6 mg/kg/wk. No subject discontinued study due to such an AE.

The most commonly reported coagulation abnormality AEs were reported with a similar incidence in placebo- and drisapersen treated subjects and were *international normalised ratio increased* (4.2% vs 4.1%), *blood fibrinogen decreased* (7.4% vs 4.1%), *prothrombin time prolonged* (4.2% vs 2.6%), *activated partial thromboplastin time prolonged* (3.2% vs 2.2%), and *fibrin D dimer increased* (2.1% vs 2.2%).

More subjects on placebo (14 of 95 subjects; 14.7%) reported an AE of coagulation abnormalities compared to those on drisapersen 6 mg/kg/wk (13 of 161 subjects; 8.1%) in the *ambulant placebo-controlled studies*. Preferred terms were similar to the all repeat-dose studies.

In addition, **MedDRA SMQs for Haemorrhages and for Embolic and Thrombotic Events** revealed more subjects (*all repeat dose studies*) with AEs on drisapersen 6 mg/kg/wk vs placebo (58% vs. 42%). This difference of drisapersen and placebo resulted from preferred terms of haematuria (16% vs. 5%), injection site bruising (13% vs. 10%), injection site haematoma (13% vs. 6%), and epistaxis (13% vs. 6%). It was noted that two SAEs fell within these search criteria: *SAE of intracranial venous sinus thrombosis* and *renal venous thrombosis and pulmonary emboli* within the diagnosis of membranous glomerulonephritis (see renal abnormalities). Thrombolic and embolic events were thought to result from inflammatory lesions caused by drisapersen as reported from preclinical experience. The laboratory assessment related to inflammation parameters revealed several abnormalities among the SAEs described for subjects no. (increased fibrinogen and haptoglobin) and no. (discrete increase in haptoglobin and elevated CRP; coagulation parameters (D dimer and PT activity) were found abnormal), although no clear pattern was found. Complement activation could not be verified in these two subjects. To further substantiate a contribution of the underlying DMD causing thromboembolic conditions in these subjects (see Kimura et al. 2015), the Applicant was asked to summarise their baseline risk factors.

Further recommendations on routine monitoring and risk minimisation measures deemed necessary to capture rare thromboembolic events.

Laboratory data relevant to coagulation abnormalities revealed small and alternating changes in aPTT and INR in subjects on placebo and drisapersen. Almost all subjects had INR values that were normal or CTCAE Grade 1 at on-treatment time-points.

Regarding shifts from baseline to post-baseline in PTT (INR) increased, more subjects on drisapersen 6 mg/kg/wk had a shift from Grade 0 to Grade 1 at weeks 24 and 48 compared to other study visits. Shifts from baseline to post-baseline in aPTT increased were similar for placebo and drisapersen treated subjects and did not follow a continuous pattern. Elevated aPTT (sec) (stopping criteria exceeded) and PTT ratio (INR) values from baseline to any post-baseline visit were found in DMD114349 for 16 (7%) of subjects. Treatment with drisapersen was interrupted and restarted after resolving of AEs.

Three subjects met the stopping criteria for **disseminated intravascular coagulation (DIC)**; these subjects had a thrombocyte count $<75 \times 10^9$/L and either fibrin split product test or D-dimer above the upper limit of the normal range. However, DIC was not confirmed on subsequent testing. These subjects were withdrawn from study due to their SAE of thrombocytopenia.

Two SAEs (INR increased; both not considered related to drisapersen) and two severe AEs (severe fibrin degradation products increased and severe fibrin D-dimer increased; severe aPTT prolonged, severe international normalised ratio increased and severe prothrombin time prolonged; both severe events considered to be related) were reported in relation to coagulation abnormalities. Both severe AEs were not resolved by the end of the study.

The outcome of the coagulation abnormalities was reported to be resolved for approximately 90% of drisapersen-treated subjects in the all repeat-dose studies as well as in the ambulant placebo-controlled trials.

Hepatic abnormalities

Hepatic toxicity of antisense oligonucleotides was reported in the rat, Cynomolgus monkey, and human (Burdick et al. 2014) due to accumulation in the liver. Preclinical studies on phosphorothioate oligonucleotides revealed increases in liver transaminases and bilirubin (Iannitti et al. 2014).

All repeat-dose studies group reported 2 of 95 subjects on placebo (2.1%) and 28 of 267 subjects on drisapersen 6 mg/kg/wk (10.5%) with AEs of hepatic abnormalities. The most commonly reported hepatic abnormality AEs in placebo and drisapersen 6 mg/kg/wk were glutamate dehydrogenase increased (0% vs 4.9%), alanine aminotransferase increased (2.1% vs 2.6%), gamma-glutamyltransferase increased (0% vs 2.6%), alanine aminotransferase (0% vs 0.4%), and aspartate aminotransferase increased (0% vs 0.4%). AEs of hepatic steatosis, hepatocellular injury, hepatomegaly, hepatotoxicity, and liver disorder occurred each in single subjects on drisapersen.

An overall similar trend emerged from the *ambulant placebo-controlled studies*.

Transaminases alanine aminotransferase (**ALT**) and aspartate aminotransferase (**AST**) are markers of hepatocellular injury but are also highly concentrated in muscle cells. Consequently, DMD as the underlying disease itself leads to an increase in transaminases (Wright et al. 2012). Hence, interpretation of changes in transaminases from very high baseline values is difficult.

Mean baseline values of ALT (more specific for liver) were found approximately near 8 x ULN and mean baseline values of AST (more specific for muscles) were slightly lower. Up to week 48 of treatment, AST and ALT slightly decreased, which was more obvious for drisapersen than for placebo (AST: **- 26.3**/- 17.4 and ALT: **- 28.1**/- 19.7). Mean GGT and GLDH were unremarkable at baseline but

increased with duration of treatment with drisapersen and not with placebo. Bilirubin baseline values were higher than clinical reference ranges but slightly decreased over time. Heterogeneous shifts from baseline were reported for ALT and AST (of note: no subject had Grade 0 ALT/AST at baseline but most subjects had Grade 3). Open-label extension study DMD114349 revealed that ALT and AST values of potential clinical concern were higher for any post-baseline visits compared to baseline assessments. No firm conclusion could be drawn from these changes. A hypothesis was that even if slight beneficial effects of ALT and AST could be seen with treatment of up to one year in the ambulant placebo-controlled studies, longer treatment durations might be again associated with increases of transaminases ALT and AST values due to potential liver impairment in accordance with other hepatic parameters. Shifts from normal at baseline to high post-baseline increasing with treatment duration were found for GLDH. At week 48, 33% of subjects shifted from normal to high. Results from DMD114349 revealed that 68% of subjects treated with drisapersen 6 mg/kg/wk had increases in GLDH above the normal reference range at any time post baseline compared with 14% at baseline. GLDH is a mitochondrial enzyme found primarily in the centrilobular region in the liver (O`Brian et al. 2002). It may therefore serve as a biomarker indicating early mitochondrial dysfunction. GLDH was also found to be higher for intermittent treatment of drisapersen compared to continuous treatment as shown in the long-term study grouping. Last but not least, reversibility of GLDH and GGT was claimed, but clear support by data is lacking.

The two protocol-defined monitoring/safety rules for liver chemistry met by a total of 31 subjects (accounting for approximately 12% of subjects on drisapersen 6 mg/kg/wk in the all repeat-dose studies) were ALT≥8 x ULN / INR > 1.5 and ALT≥8 x ULN associated with symptoms of hepatitis or hypersensitivity. Treatment has not been stopped permanently in any subject. No case of Hy's law was reported (ALT increases ≥3 x ULN with concomitant elevations in total bilirubin ≥2 x ULN).

Four **SAEs** were described including two SAEs of ALT increased (and at the same time INR increased), one SAE of hepatocellular injury and one SAE of hepatotoxicity.

Regardless of the study grouping about ¾ of the hepatic abnormalities AEs were resolved by the end of the studies. AEs not resolved were glutamate dehydrogenase increased, ALT increased, hepatic function abnormal, hepatic steatosis and hepatomegaly.

Adequate monitoring of liver transaminases may record the increases in transaminases seen in the clinical program. The Applicant revised the monitoring algorithm from every six months to once monthly liver function testing. In addition, interruption of treatment is recommended in case of signs of hepatitis and liver abnormalities (increase in bilirubin and GGT). However, a monthly routine monitoring was found additionally burdensome for the patient and according to the clinical data there seemed to be no clear clinical value for tightening the intervals to once monthly. The Applicant was requested to present **a summary** of hepatic parameters and their mean changes **up to the first two years** of treatment with drisapersen to further decide on the monitoring intervals.

Thrombocytopenia

Thrombocytopenia has been occasionally noted following antisense oligonucleotide treatment in preclinical test species but appears to be compound-specific rather than a common oligonucleotide class effect (Frazier et al. 2015). Other findings attributed thrombocytopenia to the class of AONs caused by the backbone of antisense oligonucleotides and not by a specific nucleotide sequence. The potential mechanism for thrombocytopenic episodes is not known and there is no clear understanding in the difference of this mechanism in different species (Frazier et al. 2015).

Mechanism of thrombocytopenia seen in the clinical study program with drisapersen has not been studied and it was assumed that there are separate mechanisms involved, of which one promotes early thrombocyte reduction noted as slight but continuous decreases in platelet counts from treatment

initiation on. Severe thrombocytopenia was found to involve a very sudden drop in platelet counts (by factor 10 between two platelet measurements 14 days apart) thought to be clearly immunologically mediated and appeared after at least 400 days of treatment with drisapersen (range 14 – 26 months).

No subject on placebo and 19 of 267 subjects on drisapersen 6 mg/kg/wk (7.1%) reported AEs of thrombocytopenia in the *all repeat-dose studies group***.** Preferred terms were thrombocytopenia (15 subjects [5.6%] on drisapersen 6 mg/kg/wk), platelet disorder (1 subject on drisapersen 6 mg/kg/wk), and platelet count decreased (5 subjects [1.9%] on drisapersen 6 mg/kg/wk). **Thrombocytopenia did not occur during the *ambulant placebo-controlled studies* up to one year**.

Most of the thrombocytopenia AEs were resolved by the end of the respective studies and mean duration of the resolved events were approximately three weeks.

Baseline thrombocyte counts in the *ambulant placebo-controlled trials* were similar for placebo and drisapersen 6 mg/kg/wk (306.4 x 109/L and 309.5 x 109/L; *normal reference range: 130 – 400 x 109/L*). There was a mean (SD) decrease for subjects on drisapersen 6 mg/kg/wk at Week 12 (-33.4 [46.28] x 109/L), **Week 24** (-49.8 [52.89] x 109/L; **mean reduction of 16%**), Week 36 (-56.7 [55.31] x 109/L), and **Week 48** (-67.1 [54.58] x 109/L; **mean reduction of 22%**). Mean changes for placebo were low (-0.2 [40.57], -1.3 [49.62], -6.5 [56.00], and -8.7 [37.61] x 109/L, respectively). Mean post-baseline values remained within the normal reference range. Few subjects had a shift from normal to a Grade 1 decrease in thrombocytes (value less than the lower limit of normal but ≥75 x 10^9/L) with drisapersen up to week 48 compared with no subjects treated with placebo. No shifts to Grade 2, 3, or 4 occurred in any treatment group. Long-term studies grouping (DMD114349 and parent studies) found decreases to be most significant within the first 24 weeks of treatment. Large falls of thrombocyte counts occurred in single subjects only and after at least 400 days following treatment initiation. Intermittent drisapersen treatment revealed similar results. It was concluded, that thrombocyte counts progressively decline with duration of drisapersen treatment but with different mechanisms involved.

