Report on the Deliberation Results

March 6, 2020

Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau
Ministry of Health, Labour and Welfare

Brand Name	Viltepso Intravenous Infusion 250 mg
Non-proprietary Name	Viltolarsen (JAN*)
Applicant	Nippon Shinyaku Co., Ltd.
Date of Application	September 26, 2019

Results of Deliberation

In its meeting held on February 28, 2020, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is not classified as a biological product or a specified biological product. The re-examination period is 10 years. Neither the drug product nor its drug substance is classified as a poisonous drug or a powerful drug.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.

2. Because of a limited number of Japanese patients participated in clinical studies of the product, the applicant is required to conduct a drug use-results survey involving all Japanese patients treated with the product over the re-examination period to identify the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure the proper use of the product.

3. The applicant is required to conduct clinical studies and a Japanese registry-based survey aiming to evaluate the efficacy and safety of the product, and to submit the study data and analysis results promptly upon their completion.

**Japanese Accepted Name (modified INN)*

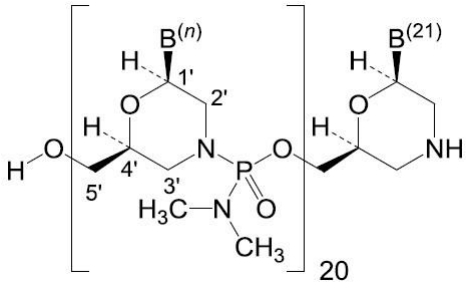
Review Report

February 19, 2020
Pharmaceuticals and Medical Devices Agency

The following are the results of the review of the following pharmaceutical product submitted for marketing approval conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Brand Name	Viltepso Intravenous Infusion 250 mg
Non-proprietary Name	Viltolarsen
Applicant	Nippon Shinyaku Co., Ltd.
Date of Application	September 26, 2019
Dosage Form/Strength	Solution for injection in vials, each (5 mL) containing 250 mg of viltolarsen
Application Classification	Prescription drug, (1) Drug(s) with a new active ingredient

Chemical Structure



$B^{(n)}$: The position of the n^{th} base from the 5'-terminal end
($B^{(21)}$ indicates the 21st base from the 5'-terminal end).

Base sequence: CCTCCGGTTC TGAAGGTGTT C
C, Cytosine; T, Thymine; G, Guanine; A, Adenine

Molecular formula: $C_{244}H_{381}N_{113}O_{88}P_{20}$

Molecular weight: 6924.82

Chemical name: *all-P-ambo*-[2',3'-Azanediyl-*P*,2',3'-trideoxy-*P*-(dimethylamino)-2',3'-seco] (2'-*N*→5')(CCTCCGGTTC TGAAGGTGTT C)

Items Warranting Special Mention

Orphan drug (Orphan Drug Designation No. 440 of 2019 [*31 yaku*], PSEHB/PED Notification No. 0820-3 dated August 20, 2019, by the

	Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare) SAKIGAKE designation drug (SAKIGAKE Drug Designation No. 2 of 2015 [*27 yaku*]; PSEHB/ELD Notification No. 1 dated October 27, 2015, by the Evaluation and Licensing Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare), SAKIGAKE comprehensive assessment consultation conducted The product is subject to Conditional Early Approval System (PSEHB/PED Notification No. 1029-3 dated October 29, 2019).
Reviewing Office	Office of New Drug III

Results of Review

On the basis of the data submitted, PMDA has concluded that the product has efficacy in the treatment of Duchenne muscular dystrophy with a deletion in the dystrophin gene amenable to exon 53 skipping therapy and that the product has acceptable safety in view of its benefits (see Attachment).

As a result of its review, PMDA has concluded that the product may be approved for the indication and dosage and administration shown below, with the following conditions.

Indication	Duchenne muscular dystrophy with a deletion in the dystrophin gene amenable to exon 53 skipping therapy
Dosage and Administration	The usual dosage is 80 mg/kg of viltolarsen injected intravenously once weekly over 1 hour.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.

2. Because of a limited number of Japanese patients participated in clinical studies of the product, the applicant is required to conduct a drug use-results survey involving all Japanese patients treated with the product over the re-examination period to identify the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure the proper use of the product.

3. The applicant is required to conduct clinical studies and a Japanese registry-based survey aiming to evaluate the efficacy and safety of the product, and to submit the study data and analysis results upon their completion.

Attachment

Review Report (1)

January 21, 2020

The following is an outline of the data submitted by the applicant and content of the review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA).

Product Submitted for Approval

Brand Name	Viltepso Intravenous Infusion 250 mg
Non-proprietary Name	Viltolarsen
Applicant	Nippon Shinyaku Co., Ltd.
Date of Application	September 26, 2019
Dosage Form/Strength	Solution for injection in vials, each (5 mL) containing 250 mg of viltolarsen.
Proposed Indication	Duchenne muscular dystrophy with a deletion in the dystrophin gene amenable to exon 53 skipping therapy

Proposed Dosage and Administration

The usual dosage is 80 mg/kg of viltolarsen injected intravenously once weekly over 1 hour.

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List of Abbreviations

See Appendix.

1. Origin or History of Discovery, Use in Foreign Countries, and Other Information

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive genetic disorder. The disease is caused by the deficiency of functional dystrophin protein resulting from deletion or duplication by mutations in the dystrophin gene on the X-chromosome (*Cell.* 1987;51:919-28). DMD is a type of designated intractable disease of "muscular dystrophy" and is an intractable and progressive muscular disease complicated by respiratory muscle and myocardial weakness as well as serious motor dysfunction, dysphagia, sputum retention, and gastrointestinal dysfunction. Children with DMD lose walking ability by around 10 years of age, with the average life-span of approximately 30 years (*Practical Guideline for Duchenne Muscular Dystrophy (DMD) 2014.* Nankodo Co., Ltd.; 2014:2-5). DMD occurs in 1 out of every 3500 newborn boys (*Neuromuscul Disord.* 1991;1:19-29), and approximately 5000 patients are estimated to be affected in Japan (*Experimental Medicine.* 2016;34:3151-8). Given that approximately 8% of patients with DMD have the genetic mutations eligible for treatment with viltolarsen (*Hum Mutat.* 2009;30:293-9), and viltolarsen is expected to be indicated for approximately 400 patients in Japan. Viltolarsen was designated as an orphan drug (Orphan Drug Designation No. 440 of 2019 [*31 yaku*]) on August 20, 2019 for the intended indication of "Duchenne muscular dystrophy with a deletion in the dystrophin gene amenable to exon 53 skipping therapy."

Viltolarsen is a synthesized morpholino oligonucleotide, developed by the applicant and the National Center of Neurology and Psychiatry. Viltolarsen binds to exon 53 of the dystrophin messenger ribonucleic acid (mRNA) precursor, thereby skipping exon 53 and leading to the expression of dystrophin protein, which is shorter-chained than the normal protein but functional.

In Japan, a clinical study was started as an investigator-initiated trial supported by Health and Labour Sciences Research Grants by the National Center of Neurology and Psychiatry in June 2013. The applicant submitted the marketing application for viltolarsen, claiming that the efficacy and safety of viltolarsen had been confirmed in patients with DMD.

In the US, the application for viltolarsen was submitted in December 2019 and is currently under review. As of December 2019, viltolarsen has not been approved in any country or region.

Approved drugs indicated for muscular dystrophy in Japan are prednisolone (for the indication of "Duchenne muscular dystrophy") and an injection of adenosine triphosphate disodium hydrate (the indication of "muscular dystrophy and related diseases").

Viltolarsen was designated to be subject to the SAKIGAKE designation system dated October 27, 2015 (SAKIGAKE Drug Designation No. 2 of 2015 [*27 yaku*]) with the intended indication of "Duchenne muscular dystrophy." Viltolarsen was also subject to the Conditional Early Approval System for Drugs (PSEHB/PED Notification No. 1029-3 dated October 29, 2019).

2. Data Relating to Quality and Outline of the Review Conducted by PMDA

2.1 Drug substance

2.1.1 Characterization

The drug substance is a synthesized oligonucleotide consisting of 21 nucleotides and occurs as a white powder. The general properties of the drug substance, including description, solubility, hygroscopicity, optical rotation, dissociation constant, distribution coefficient, pH, melting point, and crystal form, were determined. The drug substance is amorphous.

The chemical structure of the drug substance was elucidated by elemental analysis, infrared absorption spectrum (IR), nuclear magnetic resonance spectroscopy (NMR) (^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR), mass spectrum (MS), ultraviolet spectrum (UV), circular dichroism spectrum, and X-ray powder diffraction. The drug substance is a mixture of diastereomers having the asymmetric center at carbon atom on the morpholine ring and at 20 phosphorus atoms in its chemical structure.

2.1.2 Manufacturing process

The drug substance is [redacted] using [redacted],[1)] [redacted],[2] [redacted],[3)] [redacted],[4)] and [redacted] as starting materials. The [redacted] ([redacted]) in the synthesis process consists of [redacted] cycles of [redacted].[5)] After [redacted] is obtained, [redacted] are performed ([redacted]), followed by the purification process. The purification process consists of [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], and [redacted] steps.

The quality control strategy was constructed based on the following investigations using the quality by a design (QbD) approach (Table 1):

- Identification of critical quality attributes (CQAs)

- Identification of manufacturing processes and factors affecting CQAs by the failure mode effect analysis

- Identification of potential critical process parameters (CPPs) based on results from a risk analysis and establishment of standard operating conditions using a design of experiments method

1) [redacted]
2) [redacted]
3) [redacted]
4) [redacted]
5) [redacted]

Table 1. Summary of control strategy of drug substance

CQA	Control method
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process
[redacted]	Manufacturing process
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications
[redacted]	Manufacturing process, specifications

Critical steps are [redacted] ([redacted]), [redacted], [redacted], and [redacted]. In addition, [redacted] and [redacted][6] [redacted] are controlled as critical intermediates.

2.1.3 Control of drug substance

The proposed specifications for the drug substance include content, description, identification (ultraviolet-visible spectrum [UV/VIS], IR, MS, [redacted]), [redacted], pH, purity (clarity and color of solution, related substances [high performance liquid chromatography (HPLC)], residual solvents [gas chromatography (GC)]), water content, bacterial endotoxins, microbial limit, and assay (HPLC).

2.1.4 Stability of drug substance

Table 2 shows main stability studies conducted on the drug substance. The photostability testing confirmed the photostability of the drug substance.

Table 2. Stability studies of drug substance

Study	Primary batch	Temperature	Humidity	Storage package	Storage period
Long-term testing	3 pilot batches	5°C	Ambient	Polyethylene bag (double-layer) + aluminum-laminated bag	18 months
Accelerated testing	3 pilot batches	25°C	60%RH		6 months

Based on the above, a retest period of 24 months has been proposed for the drug substance when stored in a double-layer polyethylene bag, which is then placed in a multi-layered aluminum bag (nylon/aluminum/nylon/linear low-density polyethylene) at 2°C to 8°C in accordance with the ICH Q1E guideline. The long-term testing will be continued up to [redacted] months.

2.2 Drug product

2.2.1 Description and composition of drug product and formulation development

The drug product is an aqueous injection solution containing 250 mg of viltolarsen per vial (5 mL). The drug product contains sodium chloride, hydrochloric acid, sodium hydroxide, and water for injection as excipients.

6) [redacted]

2.2.2 Manufacturing process

The drug product is manufactured through a process comprising preparation, sterile filtration, filling/stoppering, crimping, packaging/labeling, and testing/storage. [redacted] and [redacted] are critical steps. Process control items and process control values are specified for [redacted], [redacted], [redacted], and [redacted] steps.

2.2.3 Control of drug product

The proposed specifications for the drug product include content, description, identification (UV/VIS, HPLC, and MS), pH, purity (related substances [HPLC]), bacterial endotoxins, extractable volume, foreign insoluble matter, insoluble particulate matter, sterility, and assay (HPLC). Furthermore, during the review process, purity ([redacted] [HPLC]) was specified.

2.2.4 Stability of drug product

Table 3 shows main stability studies conducted on the drug product. The photostability testing has shown that the drug product is photostable.

Table 3. Stability studies of drug product

Study	Primary batch	Temperature	Humidity	Storage package	Storage period
Long-term testing	3 commercial-scale production batches	5°C	Ambient	Colorless glass vial + rubber stopper + aluminum cap + carton	18 months
Accelerated testing	3 commercial-scale production batches	25°C	60%RH		6 months

Based on the above, a shelf life of 18 months was proposed for the drug product when filled in a colorless borosilicate glass vial stoppered with a butyl rubber stopper and an aluminum cap and stored in a carton at 2°C to 8°C in accordance with the ICH Q1E guideline. The long-term testing will be continued up to 36 months.

2.R Outline of the review conducted by PMDA

Based on the submitted data and the following review, PMDA concluded that the quality of the drug substance and drug product is adequately controlled.

2.R.1 Review on manufacturing process of drug substance

PMDA asked the applicant to explain their discussion at the development stage on how to assure consistent quality of the drug substance throughout its manufacturing process.

The applicant's explanation:

CQAs identified for the drug substance are [redacted], [redacted], [redacted] and [redacted], [redacted], [redacted], [redacted], [redacted] and [redacted] and [redacted], [redacted], [redacted], [redacted], and [redacted]. Table 1 shows their control methods. The applicant considered that, among the CQAs, especially [redacted] and [redacted] would affect the efficacy and safety because the drug substance is a nucleic acid medicine. In light of this, the

effects of process parameters in the processes involving the secondary generation and removal of related substances were evaluated using a failure mode effect analysis for criticality, probability, and detectability. The evaluation identified the following parameters as potential CPPs. For each of these parameters, a proven acceptable range of the operating condition was determined, and the production was to be conducted within the determined ranges.

- [redacted]
- [redacted]
- In the purification process, [redacted]
- [redacted]
- [redacted]
- [redacted]

It is difficult to separate and analyze individual oligonucleotide-related substances owing to their similar chemical properties. Because the following observations indicated that impurity profiles were consistent among batches, it is considered appropriate to control these impurities as a group of impurities that are detected as [redacted] in [redacted] HPLC analysis for purity.

- The [redacted] composition of [redacted] in pre-purification [redacted] detected in [redacted] HPLC was measured by [redacted], and the results are as shown in Table 4. In the specified synthesis process, the individual impurity profiles did not tend to differ among batches.

Table 4. Composition analysis of related substances in [redacted] determined by [redacted]

[redacted]	Compound name	[redacted] (%)[a]			
[redacted]	Compound A	[redacted]	[redacted]	[redacted]	[redacted]
	Compound B	[redacted]	[redacted]	[redacted]	[redacted]
	Compound C + Compound D[b]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Compound E	[redacted]	[redacted]	[redacted]	[redacted]
	Compound F + Compound G[c]	[redacted]	[redacted]	[redacted]	[redacted]
	Compound H	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Drug substance	100	100	100	100
[redacted]	Compound I	[redacted]	[redacted]	[redacted]	[redacted]
	Compound J	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	Compound K[d]	[redacted]	[redacted]	[redacted]	[redacted]

a) [redacted] of the related substance to the drug substance
b) Because [redacted], [redacted] is presented as [redacted].
c) Because [redacted], [redacted] is presented as [redacted].
d) [redacted] is not measurable, because [redacted].

- In addition, [redacted] is purified ([redacted]) in the purification process. Table 5 shows individual impurity profiles of the purified drug substance.

Table 5. Impurity profile of [redacted] of drug substance by [redacted]

[redacted]	Compound name	[redacted] (%)[a)]						
[redacted]	Compound A	[redacted]						
	Compound B							
	Compound C + Compound D[b)]							
[redacted]	Compound E							
	Compound F + Compound G[c)]							
	Compound H							
[redacted]	Drug substance	100	100	100	100	100	100	100
[redacted]	Compound I	[redacted]						
	Compound J							
	Compound K[d)]							

[redacted]

a) [redacted] of the related substance to the drug substance
b) Because [redacted], [redacted] is presented as [redacted].
c) Because [redacted], [redacted] is presented as [redacted].
d) [redacted] is not measurable, because [redacted] of the drug substance.

- Furthermore, the acceptance limit of each related substance [redacted] specified is as low as [redacted]% as compared with the acceptable concentration (Table 6) calculated using the clinical dose (80 mg/kg) and non-clinical dose (360 mg/kg), of which safety was confirmed (in a repeated-dose toxicity study in monkeys).