SAEs (CTCAE Grade 3 or 4 thrombocytopenia) only occurred within the drisapersen 6 mg/kg/wk group (3%; 8 subjects). All subjects were withdrawn from study due to their SAE. The course of thrombocytes to decrease with time was similar for all of the eight subjects: thrombocytes declined slightly over time and Grade 3 or 4 AEs did not emerge as early as **400 days after start of drisapersen in the parent study**. Two of eight SAEs were Grade 3 and the remaining six SAEs were Grade 4 (<25 x 10^9/L). Six of the eight subjects had reported spontaneous bleedings and had to be treated with tranexamic acid, i.v. immunoglobulin and/or steroids. Anti-thrombocyte antibodies were found positive in five of eight subjects. Additional six subjects were reported to have thrombocytopenia with thrombocytes <75 x 10^9/L. Similar to the SAEs, these additional thrombocytopenic AEs occurred not within the first year of treatment in most of the subjects. In one subject, thrombocyte antibodies were found positive.

The severe presentation of thrombocytopenia, which could finally be life-threatening due to extensive bleeding events, was not sufficiently characterised. Mechanistical explanation is lacking yet thought to be immune-mediated. There are no early signs or predictors for this event (anti drug antibodies, medical conditions). There is no information on re-challenge experiments and hence, subjects with severe thrombocytopenia have to discontinue treatment. It remained unknown if this event may become more prominent with patients on even longer drisapersen treatment.

Platelet count measures once every 14 days were thought to capture most of the steep decreases in platelets but may hardly be reasonable in real word setting of (still) ambulant patients.

Serious adverse events and deaths

No death occurred during the clinical program with drisapersen.

A total of 57 subjects reported 76 SAEs, 55 subjects in the repeat-dose studies. SAEs were found to be in accordance with AESI as defined by the Applicant and more common with drisapersen compared to placebo (all repeat-dose studies: 9 subjects on placebo, 46 subjects on drisapersen [44 subjects treated with drisapersen 6 mg/kg/wk]). Approximately half of the subjects with SAEs on drisapersen were treated in the ambulant-placebo-controlled studies.

Thrombocytopenia, reported in 8 (3.0%) subjects treated with drisapersen 6 mg/kg/wk was the most commonly reported and treatment-related SAE also leading to treatment discontinuation for all subjects. *Injection site oedema* was reported in two subjects on drisapersen. SAEs related to renal abnormalities were reported in one subject each and included *glomerulonephritis, haematuria and proteinuria*. *Hepatotoxicity* was reported as SAE in one subject. Further information on these SAEs is given in the AESI section.

Of note, seven femur fractures, three tibia fractures, one ankle fracture, one lumbar vertebral fracture and one linear fracture/head injury were reported with drisapersen and no such SAE with placebo. These were considered by the investigators as not related to treatment, since fractures are a common finding in DMD patients. In addition, patients with DMD are commonly on continuous glucocorticoid treatment with known effects on bone mineral density and the underlying disease itself is known to affect Vitamin D and calcium homeostasis (Morgenroth 2012). DEXA measurements have been conducted during most of the studies but reliability of the results was questioned due to different methodological approaches. Variability in BMC in evaluable subjects was high and could not be related to any fracture event. However, the signal of fractures was thought to deserve further attention against the background of no such SAE in the placebo group and deemed necessary to be elucidated within the risk management plan.

All other SAEs were single cases only and were discussed, wherever considered to be of clinical relevance, in the assessment of AESI.

Laboratory findings

See AESIs for main laboratory findings.

Small decreases from baseline for drisapersen compared to placebo were reported for haematological parameters haemoglobin, erythrocyte count, haematocrit, leucocytes, neutrophils, and reticulocytes. The clinical significance remained unknown. Clinical chemistry comprised electrolyte monitoring and found only small but not significant changes with few shifts only. Creatinin kinase (CK) and lactate dehydrogenase (LDH) were characteristically found high at baseline with shifts to lower grades during treatment (higher with drisapersen compared to placebo).

Only small mean changes from baseline were seen in any treatment group for total protein, albumin and globulin, and glucose.

Measurement of vital signs (systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, temperature) was not suspect of any significant difference in placebo- and drisapersen treated subjects (ambulant placebo-controlled studies). In study DMD114349, 95 (42%) subjects and 61 (27%) subjects had high systolic and diastolic blood pressure, respectively, at any time point post baseline compared with 14% and 7%, respectively, at baseline. At any visit post baseline, 61 (27%) subjects had low heart rate and 42 (18%) subjects had high heart rate compared with 13 (6%) subjects and 14 (6%) subjects, respectively, at baseline. The Applicant assumed these changes not to be of significance in this study population.

No specific pattern could be seen with ECG changes in clinical studies. Maximum on-treatment increases from baseline for QTcB and QTcF of >60 msec for 12 (5%) and 9 (4%) subjects, respectively, were noted in study DMD114349. Integrated analysis of QTc data for the all repeat-dose studies group and the ambulant placebo-controlled studies group revealed slight but not meaningful QTcB values/changes from baseline for drisapersen.

The DMD population is known to be susceptible regarding ECG changes (Thrush et al. 2009). Some ECG changes were noted in the clinical program for drisapersen and placebo, which were thought to contribute to the underlying disease.

Echocardiography was not conducted for each of the clinical studies and therefore data were presented separately (see Table S4). Few subjects were reported to have decrease in ejection fraction (EF) of ≥10% in the placebo controlled studies. The highest number was reported from study DMD114117 (placebo: 6% vs. drisapersen 6 mg/kg/wk: 28%). In the majority of recordings in these subjects the cardiologist commented that the images were suboptimal, which may have contributed to the variability in the ejection fraction measurements in some subjects.

Table S4: Summary of echocardiography data

Study Number	Placebo	Drisapersen 3 mg/kg/wk	Drisapersen 6 mg/kg/wk	Drisapersen 6 mg/kg intermittent
Number (%) subjects with a decrease in ejection fraction ≥10% at any visit post baseline				
DMD114044	5 (8%)	NA	10 (8%)	NA
DMD114117	1 (6%)	NA	5 (28%)	1 (6%)
DMD114876	0	3 (18%)	1 (6%)	NA
Mean (SD) change from baseline in ejection fraction at last visit				
DMD114044	0.41% (8.178)	NA	0.73% (6.630)	NA
DMD114117	0.53% (7.033)	NA	−1.28% (8.887)	1.67% (6.136)
DMD114876	0.65% (3.840)	0.92% (5.078)	-3.22% (4.300)	NA

Source: DMD114044 CSR, Safety Data Source Tables, Table 3.62 and Table 3.64; DMD114117 CSR, Safety Data Source Tables, Table 3.62, and Table 3.64; DMD114876 CSR, Safety Data Source Tables – Week 24, Table 3.62 and Table 3.64.
Note:
Assessment for DMD114044 and DMD114117 made at Week 48 and assessment for DMD114876 made at Week 24.

Echocardiography data from study DMD114349 showed mild decreases of EF from baseline of -0.53% at Week 24 and -1.28% at Week 48. Even if small changes appeared during the course of the respective studies, this effect may rather attribute to the underlying disease than to drisapersen.

Safety in special populations

The only **intrinsic factors** to be evaluated were age and race.

Analysis by age ≤7 years and >7 years for placebo and drisapersen (all regimens) – treated subjects revealed no significant difference between these two subsets except for the placebo groups. Subjects ≤7 years on placebo reported significantly more AESI related to renal abnormality, inflammation event and hepatic abnormality. This finding seems to be of little clinical significance.

Most of the subjects derived from the white/Caucasian/European race group (n=240), whereas only 55 subjects were of other races. Conclusions cannot be drawn from this low number of other races.

The only **extrinsic factor** to be evaluated was corticosteroid regimen. Subjects were required to be on a stable continuous or intermittent corticosteroid treatment. No additional safety concerns arose based on this subgroup analysis.

Immunological events

Immunogenicity via anti-drug antibody (ADA) presence was evaluated in 109 drisapersen-treated DMD subjects and 50 placebo-treated subjects in study DMD114044. **In 29.4% (32 out of 109 drisapersen subjects) ADAs were detected** in several plasma samples obtained during the course of the study. Most of the **positive data derive from week 24** on. Median titres were low and increased with prolonged treatment (titres ranging from 50–300 at Weeks 8 to 24, and from 1000 and 800 at Weeks 36 to 48 respectively). In contrast, only 2% (one out of 50 subjects) of the placebo subjects were confirmed positive.

ADA positivity was compared to plasma trough concentrations and muscle tissue concentrations of drisapersen. ⇒**From Week 24** onwards **drisapersen trough concentrations were higher for ADA positive subjects, compared to ADA negative subjects**. Muscle tissue concentrations did not appear to be affected by ADA formation. Statistical correlation analysis for clinical studies DMD114044, DMD114117, and DMD114349 was performed using high troughs as a surrogate for ADA presence due to a lack of ADA data in these studies. With regard to safety aspects (SAEs, AESIs and AEs that occurred in at least 5% of subjects) and laboratory parameters (thrombocyte count, hsCRP, urine protein excretion, urine cystatin C, ALT, GLDH and total bilirubin) no differences were observed between subjects with high or low trough concentrations.

Long-term safety consequences of ADA formation nevertheless remain unknown. From other antisense oligonucleotides (e.g. mipomersen) it was concluded that antibody formation might induce complement consumption, although not to a significant extent, and formation of immune-complexes could be detected in a significant number of patients with antibodies.

Regarding complement C3 decreases in study DMD114044, 33 of 125 subjects on drisapersen 6 mg/kg/wk (**26%**) had a complement factor C3 value below the reference range at any post-baseline visit. This finding may correlate with the subjects tested ADA positive in this study. The Applicant provided tabulated data on complement C3 levels for subjects in study DMD114044, who were ADA negative compared to those who were ADA positive while on drisapersen 6 mg/kg/wk treatment. Data related to a total of 124 patients. No relation between the occurrence of anti-drug antibodies and a decrease in complement C3 concentration up to 48 weeks of treatment could be found.

Safety related to drug-drug interactions and other interactions

Formal interaction studies have not been conducted with drisapersen and in-vitro data do not point towards interaction with CYP isoenzymes (drisapersen is no substrate, inducer or inhibitor). No other drug-drug interactions were found plausible.