Table 6. Qualified dose of related substance [redacted]

Related substance [redacted]	Content in drug substance[a)] (%)	Acceptable concentration (%)[b)] of each related substance [redacted] in drug substance	Acceptance limit concentration (%) of each related substance [redacted] in drug substance
RRT [redacted]	[redacted]	[redacted]	[redacted]
RRT [redacted]	[redacted]	[redacted]	[redacted]
RRT [redacted]	[redacted]	[redacted]	[redacted]
RRT [redacted]	[redacted]	[redacted]	[redacted]
Total	[redacted]	[redacted]	[redacted]

RRT, Relative retention time of peak
a) Drug substance used in repeated-dose toxicity study in monkeys (Batch No. [redacted])
b) Acceptable concentration = amount (%) of each impurity in drug substance (Batch No. [redacted]) × dose of drug substance in toxicity study (360 mg/kg) / clinical dose of drug product (80 mg/kg)

PMDA accepted the above applicant's explanation.

2.R.2 Control of [redacted] in drug substance

The drug substance is [redacted]. Given this, PMDA asked the applicant to explain [redacted].

The applicant's explanation:

- At both pilot and commercial production scales, [redacted]. Therefore, [redacted] was investigated by [redacted]. The results demonstrated that [redacted] (Table 7). In addition, [redacted] is reported to be [redacted] ([redacted]). Accordingly, [redacted] and [redacted] are considered to affect [redacted].

Table 7. [redacted]

[redacted]																					

Unit, %

- In the production of the drug substance, in order to confirm [redacted], [redacted] as process control. [redacted]. In each manufacturing process of the drug substance, [redacted] was calculated. The results showed that [redacted] remained stable and almost unchanged [redacted] in [redacted] (Table 8).

Table 8. [redacted] **of** [redacted] **in** [redacted] **process analysis**

[redacted]																					
[redacted]																					

Unit, %
a) [redacted].

- In [redacted] process, [redacted] are considered as manufacturing process parameters potentially affecting [redacted]. As the worst-case scenario, [redacted] was performed under the conditions of [redacted]. The results showed no remarkable differences in [redacted].

- Based on the above, the current manufacturing control assures consistent production in terms of [redacted]. In addition, to ensure that [redacted], the use of [redacted] is to be specified in the manufacturing process of the drug substance.

PMDA accepted the above applicant's explanation.

3. Non-clinical Pharmacology and Outline of the Review Conducted by PMDA

The applicant submitted the results of primary pharmacodynamics, secondary pharmacodynamics, and safety pharmacology studies as non-clinical pharmacology data of viltolarsen. Unless otherwise specified, numerical values are presented as the mean ± standard deviation (SD).

3.1 Primary pharmacodynamics

3.1.1 Investigation using fibroblasts derived from patients with DMD harboring exon 45-52 deletion mutation (CTD 4.2.1.1-1 to 4.2.1.1-3)

To myotube cells differentiated from fibroblasts of patients with DMD harboring exon 45-52 deletion mutation, viltolarsen (0, 0.1, 0.3, 1, 3, or 10 μmol/L) was added in the presence of a transfection reagent for 2-day culture. The exon 53 skipping efficiency (%) of viltolarsen[7)] was assessed by the reverse transcriptase-polymerase chain reaction (RT-PCR) method. Viltolarsen induced exon 53 skipping (50% effective concentration [EC_{50}], 0.63 μmol/L) (CTD 4.2.1.1-1).

To myotube cells differentiated from fibroblasts of from patients with DMD harboring exon 45-52 deletion mutation, viltolarsen (0, 0.03, 0.1, 0.3, 1, 3, or 10 μmol/L) was added in the absence of transfection reagents for 2-day culture. Then, the culture medium was replaced with one not containing viltolarsen for 5-day culture. The exon 53 skipping efficiency (%) of viltolarsen[7)] was assessed by the RT-PCR method. Viltolarsen induced exon 53 skipping (EC_{50}, 0.90 μmol/L) (CTD 4.2.1.1-2).

To myotube cells differentiated from fibroblasts of patients with DMD harboring exon 45-52 deletion mutation, viltolarsen (0, 0.03, 0.1, 0.3, 1, 3, or 10 μmol/L) was added in the absence of transfection reagents for 2-day culture. Then, the culture medium was replaced with the one not containing viltolarsen for 5-day culture. The expression of dystrophin protein was assessed by immunoblot. At the viltolarsen concentrations of ≥1 μmol/L, the assay detected a band which was considered 372 kDa dystrophin protein without the segment corresponding to exons 45-53 (CTD 4.2.1.1-3).

3.1.2 Investigation using fibroblasts derived from patients with DMD harboring exon 48-52 deletion mutation (CTD 4.2.1.1-4 to 4.2.1.1-5)

To myotube cells differentiated from fibroblasts of patients with DMD harboring exon 48-52 deletion mutation, viltolarsen (0, 0.03, 0.1, 0.3, 1, 3, or 10 μmol/L) was added in the absence of transfection reagents for 2-day culture. Then, the culture medium was replaced with one not containing viltolarsen for 5-day culture. The exon 53 skipping efficiency (%) of viltolarsen[7)] was assessed by the RT-PCR method after the culture. Viltolarsen induced exon 53 skipping (EC_{50}, 2.30 μmol/L) (CTD 4.2.1.1-4).

To myotube cells differentiated from fibroblasts of patients with DMD harboring exon 48-52 deletion mutation, viltolarsen (0, 0.03, 0.1, 0.3, 1, 3, or 10 μmol/L) was added in the absence of transfection reagents for 2-day culture. Then, the culture medium was replaced with one not containing viltolarsen

7) Polymerase chain reaction (PCR) product (A) not containing exon 53 and PCR product (B) containing exon 53 were quantified by RT-PCR, and the amount of A was divided by the amount of A + B (exon 53 skipping efficiency = A / (A + B) × 100).

for 5-day culture. After that, the expression of dystrophin protein was assessed by immunoblot. At the viltolarsen concentrations of ≥0.1 μmol/L, the assay detected a band, which was considered 390 kDa dystrophin protein without the segment corresponding to exons 48-53 (CTD 4.2.1.1-5).

3.1.3 Investigation using cynomolgus monkeys (CTD 4.2.1.1-6 and 4.2.3.2-4)

The exon 53 skipping activity[7)] of viltolarsen was assessed in skeletal muscle and myocardial tissue collected at necropsy in a 39-week repeated intravenous dose toxicity study with the 8-week recovery period in cynomolgus monkeys by RT-PCR (CTD 4.2.3.2-4). At the end of the treatment period, the exon 53 skipping efficiency in the viltolarsen 0, 10, 60, and 360 mg/kg groups, respectively, was 0.1% ± 0.2%, 0.5% ± 0.3%, 2.3% ± 1.5%, and 6.2% ± 1.8% in the skeletal muscle and 0.1% ± 0.1%, 0.2% ± 0.0%, 0.5% ± 0.2%, and 3.9% ± 3.2% in the myocardial tissue. The activity increased in the skeletal muscle in the 60 and 360 mg/kg groups and in the myocardial tissue in the 360 mg/kg group. At the end of the recovery period, the exon 53 skipping efficiency in the viltolarsen 0, 60, and 360 mg/kg groups, respectively, was 0.0% ± 0.0%, 1.4% ± 0.7%, and 4.8% ± 2.7% in the skeletal muscle, and 0.0% ± 0.0%, 0.0% ± 0.1%, and 0.6% ± 0.3% in the myocardial tissue. The activity increased in both skeletal muscle and myocardial tissue in the 360 mg/kg group.

3.2 Secondary pharmacodynamics

3.2.1 Investigation of non-dystrophin genes potentially bound by viltolarsen (CTD 4.2.1.2-1)

3.2.1.1 *In silico* analysis

Antisense oligonucleotides may act on non-target genes containing a sequence identical with or similar to its complementary sequence in a sequence-dependent manner. In addition, oligonucleotides (n ± 1 mer) with 1 deleted or repeated base may be generated in the manufacturing process of the antisense oligonucleotide as an impurity, which may act in a sequence-dependent manner as well. Therefore, genes to which viltolarsen and n ± 1 mers may bind based on their complementary sequences were analyzed in the data sets, human mRNA sequence set and precursor messenger ribonucleic acid (pre-mRNA) sequence set, prepared using Ensembl Release 86[8)] and BioMart version 0.7[9)] through the algorithm developed by the applicant. No genes containing a sequence similar to complementary sequence of viltolarsen with a difference of up to 2 bases involved in mismatch, insertion, and deletion (hereinafter referred to as ≤2-base mismatched sequence) were identified. Although no genes containing a sequence identical with any of the complementary sequences of n ± 1 mer or 1-base mismatched sequence were identified, and 3 genes identified from the human mRNA and 30 genes from the human pre-mRNA contained 2-base mismatched sequence (Table 9). Of these, 11 genes were found to be identically conserved in cynomolgus monkeys, and thus 19 genes were extracted as human-specific off-target gene candidates (CTD 4.2.1.2-1).

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8) Ensemble Release 86, genome annotation database provided by EBI (European Bioinformatics Institute, European Molecular Biology Laboratory, Hinxton, Cambridgeshire, UK) (released in October 2016)

9) BioMart version 0.7, Web tool in Ensemble

Table 9. Human genes containing ≤2-base difference sequence of n ± 1 mer of viltolarsen

	Human mRNA[a)]	Human pre-mRNA
Human-specific genes	*ZNF557*	*ALDH1A2, APCDD1, CAMKK2, CNTNAP2, FSHR, FUT1, LMTK2, LRIG1, MYT1, PCDH15, PRKCH, RP11-459O1.2, RP11-479O16.1, SLC22A10, SLC24A2, TIAM1, WDR20, WRN, ZNF557*
Genes of which sequence is identical with that of cynomolgus monkey	*RP11-649E7.5, SYCP2L*	*AC008697.1, COL18A1, EEF2K, GRIA1, GRIN2A, RP11-145G20.1, RP11-649E7.5, SLC25A18, SLIT3, SYCP2L, ZMIZ1-AS1*

a) The 3 genes identified in human mRNA were also included in the 30 genes identified in human pre-mRNA.

3.2.1.2 Gene expression analysis

3.2.1.2.1 Gene expression analysis and pathway analysis in RD cells using exon microarray (CTD 4.2.1.2-2)

To human rhabdomyosarcoma (RD) cells, viltolarsen (0, 30, or 60 μmol/L) was added in the presence of transfection reagent for 2-day culture. On the cultured cells, an expression analysis was performed for the 19 human specific off-target gene candidates (Table 9) extracted in Section 3.2.1.1 by exon microarray. There were no change in expression level in 10 genes. The assessment on the remaining 9 genes failed, because of the failures in the preparation of *RP11-459O1.2* and *RP11-479O16.1* probes and in the expression of other genes, namely, APC down-regulated 1 (*APCDD1)*, contactin-associated protein-like 2 (*CNTNAP2)*, follicle-stimulating hormone receptor (*FSHR)*, fucossyltransferase 1 (H blood group) (*FUT1)*, myelin transcription factor 1 (*MYT1)*, solute carrier family 22 member 10 (*SLC22A10)*, and solute carrier family 24 member 2 (*SLC24A2)*, in RD cells. A pathway analysis based on comprehensive gene expression variations revealed variations in 29 and 19 pathways at 30 and 60 μmol/L, respectively, but these pathways did not include off-target candidate genes showing expression variations, and thus these changes are considered unrelated to the off-target action dependent on viltolarsen hybridization.

3.2.1.2.2 Gene expression analysis and pathway analysis in HEK293 cells using exon microarray (CTD 4.2.1.2-3)

To human embryonic kidney (HEK) 293 cells, viltolarsen (0 or 120 μmol/L) was added in the presence of transfection reagent for 2-day culture. In the cultured cells, an expression analysis was performed on the 19 human specific off-target candidate genes extracted in Section 3.2.1.1 using exon microarray. The analysis showed increased expression of 1 gene (*FUT1*) and decreased expression of 3 genes (*APCDD1*, *CNTNAP2*, and *MYT1*). The assessment on remaining 6 genes failed because of the failures in the preparation of *RP11-459O1.2* and *RP11-479O16.1* probes and in the expression of other genes, namely, *FSHR*, protocadherin related 15 [*PCDH15*], *SLC22A10*, and *SLC24A2* in HEK293 cells. The pathway analysis based on comprehensive gene expression variations revealed variations in 41 pathways. However, these pathways did not include off-target candidate genes presenting expression variations, and thus these changes are considered unrelated to the off-target action dependent on viltolarsen hybridization.

3.2.1.2.3 Gene expression analysis and pathway analysis in ITO-II cells by exon microarray (CTD 4.2.1.2-4)

To human testicular tumor cells (ITO-II cells), viltolarsen (0 or 60 μmol/L) was added in the presence of transfection reagent for 2-day culture. In the cultured cells, an expression analysis was performed for

the 19 human specific off-target candidate genes extracted in Section 3.2.1.1 by exon microarray. The analysis showed increased expression of 1 gene (aldehyde dehydrogenase 1 family member A2 [*ALDH1A2*]). The assessment on 6 of remaining genes failed, because of the failures in the preparation of probes of *RP11-459O1.2* and *RP11-479O16.1* and in the expression of *CNTNAP2*, *FSHR*, *PCDH15*, and *SLC22A10* in ITO-II cells. In the pathway analysis based on comprehensive gene expression variations, no pathways with variations were observed.

3.2.1.2.4 Gene expression analysis in HEK293 cells using RT-PCR method (CTD 4.2.1.2-5)

The total RNA extracted in the gene expression analysis and pathway analysis in HEK293 cells using exon microarray (CTD 4.2.1.2-3) was subjected to quantitative RT-PCR analysis on the expression of *APCDD1*, *CNTNAP2*, *FUT1*, and *MYT1*, which expression variations were observed in the exon microarray. The analysis showed decreased expression of *APCDD1*, *CNTNAP2*, and *MYT1* and increased expression of *FUT1*.

3.2.1.2.5 Gene expression analysis in ITO-II cells using RT-PCR method (CTD 4.2.1.2-6)

The total RNA extracted in the gene expression analysis and pathway analysis in ITO-II cells using exon microarray (CTD 4.2.1.2-4) was subjected to quantitative RT-PCR analysis on the expression of *ALDH1A2*, which expression variations were observed in the exon microarray, and *FSHR* and *SLC22A10*, which expression variations were not observed. The analysis showed no expression variations of any gene.

3.3 Safety pharmacology

Table 10 is a summary of the safety pharmacology study data on viltolarsen. Rat central nervous system was affected by viltolarsen exposure (C_{max} of 1862 μg/mL), which was 5.7 fold higher than exposure (C_{max} of 329 μg/mL) at the maximum clinical dose.

Table 10. Summary of Safety pharmacology studies

Item	Test system	Evaluation item/method, etc.	Dose	Route of administration	Finding	CTD
Central nervous system	SD rat (6 males/group)	FOB	0, 125, 250, 500 mg/kg	Intravenous	500 mg/kg, Body temperature decreased	4.2.1.3-1
	Cynomolgus monkey (5 males/group)	FOB	0, 60, 200, 600 mg/kg (12 weeks, once weekly)	Intravenous	No effects	4.2.3.2-3
Cardiovascular system	CHO cells (6 preparations)	hERG channel current	0, 0.3, 1, 3 mg/mL	*In vitro*	No effects	4.2.1.3-2
	Cynomolgus monkey (4 males)	Electrocardiogram,[a] blood pressure[b]	0, 60, 200, 600 mg/kg (4 weeks, once weekly, escalating doses)	Intravenous	No effects	4.2.1.3-3
	Cynomolgus monkey (5 males/group)	Electrocardiogram,[a] blood pressure[c]	0, 60, 200, 600 mg/kg (12 weeks, once weekly)	Intravenous	No effects	4.2.3.2-3
	Cynomolgus monkey (5 males/group)	Electrocardiogram[a]	0, 100 mg/kg (12 weeks, once weekly)	Intramuscular	No effects	4.2.3.2-5
Respiratory system	Cynomolgus monkey (4 males)	Respiratory rate, blood gas parameters[d]	0, 60, 200, 600 mg/kg (4 weeks, once weekly, escalating doses)	Intravenous	No effects	4.2.1.3-4
	Cynomolgus monkey (5 males/group)	Respiratory rate	0, 60, 200, 600 mg/kg (12 weeks, once weekly)	Intravenous	No effects	4.2.3.2-3

a) Heart rate, PR interval, QRS time, QT interval, and corrected QT (QTc) [Bazett's correction formula]
b) Diastolic blood pressure, systolic blood pressure, and mean blood pressure
c) Diastolic blood pressure and systolic blood pressure
d) pH, oxygen partial pressure, carbon dioxide partial pressure, and hemoglobin oxygen saturation

3.R Outline of the review conducted by PMDA

3.R.1 Mechanism of action

PMDA asked the applicant to explain the mechanism of action of viltolarsen in light of dystrophin function *in vivo* and the development of DMD.