Discontinuation due to AES

12 out of 267 subjects (4.5%) treated with drisapersen 6 mg/kg/wk in the *all repeat-dose studies* permanently discontinued treatment and no subject in other treatment regimens (including placebo). In 10 of these subjects, discontinuation happened after longer treatment duration (beyond one year).

The only AE that led to discontinuation of more than 1 subject was *thrombocytopenia* which was reported in 7 (2.6%) subjects in the drisapersen 6 mg/kg/wk group, all during the DMD114349 long-term extension study. One subject was withdrawn because of 2 AEs (intracranial venous sinus

thrombosis and spinal pain) which were both reported as SAEs. The other AEs leading to discontinuation from study in one subject each were asthenia, glomerulonephritis, injection site oedema and proteinuria.

Dosing suspension was defined as any subject who withdrew from study treatment or who missed at least 4 consecutive doses. A total of 95 subjects had dosing with drisapersen suspended, which included 17 subjects who ultimately discontinued from a study for all reasons. The majority of suspended doses were related to laboratory abnormalities and resolved. The majority of the suspended doses required no treatment and no change in the dose regimen.

Post marketing experience

N/A

3.3.9. Discussion on clinical safety

The dossier of drisapersen contains an integrated safety database based on three main study groupings: the **ambulant placebo-controlled studies** evaluated the one-year safety data of drisapersen compared to placebo. The **all repeat-dose studies** provide information on all repeat dose studies over all study periods (PRO051-02, DMD114673, DMD114117, DMD114876, DMD114044, DMD114349) and long-term safety data up to 188 weeks derive from **DMD114349 and parent studies** (DMD114044 and DMD114117). Wherever applicable, data from single-dose studies are mentioned to support the integrated safety database.

Duchenne muscular dystrophy is a rare X-linked disease affecting 1 in 3600 – 6000 live male births (Emery et al. 1991). For this reason, the safety database for drisapersen is considered adequate at the time of marketing authorisation with an exposure of 326 subjects with DMD. Of these, 312 subjects received at least one dose of drisapersen. 302 subjects were treated in the all-repeat dose studies. 285 subjects had data on drisapersen available in the integrated analyses of all repeat-dose studies amounting to a total exposure of 490.1 subject-years. 267 of 285 subjects received the target dose of 6 mg/kg/wk (432.7 subject-years). The total exposure to placebo in the all repeat-dose studies grouping was 79.4 subject-years. Few subjects received intermittent drisapersen dosing. Subjects from the ambulant placebo-controlled studies had a higher exposure to placebo compared to drisapersen 6 mg/kg/wk at week 48. Long-term safety data are available for 223 subjects having a drisapersen exposure of at least 48 weeks (219 subjects on drisapersen 6 mg/kg/wk) with even longer exposure up to 4 years in single subjects.

Boys with a median age of 8 years (ranging from 5 to 16 years) and treated with corticosteroids constitute the group of patients represented in the clinical development of drisapersen (and hence in the safety analysis). About 46% of were younger than 7 years. The majority of subjects received corticosteroids on a continuous regimen (60-90%).Only 38 patients were on intermittent regimen. About half of the enrolled population is European. In general, this population could be considered as representative of the target ambulatory population from a demographic point of view. Common safety issues from the **all repeat-dose studies** were also adverse events of special interest and were found higher in drisapersen-treated subjects compared to placebo: **injection site related AEs** (around 80%; including injection site erythema, injection site discolouration, injection site induration, injection site pain, injection site pruritus, injection site reaction, injection site haematoma, injection site atrophy, injection site swelling, contusion, injection site bruising), **renal abnormalities** (72%; proteinuria, protein urine present, haematuria, red blood cells urine positive, cystatin C increased, urine protein/creatinine ratio increased) **and inflammation events** (around 41%; e.g. pyrexia). Exposure-adjusted incidence rates were compared for placebo and drisapersen 6 mg/kg/wk for AEs that were reported by at least 5% of subjects with higher incidences for ISRs, renal abnormalities,

thrombocytopenia, complement factor C3 decreased, GLDH increased, arthralgia, gastroenteritis, and abdominal pain upper. An overall similar picture is reflected in the **ambulant placebo-controlled studies**. Of note, incidences of pyrexia as PT and AEs from the infections and infestations SOC were similar for placebo and drisapersen 6 mg/kg/wk. Severity was most of all rated as "moderate" and "mild" for drisapersen and placebo except for "severe" AEs that were more frequently reported with drisapersen (16.1% vs. 4.2%) including AEs of thrombocytopenia, some ISRs, some fractures and renal AEs. **Adverse drug reactions** defined by the Applicant are well in accordance with AESI and comprise various injection site reactions, investigations (related to renal and hepatic parameters), haematuria, proteinuria, thrombocytopenia as well as arthralgia and alopecia.

Discontinuations due to adverse events (12 of 267 subjects, 4.5%) were mainly related to thrombocytopenia (7 of 12 subjects) and occurred exclusively during the open-label extension studies.

Few patients withdrew from the studies due to AESI (n=12; 4.5%). However, 95 subjects had to suspend the treatment (a period from 4 to 14 weeks) mainly due to laboratory abnormalities.

It remains of uncertainty if the safety profile of drisapersen would worsen in a less controlled setting.

No death was reported in the clinical program. **Serious adverse events** were found to be higher in drisapersen-treated subjects compared to placebo (16.5% vs. 9.5%) and in accordance with defined AESI: thrombocytopenia was rated as serious in 8 subjects (subjects also discontinued from study) with no subject on placebo. ALT increased and injection site oedema were also rated serious in 2 subjects each. Renally related SAEs were reported in one subject each and included *membranous glomerulonephritis, haematuria and nephrotic proteinuria*. *Hepatotoxicity* was reported as SAE in one subject. Of interest, 13 fractures occurred on drisapersen and no such SAE in placebo-treated subjects. Unless fractures are a common finding in DMD subjects (glucocorticoid treatment, Vitamin D alterations, alterations of calcium homeostasis), this signal was thought to deserve further attention within the RMP.

The relevant safety concerns for drisapersen deriving from the dossier pertain to the following **AESI**:

Injection site reactions are thought to represent an inflammatory response to the injected drug. ISRs were the most commonly reported AEs with drisapersen treatment across all study pools with similar incidence (approximately 79% of subjects on drisapersen 6 mg/kg/wk vs. 22% of subjects on placebo) and were found to be progressive. The most common PTs of ISRs in the ambulant placebo-controlled studies were injection site erythema (51%), discolouration (which describes both, hyperpigmentation and less commonly hypopigmentation, 35%), pain (16%), reaction (16%), pruritus (15%), bruising (12%) and induration (11%) (vs. incidence of 1 to 8% in the placebo group). Injection site necrosis has not been reported in any study. Two SAEs were reported of injection site oedema. IS erythema occurred within the first injections of drisapersen, whereas IS discolouration, IS pruritus, IS reaction, IS bruising and IS haematoma were found within the first 6 months of treatment. Other (more severe) ISRs were more common after longer treatment duration up to 24 months (IS induration, IS atrophy, IS sclerosis) and were additionally more likely to be persistent, which render respective injection sites unusable during long-term treatment. It may also be assumed that the risk for acquiring infections at the injection site is increased and that large-scaled inflammations and skin deficiencies may result in pruritus and even in limitation of mobility.

Recall injection site reactions have not been specifically evaluated except for study DMD115501, which is still ongoing and for which "memory responses" of ISRs were systematically prompted.

As a result, nearly 1/3 of subjects included in the study reported an ISR memory response, which could be either a hyperpigmentation or a more severe form of ISR (atrophy, rash, and erythema). Surprisingly, hardly any discontinuations were seen even though mean duration of ISR was 57.9 days, which could be assumed to impair the patients. Study DMD114673 with long-term data (up to 7 years)

of 12 patients reported ISRs of moderate to severe intensity for all subjects. Of note, injections in this study were initially administered in the abdomen for the first 9 months before rotating injection sites, which has been found to be beneficial to some degree. Some reactions were described as “sclerotic” and these were found to be persisting although no further injections were given in this area. Three subjects had treatment interruptions for this reason and s.c. injection could not be administered any longer. Moreover, it should be taken into account that all patients were on corticosteroids, what may have mitigated the clinical picture.

Photographs have been presented for n=10 subjects (of a total of n=38 subjects with moderate to severe ISRs recorded in studies DMD114673 and DMD115501). The severity of ISRs of these 10 subjects is considered played down in most of the cases presenting with wide-spread inflammatory processes. Further input on the type of ISRs and etiology would have been derived from histopathological evaluation already implemented in study protocols of the aforementioned studies. However, no biopsies have been conducted

The nature of the ISR is still not properly characterised.

Renal abnormalities were described as being a class effect of antisense oligonucleotides. Accumulation is thought to occur in the proximal tubule. The rate of AEs was twice as high for subjects on drisapersen 6 mg/kg/wk vs. placebo in the ambulant placebo-controlled studies (60.9% vs. 33.7%) with an incidence of more than 10% of subjects reporting proteinuria, haematuria, protein urine present, cystatin C increased. Four AEs were rated “serious” and comprised membranous glomerulonephritis, nephrotic proteinuria (both leading to study withdrawal), haematuria, and renal impairment. Most of the AEs (93.9%) reported in the all repeat-dose studies were resolved by the end of the studies with a mean duration of 41 days. Urinalysis data were provided and supported the clinical findings. Mean and median changes in protein urine were higher with drisapersen compared to placebo with a tendency to increase over the first 36 weeks after treatment initiation similarly to alpha1 - microglobulin (ambulant placebo-controlled studies). Shifts from normal to high in alpha1 – microglobulin were found in up to 81% of subjects (Week 36) on drisapersen 6 mg/kg/wk. Long-term study data confirmed an increase in these parameters. A tendency to recover in the drug-free periods of the intermittent dosing regimen was assumed based on a limited number of subjects. Categorical results of red and white blood cells in urine were indicative of a slight increase of these cells over time with drisapersen, whereas patients on placebo had a discrete decrease of cells in urine.

CHMP requested available data to further characterise the type of renal damage caused by drisapersen. Further predictive parameters defining the type of renal interaction are lacking (IgG urine, erythrocyte morphology). Laboratory parameters indicative of glomerular filtration impairment (serum cystatin C, creatinine and BUN) were also found increased from week 24 on in drisapersen-treated subjects compared to placebo. Regarding the provided data (including one SAE of membranous glomerulonephritis and one nephrotic range proteinuria) it cannot be ruled out that renal damage also manifests on the glomerular level caused by immunological alteration independent of accumulation properties in later stages of treatment. This uncertainty has to be addressed within the RMP.

In addition, monitoring proteinuria in clinical routine was proposed in line with the study protocols. Urine protein should be measured by dipstick at baseline and every two weeks and quantitative measurement (24 –h urine) should be initiated with more than trace amounts of urine protein to confirm proteinuria. Even though dip stick controls are considered easily to conduct, 24-h urine sampling in a substantial number of subjects may become necessary being cumbersome and fraught with error. Hence, feasibility of this subsequent risk minimisation measure is not indisputably shown and should be further addressed by the Applicant.

Inflammation events/immunological – associated toxicity was observed in non-clinical studies when drisapersen was administered. These pro-inflammatory effects have been also described for

other antisense oligonucleotides. No safety issue was found in the ambulant placebo-controlled experience but in the all repeat-dose studies (38.2% of subjects on drisapersen 6 mg/kg/wk vs. 27.4% of placebo-treated subjects) with *pyrexia* mentioned in a substantial number of subjects. Nevertheless, mean values of biomarkers (hsCRP, complement C3, MCP-1, fibrinogen, haptoglobin) and shifts from baseline were found to be altered with drisapersen compared to placebo later during treatment (around week 48). One fourths of subjects on drisapersen 6 mg/kg/wk in the open-label extension study DMD114349 reached the stopping criteria for inflammatory parameters. Inflammation AEs were reported to a slightly higher degree with drisapersen compared to placebo (nasopharyngitis, gastroenteritis, rhinitis, influenza). In this context the role of corticosteroids (all patients were treated) is difficult to assess. The aforementioned events need to be additionally monitored in corticosteroids-naïve patients. In the ambulant placebo-controlled studies, complement C3 was found to decrease around 8% from baseline to Week 48. For study DMD114349, a mean decrease of 2.4% up to week 104 was found and compared to the original baseline complement C3 values of the ambulant placebo-controlled parent studies; there was a mean decrease of 6% in total in study DMD114349.It could hence be ascertained that complement C3 concentration not further decreased in a time interval of approximately two years. Duchenne muscular dystrophy itself has been reported to present with enhanced coagulation and fibrinolysis (measured by D dimer) secondary to degeneration of the muscle (Saito et al. 2001). **Coagulation abnormalities** did however not constitute a main safety issue in either of the safety pools with similar incidences of respective AEs. Two SAEs of INR increased occurred in the drisapersen 6 mg/kg/wk group. Adverse events related to *haemorrhages* (haematuria, injection site bruising, injection site haematoma, epistaxis) were found at a higher incidence in drisapersen-treated patients vs. placebo. Two SAEs fell within the MedDRA SMQs for haemorrhages and for embolic and thrombotic events: *SAE of intracranial venous sinus thrombosis* and *renal venous thrombosis and pulmonary emboli* (within the diagnosis of membranous glomerulonephritis, see *renal abnormalities*). Both SAEs were found to be associated with coagulation abnormalities, which are usually not typical with drisapersen. Increases in several inflammation parameters without a clear pattern have been reported for these subjects. Against the background of inflammatory properties of drisapersen probably elicited by immunological alterations, these two SAEs deserve special attention and further action is deemed necessary to refer to this potential risk.

Laboratory findings of aPTT prolongation and INR increased were noted in placebo- and drisapersen-treated subjects during the course of the studies but with no specific pattern over time and by treatment regimen.

Accumulation of phosphorothioate oligonucleotides was shown to affect the **liver**. Preclinical studies on POs revealed increase in liver transaminases and bilirubin (Iannitti et al. 2014). Hepatic abnormalities were found to be higher for drisapersen compared to placebo mainly deriving from liver transaminase increases of glutamate dehydrogenase increased and gamma-glutamyltransferase (GGT) increased relative to baseline. The Applicant stated that GGT and GLDH increases were found to be reversible in the off-drug phase of intermittent drisapersen treatment based on few subject data only (n=2 for reversibility of GGT). However, three events of GLDH were reported not to be resolved by the end of the respective study. Reversibility of transaminases has been hypothesised based on single subjects and cannot be generalised for drisapersen-treated subjects. Gradual GLDH shifts from baseline to high were found in the 48 week studies and even higher in the open-label DMD114349 (68% at any time post-baseline compared to 14% at baseline. GLDH increased may be related to early mitochondrial dysfunction. Therefore, interpretation in regard to ALT and AST values should be presented. Of note, the significance of increases of ALT and AST is hampered by disease-dependant very high baseline values (>8 x ULN). Four SAEs were described including two SAEs of ALT increased (and at the same time INR increased), one SAE of hepatocellular injury and one SAE of hepatotoxicity. The SAE of hepatocellular injury presented with low complement factors. The SAE of hepatotoxicity needs further clarifying data. No case of Hy's law was reported.

The AESI found to be serious in nature and most often leading to study withdrawal is **thrombocytopenia**. No such AE was reported in the ambulant placebo-controlled studies. Thrombocytopenia affected 19 of 267 subjects on drisapersen 6 mg/kg/wk (7.1%). Mean thrombocyte counts slightly but progressively decreased during the course of the studies shortly after treatment initiation. Mean baseline and post-baseline thrombocyte counts were all within the normal reference range although variability was high. Stopping criteria for thrombocytopenia was thrombocyte counts <75 x 109/L or thrombocyte count fallen >25% from previous count and at the same time is <100 x 109/L. In addition to mild but progressive decreases within the first year of drisapersen treatment, large platelet count drops occurred in single subjects probably caused by a different (immunologically - mediated) mechanism. An SAE of thrombocytopenia was reported for 8 subjects followed by discontinuation from study. The course of thrombocytes to decrease with time was similar for all of the eight subjects: thrombocytes declined slightly over time and Grade 3 or 4 AEs did not emerge as early as 400 days after treatment initiation. Two of eight SAEs were Grade 3 (<50 x 109/L) and the remaining six SAEs were Grade 4 (<25 x 109/L that is considered a severe thrombocytopenia with the risk of potential fatal complications, like intracranial haemorrhages. Six of the eight subjects had reported spontaneous bleedings and had to be treated with tranexamic acid, i.v. immunoglobulin and/or steroids. Anti-thrombocyte antibodies (anti GP antibodies) were found positive in five of eight subjects. Additional six subjects were reported to have thrombocytopenia with thrombocytes <75 x 109/L. Similar to the SAEs, these additional thrombocytopenic AEs occurred not within the first year of treatment in most of the subjects. In one subject, antibodies were found positive. It cannot be ruled out that the immune system is activated by platelet autoantigens resulting in immune mediated platelet destruction or suppression of platelet production relevant for the sudden decreases in platelet counts after approximately the first year of treatment. No re-challenge has been conducted and it is not known if longer treatment durations generally increase the risk for experiencing thrombocytopenic episodes with drisapersen.

In addition to the AESI defined for drisapersen, almost 30% of patients treated with drisapersen in study DMD114044 were **positive to anti-drug antibodies** compared to a maximum of 2% treated with placebo. Most of the positive data derive from week 24 on. Drisapersen plasma trough concentrations were found to be higher in subjects tested ADA positive. However, muscle tissue concentrations were not affected. A relation between ADA occurrence and activation of the complement system (including decreases in complement C3 levels) can only be precluded for the 48-week evaluation of study data.

Specific AESIs/SAEs/AEs including membranous glomerulonephritis, thrombocytopenia, and ISRs may develop from immune-mediated reactions caused by drisapersen with long-term effects being elusive.

3.3.10. Conclusions on clinical safety

The safety database at present seems comprehensive for the target population keeping in mind that DMD affects 1 in 3600 – 6000 live male births and drisapersen is indicated for a subpopulation of approximately 13 % of DMD patients with out-of-frame exon deletions in the *"hot spot"* region adjacent to exon 51.

The overall safety profile of drisapersen suggests that there may be an effect on the immune system stimulating a pro-inflammatory status that may affect parts of these organs and systems with long-term consequences unknown. Further clarification and discussion has been requested and provided as part of a major objection on clinical safety on immunological parameters and their contribution to certain AESIs like ISRs, renal abnormalities, hepatic abnormalities and thrombocytopenia. It may be assumed that – albeit mechanistically clarification could not been given for most of the AESI – renal and hepatic abnormalities as well as coagulation abnormalities may be handled with adequate routine

monitoring algorithms and risk minimisation measures post-marketing although they will represent an extra burden for patients suffering from a very demanding disease.

However, there are safety issues, which are thought to set some subjects at an unpredictable risk while being on long-term treatment with drisapersen with unknown implications on treatment continuation:

- a high incidence of partly moderate to severe, progressing and persisting **ISRs**, likely to adversely affect the quality of life of patients and to limit the viability of drisapersen treatment in the long-term
- **Thrombocytopenia**. The mechanism for thrombocytopenia is not clear and seems to differ between early progressive declines of platelet counts and later steep and sudden decreases of thrombocytes (with likely immunological aetiology) leading to permanent treatment discontinuation. Particularly worrisome is that 6 patients (almost one third of those presenting thrombocytopenia) had <20 x 109/L that is considered a severe thrombocytopenia with the risk of potential fatal complications, like intracraneal hemorrhages. Routine monitoring of platelet counts every 14 days seems to capture life - threatening episodes of thrombocytopenia albeit hardly feasible in this patient population.

It is hence assumed that the major objectionon clinical safety especially pertaining to injection site reactions and thrombocytopenia cannot be sufficiently solved to address the unknown effects of long-term application of drisapersen, which is intended in DMD. For this reason, the proposed risk minimisation measures are not indisputably considered feasible or even fully effective.

For these reasons, the safety profile is considered hardly acceptable against the background of the intended long-term administration of drisapersen. Particularly worrisome are the unpredictable severe thrombocytopenia cases and the injection site reactions (despite injection rotation) that cannot be mitigated with risk minimisation measures. It is the CHMP view that this adverse safety profile could only be acceptable if a relevant effect on efficacy can be observed.

3.4. Pharmacovigilance system

3.5. Risk management plan

PRAC Outcome

The PRAC considered the following:

The PRAC noted the 2 current major objections on grounds of safety and efficacy for this initial application.

PRAC concluded that there are a number of significant risks identified from the clinical programme of drisapersen that will not be adequately mitigated by the proposed risk minimisation activities. These risks include renal toxicity, thrombocytopenia and injection site reactions. The proposed routine and additional risk minimisation measures also impose a significant burden on patients, their carers and healthcare systems.

The following recommendations were made:

- The Registry study should be a disease registry.
- The current RMP does not mitigate a number of important risks that could be life-threatening.

Details are provided in the PRAC endorsed PRAC Rapporteur pre-day 180 Updated Assessment Report.

Following the PRAC Outcome, the CHMP considers that the risk management plan version 2.0 is not acceptable. The Applicant should submit an updated RMP addressing all points raised in the list of outstanding issues.