The applicant's explanation:

- Dystrophin binds to intracellular actin and forms a complex of dystrophin/dystrophin-glycoprotein with glycoprotein in the cell membrane, which connects with the basal lamina through laminin α2, an extracellular matrix protein. Dystrophin secures the basal lamina and cytoskeleton of the muscle fiber and functions to maintain the muscle fiber structure (*Dis Model Mech.* 2015;8:195-213, *Nat Rev Mol Cell Biol.* 2006;7:762-73). The actin-binding N-terminal domain and β-dystroglycan-binding region are considered important for dystrophin to function (*Dis Model Mech.* 2015;8:195-213).

- In patients with DMD, some exons are deleted owing to dystrophin gene mutation. The total number of bases of the deleted exons that is not a multiple of 3 causes the misalignment of amino-acid reading frames (out-of-frame), resulting in the premature termination of translation due to the introduction of the stop codon, thereby producing structurally unstable dystrophin protein.

- Viltolarsen has a sequence complementary to exon 53 of dystrophin gene pre-mRNA and thus is considered to modulate splicing, a process in which pre-mRNA is transformed into mRNA, by preventing spliceosome's recognition of specific pre-mRNA region and facilitate exon 53 skipping. The viltolarsen's target sequence within exon 53 of dystrophin pre-mRNA is conserved in humans

and cynomolgus monkeys, but not in rodents. Viltolarsen, therefore, does not have any pharmacological action on dystrophin pre-mRNA in rodents.

- Viltolarsen is an exon 53 skipping drug, and is expected to be indicated for patients with DMD with deleted exon 43-52, 45-52, 47-52, 48-52, 49-52, 50-52, or 52, etc. In these patients, viltolarsen promotes exon 53 skipping so that the number of bases of the deleted exon is multiples of 3 (in-frame deletion), restoring the amino-acid reading frames. Exon 53 skipping with the in-frame deletion of dystrophin mRNA produces dystrophin protein, which total length is shorter than the normal protein but retains the structure of both ends (*Nucleic Acid Ther.* 2014;24:37-47).

- In patients with Becker muscular dystrophy (BMD), which is caused by dystrophin gene mutation as with DMD, the number of bases of the deleted exons is multiples of 3 and the amino-acid reading frame is maintained, allowing the production of functional dystrophin protein. For this reason, BMD is characterized by milder symptoms as compared with DMD (*Am J Hum Genet.* 1989;45:498-506). Viltolarsen is expected to increase the expression of dystrophin protein which has shorter chains than full-length one, turning DMD into BMD that is milder in pathological condition, thereby suppressing disease progression and alleviating the symptoms (*Brain.* 2011;134:3547-59).

PMDA accepted the above applicant's explanation.

3.R.2 Safety related to interactions of viltolarsen with genes

Viltolarsen is an antisense oligonucleotide, which is thought to have the following gene expression-related actions: (a) the action on the target sequence; and (b) the off-target action caused by viltolarsen's hybridization with non-target sequence (off-target action attributable to hybridization). PMDA asked the applicant to explain the safety of viltolarsen from the viewpoints of (a) and (b).

The applicant's explanation:

For the following reasons, the action (a) of viltolarsen is unlikely to cause serious adverse events on the target sequence:

- Dystrophin protein is known to be expressed in myocytes as well as in the central nervous system, retina, and kidney (*Cell Mol Life Sci.* 2006;63:1614-31). Viltolarsen is distributed into the retina and kidney, but scarcely into the central nervous system [see Section 4.2.1]. Increased expression of dystrophin protein by the administration of viltolarsen may affect the retina and kidney.

- The dystrophin-glycoprotein complex has been identified especially in photoreceptors, neurons, Muller glial cells, and blood vessels in the retina (*Cell Mol Life Sci.* 2006;63:1614-31). The effect of viltolarsen on their function, if any, may affect vision. The toxicity studies in cynomolgus monkeys with a dystrophin gene sequence complementary to viltolarsen revealed no changes in the retina. In the clinical studies of viltolarsen, keratitis and conjunctivitis allergic were reported as adverse events that fall under eye disorders, but none of them involved the retina. Thus, viltolarsen is considered to have a low risk in the retina.

- The dystrophin-glycoprotein complex has been identified in nephron epithelial cells in the kidney. (*Cell Mol Life Sci.* 2006;63:1614-31), suggesting viltolarsen may affect the renal function. The toxicity studies in cynomolgus monkeys with dystrophin gene sequence complementary to viltolarsen revealed changes mainly in renal tubules (dilation of renal tubules/epithelial vacuolation, basophilic changes, etc.) [see Section 5.2]. These changes are, however, generally observed in the use of nucleic acid medicine, and therefore considered unlikely to have occurred because of hybridization with the target sequence (*Int J Toxicol.* 2011;30:313-21, *Nucleic Acid Ther.* 2016;26:199-209). In addition, renal disorder-related adverse events reported in the clinical studies of viltolarsen were minor in severity.

The applicant's explanation about the justification of the *in silico* analysis performed to assess the safety related to the action (b), the off-target action caused by hybridization:

- In the *in silico* analysis to identify gene transcripts potentially exerting the off-target action caused by hybridization [see Section 3.2.1.1], the human mRNA sequence set and pre-mRNA sequence set were prepared using Ensembl Release 86 (released in October 2016) and BioMart version 0.7. A similar analysis was performed using recent database, Ensemble Release 94 (released in October 2018). The later analysis additionally identified IQ motif and sec7 domain ArfGEF 1 [*IQSEC1*] and *AL031590.1* as off-target gene candidates. The exon microarray showed that gene expression of *IQSEC1* was not affected. *AL031590.1* is a non-coding RNA and its genetic polymorphisms are associated with ankylosing spondylitis, Crohn's disease, psoriasis, primary sclerosing cholangitis, and colitis ulcerative according to genome-wide association studies (https://www.ebi.ac.uk/gwas/genes/AL031590.1). However, these polymorphisms are not directly involved in the development of such diseases. The clinical studies of viltolarsen did not suggest the development of these chronic inflammatory diseases. Accordingly, viltolarsen is considered unlikely to exert the off-target action through additionally identified *IQSEC1* or *AL031590.1*.

- The pyGenome Version 2.2.2, the algorithm used to identify off-target gene candidates in the *in silico* analysis [see Section 3.2.1.1], was validated for search integrity using random query sequences or the off-line GGGenome. Furthermore, the *in silico* analysis was repeated using the on-line GGGenome, an algorithm in the public domain, but no additional off-target gene candidates were identified.

- In the *in silico* analysis, off-target gene candidates were identified [see Section 3.2.1.1] by searching similar sequences with a total of ≤2 mismatched bases.[10)] This search condition is justified by a report (*Nucleic Acid Ther.* 2013;23:21-8), which showed that the antisense effect of oligonucleotides would be attenuated completely or almost completely on the target sequence with a total of ≥2 mismatched bases. The antisense effects of other antisense oligonucleotides in the same morpholino class of viltolarsen are also known to be attenuated completely or almost completely in the presence of ≥3 mismatched bases (*Biochim Biophys Acta.* 1999;1489:141-58, *Antisense Nucleic Acid Drug Dev.* 1997;7:151-7). Based on the above, searching for sequences with a total of 2-mismatched bases is considered appropriate.

[10)] Including mismatch, deletion, and insertion

The *in silico* analysis identified 30 off-target gene candidates, and of these, 19 genes were found to have no common sequences in cynomolgus monkeys. Exon microarray and RT-PCR assays identified expression variations in 4 genes (*CNTNAP2*, *MYT1*, *FUT1,* and *APCDD1*) among the 19. The applicant further explained that the 4 genes are unlikely to have the off-target action dependent on viltolarsen hybridization in humans and have low possibility to raise the safety concerns for the following reasons:

- While *CNTNAP2* and *MYT1* are known to be expressed in the nervous system (*J Psychiatr Res.* 2013;47:1349-56, *Neurogenesis.* 2017;4:e1329683, etc.), viltolarsen is scarcely distributed into the nervous system [see Section 4.2.1]. No serious adverse events occurred in the central nervous system during the clinical studies of viltolarsen, and all relevant events were Grade ≤2 [see Section 7.R.4.2].

- *FUT1* mRNA expression, in the exon microarray, increased 1.53-fold at 120 μmol/L of viltolarsen in the presence of the transfection reagent, which concentration is 2.5 fold higher than C_{max} (329 μg/mL)[11] at a clinical dose of viltolarsen (80 mg/kg) in humans. Because the transfection reagent increases the transfection efficiency of morpholino nucleic acids into cells 10- to 20-fold (*Mor Ther.* 2013;21:210-6), viltolarsen is unlikely to increase *FUT1* gene expression and to affect physiological functions in humans. Studies on diseases related to increased expression of *FUT1* gene using cancer cells revealed the proliferation, metastasis, drug-resistance acquisition of cancer cells, etc. (*Biochimie.* 2014;107:286-92, *Oncol Rep.* 2016;35:3025-33, etc.), but no investigations using normal cells have been reported.

- *APCDD1* mutation is related to congenital hypotrichosis and is known to be frequently associated with discoloration or low pigmentation of hair shafts (*Cell.* 2011;145:941-55, *Nature.* 2010;464:1043-7). A clinical study of viltolarsen reported hair colour change in 1 patient, but neither the affected patient nor the rest of all patients experienced hypotrichosis. The relationship between hypotrichosis and viltolarsen remains unclear, including the off-target action dependent on viltolarsen hybridization.

Among the 19 genes found to have no common sequences in cynomolgus monkeys, 13 genes were demonstrated to show no expression variations in exon microarray or RT-PCR assays. They are, namely, *ALDH1A2*, calcium/calmodulin-dependent protein kinase 2 (*CAMKK2*), *FSHR*, lemur tyrosine kinase 2 (*LMTK2*), leucine-rich repeats and immunoglobulin-like domains 1 (*LRIG1*), *PCDH15*, protein kinase C eta type (*PRKCH*), *SLC22A10*, *SLC24A2*, T-cell lymphoma invasion and metastasis-inducing protein 1 (*TIAM1*), WD repeat domain 20 (*WDR20)*, Werner syndrome RecQ like helicase (*WRN*), and zinc finger protein 557 (*ZNF557*). For the remaining 2 genes (*RP11-459O1.2* and *RP11-479O16.1*), no information is available about non-coding RNA function, physiological function, or related diseases, or no assessment has not been performed based on exon microarray and RT-PCR assay.

The *in silico* analysis identified 30 off-target gene candidates, and of these, 11 genes were found to have common sequences in cynomolgus monkeys. The applicant further explained that the 11 genes are

[11] C_{max} (329 μg/mL) at the final dose of 80 mg/kg in a Japanese Phase I/II study (CTD 5.3.5.1-2, Study NS065/NCNP01-P1/2 [Study P1/2])

unlikely to have the off-target action dependent on viltolarsen hybridization in humans and have low possibility to pose the safety concerns for the following reasons:

- A re-analysis of exon microarray data using human cells showed no expression variations of 5 genes (collagen type XVIII alpha 1 chain [*COL18A1*], eukaryotic elongation factor 2 kinase [*EEF2K*], solute carrier family 25 member 18 [*SLC25A18*], slit guidance ligand 3 [*SLIT3*], and synaptonemal complex protein 2 like [*SYCP2L*]). The non-clinical safety studies in cynomolgus monkeys revealed no relevant toxicological findings.

- Two genes (glutamate ionotropic receptor AMPA type subunit 1 [*GRIA1*] and glutamate ionotropic receptor NDMA type subunit 2A [*GRIN2A*]) are expressed mainly in the brain, and viltolarsen is scarcely distributed into the nervous system [see Section 4.2.1]. The non-clinical safety studies in cynomolgus monkeys revealed no neurologic symptoms or other changes suspected of expression variations of the 2 genes.

- The biological properties of 4 genes (zinc finger MIZ-type containing 1 antisense RNA 1 [*ZMIZ1-AS1*], *AC008697.1*, *RP11-145G20.1*, and *RP11-649E7.5*) remain unclear. Nevertheless, the findings from the non-clinical safety studies in cynomolgus monkeys are common changes attributable to renal disorders in the use of nucleic acid medicines, and there were no changes suspected of expression variations of these genes.

PMDA accepted the above applicant's explanation, however, the effects of viltolarsen on the renal function and central nervous system will continue to be discussed in Sections 7.R.4.1 and 7.R.4.2.

4. Non-clinical Pharmacokinetics and Outline of the Review Conducted by PMDA

The applicant submitted the result of studies on the absorption, distribution, metabolism, and excretion of in viltolarsen mice, rats, and cynomolgus monkeys as non-clinical pharmacokinetics data. Because the current application is intended for DMD, which is an X-linked recessive genetic disorder, non-clinical pharmacokinetic studies of viltolarsen were conducted only in male animals.

Unchanged viltolarsen concentrations in biological samples were determined by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) (lower limit of quantitation, 0.04-0.5 μg/mL), and impurity concentrations were determined by HPLC (lower limit of quantitation, 1000 μg/mL). Radioactivity concentrations in biological samples from studies using ^{14}C-viltolarsen were determined with a liquid scintillation counter (lower limit of quantitation, 0.002-0.152 μg eq./mL). Anti-drug antibody concentrations were measured by enzyme-linked immunoassay.

Unless otherwise specified, of the pharmacokinetic parameters, t_{max} values are presented as the median, and the other values are presented as the mean or mean ± SD.

4.1 Absorption

4.1.1 Single-dose studies

4.1.1.1 Rats

Table 11 shows the pharmacokinetic parameters of total plasma radioactivity in male rats (n = 3/group) which intravenously received a single dose of ^{14}C-viltolarsen at 6, 20, or 60 mg/kg (CTD 4.2.2.2-1). Animals administered 60 mg/kg tended to show $t_{1/2}$ longer than those administered the other doses. The applicant explained that the trend is attributable to the blood collection period of 24 hours in the 60 mg/kg group, which was longer than 10 hours in the 6 and 20 mg/kg groups.

Table 11. Pharmacokinetic parameters in male rats following a single intravenous dose of ^{14}C-viltolarsen

Dose (mg/kg)	C_{5min} (μg eq./mL)	$AUC_{0-\infty}$ (μg eq·h/mL)	$t_{1/2}$ (h)	CL_{tot} (mL/h/kg)	Vd_{ss} (mL/kg)
6	32.4 ± 10.2	17.5 ± 4.0	1.19 ± 0.74	358 ± 95	201 ± 61
20	115 ± 4	61.4 ± 6.4	1.19 ± 0.13	328 ± 33	184 ± 1
60	339 ± 83	207 ± 44	10.5 ± 3.0	298 ± 60	217 ± 54

Mean ± SD; number of animals, n = 3/group

4.1.1.2 Monkey

Male cynomolgus monkeys (n = 3/group) received a single intravenous dose of viltolarsen 6, 20, or 60 mg/kg, a single intramuscular dose of viltolarsen 20 mg/kg, or a single intravenous dose of ^{14}C-viltolarsen 20 mg/kg. Table 12 shows the pharmacokinetic parameters of unchanged viltolarsen in plasma and total plasma radioactivity. The bioavailability of a single intramuscular dose of viltolarsen was 113% (CTD 4.2.2.2-2).

Table 12. Pharmacokinetic parameters in male cynomolgus monkeys following a single intravenous or intramuscular dose of viltolarsen or a single intravenous dose of ^{14}C-viltolarsen

	Route of administration	Dose (mg/kg)	C_{5min}[a] (μg/mL)[b]	$AUC_{0-\infty}$ (mg·h/mL)[c]	$t_{1/2}$ (h)	t_{max} (h)	CL_{tot} (mL/h/kg)	Vd_{ss} (mL/kg)
Viltolarsen	Intravenous	6	67.5 ± 14.9	38.3 ± 6.8	2.1 ± 0.2		160 ± 32	185 ± 44
		20	220 ± 44	124 ± 19	2.0 ± 0.3		164 ± 28	179 ± 39
		60	643 ± 303	430[d]	3.4[d]		157[d]	196[d]
	Intramuscular	20	24.3 ± 6.9	140 ± 21	2.7 ± 0.4	1.0 [1.0,2.0]	NC	NC
^{14}C-viltolarsen	Intravenous	20	300 ± 83	163 ± 38	1.7 ± 0.1		NC	NC

Mean or mean ± SD; medium [range] for t_{max}; n = 3/group; NC, Not calculated
a) C_{max} for intramuscular administration
b) μg eq./mL for administration of ^{14}C-viltolarsen
c) μg eq·h/mL for administration of ^{14}C-viltolarsen
d) n = 2

4.1.2 Repeated-dose studies (toxicokinetics)

Toxicokinetics was investigated in repeated-dose toxicity studies in male mice and male cynomolgus monkeys. Table 13 shows pharmacokinetic parameters in major studies (CTD 4.2.3.2-2, CTD 4.2.3.2-4, CTD 4.2.3.2-5, CTD 4.2.3.7.6-1, and CTD 4.2.3.7.6-2).

Table 13. Pharmacokinetic parameters of viltolarsen repeatedly administered

	Route of administration	Dose (mg/kg)	n	Time point	C_{max} (μg/mL)	AUC_{0-24} (mg·h/mL)	CTD
Mice[a)]	Intravenous	15	3	Day 0	57.7	20.3	4.2.3.2-2
			3	Day 175	47.1	17.6	
		60	3	Day 0	229.7	75.8	
			3	Day 175	210.2	67.5	
		240	3	Day 0	981.1	654.9	
			3	Day 175	682.4	500.6	
		1000	3	Day 0	4660	7458	
			3	Day 175	4352	17280	
Cynomolgus monkeys	Intravenous	10	3	Week 1	137.6 ± 6.0	50.3 ± 7.9	4.2.3.2-4
			3	Week 38	122.8 ± 19.6	54.7 ± 15.2	
		60	3	Week 1	805.9 ± 56.5	269.3 ± 31.1	
			3	Week 38	613.9 ± 106.8	261.1 ± 24.4	
		360	3	Week 1	3665 ± 672	2452 ± 1160	
			3	Week 38	3711 ± 63	2294 ± 554	
Cynomolgus monkeys	Intramuscular	100	5	Week 1	85.9 ± 21.3	621.4 ± 120.6	4.2.3.2-5
			5	Week 12	49.3 ± 11.1	540.0 ± 85.2	

Mean or mean ± SD

a) Day of the first dose was defined as Day 0.

4.2 Distribution

4.2.1 Tissue distribution

Male wild-type mice and male X chromosome-linked muscular dystrophy mice (mdx mice)[12)] intravenously received a single dose of ^{14}C-viltolarsen 20 mg/kg. The radioactivity was rapidly distributed into tissues other than the brain, spinal cord, eyeballs, vesicular gland, and bile. The concentration peaked by 15 minutes post-dose. Radioactivity concentrations were high particularly in the kidney, bladder wall, and bulbourethral gland. Radioactivity concentrations in the brain, spinal cord, eyeballs, vesicular gland, and bile were lower than those in the other tissues, indicating low distribution into these tissues. Plasma and blood radioactivity concentrations peaked at 5 minutes post-dose (first time point) before a rapid decrease, but the elimination was slow in most tissues. The radioactivity was still detected even at 168 hours post-dose, the last time point. Radioactivity concentration in the muscle tissue of mdx mice was remarkably higher at 1 hour post-dose than those in wild-type mice (CTD 4.2.2.3-2).

Male cynomolgus monkeys intravenously received a single dose of ^{14}C-viltolarsen 20 mg/kg. The radioactivity was rapidly distributed into tissues other than the cerebrum, cerebellum, spinal cord, and eyeballs. The concentration peaked by 15 minutes post-dose. Radioactivity concentrations were high particularly in the renal cortex, adrenal gland, thyroid, liver, and spleen. Radioactivity concentrations in the cerebrum, cerebellum, spinal cord, and eyeballs were lower than those in the other tissues, indicating low distribution into these tissues. Plasma and blood radioactivity concentrations peaked at 15 minutes post-dose (first time point) before a rapid decrease, but the elimination was slow in most tissues. The radioactivity was still detected in the following tissues even at 504 hours post-dose: renal cortex, renal medulla, liver, spleen, adrenal cortex, adrenal medulla, mesenteric lymph node, turbinate, submandibular gland, thymus, penis, prostate gland, vesicular gland, testis, bone marrow, brown fat, gastrocnemius, skin (non-pigmented part), small intestine wall, and bladder wall. In male cynomolgus monkeys receiving intravenous repeated doses of ^{14}C-viltolarsen 20 mg/kg for 8 weeks, the tissue

12) Muscular dystrophy model mouse

distribution was similar to that of the single dose, and no accumulation due to repeated doses was observed (CTD 4.2.2.3-3 and CTD 4.2.2.3-4).

4.2.2 Serum protein binding and distribution in blood cells

To serum specimens from mice, rats, and cynomolgus monkeys, ^{14}C-viltolarsen 1000 or 10000 ng/mL was added for ultra-centrifugation to investigate the serum protein binding. The binding was 23.0% to 25.7%, 29.7% to 32.2%, and 36.1% to 36.2%, respectively (CTD 4.2.2.3-1 and CTD 5.3.2.1-1).

To blood specimens from rats and cynomolgus monkeys, ^{14}C-viltolarsen 1000 or 10000 ng/mL was added to investigate the distribution in blood cells. The distribution was 0.4% to 2.5% and 4.1% to 6.7%, respectively (CTD 5.3.2.3-1).

4.3 Metabolism

4.3.1 *In vitro* metabolism

To serum specimens from mice, rats, and cynomolgus monkeys, ^{14}C-viltolarsen 0.1 mg/mL was added for 1-hour incubation at 37°C. No metabolites were observed in any specimen. To liver microsome and S9 specimens from mice, rats, and cynomolgus monkeys, ^{14}C-viltolarsen 0.1 mg/mL was added for 2-hour incubation at 37°C in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). No metabolites were observed in any specimen (CTD 4.2.2.3-1 and CTD 5.3.2.2-1).

4.3.2 *In vivo* metabolism

Male rats received a single dose of ^{14}C-viltolarsen 20 mg/kg intravenously and metabolites in the kidney was investigated. Unchanged viltolarsen was mainly observed in the kidney (CTD 4.2.2.4-1).

Male cynomolgus monkeys received a single dose of ^{14}C-viltolarsen 20 mg/kg intravenously. Unchanged viltolarsen was mainly observed in plasma and urine (CTD 4.2.2.4-2).

4.4 Excretion

Male rats received a single dose of ^{14}C-viltolarsen 20 mg/kg intravenously. By 168 hours post-dose, 89.6% and 7.7% of the total administered radioactivity were excreted into urine and feces, respectively (CTD 4.2.2.5-1).

Male cynomolgus monkeys received a single dose of ^{14}C-viltolarsen 20 mg/kg intravenously. By 168 hours post-dose, 75.8%[13)] and 1.2% of the total administered radioactivity were excreted into urine and feces, respectively (CTD 4.2.2.2-2).

4.R Outline of the review conducted by PMDA

PMDA concluded that there were no particular problems in the submitted non-clinical pharmacokinetic study data.

[13)] By 168 hours post-dose, 28.4%, 27.3%, and 20.1% of the total administered radioactivity were excreted into urine, cage washings, and cage sediment, respectively, and the radioactivity in the cage washings and cage sediment was considered to be from urine.

5. Toxicity and Outline of the Review Conducted by PMDA

The applicant submitted the results of single-dose toxicity, repeated-dose toxicity, genotoxicity, reproductive and developmental toxicity, and other studies (immunotoxicity studies and safety evaluation of impurities) as toxicity data of viltolarsen. Unless otherwise specified, physiological saline was used as the vehicle in *in vivo* studies. Because the current application is intended for DMD, an X-linked recessive genetic disorder, toxicity studies of viltolarsen were conducted only in male animals.

5.1 Single-dose toxicity

Acute toxicity of viltolarsen was evaluated based on results from the single intravenous dose toxicity study and the single intramuscular dose toxicity study in monkeys (Table 14). An approximate lethal dose of viltolarsen in monkeys was determined to be >600 mg/kg for an intravenous dose and >100 mg/kg for an intramuscular dose.

Table 14. Summary of single dose toxicity study results

Test system	Route of administration	Dose (mg/kg)	Major findings	Approximate lethal dose (mg/kg)	CTD
Male cynomolgus monkey	Intravenous	0, 600	600: Transient increase in blood IL-6 and vacuolation of proximal renal tubular epithelium	>600	Reference 4.2.3.1-1
Male cynomolgus monkey	Intramuscular	0, 1, 10, 100	≥10: Increased blood AST; increased blood CK-MM; and muscle fiber degeneration/necrosis in the quadriceps femoris at and adjacent to the injection site 100: Inflammatory cell infiltration, edema, and haemorrhage at the injection site	>100	4.2.3.1-2

5.2 Repeated-dose toxicity

Repeated-dose toxicity studies were conducted in mice (13 and 26 weeks) and cynomolgus monkeys (12 and 39 weeks) (Table 15). A major finding was renal toxicity. The exposure ($AUC_{0\text{-}24h}$) to viltolarsen at the no observed adverse effect level (NOAEL) was 17,640 ng·h/mL in male mice (26 weeks) and 261,100 ng·h/mL in male cynomolgus monkeys (39 weeks), which were 0.03- and 0.51-fold, respectively, exposure ($AUC_{0\text{-}\infty}$, 508,000 ng·h/mL) at the clinical dose (80 mg/kg/week) (CTD 5.3.5.1-2, Study NS065/NCNP01-P1/2 [Study P1/2]).

Table 15. Summary of repeated-dose toxicity study results

Test system	Route of administration	Treatment period	Dose (mg/kg)	Major findings	NOAEL (mg/kg/day)	CTD
Male mouse (CD-1)	Intravenous	13 weeks (once weekly) + Withdrawal 4 weeks	0, 60, 240, 1000	≥240: Increased blood MCP-1; increased kidney weight; and vacuolation of distal renal tubular/collecting tubular epithelium, accumulation and dilation of basophilic substances of the kidney 1000: Decrease in locomotor activity; decreases in hemoglobin, corpuscular volume, and mean corpuscular hemoglobin; increases in blood urea nitrogen, creatinine, C-reactive protein, and cystatin C; decreased blood total bilirubin, increases in blood IL-6·and TNF-α; decreased thymus weight; vacuolation of proximal renal tubular epithelium; and regeneration of muscle fiber of the diaphragm Reversibility: reversible	60	4.2.3.2-1
Male mouse (CD-1)	Intravenous	26 weeks (once weekly) + Withdrawal 8 weeks	0, 15, 60, 240, 1000	Death: 1000[a)] (1 of 30 males) ≥60: Cytoplasmic eosinophilic substances in the bladder transitional epithelium ≥240: Decreased urine pH; vacuolation of distal renal tubular/collecting tubular epithelium, accumulation and dilation of basophilic substances of the kidney; and epithelial vacuolation of proximal renal tubule 1000: Decrease in locomotor activity; decreased body weight; decreases in hemoglobin, corpuscular volume, mean cell volume, and mean corpuscular hemoglobin; increases in blood AST, blood urea nitrogen, creatinine, and cystatin C; increases in blood IL-6, MCP-1, and TNF-α; change in blood C3[b)]; increased kidney weight; fibrogenesis of proximal renal tubular epithelium; low electron density substances and high electric substances in the collecting tubular lumen; low electron density substances in the distal renal tubular lumen; cytoplasmic membrane-bound vacuoles in the collecting, distal renal, and proximal renal tubular epithelium; tegmentum cell cytoplasmic granules and large vacuoles containing vesicles in the bladder transitional epithelium; and macrophages engulfing basophilic substances in the testis Reversibility: reversible (except for erythrocyte parameters)	15	4.2.3.2-2
Male cynomolgus monkey	Intravenous	12 weeks (once weekly) + Withdrawal 4 weeks	0, 60, 200, 600	≥200: Increases in blood AST, CK, and total bilirubin; increased spleen weight; and basophilic change and vacuolation of epithelium in proximal renal tubular 600: Decreases in red blood cell count, corpuscular volume, and hemoglobin; increased reticulocyte rate; increases in blood urea nitrogen and C-reactive protein; increased kidney weight; mononuclear cell infiltration and edema of medullary ray interstitium in the proximal renal tubular epithelium; and subcutaneous neutrophil infiltration and edema at the injection site and neutrophil infiltration in the vascular wall Reversibility: reversible	60	4.2.3.2-3
Male cynomolgus monkey	Intravenous	39 weeks (once weekly) + Withdrawal 8 weeks	0, 10, 60, 360	≥10: Deposition of basophilic granules in the renal tubular epithelium 360: Vacuolation of proximal renal tubular epithelium and renal tubular dilation, basophilic change in the proximal renal tubular straight part, and cytoplasmic membrane-bound vacuoles in the renal tubular epithelium and collecting tubular epithelium Reversibility: reversible	60	4.2.3.2-4

Table 15. Summary of repeated-dose toxicity study results

Test system	Route of administration	Treatment period	Dose (mg/kg)	Major findings	NOAEL (mg/kg/day)	CTD
Male cynomolgus monkey	Intramuscular	12 weeks (once weekly) + Withdrawal 4 weeks	0, 100	100: Increases in blood AST, CK-MM, C-reactive protein; white, induration, and red focal at injection site; and muscle fiber degeneration/necrosis, regeneration, inflammatory cell infiltration, fibrogenesis, edema in the epimysium, fibrin effusion, and perivascular mononuclear cell infiltration in the quadriceps femoris at and adjacent to the injection site Reversibility: reversible	<100	4.2.3.2-5

a) Considered attributable to renal disorder
b) Decreasing trend at 1 hour after the first dose and at 1 and 4 hours after the last dose, and increasing trend at 24 hours after the last dose

5.3 Genotoxicity

The following studies were conducted, and no genotoxicity was observed (Table 16).

Table 16. Summary of genotoxicity study results

Study type		Test system	Metabolic activation (treatment)	Concentration or dose[a)]	Result	CTD
In vitro	Bacterial reverse mutation assay	*Salmonella typhimurium*: TA100, TA1535, TA98, TA1537 *Escherichia coli*: WP2*uvrA*	S9-/+	0, 156, 313, 625, 1250, 2500, 5000 (μg/plate)	Negative	4.2.3.3.1-1
	Chromosomal aberration assay in mammalian cells	Chinese hamster lung fibroblasts (CHL/IU cells)	S9- (24 hours)	0, 1250, 2500, 5000 (μg/mL)	Negative	4.2.3.3.1-2
			S9+ (6 hours)	0, 1250, 2500, 5000 (μg/mL)		
			S9- (6 hours)	0, 1250, 2500, 5000 (μg/mL)		
In vivo	Bone marrow micronucleus assay in rodents	Male mouse (CD-1) Bone marrow		0, 250, 500, 1000 (mg/kg) (intravenous, once daily for 2 days)	Negative	4.2.3.3.2-1

a) Drug substance synthesized by a solid-phase method was used.

5.4 Carcinogenicity

No results from carcinogenicity studies were included in this application.

5.5 Reproductive and developmental toxicity

A study for male fertility in mice and repeated-dose toxicity study in juvenile mice were conducted (Tables 17 and 18).

Table 17. Summary of reproductive and developmental toxicity study results

Study type	Test system	Route of administration	Treatment period	Dose (mg/kg)	Major findings	NOAEL (mg/kg/day)	CTD
Study for fertility	Male mouse (CD-1)	Intravenous	Male: From 9 weeks before mating to mating period (12 weeks in total) (once weekly)	0, 60, 240, 1000	1000: Increased BUN	Parental animal (general toxicity): 240[a)] Parental animal (fertility): 1000	4.2.3.5.1-1

a) A histopathological examination of the kidney was not performed. The toxicity was determined based on the increased BUN, which was suggestive of a renal disorder, by reference to increased BUN associated with a histopathological change indicative of a renal disorder observed in a 13-week repeated intravenous dose toxicity study in mice.