Safety Specification

The applicant identified the following safety concerns in the RMP version 2.0:

Table RMP1: Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	• Injection site reactions • Thrombocytopaenia, including development of anti-thrombocyte antibodies • Renal toxicity, including glomerulonephritis due to immunological effects • Hepatotoxicity • Immunogenicity, including anti-drug antibody development
Important potential risks	• Prolongation of activated partial thromboplastin time (aPTT) • Pro-inflammatory effects and complement activation, including vasculitis • Thromboembolic events • Off-label use • Medication error
Missing information	• Use in females (including use in pregnancy and during lactation) • Use in boys less than 5 years of age • Use in patients with pre-existing renal impairment • Use in patients with pre-existing hepatic impairment • Use in patients without concomitant glucocorticoids • Long term adverse effects with regard to tissue accumulation

Having considered the data in the safety specification the CHMP considers that the following should be addressed:

- In order to capture all renal disorders a broader term should be included as important identified risk. Therefore it is proposed to include renal dysfunction (including glomerulonephritis due to immunological effects) as important identified risk in the RMP.
- Furthermore the risk of fractures needs further evaluation within the risk management plan. The wording may be slightly varied to risk of fractures as an important potential risk.

The following safety concerns should be included in the RMP (requested changes underlined):

Important identified risks
• Injection site reactions • Thrombocytopenia, including development of anti-thrombocyte antibodies • Renal <u>dysfunction</u>, including glomerulonephritis due to immunological effects • Hepatotoxicity • Immunogenicity, including anti-drug antibody development
Important potential risks
• Prolongation of activated partial thromboplastin time (aPTT) • Pro-inflammatory effects and complement activation, including vasculitis • Thromboembolic events

• Risk of fractures • Off-label use • Medication error
Missing information
• Use in females (including use in pregnancy and during lactation) • Use in boys less than 5yearsofage • Use in patients with pre-existing renal impairment • Use in patients with pre-existing hepatic impairment • Use in patients without concomitant glucocorticoids • Long term adverse effects with regard to tissue accumulation

Pharmacovigilance plan

The Pharmacovigilance Plan proposed in the RMP requires revision to address all outstanding points raised in the PRAC Rapporteur pre-day 180 Updated Assessment Report.

Risk minimisation measures

The Risk Minimisation Plan proposed in the RMP requires revision to address all outstanding points raised in the PRAC Rapporteur pre-day 180 Updated Assessment Report.

Public summary of the RMP

The public summary of the RMP requires revision to address all outstanding points raised in the PRAC Rapporteur pre-day 180 Updated Assessment Report.

4. Orphan medicinal products

Drisapersen (under the name of "exon 51 specific phosphorothioate oligonucleotide") was designated as an Orphan Medicinal Product for the *"Treatment of Duchenne muscular dystrophy (DMD)"* (EU/3/08/599) on 27thFebruary 2009.

5. Benefit risk assessment

Duchenne Muscular Dystrophy (DMD) is a rare, disabling, progressive and ultimately fatal X-linked genetic disorder caused by mutations in the gene for dystrophin. Functional dystrophin is critical for the structural stability of myofibers in skeletal, diaphragm and cardiac muscle and is also of importance for smooth muscles.

DMD is caused by several types of mutations in the dystrophin gene such as deletions, duplications and point mutations, which produce a shift in the open reading frame of the dystrophin mRNA leading to the absence of functional dystrophin protein. The disease primarily affects males with an incidence of 1 in 3600 – 6000 male newborns worldwide (Bushby *et al*., 2010). Initial signs of muscle weakness begin at the age of 2 and then progressively deteriorate, so that DMD patients become wheelchair-bound before the age of 12 and later die from respiratory failure and cardiomyopathy in their early twenties.

For a subpopulation of DMD patients aged 5 years and above, in which mutation created a nonsense stop codon in the dystrophin mRNA resulting in premature termination of translation and, hence, a truncated, non-functional protein, the API ataluren was granted central MA across the EU on 31st July 2014 (EMEA/H/C/2720, *"Translarna"*). Apart from ataluren, the current management of the disease focuses on prevention and management of complications. In addition, corticosteroids (e.g. prednisone or deflazacort) have been shown to temporarily reduce the decline in motor function in DMD patients.

The current application of drisapersen is for Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing. Exon 51-skipping amenable mutations occur in approximately 13% of DMD boys. Drisapersen is a 20-mer chemically-modified antisense oligonucleotide with a sequence designed to induce the skipping of exon 51 from the human dystrophin pre-messenger ribonucleic acid (mRNA) during the splicing process, restore the reading frame in mutations causing truncation of translation, and thereby increase truncated dystrophin expression. This truncated dystrophin synthesis is thought to convert the severe DMD into a milder phenotype.

Benefits

Beneficial effects

Non-clinical data and preliminary pharmacodynamic results showed that drisapersen induced an increase of dystrophin expression and exon 51 skipping. An increased dystrophin expression in muscle (muscle biopsy) was observed in initial studies in about 60% of patients after 25 weeks of drisapersen treatment compared with pre-treatment levels and with placebo (increase in 6%). Some results also suggest certain dose relationship, higher dystrophin expression achieved with increasing doses. The results from Phase II and III studies appear less convincing, due to the wide inter-subject variability in dystrophin intensity and the overlapping images between placebo and the different doses. Effective skipping of exon 51 was confirmed in the majority of subjects (>75%) with drisapersen, which further supports the claimed mechanism of action.

The clinical programme for drisapersen consisted of three clinical studies, i.e. study DMD114117, study DMD114876, both exploratory phase II studies, and one pivotal phase III study, study DMD114044 and two open-label extension studies. The two phase II studies included a Duchenne patient population representing patients with rather mild disease progress.

Study DMD114117:

In the primary analysis of change from baseline in 6MWD (m) at Week 25, a statistically significant difference was demonstrated for the drisapersen 6 mg/kg/week continuous regimen when compared against the combined placebo group (p = 0.014) representing a mean difference of 35.09 meters on the 6MWT. A 30 m change in the 6MWT was earlier accepted for Duchenne clinical programmes as a clinically relevant effect.

Study DMD114876:

In the primary analysis of change from baseline in 6MWD (m) at Week 24, no statistically significant difference was demonstrated for the drisapersen 6 mg/kg group when compared against the combined placebo group (p=0.069).

Study DMD114044:

The primary analysis of change from baseline in 6MWD (m) at Week 48 failed to show statistical significance when the drisapersen 6 mg/kg/week treatment group was compared against placebo (p=0.415). The 10.3 m treatment difference over placebo observed for the drisapersen treatment

group is considered to be not clinically relevant. Mean decreases from baseline in 6MWD were observed for both the placebo and the drisapersen 6mg/kg/week treatment group, indicating a decline in ambulatory function over 48 weeks.

Patients treated with drisapersen showed a decrease in creatine kinase levels, as a marker of muscle damage, over time.

Uncertainty in the knowledge about the beneficial effects

Presence of dystrophin as well as changes in dystrophin expression has also been reported in placebo subjects, although at levels lower than those measured in drisapersen treated patients. These residual amounts of dystrophin have been reported in the literature, either as dystrophin-positive (revertant) fibres or traces of exon 51 excision, consistent with spontaneous exon skipping activity. In this regard, the overlapping images between placebo and the different doses of drisapersen, and the wide inter-subject variability in dystrophin intensity questioned the validity of the measures.

Anti-drisapersen antibodies were detected in 29.4% of drisapersen treated subjects. Antibody formation had been already reported for mipomersen (other antisense oligonucleotide) although to a lesser extent (5% of mipomersen treated patients). ADAs are preferentially detected beyond Week 24 and correlated with high trough plasma concentrations. Although no significant signal on efficacy or safety has been identified, the role of these anti-drisapersen antibodies and their potential impact on the effect are not fully elucidated.

Uncertainties refer to the evidence of efficacy in terms of a rather marginal and inconsistent beneficial effect observed on decline of ambulation as the confirmatory phase III study, study DMD114044, failed to show a statistically significant difference for the primary endpoint, change from baseline in muscle function using the 6 Minute Walking Distance (6MWD) test, assessed at Week 48 (p=0.415). With respect to the +10.33 metres of difference observed in this study, a mean change from baseline between drug and placebo of 30 meters was assumed as clinically relevant. This difference has been recently reported and considered as a predictive factor of disease progression when Translarna (containing ataluren) was approved for the treatment of nonsense mutation in DMD. The difference achieved by drisapersen is far from the minimum distance a priori defined as clinically relevant. Only in one clinical study (exploratory) a difference of 30 metres versus placebo was observed.

For the primary endpoint the applicant provided further selected subgroup analyses post-hoc with different age ranges (≤ 7 years and >7 years) and baseline 6MWD (> 330 m and ≤ 330 m). For the combined subgroup ≤ 7 years and baseline 6 MWD about ≤ 330, the most promising numerical differences on the 6MWD in comparison to placebo were achieved. A greater treatment difference in change from baseline in 6MWD over placebo was observed for the drisapersen treatment group at Week 48 in subjects ≤7 years (21.5 metres) compared with subjects >7 years (6.9 metres). A greater treatment difference for the drisapersen group compared with placebo for the change from baseline in 6MWD at Week 48 was observed in the ≤330 m subgroup (18.4 m) than in the >330 m subgroup (7.4 m), however neither treatment differences were considered clinically meaningful. Although not statistically significant, the results seem to suggest that a better effect is observed in younger (i.e. <7 years of age) and less advanced in their disease patients, while at the same time the analysis of the other sub-group, based on the 6MWD suggests the opposite – a numerically better effect is seen in the patients with more advanced disease (and 6MWD <330m). However, patients were not stratified to ensure balance across all treatment groups with respect to age and baseline. The subgroup of patients with baseline 6MWD >330 meters consisted for the placebo group of patients that tended to be younger: 66% (25/38) of placebo subjects were ≤7 years old, as compared to 49% (33/67) in the drisapersen group. In the applicant`s view this might have confounded the estimates for the ≤330 meters and the >330 meters subgroups. Although this analysis might support the arguments of a

better effect of drisapersen in milder affected DMD patients, it should be kept in mind that the robustness of these results is considerably influenced by the low number of patients in some subgroups resulting from stratification.

Within the answer to the day 120 LoQ the applicant provided one 6MWD based subgroup analysis as sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters, which suggests that the effect estimates could considerably vary with different cut-off values. It showed a treatment effect ranging from 67.8 - 27.8 meters across the different studies, with a markedly higher estimate in study DMD114044 and a lower estimate in study DMD114876 compared to those analyses with a baseline window from 313 m to 419 m (50% of subjects in reference to baseline 6MWD). In study DMD114044 the difference was 27.8 meters, representing an almost clinical relevant difference in reference to the recently as clinically relevant accepted effect of 30 meters.

As the phase III study included a broader patient population compared to those included in the two phase II studies, the applicant now provided further subgroup analyses defined by "rise from floor time" and "6MWD" and one sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters to determine a subset of patients that would show a consistent treatment effect in all three placebo controlled studies.