Table 18. Summary of juvenile animal study results

Study type	Test system	Treatment period	Dose (mg/kg)	Items	Major findings	CTD
Repeated subcutaneous and intravenous dose toxicity study in juvenile mice	Male mice aged 7 days (CD-1)	10 weeks (once weekly) (first dose, subcutaneous; second and subsequent doses, intravenous)	0, 15, 60, 240, 1200	Body weight and food consumption, clinical observations, sexual maturation, functional observational battery, ophthalmology, haematology, clinical chemistry, urinalysis, bone examination, neuroethology, neuropathology, histopathology, anti-viltolarsen antibody, and toxicokinetics Reversibility 10 weeks after administration	Death: 1200 (5 of 33 males)[a)] ≥15: Hypertrophy of renal tubular epithelium and inflammatory cell infiltration around the injection site (tail vein) ≥240: Renal tubular degeneration, basophilic renal tubule, and vacuolation 1200: Incomplete eyelid opening, inactive, increased vocalization, piloerection, decreased body weight, decreased prostate weight, renal tubular dilation and cast, and decreased lymphocytes associated with decreased thymus weight Reversibility: reversible NOAEL (general toxicity): 60 mg/kg NOAEL (bone growth and neurotoxicity): 1200 mg/kg	4.2.3.5.4-3

a) Considered attributable to renal disorder

5.6 Local tolerance

Local tolerance of intravenous viltolarsen was evaluated during the 12-week repeated intravenous dose toxicity study in monkeys with a 4-week recovery period (CTD 4.2.3.2-3) and the 39-week repeated intravenous dose toxicity study in monkeys with a 8-week recovery period (CTD 4.2.3.2-4). Local tolerance of intravenous viltolarsen was observed at up to 360 mg/kg (CTD 4.2.3.2-4). Local tolerance of intramuscular viltolarsen was evaluated during the 12-week repeated intramuscular dose toxicity study in monkeys with a 4-week recovery period (CTD 4.2.3.2-5). An inflammatory change in skeletal muscle was observed at the site where viltolarsen 100 mg/kg was intramuscularly injected.

5.7 Other toxicity studies

5.7.1 Immunotoxicity evaluation

Anti-drug antibody concentrations in serum were measured by enzyme-linked immunoassay, using serum specimens obtained from the 12-week repeated intravenous dose toxicity study in monkeys with a 4-week recovery period (CTD 4.2.3.2-3) and the 12-week repeated intramuscular dose toxicity study in monkeys with a 4-week recovery period (CTD 4.2.3.2-5). Anti-drug antibodies against viltolarsen were identified in a Day-29 specimen from 1 of 5 male monkeys in the 200 mg/kg group of the 12-week repeated intravenous dose toxicity study with a 4-week recovery period and in a Day-77 specimen from 1 of 5 male monkeys in the 100 mg/kg group of the 12-week repeated intramuscular dose toxicity study with a 4-week recovery period.

5.7.2 Toxicity comparison between pre- and post-manufacturing process change

In response to changes in the manufacturing process of viltolarsen, to compare toxicity among [redacted] drug substance ([redacted] drug substance), [redacted] drug substance ([redacted] drug substance), and [redacted] drug substance ([redacted] drug substance), a 5-week repeated intravenous dose toxicity study in monkeys, bacterial reverse mutation assay, and chromosomal aberration assay in mammalian cells were conducted using these drug substances. The toxicity findings did not differ among these drug substances (Tables 19 and 20).

Table 19. Summary of toxicity study results of products pre- and post-manufacturing process change

Test system	Route of administration	Treatment period	Dose (mg/kg)	Major findings	CTD
Male cynomolgus monkey	Intravenous	5 weeks (once weekly)	[redacted] drug substance 0, 200, 600 [redacted] drug substance 0, 200, 600	[redacted] drug substance ≥200: Deposition of basophilic granules in the renal tubular epithelium 600: Increased blood BUN, increased urinary protein, epithelial vacuolation in proximal renal tubular curve and straight parts, and dilation of distal renal tubule [redacted] drug substance ≥200: Deposition of basophilic granules in the renal tubular epithelium 600: Increased blood BUN, increased urinary protein, epithelial vacuolation in proximal renal tubular curve and straight parts, and dilation of distal renal tubule	4.2.3.7.6-1
Male cynomolgus monkey	Intravenous	5 weeks (once weekly)	[redacted] drug substance 0, 200, 600 [redacted] drug substance 0, 200, 600	[redacted] drug substance ≥200: Increased urinary protein, deposition of basophilic granules in renal tubular epithelium, and epithelial vacuolation in proximal renal tubular curve and straight parts 600: Increased blood BUN, increased kidney weight, swelling of kidney, and dilation of distal renal tubule [redacted] drug substance ≥200: Increased urinary protein, deposition of basophilic granules in renal tubular epithelium, and epithelial vacuolation in proximal renal tubular curve and straight parts 600: Increased blood BUN and dilation of distal renal tubule	4.2.3.7.6-2

Table 20. Summary of genotoxicity study results of product post-manufacturing process change

Study type		Test system	Metabolic activation (treatment)	Viltolarsen concentration or dose[a)]	Result	CTD
In vitro	Bacterial reverse mutation assay	*Salmonella typhimurium*: TA100, TA1535, TA98, TA1537 *Escherichia coli*: WP2*uvrA*	S9-/+	[redacted] drug substance 0, 156, 313, 625, 1250, 2500, 5000 (μg/plate)	Negative	4.2.3.7.6-3
	Bacterial reverse mutation assay	*Salmonella typhimurium*: TA100, TA1535, TA98, TA1537 *Escherichia coli*: WP2*uvrA*	S9-/+	[redacted] drug substance 0, 156, 313, 625, 1250, 2500, 5000 (μg/plate)	Negative	4.2.3.7.6-4
	Chromosomal aberration assay in mammalian cells	Chinese hamster lung fibroblasts (CHL/IU cells)	S9- (24 hours)	[redacted] drug substance 0, 1250, 2500, 5000 (μg/mL)	Negative	4.2.3.7.6-5
			S9+ (6 hours)	[redacted] drug substance 0, 1250, 2500, 5000 (μg/mL)		
			S9- (6 hours)	[redacted] drug substance 0, 1250, 2500, 5000 (μg/mL)		
	Chromosomal aberration assay in mammalian cells	Chinese hamster lung fibroblasts (CHL/IU cells)	S9- (24 hours)	[redacted] drug substance 0, 1250, 2500, 5000 (μg/mL)	Negative	4.2.3.7.6-6
			S9+ (6 hours)	[redacted] drug substance 0, 1250, 2500, 5000 (μg/mL)		
			S9- (6 hours)	[redacted] drug substance 0, 1250, 2500, 5000 (μg/mL)		

5.R Outline of the review conducted by PMDA

5.R.1 Effects on kidney

Toxicity studies in mice, rats, and cynomolgus monkeys showed the dilation of renal tubule/epithelial vacuolation and the deposition of basophilic substances. PMDA asked the applicant to explain the mechanism of these changes and safety in humans.

The applicant's explanation:

- Basophilic granules were observed in renal tubule of animals administered other nucleic acid medicine as well. The images show oligonucleotide administered was taken into lysosomes or phagolysosomes (*Int J Toxicol.* 2011;30:313-21, *Nucleic Acid Ther.* 2016;26:199-209). Basophilic

substances in renal tubular lumen identified in the toxicity studies of viltolarsen in mice and monkeys showed similar stainability to that of basophilic granules observed after the administration of the other oligonucleotide, and thus they are expected to be similar substances.

- The study on the other nucleic acid medicines showed that kidney tissue concentration of oligonucleotide was correlated to toxicity development (*Nucleic Acid Ther.* 2016;26:199-209). Because viltolarsen is distributed in the kidney at high concentrations [see Section 4.2.1], the toxicological changes in the kidney are considered related to the distribution of viltolarsen in the kidney at a high concentration.

- The changes in the kidney observed in the non-clinical safety studies were considered histopathologically minor at a dose corresponding to the human clinical dose (80 mg/kg) and were non-serious changes without accompanying necrosis or changes in clinicopathological parameters.

- However, an exposure-based safety margin is inadequate [see Section 5.2]. Viltolarsen is renally excreted, and a high concentration of viltolarsen in the kidney may cause an adverse event. Given these, the effect of viltolarsen on the kidney should be communicated through the package insert.

PMDA will further discuss the effects of viltolarsen on the kidney in Section 7.R.4.1, including the occurrence of adverse events in the clinical studies.

5.R.2 Carcinogenicity evaluation

Data from carcinogenicity studies of viltolarsen were not submitted [see Section 5.4]. PMDA asked the applicant to explain their plan on carcinogenicity studies and their view on carcinogenicity risks based on the currently available findings.

The applicant's explanation:
Viltolarsen targets the intractable and progressive disease. The applicant considered that the submission of carcinogenicity study data after marketing authorization be possible based on "Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals" (PFSB/ELD Notification No. 0219-4 dated February 19, 2010) and "Revision of the Guideline for Carcinogenicity Studies for Pharmaceuticals" (PFSB/ELD Notification No. 1127001 dated November 27, 2008).

Carcinogenicity study data are to be submitted after marketing authorization, based on the final report of a 26-week repeated intravenous dose carcinogenicity study in rasH2 mice (to be completed in Fiscal Year 2020) and a 104-week repeated intravenous dose carcinogenicity study in rats (to be completed in Fiscal Year 2023).

The applicant's additional explanation about the carcinogenicity risk of viltolarsen based on the currently available information:
- Viltolarsen was tested negative for genotoxicity [see Section 5.3]. The non-clinical toxicity studies of viltolarsen, including the 26-week repeated intravenous dose toxicity study in mice with a 8-week

recovery period and the 39-week repeated intravenous dose toxicity study in monkeys with a 8-week recovery period revealed no changes indicative of preneoplastic lesions, tumor lesions, or findings in the endocrine organs related to hormonal effects. The clinical studies of viltolarsen identified no adverse events suggestive of carcinogenicity.

- Golodirsen (unapproved in Japan and approved overseas) and cacimersen (unapproved either in Japan or overseas) are morpholino nucleic acid medicines of the same skeleton with viltolarsen. Repeated-dose toxicity studies of these drugs suggested no carcinogenicity risks (*J Neuromuscul Dis.* 2016;3:381-93).

- Eteplirsen (unapproved in Japan and approved in the US) is a morpholino nucleic acid medicine as with viltolarsen. The clinical and non-clinical studies of eteplirsen did not suggest a clear carcinogenicity risk (https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/206488lbl.pdf, https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206488Orig1s000PharmR.pdf).
  However, a 13-week repeated-dose toxicity study in rats showed hypertrophy of bladder epithelium consisting of atypical, large, and irregular cells in the bladder. Eteplirsen and viltolarsen, however, differ in base sequence, base length, and 5'-terminal structure, and thus these findings are considered not suggestive of the carcinogenicity risks of viltolarsen. In addition, golodirsen, cacimersen, and eteplirsen were all tested negative for genotoxicity (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206488Orig1s000PharmR.pdf, https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/PediatricAdvisoryCommittee/UCM557907.pdf).

- Mipomersen and inotersen (unapproved in Japan and approved overseas) are oligonucleotides with chemical modification (2'-MOE) not found in viltolarsen or eteplirsen. The carcinogenicity studies of these drugs did not suggest a carcinogenicity risk in humans. Furthermore, these drugs were tested negative for genotoxicity as well (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/203568Orig1s000PharmR.pdf, https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/211172Orig1s000PharmR.pdf).

- Based on the above, there are no findings suggestive of the carcinogenicity risk of viltolarsen currently available.

The applicant submitted a clinical trial adverse drug reaction report dated [redacted], which included the following preliminary findings from the ongoing 26-week repeated intravenous dose carcinogenicity study in rasH2 mice: Mass or hypertrophy found in the urinary duct in 1 and 2 animals at 50 and 150 mg/kg, respectively, at necropsy; and transitional cell carcinoma found in the urinary duct on a histopathological examination.

The applicant's explanation:
The transitional cell carcinoma in the urinary duct observed in the 26-week repeated intravenous dose carcinogenicity study in rasH2 mice is likely due to viltolarsen that was insolubilized in the urinary duct and physically stimulated the transitional epithelium of the urinary duct wall in a continuous manner,

leading to repeated injury and regeneration, which potentially resulted in the neoplastic transformation. This mechanism of development, however, is considered unlikely to be relevant to humans because the urinary duct diameter differs between humans and rodents (approximately 3.4 mm in internal diameter in humans, approximately 0.3 mm in external diameter in rodents), and clinical studies did not yield findings suggestive of injury in the urinary system due to deposit formation.

PMDA is currently reviewing the carcinogenicity of viltolarsen in details based on the preliminary report on the 26-week repeated intravenous dose carcinogenicity study in rasH2 mice. This matter will be discussed in the Review Report (2).

6. Summary of Biopharmaceutic Studies and Associated Analytical Methods, Clinical Pharmacology, and Outline of the Review Conducted by PMDA

6.1 Summary of biopharmaceutic studies and associated analytical methods

Unchanged viltolarsen concentrations in plasma and viltolarsen concentrations in urine were determined by LC-MS/MS (lower limit of quantitation, 20.0 ng/mL in plasma and 500 ng/mL in urine). In addition, muscular dystrophin was detected or measured by Western blot, immunofluorescent staining, and LC-MS/MS, and serum anti-viltolarsen antibodies were determined by enzyme-linked immunoassay.

6.2 Clinical pharmacology

Submitted evaluation data cover a study using human biological samples,[14)] Phase I/II study in Japanese patients with DMD (CTD 5.3.5.1-2, Study P1/2), Phase II study in non-Japanese patients with DMD (CTD 5.3.5.1-1, Study NS-065/NCNP-01-201 [Study 201]), and Japanese Phase I investigator-initiated trial in Japanese patients with DMD (CTD 5.3.5.2-1, Study NCNP/DMT01 [Study DMT01]). Unless otherwise specified, of a pharmacokinetic parameter, t_{max} values are presented as the median, and the other values are presented as the mean ± SD. Only the major pharmacokinetic study results are presented.

6.2.1 Study using human biological samples

To human serum, ^{14}C-viltolarsen 1000 or 10000 ng/mL was added to investigate the serum protein binding by ultra-centrifugation. The binding was 39.4% to 40.3% (CTD 5.3.2.1-1).

To human blood, ^{14}C-viltolarsen 1000 or 10000 ng/mL was added to investigate the distribution in blood cells. The distribution was 2.1% to 3.5% (CTD 5.3.2.3-1).

To human serum, human liver microsomes, human liver S9, DNase I, or PDE1, ^{14}C-viltolarsen 0.1 mg/mL was added for incubation at 37°C for ≥30 minutes. No metabolites were observed in any specimen (CTD 5.3.2.2-1 and CTD 5.3.2.2-2).

14) CTD 5.3.2.1-1, Study BP-065-006; CTD 5.3.2.3-1, Study BP-065-020; CTD 5.3.2.2-1, Study BP-065-010; CTD 5.3.2.2-2, Study BP-065-012; CTD 5.3.2.2-3, Study BP-065-021; CTD 5.3.2.2-4, Study BP-065-041; CTD 5.3.2.2-5, Study BP-065-043; CTD 5.3.2.2-6, Study BP-065-004; CTD 5.3.2.2-7, Study BP-065-032; CTD 5.3.2.2-8, Study BP-065-042; CTD 5.3.2.2-9, Study BP-065-038; CTD 5.3.2.2-10, Study BP-065-033; and CTD 5.3.2.2-11, Study BP-065-044

Using substrates[15] specific to cytochrome P450 (CYP) 1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5, the inhibition and time-dependent inhibitory effect of viltolarsen (1-3000 μmol/L) against CYP isoforms in human liver microsomes were investigated. Viltolarsen inhibited CYP3A4 (50% inhibition concentration [IC_{50}], 1.44-1.48 mmol/L), but no time-dependent inhibitory effect was observed (CTD 5.3.2.2-3 and CTD 5.3.2.2-4).

Using substrates[16] specific to uridine diphosphate glucuronosyltransferase (UGT) 1A1 and UGT2B7, the inhibition of viltolarsen (0.1-3 mmol/L) against UGT isoforms in human liver microsomes was investigated. Viltolarsen inhibited UGT1A1 (IC_{50}, 0.519 mmol/L) (CTD 5.3.2.2-5).

To human hepatocytes, viltolarsen 0.03, 0.1, 0.3, 0.5, 1 or 3 mmol/L was added to investigate its induction of CYP1A2, CYP2B6, and CYP3A4. No clear induction was observed on these metabolic enzymes (CTD 5.3.2.2-8).

To LLC-PK1 cells expressing P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), ^{14}C-viltolarsen 10 μmol/L was added to investigate a transport ratio of viltolarsen (P_{app} ratio in cells expressing transporters[17]/P_{app} ratio in control cells[17]). The ratio was 1.0 for P-gp and 0.9 for BCRP, and inhibitors specific to these transporters did not affect the ratio, suggesting that viltolarsen was not a substrate of P-gp or BCRP. To HEK293 cells expressing organic anion transporter (OAT) 1, OAT3, organic cation transporter (OCT) 2, multidrug and toxin extrusion (MATE) 1, or MATE2-K, ^{14}C-viltolarsen 100 μmol/L was added to investigate an uptake ratio of cells expressing transporters to control cells. The ratio was <2 for any cells expressing these transporters and was not affected by specific inhibitors, suggesting that viltolarsen was not a substrate of OAT1, OAT3, OCT2, MATE1, or MATE2-K (CTD 5.3.2.2-9).

To membrane vesicles prepared from LLC-PK1 cells expressing P-gp and BCRP, HEK293 cells expressing organic anion transporting polypeptide (OATP) 1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, or MATE2-K, and cells expressing bile salt export pump (BSEP), viltolarsen 30 to 3000 μmol/L was added. Viltolarsen inhibited BCRP, OATP1B1, OATP1B3, and OAT3-mediated transport ($IC_{50;}$ 1970, 485, 448, and 176 μmol/L, respectively) (CTD 5.3.2.2-11).

6.2.2 Investigations in patients

Table 21 shows pharmacokinetic parameters of viltolarsen in Japanese patients with DMD (16 patients included in the pharmacokinetic analysis) who intravenously received viltolarsen 40 or 80 mg/kg once weekly for 24 weeks. The excretion rate into urine until 24 hours after the first dose and the dose at Week 24 was 92.8% ± 9.8% and 95.6% ± 8.7% at 40 mg/kg, and 92.0% ± 12.5% and 93.1% ± 8.0% at 80 mg/kg, indicating that viltolarsen was excreted into urine as the unchanged form. (CTD 5.3.5.1-2, Study P1/2).

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15) CYP1A2, phenacetin; CYP2A6, coumarin; CYP2B6, bupropion; CYP2C8, paclitaxel and amodiaquine; CYP2C9, diclofenac; CYP2C19, S-mephenytoin; CYP2D6, (±)-bufuralol; CYP2E1, lauric acid; and CYP3A4/5, testosterone and midazolam

16) UGT1A1, β-estradiol; and UGT2B7, zidovudine

17) Ratio of apparent membrane permeability coefficient from basolateral to apical surface to that from apical to basolateral surface (apparent membrane permeability coefficient from basolateral to apical surface/that from apical to basolateral surface)

Table 21. Pharmacokinetic parameters in Japanese patients with DMD who intravenously received multiple doses of viltolarsen (Japanese Study P1/2)

Time point	Dose		n	C_{max} (μg/mL)	$AUC_{0\text{-last}}$ (mg·h/mL)	$t_{1/2}$ (h)	CL_{tot} (mL/h/kg)	Vd_{ss} (mL/kg)
First dose	40 mg/kg		8	147 ± 34.5	235 ± 60.0	2.4 ± 0.5	179 ± 37.7	238 ± 39.2
	80 mg/kg		8	321 ± 74.8	490 ± 125	2.5 ± 0.2	164 ± 51.5	223 ± 69.4
Week 24	40 mg/kg		8	165 ± 79.0	240 ± 93.5	2.0 ± 0.7	185 ± 58.0	224 ± 55.4
	80 mg/kg		8	329 ± 91.0	508 ± 111	2.5 ± 0.1	165 ± 38.1	234 ± 50.4

Mean ± SD

Table 22 shows the pharmacokinetic parameters of viltolarsen in non-Japanese patients with DMD (16 patients included in the pharmacokinetic analysis) who intravenously received placebo or viltolarsen at 40 or 80 mg/kg once weekly for 4 weeks followed by viltolarsen at 40 or 80 mg/kg once weekly for 20 weeks (CTD 5.3.5.1-1, Study 201).

Table 22. Pharmacokinetic parameters in non-Japanese patients with DMD who intravenously received multiple doses viltolarsen (foreign Study 201)

Time point	Dose	n	C_{max} (μg/mL)	$AUC_{0\text{-last}}$ (mg·h/mL)	$t_{1/2}$ (h)	CL_{tot} (mL/h/kg)	Vd_{ss} (mL/kg)
First dose	40 mg/kg	6	105.2 ± 9.6	174.5 ± 31.4	1.9 ± 0.7	234.3 ± 41.4	296.5 ± 17.9
	80 mg/kg	5	238.8 ± 37.0	435.8 ± 135.7	2.2 ± 0.5	195.4 ± 50.1	283.4 ± 41.9
Week 24	40 mg/kg	8	121.8 ± 7.0	197.1 ± 20.4	2.2 ± 0.7	204.5 ± 22.6	257.5 ± 33.4
	80 mg/kg	8	227.5 ± 35.8	387.4 ± 105.8	2.5 ± 0.2	217.3 ± 48.0	301.4 ± 43.5

Mean ± SD

6.R Outline of the review conducted by PMDA

6.R.1 Ethnic differences

PMDA asked the applicant to explain ethnic factors that might affect the pharmacokinetics of viltolarsen.

The applicant's explanation:

In a Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2) in Japanese subjects, the exposure (C_{max} and AUC) to viltolarsen tended to be higher in subjects receiving 40 or 80 mg/kg than in non-Japanese subjects in a foreign Phase II study (CTD 5.3.5.1-1, Study 201) [see Section 6.2.2]. Subject characteristics such as age, body weight, and use of systemic corticosteroid preparations were investigated to identify possible contributing factors to such difference. The results showed that aspartate aminotransferase (AST) and ALT tended to be higher in non-Japanese subjects than in Japanese subjects. However, the difference was unlikely to be influential because viltolarsen is mainly excreted into urine as unchanged form. The factor for the higher exposure in Japanese subjects than in non-Japanese subjects remained unclear. Relationship between the exposure to viltolarsen following a dose of 80 mg/kg with dystrophin protein expression, time to walk/run 10 meters, time to stand, or 6-minute walk test (6MWT) in Studies P1/2 and 201 were investigated based on the results of individual subjects, but no particular trend was found. In addition, there were no large differences in the safety profile between Study P1/2 and Study 201 [see Section 7.R.4]. In Study P1/2, 2 Japanese subjects were found to have relatively high exposure following a dose of 80 mg/kg, ($AUC_{0\text{-}\infty}$, 635 μg·h/mL and 656 μg·h/mL), their adverse events were, however, all non-serious.

Accordingly, the ethnic factors were considered unlikely to have clinically significant effects on the pharmacokinetics of viltolarsen.

PMDA accepted the above applicant's explanation.

6.R.2 Pharmacokinetics of viltolarsen in patients with renal impairment

Although viltolarsen is mainly excreted into urine, no clinical studies were conducted to investigate the effects of the renal function on the pharmacokinetics of viltolarsen. PMDA asked the applicant to explain the possibility of different pharmacokinetics of viltolarsen in patients with renal impairment and the necessity of cautionary advice.

The applicant's explanation:

Viltolarsen may affect normal gene expression. From a viewpoint of safety, it is difficult to conduct a clinical study in patients with non-DMD with renal impairment. At the same time, the following observations indicate possible different pharmacokinetics in patients with renal impairment from that in patients with normal renal function. Thus viltolarsen may pose a risk of delayed excretion to patients with renal impairment, and the risk should be communicated via the package insert.

- Because mainly unchanged viltolarsen is excreted into urine, patients with renal impairment can be exposed to higher levels of viltolarsen than patients with normal renal function. In fact, in a Japanese Phase I study (CTD 5.3.5.2-1, Study DMT01), a patient with DMD who had a sporadically high serum cystatin C, a renal function indicator, and their pharmacokinetic parameters of viltolarsen 5 mg/kg, i.e., $AUC_{0\text{-}t}$ at Week 12, tended to be higher than those in the other subjects receiving the same dose (Table 23). The subject experienced no clinically relevant adverse events. In Japanese and foreign clinical studies (CTD 5.3.5.1-2, Study P1/2; CTD 5.3.5.1-1, Study 201), No patients had the serum cystatin C value exceeding the upper limit of normal even once throughout the study period.

Table 23. Pharmacokinetic parameters of viltolarsen at 5 mg/kg in each subject (Study DMT01)

	Time point	C_{max} (μg/mL)	t_{max} (h)	$AUC_{0\text{-}t}$ (mg·h/mL)	$t_{1/2}$ (h)	CL_{tot} (mL/h/kg)
Subject 1 (with high serum cystatin C)	Day 1	18.9	0.5	31.0	1.68	159
	Week 12	20.2	0.5	37.8	1.56	130
Subject 2	Day 1	26.9	1.0	30.9	1.83	160
	Week 12	20.5	0.5	22.6	1.55	219
Subject 3	Day 1	19.7	1.0	24.2	1.44	205
	Week 12	17.9	1.0	22.6	1.46	220

- In Study P1/2, of subjects who received 80 mg/kg (16 subjects), 2 Japanese subjects had relatively high exposure ($AUC_{0\text{-}\infty}$, 635 μg·h/mL and 656 μg·h/mL), and their adverse events were all non-serious. In addition, an incidence of adverse events in Study P1/2 was 87.5% (7 of 8) in the viltolarsen 40 mg/kg group and 87.5% (7 of 8) in the 80 mg/kg group. A serious adverse event (upper respiratory tract infection) occurred in 1 subject in the 80 mg/kg group, but a causal relationship to viltolarsen was ruled out for the event. That is, there were no events occurring dose-dependently, and thus the difference in exposure is considered unlikely to have a clinically relevant effect on the safety of viltolarsen.

- Based on the above, exposure to viltolarsen may be increased in patients with DMD with renal impairment owing to its delayed excretion, but no effect on the safety were observed, and the effect of viltolarsen on the pharmacokinetics remains unclear. Dose adjustment is therefore considered unnecessary.

PMDA accepted the applicant's explanation. However, the appropriateness of caution advice on patients with renal impairment is to be determined in Section 7.R.4.1 in light of the effect of viltolarsen on the renal function.

6.R.3 Drug interactions

PMDA asked the applicant to explain pharmacokinetic interactions of viltolarsen.

The applicant's explanation:

For the following reasons, viltolarsen is unlikely to cause clinically significant pharmacokinetic interactions, and thus specific cautionary advice is unnecessary:

- The investigation of viltolarsen's inhibitory effects against various CYP isoforms revealed the inhibitory effect on CYP3A4 [see Section 6.2.1] with the inhibition constant (K_i) value of 1090 μmol/L. The ratio of AUC of a CYP3A4 substrate drug administered concomitantly with viltolarsen to that administered alone, estimated from the mean C_{max} (47.5 μmol/L or 329 μg/mL) in the Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2) and the above K_i value, was 1.05,[18)] indicating that viltolarsen is unlikely to have a clinically significant effect on the pharmacokinetics of a CYP3A4 substrate drug.

- The investigation of viltolarsen's inhibitory effects against UDP glucuronosyltransferase revealed the inhibitory effect on UGT1A1 [see Section 6.2.1] with the K_i value of 642 μmol/L. The ratio of AUC of a UGT1A1 substrate drug administered concomitantly with viltolarsen to that administered alone, estimated from the mean C_{max} (47.5 μmol/L) in Study P1/2 and the above K_i value, was 1.10,[18)] indicating that viltolarsen is unlikely to have a clinically significant effect on the pharmacokinetics of a UGT1A1 substrate drug.

- The investigation of viltolarsen's inhibitory effect against various transporters revealed the inhibitory effect on OATP1B1, OATP1B3, OAT3, and BCRP with IC_{50} of 485, 448, 176, and 1970 μmol/L, respectively [see Section 6.2.1]. The ratio of AUC of a transporter's substrate drug administered concomitantly with viltolarsen to that administered alone, estimated from the mean C_{max} (47.5 μmol/L) in Study P1/2 and the above IC_{50} value, was 1.16,[19)] even for the OAT3 substrate, on which viltolarsen showed the strongest inhibitory effect. The result indicated that viltolarsen is unlikely to have a clinically significant effect on the pharmacokinetics of an OATP1B1, OATP1B3, OAT3, or BCRP substrate drug.

- An investigation was conducted on whether any of the isoforms found to be inhibited by viltolarsen in the above investigations can be a concomitant drug in clinical use of viltolarsen, with reference to drugs listed in the DMD clinical practice guidelines (*Practical Guideline for Duchenne Muscular*

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18) Calculated according to AUC ratio = $(1 + [I] / K_i)^2$. Where, [I] is a concentration of the unbound form at C_{max} and is calculated by multiplying the mean C_{max} (47.5 μmol/L) at Week 24 in the Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2) by 60.6% of the unbound form percentage (28.8 μmol/L).

19) Calculated according to AUC ratio = $1 + [I] / IC_{50}$. Where, [I] is a concentration of the unbound form at C_{max} and is calculated by multiplying the mean C_{max} (47.5 μmol/L) at Week 24 in the Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2) by 60.6% of the unbound form percentage (28.8 μmol/L).

Dystrophy (DMD) 2014. Nankodo Co., Ltd.; 2014, *The Guide for Families with a child with Duchenne Muscular Dystrophy*. National Center of Neurology and Psychiatry; 2011) and drugs used as concomitant drugs in the Japanese clinical studies. There were no drugs that could be an UGT1A1 substrate, but there were 6 drugs[20)] that could be a CYP3A4 substrate and 6 drugs[21)] that could be an OAT3 substrate. The investigation of viltolarsen's effect on the pharmacokinetics of these drugs revealed that viltolarsen is likely to increase AUC of a CYP3A4 substrate drug by up to 1.05-fold and that of an OAT3 substrate drug by up to 1.16-fold. In light of the rapid elimination of intravenous viltolarsen from plasma, viltolarsen was considered unlikely to have a clinically significant effect on the pharmacokinetics of a potential concomitant drug in clinical use.

PMDA accepted the above applicant's explanation.

6.R.4 Risk of QT/QTc interval prolongation

PMDA asked the applicant to explain a risk of the prolongation of QT/corrected QT (QTc) interval posed by viltolarsen.

The applicant's explanation:

As explained earlier, viltolarsen may affect normal gene expression, and a study to assess QT/QTc intervals in healthy subjects was infeasible in accordance with the ICH E14 guideline from a safety viewpoint. The following are observations on a risk of QT/QTc interval prolongation posed by viltolarsen.

- In a human ether-a-go-go related gene (hERG) study, viltolarsen up to 3 mg/mL did not affect the hERG current. In a safety pharmacology study for the cardiovascular system in monkeys (CTD 4.2.1.3-3, CTD 4.2.3.2-3, and CTD 4.2.3.2-5), blood pressure, heart rate, and electrocardiogram (ECG) parameters were not affected.

- Table 24 shows results from a QTcF categorical analysis on ECG measurement data[22)] at the last evaluation point in Japanese and foreign clinical studies.[23)] Fridericia-corrected QT Interval (QTcF interval), which was recommended to be used for children with high heart rate in the foreign Phase II study (CTD 5.3.5.1-1, Study 201) and its extension study (Study NS-065/NCNP-01-202 [Study 202]), showed the mean change from baseline of <20 milliseconds in any subject, and there were no subjects whose QTcF interval was >450 milliseconds or change from baseline was >60 milliseconds.

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20) Eplerenone, felodipine, midazolam, nisoldipine, tolvaptan, and triazolam

21) Cefaclor, ceftizoxime, ciprofloxacin, furosemide, oseltamivir, and penicillin

22) Study P1/2: 12-Lead ECG examination was performed 14 days before the first dose and on Days 1, 85, 162, and 169 or day of discontinuation. On Days 1 and 162, the measurement was performed at 1 hour, 40 and 20 minutes before administration, and 30 minutes after administration, just after the end of administration, and 1, 2, and 4 hours after the end of administration. The ECG parameters were centrally measured.
Study 201: 12-Lead ECG examination was performed 21 days before the first dose, on Day 1, Weeks 13 and 25, or day of discontinuation.
Study 202 (extension from Study 201): 12-Lead ECG examination was performed at Weeks 37, 49, 73, 97, 121, 145, 169, and 193 or day of discontinuation.

23) Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2), foreign Phase II study (CTD 5.3.5.1-1, Study 201), and foreign Phase II study (Study 202)

Table 24. Results from QTcF interval categorical analysis in Japanese and foreign clinical studies

		Study P1/2		Study 201		Study 202	
		40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg
Number of subjects evaluated		8	8	8	8	8	8
Absolute QTcF interval (msec)	>450	0	0	0	0	0	0
	>480	0	0	0	0	0	0
	>500	0	0	0	0	0	0

Number of applicable subjects (percentage, %)

- As an adverse event related to QT interval prolongation,[24] sinus arrhythmia occurred in 1 subject at 40 mg/kg in the foreign Study 202, but it resolved within the day while viltolarsen treatment was being continued, and a causal relationship to viltolarsen was ruled out.