However, the provided data showed that results cannot be regarded as consistent concerning the size of the effect for the primary endpoint across the three studies. While the sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters demonstrated a treatment difference about 27.8 meters that should be considered as almost clinical relevant, the treatment effect in the subgroup analyses for the middle 50% of patients with respect to baseline 6MWD and RFF was away from what was considered clinically relevant in the past. From all the post-hoc analyses provided by the Applicant it can be concluded that the large variability of the observed effect makes the results problematic to interpret: as for Study DMD114044 when the baseline 6MWD cut-off point is moved from 313-419 metres (middle 50%) to 300-400 meters, the treatment effect versus placebo changes from 19.9 metres (95% CI -8.8, 48.7) to 27.8 (95% CI -7.5, 63.1). The confidence intervals are even wider for Phase II trials estimations. These results appear highly dependent on the selected cut-off point of 6MWD and are thus far from being robust.

Although the treatment effect in one subgroup defined by baseline 6MWD even approaches an effect size that should be considered clinically relevant, the overall currently provided subgroup results are insufficient to provide robustness of efficacy and therefore the efficacy of Kyndrisa is still questionable. In this context, also the draft Guideline on the investigation of subgroups in confirmatory clinical trials (EMA/CHMP/539146/2013) should be taken into account. As mentioned in this draft guideline, once a study has failed its primary endpoint it will be exceptionally difficult to confirm efficacy within a subgroup. Whether the subgroup of interest is a well defined and clinically relevant entity still needs further discussion.

The lack of consistent favourable results on complementary meaningful clinical outcomes such as timed-function tests, the North Star Ambulatory Assessment (NSAA) total score, muscle strength or activities of daily living were observed across the overall patient population. Thus, the effect on ambulation was not supported by an impact on secondary endpoints directly linked to the daily living activities or those reflecting the negative impact of the condition (e.g. accidental falls, time to loss of ambulation, pulmonary function parameters) what reinforces the lack of effect observed in the primary endpoint. Only a decrease in creatine kinase was observed, which interpretation appears difficult without any further support. No perception of benefit was reported by patients or parents.

Only study DMD110117 included a loading dose at the beginning. Whether results of this single positive study were caused by the initial loading dose or a chance finding needed to be discussed. Although the provided analyses support the assumption that the positive results received in study

DMD114117 are caused by the initial use of a loading dose the applicant`s answer does not fully resolve all doubts based on the presented exposure models. Some uncertainties also exist in reference to the positive results observed at week 24 in study DMD114876 although no loading dose has been used. Although the included study population in study DMD114117 and DMD114876 was comparable in reference to baseline characteristics, results obtained under placebo treatment were not that comparable. The best evidence to assess the influence of a loading dose would have been derived from a study that uses the same treatment regimen across two treatment arms and that in addition includes a loading dose in one of these arms.

Risks

Unfavourable effects

Drisapersen elicited prominent **pro-inflammatory effects** in toxicity studies in all animal species that are related to its extensive and persistent distribution into multiple tissues and culminated as target organ toxicities like glomerulopathies of the kidneys, increased metabolic activity of the liver with single hepatocellular necrosis, transient prolongations of aPTT and PT, reductions in thrombocytes and local intolerabilities at the injection sites. In a 26 week chronic toxicity in monkeys, mortalities due to thrombocytopenia with haemorrhages in various organs were evident, whereas the decrease in thrombocytes in a subsequent 39 week chronic toxicity study in this species did not lead to mortality despite similar dosages. In addition, drisapersen evoked irreversible multi-organ perivascular inflammation with intimal and endocardial thickening in rats and monkeys. Moreover, three thromboembolic events occurred in the two chronic toxicity studies in monkeys, which coincide with two clinical SAEs. Although coagulation abnormalities and thromboembolism frequently occur in DMD patients, persistent vasculitis and thromboembolism are potential risks of long-term drisapersen treatment.

In line with non-clinical findings, drisapersen also provoked immunological reactions of different signs and symptoms in clinical studies, which include, but are not necessarily limited to, ISR, renal abnormalities, hepatotoxicity and haematological changes (thrombocytopenia). A single study including ADA assessment (DMD114044) revealed about 30 % of drisapersen - treated patients with anti – drug antibodies during treatment.

Thrombocytopenia emerges as a safety issue with treatment durations of more than one year (in the all repeat-dose studies) and concerned 19 of 267 patients (7.1%) on drisapersen 6 mg/kg/wk. Mean reduction of thrombocyte counts from baseline to week 48 was 22%but seemed to be slightly lower thereafter and always within the reference range. Eight subjects reported a SAEs of Grade 3 or Grade 4 thrombocytopenia after at least 400 days (range 14 to 26 months) of continuous drisapersen treatment followed by discontinuation from study. There was high consistency between presentations of these SAEs including the onset of the event, a sudden drop of thrombocyte counts of factor 10 within 14 days. Six of the eight SAEs were accompanied by spontaneous bleeding episodes. Particularly worrisome is that 6 patients (almost one third of those presenting thrombocytopenia) had $<20 \times 10^9$L that is considered a severe thrombocytopenia with the risk of potential fatal complications, like intracraneal hemorrhages. Moreover, the unpredictable nature of the platelet decrease does not allow excluding the risk despite a close and intensive monitoring. Five of eight SAEs were found positive for anti-thrombocyte antibodies (anti -GP antibodies)

The mechanism(s) of thrombocytopenia have not been elucidated.

ISRs (injection site reactions) were the most frequently mentioned AEs associated with drisapersen (incidence approximately 80% up to two years of drisapersen treatment with a trend to increase up to 100% in subjects with longer drisapersen treatment) and appear to increase over time.

From a mechanistical perspective, ISRs resemble a localised pro-inflammatory process triggered by exogenous nucleic acid consistent with stimulation of innate immunity in line with other drug candidates of the class of phosphorothioate oligonucleotides (Frazier 2014). It should be taken into account that all patients were on corticosteroids, what may have mitigated the clinical picture.

The onset for some of the ISRs (IS erythema and discoloration) was early in treatment (from the first month on), whereas more severe ISRs (IS induration, IS atrophy, IS sclerosis) occurred later (24 weeks and thereafter). ISRs reported in the open-label studies with longer treatment duration were often found **progressing in nature and persisting** (even without further injections) and included lipoatrophy, lipodystrophy, sclerosis, fibrosis, injection site oedema, injection site nodule, injection site microcalcification and injection site ulcer. Injection site ulcer was additionally found to progress with bacterial infections.

Rotation of injection sites was not systematically evaluated but retrospective analyses of open-label long-term studies DMD114349 and DMD114673 revealed beneficial effects of rotation for at least a subset of ISRs (e.g. injection site induration).

“Memory responses” of ISRs representing injection site recall reactions were systematically prompted in study DMD115501and found in 1/3 of subjects from this study. Memory responses / recall ISRs presented with either a hyperpigmentation or a more severe form of ISR (atrophy, rash, and erythema). In addition, one subject from study DMD114673 , an ulcer has been reported to occur at an injection site at which no injection was administered for 8 months.

Medical photographs of subjects with moderate to severe ISRs recorded in studies DMD114673 and DMD115501 reveal wide-spread inflammatory processes with drisapersen. Worsening of some of the ISRs in drug-free intervals has been stated which could be predictive for broad inflammatory and also immunological processes.

Renal abnormalities were frequently associated with drisapersen (incidence in ambulant placebo-controlled studies 61% as compared to 34% on placebo) and mainly presented with proteinuria, haematuria and cystatin C increased.

Protein urine and α1 – microglobulin (urinalysis) were found to increase with study duration mainly over the first year of drisapersen treatment. Mechanistically, low molecular weight proteinuria presented as tubular interaction rather than as a consequence of tubular damage caused by drisapersen. In analogy with preclinical findings, drisapersen seems to inhibit tubular reabsorption of e.g. alpha-1 microglobulin via competition for reabsorption.

Mean values of serum parameters (cystatin C, could be predictive for glomerular function loss) and urinalysis (alpha-1 microglobulin, which is more pronounced with tubular function abnormalities) were found to increase during the ambulant placebo-controlled studies with drisapersen but tended to plateau afterwards with no clear trend to increase further.

Liver enzymes were found to be increased in drisapersen-treated subjects. ALT and AST were found high at baseline in accordance with the underlying muscle damage in DMD. A slight decrease observed during the 48 week period of the ambulant placebo-controlled studies was found to be in contrast to higher post-baseline ALT and AST values in the open-label extension studies. Mean GGT and GLDH were normal at baseline but increased with duration of drisapersen treatment and not with placebo. Both parameters were also reported as ADRs. Four SAEs related to hepatic abnormalities were reported and included ALT increased as well as hepatocellular injury and hepatotoxicity. Hepatotoxicity was accompanied by low complement factors.

Uncertainty in the knowledge about the unfavourable effects

The 6 mg/kg/wk dose is the only administered dose and no safety margin is available from higher exposures.

With regard the potential target population, non-ambulant patients have not been included in the integrated safety analysis. However, the safety of drisapersen in the non-ambulant population even although limited appears of relevance. The lack of data from safety point of view may pose some difficulties for the extrapolation of the results.

Few patients withdrew from the studies due to AESI (n=12; 4.5%). However, 95 subjects had to suspend the treatment (a period from 4 to 14 weeks) mainly due to laboratory abnormalities.

Uncertainty remains on the **different presentations of thrombocytopenia**. Early platelet decreases may have a different mechanism compared to the sudden onset of steep decreases not earlier than 400 days after treatment initiation. At least the latter one is suspected to have an immunological origin. Assumption is further supported by presentation of two of eight subjects with an SAE with larger-then-normal megakaryocytes upon blood smear. One of these subjects was diagnosed with *idiopathic thrombocytopenic purpurea (ITP)*. Drug re-challenge has not been conducted in the clinical studies and hence no information is available if thrombocytes would again decrease after recovery.

Thrombocytopenia has been hypothesised to be related to transient sequestration of platelets but aetiology is not clear and mechanistic information should be provided. It cannot be ruled out that the immune system is activated by platelet autoantigens resulting in immune mediated platelet destruction or suppression of platelet production. Of note, when antiplatelet antibodies were performed a positive test was reported in 4 out of 5. No abnormal findings were observed when spleen echogenicity was performed in DMD114876 where patients were treated only 24 weeks. The Applicant has discussed four potential mechanisms causing thrombocytopenia (immune-mediated thrombocytopenia, heparin-induced-thrombocytopenia like mechanism, direct drug-induced platelet activation and indirect platelet activation vs complement activation). The exact mechanism cannot be completely elucidated from this discussion but the presence of anti-glycoprotein antibodies in 5 out of the 8 patients with severe thrombocytopenia points out to an immune thrombocytopenia. It seems that the antibody test was not performed in two subjects and it was negative in the remaining one. Nevertheless, assay methods may not be sufficiently sensitive to detect antibodies in all cases. For the time being, the mechanism of the thrombocytopenia is considered not sufficiently characterised and needs to be further investigated. The risk minimisation measures proposed by the Applicant (platelet count measurement every two weeks and immediately if signs/symptoms of thrombocytopenia develop) does not seem realistic in the daily clinical practice.

The long-term incidences of severe thrombocytopenia cannot be deduced from the available experience with drisapersen and may be higher as seen in the clinical study program.