Based on the above, the applicant explained that viltolarsen was considered unlikely to affect QT/QTc interval.

PMDA accepted the above applicant's explanation.

6.R.5 Anti-viltolarsen antibody

PMDA asked the applicant to explain the effect of anti-viltolarsen antibody on the pharmacokinetics of viltolarsen and immunogenicity of viltolarsen.

The applicant's explanation:

In Japanese and foreign clinical studies,[25] no anti-viltolarsen antibody was identified. In the 12-week repeated intravenous dose toxicity study in cynomolgus monkeys (CTD 4.2.3.2-3) and 12-week repeated intramuscular dose toxicity study in cynomolgus monkeys (CTD 4.2.3.2-5), the pharmacokinetics did not clearly differ between animals tested positive for anti-viltolarsen antibody and those tested negative (Table 25), and clinical observations and the histopathological examination did not present changes suggestive of immunogenicity such as the deposition of immune complexes.

Table 25. Toxicokinetics at Week 12 in repeated-dose toxicity studies in cynomolgus monkeys between animals tested positive for anti-viltolarsen antibody and those tested negative

Anti-viltolarsen antibody	No.	12-week repeated intravenous dose toxicity study[a]		12 week repeated intramuscular dose toxicity study[b]		
		C_{max} (μg/mL)	$AUC_{0\text{-}24h}$ (mg·h/mL)	C_{max} (μg/mL)	T_{max} (h)	$AUC_{0\text{-}24h}$ (mg·h/mL)
Positive	1	2269	863.5	51.78	4.0	531.3
Negative	2	2173	746.9	56.52	4.0	528.9
	3	2144	863.0	54.43	4.0	572.2
	4	2950	1379	54.06	1.0	651.4
	5	2305	964.1	29.62	8.0	416.0

a) Dose, 200 mg/kg; b) Dose, 100 mg/kg

Based on the above, the applicant explained that the possibility is unlikely that anti-viltolarsen antibody affects the pharmacokinetics of viltolarsen or immunogenicity of viltolarsen poses a problem.

[24] MedDRA Standardized MedDRA Query (SMQ) "Torsade de pointes/QT prolongation" and "Cardiac arrhythmias"

[25] Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2), foreign Phase II study (CTD 5.3.5.1-1, Study 201), foreign Phase II study (Study 202), and Japanese Phase I study (CTD 5.3.5.2-1, Study DMT01)

Although PMDA accepted the above applicant's explanation, subjects who received viltolarsen in the clinical studies are extremely limited in number, and thus information about the immunogenicity of viltolarsen should be continuously collected in the post- marketing setting.

7. Clinical Efficacy and Safety and Outline of the Review Conducted by PMDA

The applicant submitted the results of clinical studies listed in Table 26 (1 Japanese Phase I study, 1 Japanese Phase I/II study, and 1 foreign Phase II study) as efficacy and safety evaluation data. The major study results are shown below.

Table 26. List of clinical studies for efficacy and safety

Data category	Region	Study CTD	Phase	Study population	No. of subjects treated	Dosage regimen	Major endpoints
Evaluation	Japan	Study DMT01 5.3.5.2-1	I	Patients with DMD aged 5-17 years	10	Intravenous infusion of viltolarsen at 1.25, 5, or 20 mg/kg once weekly for 12 weeks	Safety Efficacy Pharmacokinetics
	Japan	Study P1/2 5.3.5.1-2	I/II	Patients with DMD aged 5-17 years	16	Intravenous infusion of viltolarsen at 40 or 80 mg/kg once weekly for 24 weeks	Safety Efficacy Pharmacokinetics
	Foreign	Study 201[a)] 5.3.5.1-1	II	Patients with DMD aged 4-9 years	16	Double-blind period: Intravenous infusion of placebo or viltolarsen at 40 or 80 mg/kg once weekly for 4 weeks Open-label period: Intravenous infusion of viltolarsen at 40 or 80 mg/kg once weekly for 20 weeks	Efficacy Safety Pharmacokinetics

a) The extension study (Study 202) is ongoing, and the safety analysis results have been submitted (CTD 5.3.5.3-1).

7.1 Phase I studies

7.1.1 Japanese Phase I investigator-initiated study (CTD 5.3.5.2-1, Study DMT01, June 2013 to September 2014)

An open-label, uncontrolled study was conducted in Japanese boys[26)] with DMD aged ≥5 and <18 years with a deletion in the dystrophin gene amenable to exon 53 skipping therapy (target sample size, 9-10 subjects [3 in the 1.25 mg/kg group, 3 in the 5 mg/kg group, 3-4 in the 20 mg/kg group]) to investigate the safety, efficacy, and pharmacokinetics of viltolarsen as an investigator-initiated study supported by Health and Labour Sciences Research Grants.

Viltolarsen 1.25, 5, or 20 mg/kg was intravenously administered once weekly for 12 weeks over 1 hour.

All of the 10 randomized subjects (3 in the 1.25 mg/kg group, 3 in the 5 mg/kg group, 4 in the 20 mg/kg group) received the study drug and were included in the safety and efficacy analyses. No subjects discontinued the study.

Table 27 shows results on the efficacy endpoints, i.e., exon 53 skipping efficiency[7)] and dystrophin protein expression (Western blot and immunofluorescent staining) in muscle biopsy specimens. The Western blot assay identified dystrophin protein expression only in 1 subject in the 20 mg/kg group, and results on other endpoints tended to increase with the dose.

26) Patients meeting the following major inclusion criteria were included:
- The presence of the out-of-frame deletion mutation in the dystrophin gene that can be amended into in-frame deletion mutation by exon 53 skipping therapy confirmed by Genetic diagnosis tests by multiplex ligation-dependent probe amplification (MLPA), comparative genomic hybridization (CGH), etc.

Table 27. Summary of dystrophin expression and exon 53 skipping efficiency

Dose			1.25 mg/kg			5 mg/kg			20 mg/kg			
RT-PCR[a)]	Change in exon 53 skipping efficiency	Individual[b)]	0.7	1.5	0.3	3.6	2.7	1.6	47.5	1.8	7.3	2.6
		Mean	0.8			2.6			14.8			
Immunofluorescent staining[c)]	Change in fluorescence intensity ratio of dystrophin to spectrin (Dys/Spec) per image area	Individual[b)]	-0.2	0.4	0.0	1.9	2.0	0.2	16.9	1.7	0.6	0.0
		Mean	0.1			1.3			4.8			
	Change in fluorescence intensity ratio of Dys/Spec per muscle fiber	Individual[b)]	-0.3	0.3	0.1	2.0	1.9	0.2	18.0	1.7	0.6	0.1
		Mean	0.0			1.4			5.1			
	Change in number of dystrophin-positive muscle fibers from baseline	Individual[b)]	-0.4	0.0	0.0	0.0	0.0	0.0	6.3	0.9	0.0	0.0
		Mean	-0.1			0.0			1.8			
Western blot[d)]	Dystrophin/spectrin ratio to normal control	Individual[b)]	ND	ND	ND	ND	ND	ND	8.1	ND	ND	ND

Unit, %; ND, Not detected

a) Difference in muscle biopsy specimens between baseline and after the end of study drug administration was calculated. Measurement was performed on lysate from the same specimen in multiple runs both at baseline and the end of administration.
b) Mean of multiple measured values obtained from each subject
c) Difference in muscle biopsy specimens between baseline and after the end of study drug administration was calculated. Both at baseline and end of the administration, multiple slices were prepared from each subject, and multiple images were obtained for each slice.
d) Measured values in muscle biopsy specimens collected after the end of study drug administration. Measurement was performed more than once on lysate from the same specimen.

Adverse events (including abnormal laboratory values) occurred in all subjects, but neither deaths nor other serious adverse events occurred. All subjects experienced adverse events for which a causal relationship to the study drug could not be ruled out, which included beta-N-acetyl-D-glucosaminidase increased (3 in the 1.25 mg/kg group, 3 in the 5 mg/kg group, 3 in the 20 mg/kg group), protein urine present (1, 3, 4), albumin urine present (2, 2, 3), anaemia (1, 3, 3), interleukin level increased (2, 1, 2), complement factor Complement 3 (C3) increased (2, 0, 0), brain natriuretic peptide increased (1, 1, 0), blood pressure diastolic increased (0, 2, 0), and beta 2 microglobulin increased (0, 0, 2).

Adverse events related to vital signs (blood pressure, oxygen saturation, respiratory rate, and ejection fraction) were blood pressure diastolic increased in 2 subjects, and oxygen saturation decreased, respiratory rate increased, and ejection fraction decreased in 1 subject each. A causal relationship to the study drug could not be ruled out for all these events.

7.2 Phase II studies

7.2.1 Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2, [redacted] 2016 to [redacted])

An open-label, dose-finding study was conducted in Japanese boys[27)] with DMD aged ≥5 and <18 years with a deletion in the dystrophin gene amenable to exon 53 skipping therapy (target sample size, 16 subjects [8 each in the 40 and 80 mg/kg groups]) to investigate the efficacy, safety, and pharmacokinetics of viltolarsen (for the pharmacokinetics, see Section 6.2.2).

Viltolarsen 40 or 80 mg/kg was intravenously administered once weekly for 24 weeks over 1 hour.[28)]

27) Patients meeting the following major inclusion criteria were included:
- Patients with dystrophin gene deletion amenable to exon 53 skipping therapy (exons 43-52, 45-52, 47-52, 48-52, 49-52, 50-52, and 52 deletion mutations, etc.) confirmed by MLPA genetic testing
- Ability to walk was not asked.

28) The dose was to be reduced or interrupted as appropriate at the discretion of the (sub-) investigator for a safety reason such as an adverse event.

All of 16 subjects allocated[29] (8 each in the 40 and 80 mg/kg groups) received the study drug and were included in the full analysis set (FAS) for the safety and efficacy analyses. No subjects discontinued the study.

Table 28 shows results of the primary endpoints, dystrophin protein expression and exon 53 skipping efficiency.[7] As compared with baseline, viltolarsen 40 mg/kg and 80 mg/kg significantly increased exon 53 skipping efficiency, and viltolarsen 80 mg/kg significantly increased dystrophin protein expression measured by Western blot (paired t-test, multiplicity of each test was not adjusted).

Table 28. Changes in dystrophin protein expression and exon 53 skipping efficiency from baseline (FAS)

Dose (number of subjects evaluated)[a]	Immunofluorescent staining[b]						Western blot[c]		RT-PCR[d]	
	Fluorescence intensity ratio of dystrophin to spectrin (dystrophin/spectrin) (%)				Percentage of dystrophin-positive muscle fibers in number (%)		Dystrophin assay value (%)		Exon 53 skipping efficiency (molar concentration ratio, %)	
	Per image area		Per muscle fiber[e]							
	Change	*P* value[f]	Change	*P* value[f]	Change	*P* value[f]	Change	*P* value[f]	Change	*P* value[f]
40 mg/kg (8)	0.0 ± 3.4	0.9949	0.0 ± 3.3	0.9691	0.1 ± 0.6	0.6668	0.126 ± 2.769	0.9009	21.77 ± 10.86	0.0008
80 mg/kg (8)	1.5 ± 4.6	0.3770	2.7 ± 5.1	0.1793	1.3 ± 2.2	0.1445	2.785 ± 3.051	0.0364	42.40 ± 11.26	<0.0001

Mean ± SD

a) Combined number of subjects assayed at different time points (Weeks 12 and 24)
b) Multiple slices were prepared from each subject, and multiple images were obtained for each slice.
c) Percentage with respect to the normal control. Each specimen was measured 3 times, and the mean was used as the measured value of the specimen.
d) For each specimen, 3 lanes in the same gel were used for electrophoresis, and the mean was used as the measured value of the concerned specimen.
e) For each muscle fiber in the image area, fluorescence intensity was measured, and the mean was calculated.
f) Paired t-test

Adverse events occurred in 87.5% (7 of 8) of subjects in the 40 mg/kg group and in 87.5% (7 of 8) of subjects in the 80 mg/kg group. No deaths occurred. A serious adverse event other than death occurred in 1 subject in the 80 mg/kg group (upper respiratory tract infection), for which a causal relationship to viltolarsen was ruled out.

Adverse events for which a causal relationship to the study drug could not be ruled out occurred in 37.5% (3 of 8) of subjects in the 40 mg/kg group and 75.0% (6 of 8) of subjects in the 80 mg/kg group. Major events were brain natriuretic peptide increased (1, 1), interleukin level increased (1, 1), pyrexia (0, 2), beta-N-acetyl-D-glucosaminidase increased (0, 2), ejection fraction decreased (0, 2), and urticaria (0, 2).

There were no clinically relevant changes in vital signs (blood pressure and pulse rate) and ECG.

[29] Subjects were allocated according to a stratification factor of walking function (ambulatory, non-ambulatory) so that the numbers of subjects in the following 4 groups were roughly the same: (a) 40 mg/kg group ×muscle biopsy at Week 12 week, (b) 40 mg/kg group × muscle biopsy at Week 24, (c) 80 mg/kg group × muscle biopsy at Week 12, (d) 80 mg/kg group × muscle biopsy at Week 24. In the allocation, the priority was given to muscle biopsy at Week 24 [(b) and (d)] and 40 mg/kg group [(a) and (b)].

7.2.2 Foreign Phase II study (CTD 5.3.5.1-1, Study 201, December 2016 to ■ 2018)

In the US and Canada, a placebo-controlled, randomized, double-blind, parallel-group study was conducted in non-Japanese boy[30] with DMD aged ≥4 and <10 years with a deletion in the dystrophin gene amenable to exon 53 skipping therapy (target sample size, 16 subjects) to investigate the efficacy, safety, and pharmacokinetics of viltolarsen [for the pharmacokinetics, see Section 6.2.2]. This study consisted of a low-dose cohort (8 subjects) and a high-dose cohort[31] (8 subjects), and each cohort started with the double-blind period (4 weeks) followed by the open-label period (20 weeks).[32] For the double-blind period, subjects in the low-dose cohort were randomized to the placebo group or the viltolarsen 40 mg/kg group at the ratio of 1:3, and subjects in the high-dose cohort were randomized to the placebo group or the viltolarsen 80 mg/kg group at the ratio of 1:3. During the open-label period, all subjects received viltolarsen (40 mg/kg in the low-dose cohort, 80 mg/kg in the high-dose cohort) for 20 weeks.

The placebo or viltolarsen (40 or 80 mg/kg) during the double-blind period or viltolarsen 40 or 80 mg/kg during the open-label period was intravenously administered once weekly over 1 hour.

All 16 randomized subjects (8 each in the low-dose and high-dose cohorts) received viltolarsen and were included in the safety and efficacy analyses. No subjects discontinued the study. In order to compare motor function test results for the efficacy evaluation, the study had a comparator group of 65 patients (9 patients in the exon 53 skipping group, 56 in the non-exon 53 skipping group) from a natural history study[33] conducted by the Cooperative International Neuromuscular Research Group (CINRG). They were selected[34] based on baseline data.

Table 29 shows changes from baseline in dystrophin protein expression (measured by Western blot) in muscle biopsy specimens, the primary endpoint.[35] When standardized with the reference protein of either myosin heavy chain or α-actinin, the dystrophin protein expression increased from baseline

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30) Patients meeting the following major inclusion criteria were included:
- MLPA, CGH, etc. genetic testing identified the presence of DMD mutation (including determination of a clearly defined exon boarder) that is amenable to exon 53 skipping therapy to restore the dystrophin mRNA reading frame in the dystrophin gene.
- Able to walk independently without assistive devices
- Able to undergo testing for time to rise, time to walk 10 meters, time to climb 4 steps test as confirmed by clinical assessor at the time of screening
- The dose of glucocorticoid remains unchanged for at least 3 months prior to study entry and is not expected to be changed throughout the study period

31) Administration in the high-dose cohort was started after all the subjects in the low-dose cohort had completed the double-blind period with the safety confirmed.

32) After end of the open-label period, subjects could participate in the extension study (NS-065/NCNP-01-202) if desired, and subjects who did not participate were subjected to follow-up for 30 days.