There is uncertainty on the **long-term effects of injection site reactions** probably triggered by immunological alterations. The clinical relevance of ISRs seems to be crucial for long-term tolerability and safety and it should be mentioned that late onset of ISRs associated with a more severe outcome could be even higher as shown in the clinical program. Only few patients (n=12 in study DMD114673) have been treated for an extended period of up to 6-7 years.

The etiology of ISRs could further be elucidated by histopathological evaluation. Skin biopsies were mentioned in the protocols of the ongoing long-term studies but data has not been presented. Therefore, it cannot be confirmed if calcification is indeed a calcification, and if sclerosis is indeed a sclerosis and which kind of granulomas are prevailing with drisapersen treatment. Similarly, dermatological expert consultation was stated to be conducted but no evaluation of single (more

severe) ISRs has been presented. Long-term administration of drisapersen may be hampered by occurrence of injection site infections, pruritus and limitation of available injection sites and even mobility due to large-scaled skin deficiencies. Even local tumorigenic changes cannot be excluded from chronic inflammation sites.

In addition, there is uncertainty regarding the occurrence of ISRs at study site no. 091354 reported subsequent to a routine GCP inspection. ISRs have not been adequately documented and concern is raised on the overall number of reported ISRs and adverse events. The frequency of ISRs was significantly lower at this study site compared to the overall frequency of ISRs reported for the all-repeat dose studies.

Even though low molecular proteinuria has been identified to be the main **renal safety finding** with drisapersen due to competitive inhibition of tubular reabsorption of alpha-1 microglobulin and albumin by drisapersen, one SAE of *membranous glomerulonephritis* and one SAE of nephrotic range proteinuria were reported. The Applicant stated that the SAE of membranous glomerulonephritis is consistent with an immune-mediated mechanism. It cannot be ruled out that renal damage is also present on the glomerular level and probably caused by immunological alteration especially with longer treatment duration. Risk minimisation measure of routine dip stick controls every 14 days may adequately account for the high number of proteinuria seen in the clinical studies; however, subsequent high number of 24-h urine collection is thought to be cumbersome and fraught with error and hence questions the overall feasibility of renal monitoring in clinical practice.

Inflammation abnormalities were not an issue during the ambulant placebo-controlled studies with only a slight increase of AEs related to *"Infections and Infestations"* in the drisapersen treatment group compared to placebo (nasopharyngitis, gastroenteritis, rhinitis, influenza). In this context the potential role of concomitantly administered corticosteroids is unclear. These events should also be carefully monitored in corticosteroids-naïve patients.

Uncertainty remains on the interpretation of single inflammatory biomarkers (hsCRP, complement C3, MCP-1, fibrinogen, haptoglobin, immunoglobulin IgG) and their correlation in regard to the long-term outcome of several safety issues (ISRs, renal abnormalities, thrombocytopenia, coagulation abnormalities). The Applicant should present further data on the assumption that there is no correlation of persistently low C3 levels and other parameters indicative of inflammation processes. Summary of the results of complement split products as measured in phase I and II studies has been presented. No strong evidence emerges from the two small phase I/II studies that drisapersen triggers formation of complement split products. However, complement activation may be different with long-term drisapersen administration, which can be assumed for various safety issues (ISRs, thrombocytopenia, renal abnormalities). For subjects on long-term treatment with drisapersen, no measurement of complement split products could be provided.

Coagulation abnormalities, though being mentioned as AESI due to non-clinical findings, did not clearly present a safety issue for drisapersen. There are nonetheless uncertainties regarding the correlation of two thrombotic events that were reported as SAEs (intracranial venous sinus thrombosis / renal venous thrombosis and pulmonary emboli) with immunologically mediated inflammation. The laboratory assessment on inflammation parameters revealed several abnormalities for these SAEs although without a clear pattern.

The **long-term hepatic safety** is not known. It may be hypothesised that even if slight beneficial effects of ALT and AST could be seen with treatment of up to one year in the ambulant placebo-controlled studies, longer treatment durations might be again associated with increases of transaminases ALT and AST values due to potential liver toxicity. Reversibility of GGT and GLDH as stated by the Applicant has not been verified by sound clinical data. Instead, intermittent treatment

with drisapersen showed higher GLDH levels compared to continuous treatment, which contradicts reversibility of transaminases.

Uncertainty is also expressed on seven femur **fractures**, three tibia fractures, one ankle fracture, one lumbar vertebral fracture and one linear fracture/head injury were reported with drisapersen. This common finding in DMD patients (known to be susceptible in regard to alterations of Vitamin D levels and calcium homeostasis) is additionally triggered by continuous glucocorticoid treatment with known effects on bone mineral density. DEXA measurements have been conducted during most of the studies but reliability of the results is questioned due to different methodological approaches. Variability in BMC in evaluable subjects was high and could not be related to any fracture event. However, the signal of fractures deserves attention against the background of no such SAE in the placebo groups and should be further evaluated within routine pharmacovigilance.

In the open-label study DMD114349, there were more subjects with high systolic and diastolic pressure post-baseline compared to baseline. It could be clarified that systolic and diastolic BP values of potential concern did not substantially change over the 104 - weeks study period compared to baseline values. Single increases in blood pressure may also be attributed to the concomitant treatment with glucocorticoids.

Complement activation was not systematically studied in the clinical programme to further substantiate the immunogenic potential of drisapersen. A progressive decrease in complement factor C3 was observed during phase 2 and 3 studies. The Applicant clarified that no relation between the occurrence of anti-drug antibodies (30% of subjects in study DMD114044) and a decrease in complement C3 concentration up to 48 weeks of treatment could be found (based on data from study DMD114044).

Effects Table

Effects Table for drisapersen for treatment of Duchenne muscular dystrophy (DMD) with mutations in the dystrophin gene that are amenable to treatment with exon 51 skipping as determined by genetic testing (data cut-off: 31st August 2014).

Effect	Short Description	Unit	DRIS	PBO	Uncertainties / Strength of evidence	References
Favourable Effects						
6MWD (primary EP)	Change from baseline at week 48 (prim. analysis)	meter	6mg/kg -42.32	-52.7	No stat. sign. difference (10.334meters) for drisapersen 6mg/kg against placebo, difference not clinically relevant	(E3)
	Adjusted mean change from baseline at week 25 (prim. analysis)	meter	6mg/kg contin: 31.5* 6mg/kg interm: -0.1	-3.6	Exploratory phase II study, not designed for sufficient power to show statistical significance of clinically important effect size. *Stat. sign. difference (35.09 meters) for drisapersen 6m/kg continuous treatment against placebo, difference clinically relevant (p = 0.014) No stat. sign. difference (3.51 meters) for drisapersen 6mg/kg interm against placebo.	(E1)

Effect	Short Description	Unit	DRIS	PBO	Uncertainties / Strength of evidence	References
	Adjusted mean change from baseline at week 49	meter	6mg/kg contin: 11.2* 6mg/kg interm: 2.4	-24.7	* Difference of 35.84 meters for drisapersen 6m/kg continuous treatment against placebo clinically relevant (p=0.051)	(E1)
	Adjusted mean change from baseline at week 24 (prim. analysis)	meter	3mg/kg -19,93 6mg/kg 16.12*	-10.9	Exploratory phase II study, not designed for sufficient power to show statistical significance of clinically important effect size. No stat. sign. differences against placebo, * difference of 27.099 meters for drisapersen 6mg/kg against placebo almost clinically relevant	(E2)
	Adjusted mean change from baseline at week 48	meter	3mg/kg -37.92 6mg/kg 14.69*	-13.2	Analysis at week 48, after patients had been stopped treatment with drisapersen for 24 weeks. *Difference of 27.866 meters for drisapersen 6mg/kg against placebo almost clinically relevant	(E2)
NSAA (relevant secondary endpoint)	Change from baseline at week 48	points	6mg/kg -7.2	-6.7	No stat. sign. or clinically meaningful difference for drisapersen 6mg/kg against placebo.	(E3)
6MWD	Change from baseline at week 48 in subjects with baseline 6MWD middle 50% (baseline 6MWD 313-419 meters)	meter	Treatm diff.: (Dris-plbo: 19.9		Post hoc analysis involving around 50% of patients. Non clinically relevant	(E3)
	Change from baseline at week 48 in subjects with baseline 6MWD ≥ 300 to ≤ 400 meters	meter	Treatm diff.: (Dris-plbo: 27.8		Post hoc analysis, almost clinically relevant	(E3)
Unfavourable Effects						
ISRs	Incidence of injection site reactions	%	77.0	22.1	For subjects on long-term treatment up to 7 years: Large-scaled inflammatory processes. Histopathological evaluation is lacking; hence, aetiology cannot be determined; Long-term effects on chronic inflammation at injection sites are unknown. ISRs were found to be progressing in nature and more severe ISRs were found to be persistent. Occurrence of injection -site recall reactions.	(S1)

Effect	Short Description	Unit	DRIS	PBO	Uncertainties / Strength of evidence	References
	Incidence of injection site erythema	%	50.9	8.4	Early onset (within the first months of treatment)	(S1)
	Incidence of injection site discolouration	%	34.8	5.3	Early onset (within the first months of treatment)	(S1)
Renal abnormalities	Incidence of renal abnormalities	%	60.9	33.7	Renal abnormalities mainly comprise low molecular weight proteinuria (alpha-1 microglobulin). One SAE of membranous glomerulonephritis and one SAE of nephrotic range proteinuria questions pure tubular interaction/damage caused by drisapersen.	(S1)
	Incidence of proteinuria	%	43.5	23.2	Low molecular weight proteinuria (alpha-1 microglobulin) mostly from random urine samples; 24-h urine samples confirmed proteinuria for 14% of subjects.	(S1)
	Incidence of haematuria	%	16.1	10.5	sporadic, not progressive and not associated with other renal events.	(S1)
Inflammation events	Incidence of inflammation events	%	29.8	27.4	inflammatory markers (hsCRP, MCP-1, fibrinogen, haptoglobin, immunoglobulin IgG) are not consistently elevated; no clear pattern; complement C3 decreases seem not to be associated with certain AEs; complement split products have only been measured in few subjects in short-term studies (single dose or 5 weeks); uncertainty: no long-term data available.	(S1)
Coagulation abnormalities	Incidence of coagulation abnormalities	%	8.1	14.7	2 SAEs of thrombotic and embolic events; DMD is associated with thromboembolic events (risk factors in this population); however, further information requested.	(S1)
Hepatic abnormalities	Incidence of hepatic abnormalities	%	6.2	2.1	Interpretation of AST/ALT values difficult since baseline values were already very high (disease characteristic). Increases of GGT and GLDH over time. Reversibility of GGT and GLDH has not been verified by sound clinical data.	(S1)
Thrombocyto-penia	Incidence of thrombocytopenia	%	7.1	0	Early progressive decreases in platelets with mean values in the normal range. In addition, sudden onset (within 14 days) of steep decreases of platelets (factor 10) not earlier than 400 days after treatment initiation; mechanisms	(S2)

Effect	Short Description	Unit	DRIS	PBO	Uncertainties / Strength of evidence	References
					unknown; long-term incidences not known; thrombocytopenia may become life-threatening	

Notes: (E1): Data from study DMD114117, (E2): Data from study DMD114876, (E3): Data from study DMD114044

(S1) Data from the ambulant placebo-controlled studies grouping; (S2) Incidence from the all repeat-dose studies grouping: no subject in the ambulant placebo-controlled studies reported thrombocytopenia.

Balance

Importance of favourable and unfavourable effects

There is some evidence for a PD effect of drisapersen, i.e. that it can reach the target tissue and induce production of a shortened dystrophin. This is also complemented with some exploratory data showing positive change in muscle fibres with respect to fat replacement, muscle fibre membrane integrity, oedema and inflammation. The question is if this leads to clinical improvements of muscle function, which is progressively deteriorating in DMD patients.

By measuring endurance and the ability to walk, the 6MWT measures walking parameters that are relevant in the ambulant stage of DMD. There are however some issues identified with using the 6MWT as an outcome measure, including a learning effect, inter- and intra-personal variability and the impact of age at baseline. Therefore, the results on the 6MWT should be supported by consistent favourable results on complementary meaningful clinical outcomes such as timed-function tests, the North Star Ambulatory Assessment (NSAA) total score, muscle strength or activities of daily living. Consistency of effect with other relevant efficacy endpoints would confirm results based on the 6MWT and would allow a better interpretation in terms of clinical relevance. A 30 m change in the 6MWT was earlier accepted for Duchenne clinical programmes as a clinically relevant effect.

The efficacy data as presented by the applicant are considered unconvincing. Taking into consideration the positive phase II study, study DMD114117, there are some promising results showing that drisapersen may reduce decline in the 6MWT if given 6 mg/kg/wk (and initial loading dose) in younger and less advanced DMD patients (after 24 weeks treatment), however this was not confirmed when 6 mg/kg/wk without a loading dose was given to a broader DMD population and with a longer treatment duration of 48 weeks (study DMD114044). Although in study DMD114117, initially designed to be exploratory, the primary endpoint, change from baseline in the 6MWT was statistically significant and provided a clinically relevant difference of 35.09 meters in comparison to placebo and also the phase II study DMD114876 provided results that pointed into this direction it also has to be considered that the phase III study failed.

Also the effects on other clinically relevant endpoints and patient outcomes were far from convincing. The lack of consistent results on complementary meaningful clinical outcomes such as timed-function tests, the North Star Ambulatory Assessment (NSAA) total score, muscle strength or activities of daily living casts doubts on the weak observed effect on walking performance.

As the phase III study included a broader patient population compared to those included in the two phase II studies, the applicant now provided further subgroup analyses and one sensitivity analysis to determine a subset of patients that would show a consistent treatment effect in all three placebo controlled studies.

These additional analyses in subgroups defined by “rise from floor time“ and “6MWD“ are considered useful and reasonable to assess the consistency between the Phase II and Phase III data for the primary endpoint. It is acknowledged that the subgroup definitions are based on subgroup ranges of single baseline variables rather than a combination of criteria. It is also noted that these analyses are post-hoc subgroup analyses and interpretation of the results has to be made with caution.

However, the provided data showed that results cannot be regarded as consistent concerning the size of the effect for the primary endpoint across the three studies. While the sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters demonstrated a treatment difference about 27.8 meters that should be considered as almost clinical relevant, the treatment effect in the subgroup analyses for the middle 50% of patients with respect to baseline 6MWD (313 m to 419 m) and RFF was away from what was considered clinically relevant in the past.

As the results based on the middle 50% of subjects according to baseline 6MWD were different from those of the sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters, the two analyses suggest that results could be sensibly dependent on the choice of the cut-off value and that small differences in it (e.g. 313 versus 300 and 419 versus 400 meters) provide very different estimations (19.9 versus 27.8 meters). Thus, a set of sensitivity analyses for different cut-off values is requested to evaluate how results are influenced by the cut-off value.

Although the provided analyses support the assumption that the positive results received in study DMD114117 are caused by the initial use of a loading dose the applicant`s answer based on the presented exposure models does not fully resolve all doubts. Some uncertainties also exist in reference to the positive results observed at week 24 in study DMD114876 although no loading dose has been used. Although the included study population in study DMD114117 and DMD114876 was comparable in reference to baseline characteristics, results obtained under placebo treatment were not that similar.

The best evidence to assess the influence of a loading dose would have been derived from a study that uses the same treatment regimen in two treatment arms and that includes in addition a loading dose in one of these arms. However, in reference to the treatment administration in general, the three additional drisapersen administrations during the first three weeks of treatment are not considered relevant for the long-term treatment effect of this chronic disease. Whether the results received in study DMD114117 were caused by the use of a loading dose cannot totally be resolved from the information provided. Uncertainties about the optimal dose regimen still remain (e.g. with or without loading dose).

Regarding **clinical safety**, knowledge on the long-term implications of immunological and pro-inflammatory effects that may be attributed to drisapersen is limited and the risk minimisation measures proposed by the Applicant may not completely rule out the risks. This holds true for at least two important safety issues with unknown outcome during long-term drisapersen treatment.

The pathogenesis of sudden onset of platelet count decreases (Grade 3/Grade 4 thrombocytopenia) emerging as “common” adverse drug reaction after treatment duration of more than 400 days is unknown but strongly assumed to be related to immunological processes probably causing platelet destruction. There are no early signs or predictors for this event (anti - drug antibodies, medical conditions) affecting about 3% of the overall drisapersen population. In addition, this uncertainty on the incidence of thrombocytopenia while patients are on long-term treatment beyond two years should be considered. The steep increases in thrombocytes by factor 10 within a time interval of not more than 14 days may set the patient at high risk for serious and even life-threatening bleeding episodes. Grade 3 thrombocytopenia has been experienced by 2 subjects and Grade 4 thrombocytopenia (per definition relates to “life-threatening consequences”) was experienced by six subjects. The Applicant proposed a tightened routine platelet monitoring for all subjects on drisapersen at a 14 – days interval according to monitoring algorithm in the clinical studies, which is thought to capture most of the

thrombocytopenic events in a timely manner. However, conduction is hardly feasible taking into account that additional medical appointments are necessary for subjects still ambulant by indication.

Injection site reactions constitute the adverse event with the highest frequency affecting the majority of patients treated with drisapersen (80% - 100% with long-term treatment) and are hence of special importance. ISRs are progressing in nature (milder ISRs within the first months of treatment and more severe and persistent ISRs emerging thereafter), may affect sites previously injected (so - called memory responses or injection site recall reactions), and are persistent in terms of IS discoloration, IS atrophy, and IS sclerosis. It may be assumed that more severe ISRs occurring later in treatment may also increase the risk for acquiring infections at the injection site and resulting in pruritus and even in limitation of available injection sites and furthermore mobility. Especially in the very long-term studies DMD114673 and DMD115501, all adverse events mentioned to be moderate or severe remained unresolved. It is hence concluded, that the impact of ISRs on the long-term safety profile of drisapersen is still insufficiently characterized at this time and hard to handle. Risk minimisation measures include informing patients and caregivers of the progression and severity of ISRs prior to treatment start and to train the treating physician for drisapersen administration (e.g. rotating injection sites). Furthermore, annual dermatological assessment is recommended. IV dosing is additionally evaluated but no results are available so far. For the latter two measures, no data from clinical studies are available.

Renal (tubular) abnormalities may be managed by tight routine monitoring, which has been proposed by the Applicant although they will represent an extra burden (related to frequent dip stick testing and subsequent 24-h urine collection) for patients suffering from a very demanding disease. However, glomerular damage/nephrotic range proteinuria as a consequence of the inflammatory properties of drisapersen cannot be excluded and could present a rare but serious safety problem.

Intermittent drisapersen regimen was not proven to have a more benign safety profile for most of the AESI.

Benefit-risk balance

Discussion on the benefit-risk assessment

The data derived from the presented clinical programme have several deficiencies which affect the benefit-risk balance. The main deficiency is the insufficient evidence of efficacy in terms of a rather marginal and inconsistent beneficial effect observed on decline of ambulation as a sufficient effect on the primary endpoint only was shown in one exploratory phase II study, but the confirmatory phase III study and a second phase II study failed. Consistent results on complementary meaningful clinical outcomes were not achieved. Therefore, the identification of a subset of patients that would show a consistent efficacy effect in all three placebo controlled studies is reasonable to elucidate any effect if it exists.

As the phase III study included a broader patient population compared to those included in the two phase II studies, the applicant now provided further subgroup analyses defined by “rise from floor time“ and “6MWD“ and one sensitivity analysis to determine a subset of patients that would show a consistent treatment effect in all three placebo controlled studies. However, the provided data showed that results cannot be regarded as consistent concerning the size of the effect for the primary endpoint across the three studies. While the sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters demonstrated a treatment difference about 27.8 meters that should be considered as almost clinical relevant, the treatment effect in the subgroup analyses for the middle 50% of patients with respect to baseline 6MWD (313 to 419 meters) and RFF (4.2 and 13.3 seconds) was away from what was considered clinically relevant in the past. As the results based on the middle 50% of subjects according

to baseline 6MWD were different from those of the sensitivity analysis with baseline 6MWD ≥ 300 to ≤ 400 meters, the two analyses suggest that results could be sensibly dependent on the choice of the cut-off value and that small differences in it (e.g. 313 versus 300 and 419 versus 400 meters) provide very different estimations (19.9 versus 27.8 meters). Thus, a set of sensitivity analyses for different cut-off values is requested to evaluate how results are influenced by the cut-off value.

The safety profile is still not fully characterized in regard to the contribution of immunological alterations and pro-inflammatory effects on the main safety issues. The safety profile, especially regarding the intended long-term administration of drisapersen is hardly acceptable. Based on the outcome of the additional data provided by the Applicant, hepatic abnormalities may be manageable by means of the proposed risk minimisation measures (routine monitoring and treatment interruption recommendations) although representing an extra burden for patients suffering from a very demanding disease. Feasibility of renal monitoring, especially the high number of 24-h urine collection subsequent to dip stick proteinuria results is however not sufficiently clear. Additional data are not sufficient to reasonably lower the risk for progressing and persisting ISRs adversely affecting the quality of life of patients and limiting long-term treatment with drisapersen. The same holds true for the unpredictable onset of severe thrombocytopenia, which could have life-threatening consequences related to serious bleeding complications and for which monitoring schedules seem not feasible in a still ambulant population.

For these reasons, the safety profile is considered hardly acceptable against the background of the intended long-term administration of drisapersen. Particularly worrisome are the unpredictable severe thrombocytopenia cases and the injection site reactions (despite injection rotation) that cannot be mitigated with risk minimisation measures. It is the CHMP view that this adverse safety profile could only be acceptable if a relevant effect on efficacy can be observed.

5.1. Conclusions

The overall B/R of drisapersen (“Kyndrisa”) is negative.