33) The study included approximately 440 patients with DMD for longitudinal natural history research and collected data from 2006 to 2016. Timed function tests, muscle strength test, questionnaire-based function test, pulmonary function test, and quality-of-life assessment were performed at baseline, 4 times in the first year, twice in the second year, and then once a year for a period up to 10 years.

34) Patients meeting the following criteria were included:
- Data from timed function tests covering a period of at least 12 months are available (requiring data on time to stand, time to walk/run 10 meters, and time to climb 4 steps at baseline)
- Aged ≥4 and <10 years at baseline
- Resident in the North America (US and Canada)
- Glucocorticoid has been administered for at least 3 months, and its use has been continued throughout the 12-24-month observation period.
- Not registered in clinical studies of the other exon skipping drugs
- The comparator group included patients with duplication mutation, inducing nonsense mutation or frame shift
- Patients with a mutation between the promoter and exon 8 and patients who might be eligible for exon 44 skipping therapy should be excluded.
- Patients with an in-frame mutation and patients with a mutation at a site uncertain of reading-frame should be excluded.

35) The primary endpoint was dystrophin expression measured by mass spectrometry at the beginning of the study. It was then changed to dystrophin expression measured by Western blot before the measurement was performed for each subject.

statistically significantly in both viltolarsen 40 and 80 mg/kg groups (paired t-test, multiplicity of each test was not adjusted).

Table 29. Change in dystrophin protein expression measured by Western blot from baseline

Dose (number of subjects evaluated)	Standardized with myosin heavy chain				Standardized with α-actinin			
	Baseline	Week 25	Change from baseline	*P* value[a)]	Baseline	Week 25	Change from baseline	*P* value[a)]
40 mg/kg (8)	0.3 ± 0.10	5.7 ± 2.37	5.4 ± 2.40	0.0004	0.2 ± 0.22	5.4 ± 2.79	5.2 ± 2.83	0.0012
80 mg/kg (8)	0.6 ± 0.82	5.9 ± 4.50	5.3 ± 4.48	0.0123	0.4 ± 0.67	3.7 ± 2.37	3.3 ± 2.47	0.0074

Mean ± SD, unit (%)
Each measured value represents the percentage with respect to the normal control. Each specimen was measured 3 times, and the mean was used as the measured value of the specimen.
a) Paired t-test

The secondary endpoints included changes in results from timed function tests (time to walk/run 10 meters [velocity second], time to stand [velocity, second], 6-minute walk distance [meter], North Star Ambulatory Assessment [NSAA], and time to climb 4 steps [velocity, second]) from baseline to Week 25 in the viltolarsen group (pooled) and the natural history patient population. As Table 30 shows, the viltolarsen group showed improvement in time to walk/run 10 meters (velocity, second), time to stand (second), and 6-minute walk distance as compared with the natural history patient population.[36)]

Table 30. Results on efficacy endpoints in foreign Study 201 and natural history population (mITT, MMRM)

	Population[a)]	Baseline	Week 25	Change	Comparison with natural history population[a)]
					Difference (95% CI)
Time to walk/run 10 meters (velocity)	Natural history	1.91 ± 0.465 (65)	1.89 ± 0.464 (43)	-0.04 ± 0.327	
	Viltolarsen	1.77 ± 0.374 (16)	2.00 ± 0.443 (16)	0.23 ± 0.251	0.2665 [0.0953, 0.4377]
Time to walk/run 10 meters (sec)	Natural history	5.61 ± 1.671 (65)	5.64 ± 1.500 (43)	0.08 ± 1.414	
	Viltolarsen	5.93 ± 1.469 (16)	5.27 ± 1.319 (16)	-0.66 ± 1.047	-0.6622 [-1.3126, -0.0117]
Time to rise (velocity)	Natural history	0.22 ± 0.089 (65)	0.21 ± 0.108 (42)	-0.01 ± 0.074	
	Viltolarsen	0.25 ± 0.074 (16)	0.28 ± 0.102 (16)	0.02 ± 0.075	0.0395 [-0.0023, 0.0813]
Time to rise (second)	Natural history	5.55 ± 3.041 (65)	5.80 ± 2.867 (41)	0.66 ± 1.845	
	Viltolarsen	4.44 ± 1.956 (16)	4.25 ± 2.148 (16)	-0.19 ± 1.141	-1.0009 [-1.9372, -0.0646]
6-Minute walk distance (meter)	Natural history	408.0 ± 167.16 (21)	358.3 ± 139.28 (13)	-65.3 ± 162.60	
	Viltolarsen	372.4 ± 78.59 (16)	407.3 ± 83.12 (15)	28.9 ± 36.31	88.9907 [1.2646, 176.7167]
Time to climb 4 steps (velocity)	Natural history	0.28 ± 0.112 (65)	0.30 ± 0.162 (42)	0.01 ± 0.090	
	Viltolarsen	0.30 ± 0.082 (16)	0.33 ± 0.122 (16)	0.03 ± 0.088	0.0217 [-0.0278, 0.0711]
Time to climb 4 steps (second)	Natural history	4.30 ± 1.865 (65)	4.22 ± 1.992 (42)	0.15 ± 1.282	
	Viltolarsen	3.61 ± 0.954 (16)	3.44 ± 1.233 (16)	-0.17 ± 0.897	-0.4645 [-1.1215, 0.1925]
NSAA (total score)	Natural history	25.7 ± 5.37 (22)	24.2 ± 7.27 (15)	-1.1 ± 4.28	
	Viltolarsen	24.3 ± 5.36 (16)	25.1 ± 5.22 (16)	0.8 ± 2.86	2.1216 [-0.6246, 4.8678]

Mean ± SD (No. of subjects evaluated)
a) Based on mixed model for repeated measures (MMRM) (within-subject variance-covariance matrix, unstructured), treatment, visit week, and interactions between visit week and treatment were used as factors, and age (at the enrollment of Study 201, baseline age for natural history population) and baseline values as covariates.

Adverse events occurred in 60.0% (3 of 5) of subjects in the placebo group (pooled) and 66.7% (4 of 6) of subjects in the 40 mg/kg group, and 80.0% (4 of 5) of subjects in the 80 mg/kg group during the double-blind period; and 62.5% (5 of 8) of subjects in the 40 mg/kg group and 87.5% (7 of 8) of subjects in the 80 mg/kg group during the open-label period. Neither deaths nor other serious adverse events occurred, and there were no adverse events for which a causal relationship to the study drug could not be ruled out.

36) Although these were the secondary endpoints compared in an exploratory manner, motor function tests in Study 201 were conducted at a center participating in the natural history study conducted by CINRG according to the same procedures as the CINRG study.

A vital sign-related event (blood pressure, pulse rate, respiratory rate, and body temperature) observed was pyrexia in 1 subject in the viltolarsen 80 mg/kg group during the open-label period. There were no clinically significant changes in ECG.

7.R Outline of the review conducted by PMDA

7.R.1 Clinical positioning

PMDA asked the applicant to explain the clinical positioning of viltolarsen.

The applicant's explanation:

- There are no radical medications for DMD, and the clinical practice guideline in Japan (*Practical Guideline for Duchenne Muscular Dystrophy (DMD) 2014.* Nankodo Co., Ltd.; 2014:58-70) recommends steroids as the sole medication with evidence on the prevention of DMD progression. Prednisolone was approved for the treatment of DMD in 2013. The guideline, at the same time, notes that the steroid therapy does not have sufficient evidence on improvement in long-term prognosis in patients with DMD and calls attention to adverse drug reactions such as obesity. Besides, adenosine triphosphate disodium hydrate is indicated for "muscular dystrophy and related diseases" in Japan but is rarely used at present. Available non-drug therapies include surgery for joint contracture or scoliosis, physical therapy, respiratory support, and drug therapy for cardiomyopathy. These approaches, however, only help delay disease progression or serve as symptomatic therapy.

- In the US, medical experts in DMD published guidelines for diagnosis and management of DMD in 2010 with support of the Centers for Disease Control and Prevention, and the updated version was published in 2018 (*Lancet Neurol.* 2018;17:251-67, 347-61, 445-55). In these publications, steroid therapy is recognized as the center of DMD management along with physical therapy, and is recommended to be continued even after the loss of ability to walk. In the US, prednisolone, prednisone, or deflazacort is used as steroid therapy.

- Other therapeutic drugs available in the US include eteplirsen, a morpholino nucleic acid medicine as with viltolarsen, to which accelerated approval[37)] was granted for the indication of DMD amenable to exon 51 skipping therapy in September 2016. Accelerated approval was granted to golodirsen as well for the indication of DMD amenable to exon 53 skipping therapy in December 2019. In Europe, ataluren, a small molecule drug that induces read-through of nonsense mutation, received a conditional approval[38)] for the indication of nonsense mutation DMD in August 2014. In the foreign clinical practice guidelines, these drugs are not listed as of now. In addition, these drugs are not approved in Japan.

- Viltolarsen is a new therapeutic drug with its action mechanism different from steroids, and dystrophin protein expression and exon 53 skipping were confirmed in patients treated with viltolarsen [see Section 7.R.3]. Viltolarsen, therefore, can be a new treatment option for patients with DMD harboring a mutation in the dystrophin gene, who are eligible for viltolarsen.

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37) The approval condition of eteplirsen requires a double-blind study with the primary endpoint of NSAA in which eteplirsen is administered at 2 doses, the approved dose and higher dose, for 96 weeks

38) The approval condition of ataluren requires a placebo-control study (18 months) and open-label extension study (18 months).

PMDA accepted the applicant's explanation and considers that viltolarsen will provide a new treatment option to patients with DMD harboring a deletion in the dystrophin gene amenable to exon 53 skipping therapy.

7.R.2 Clinical data package of viltolarsen

PMDA asked the applicant to explain the appropriateness of clinical data package of viltolarsen based on positioning of each clinical study.

The applicant's explanation:

- DMD is caused by a deficiency in dystrophin protein that sustains the structure of muscle fibers. Viltolarsen is expected to have efficacy in facilitating exon-53 skipping that enhances the expression of functional dystrophin protein, although which is shorter-chained than the normal protein [see Section 3.R.1]. The applicant conducted a Japanese Phase I study (CTD 5.3.5.2-1, Study DMT01), Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2), and foreign Phase II study (CTD 5.3.5.1-1, Study 201), considering the efficacy endpoint should be related to dystrophin expression in the early stage of clinical studies on viltolarsen.

- In both Japanese Study P1/2 and foreign Study 201, viltolarsen increased dystrophin protein (Tables 28 and 29). Given that there was no large difference in expression levels between the studies or in the safety between Japanese and non-Japanese subjects, the results of these 2 studies should be regarded as the core of the clinical data package for the application in Japan.

- The pharmacokinetics of viltolarsen does not show large ethnic differences [see Section 6.R.1]; and diagnostic criteria and treatment approaches for DMD do not largely differ in Japan and overseas. The use of the results from foreign Study 201 is thus possible. In the foreign Study 201, motor functions tended to improve in the viltolarsen group as compared with the natural history population, the external comparator, although the comparison was only exploratory (Table 30).

- Viltolarsen increased dystrophin protein in the Japanese and foreign clinical studies, and motor functions tended to improve in the viltolarsen group as compared with the CINRG natural history population, the external comparator, in the foreign Study 201. These results demonstrated the efficacy of viltolarsen to a certain extent. In the US, eteplirsen, an oligonucleotide drug with a similar action mechanism to viltolarsen, granted accelerated approval based on dystrophin expression as the primary efficacy evaluation.

- A clinical study aiming to verify the efficacy of viltolarsen based on clinical endpoints such as motor functions, even if conducted as a global study, may require around [redacted] years for the enrollment of subjects and approximately 5 years for the entire study.

- Viltolarsen is targeted for rare and serious disease with limited effective therapeutic options. The approval application should be filed based on the currently available clinical data package (Table 26) under the Conditional Early Approval System.[39)]

PMDA accepted the above applicant's explanation. However, the efficacy of viltolarsen is to be discussed in Section 7.R.3.

7.R.3 Efficacy

7.R.3.1 Appropriateness of endpoints

In Japanese Phase I/II study (CTD 5.3.5.2-1, Study P1/2) and foreign Phase II study (CTD 5.3.5.1-1, Study 201), the primary efficacy endpoint was dystrophin protein expression. PMDA asked the applicant to explain the appropriateness of this endpoint.

The applicant's explanation:

- In the development of a medication for DMD, a 6-minute walk distance is the standard primary endpoint in clinical studies especially at its late stage. Nevertheless, none of the products of this category have been proved to be superior to placebo in this primary endpoint. A guidance for the development of drugs for the treatment of DMD[40)] issued by the Food and Drug Administration (FDA) mentions that, in the drug development process for dystrophinopathies (DMD, BMD, dystrophin gene-associated dilated cardiomyopathy, etc.), biomarkers that reliably reflect the amount of skeletal muscle at a tissue level, etc. may be useful as surrogate endpoints to support accelerated approval, if backed by sufficient scientific evidence and acceptable analytical methods. When Japanese Study P1/2 and foreign Study 201 were being planned for viltolarsen, the development of drisapersen and eteplirsen was underway outside Japan for the indication of DMD amenable to exon 51 skipping therapy. The clinical studies on these drugs were conducted with dystrophin protein expression as the primary endpoint, and their applications were submitted based on results of these studies. Drisapersen was under review in the US, and the step-wise application began for eteplirsen. Up to the present date, accelerated approval of eteplirsen, an oligonucleotide drug with a similar action mechanism to viltolarsen, has been granted, as a result of efficacy evaluation primarily based on dystrophin expression. Meanwhile, the guidance recommends a primary endpoint related to clinical function evaluation for confirmatory clinical studies, but does not mention any specific one.

- Viltolarsen is expected to exert its efficacy by facilitating exon-53 skipping in patients with DMD and increasing expression of dystrophin protein, although which is shorter-chained than the normal protein [see Section 3.R.1]. In addition, according to published literature on patients with BMD expressing non-full-length dystrophin comparable to one produced in patients with DMD on viltolarsen (exon 43-53, 45-53, 47-53, 48-53, 49-53, 50-53, or 52-53 deleted dystrophin protein), all patients with BMD had the ability to walk, and some were found to be asymptomatic or mild in severity (*Brain.* 2011;134:3547-59, *Brain.* 1994;117:1-14, etc.). The dystrophin protein produced in patients with DMD receiving viltolarsen is, therefore, considered physiologically functional.

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39) PSEHB/PED Notification No. 1020-1 dated October 20, 2017

40) Guidance for Industry, Duchenne Muscular Dystrophy and Related Dystrophinopathies: Developing Drugs for Treatment; February 2018 (https://www.fda.gov/media/92233/download)

- Female carriers have normal and abnormal dystrophin genes on X chromosomes. They present with symptoms when the X chromosome with a normal dystrophin gene is preferentially inactivated, causing decreased dystrophin expression. According to a report on the evaluation of dystrophin expression, clinical phenotype, and the relationship with X chromosome inactivation in 14 symptomatic female carriers (*Neurology.* 1995;45:677-90), dystrophin expression levels widely varied from 3% to 76% of the normal level. A total of 4 symptomatic carriers had dystrophin expression at 3% to 5% of the normal level. Their pathological condition was severe in 1 carrier, moderate in 2, and mild in 1. Some had a milder condition than typical DMD. Accordingly, even a low level of dystrophin expression, i.e., 3% to 5% of the normal level, is expected to improve the prognosis of DMD, while strict comparison remains difficult because of different measurement methods.

- Based on the above, the primary endpoint related to dystrophin expression and the secondary endpoints based on motor functions are appropriate for both Japanese Study P1/2 and foreign Study 201.

PMDA asked the applicant to explain the appropriateness of the Western blot-based method to measure dystrophin protein, the primary endpoint in Japanese Study P1/2 and foreign Study 201.

The applicant's explanation:

- Table 31 is the summary of Western blot in Japanese Study P1/2 and foreign Study 201. The Western blot was performed in Study P1/2 by reference to the Japanese Phase I study conducted as an investigator-initiated study (CTD 5.3.5.2-1, Study DMT01). Specifically, the expression of spectrin, a reference protein, was determined, then the ratio of normal control dystrophin with respect to spectrin was assessed (old method). By the old method, the lower limit of quantitation was [redacted]% (based on the expression level of the normal control of 100%) while the dystrophin expression level with eteplirsen, a similar drug, was reported to be 0.93% of the normal control. In response, another Western blot method was established by the use of a different sample buffer, etc. for better sensitivity and quantitative performance (new method). Dystrophin expression level was measured again by the new method in a blinded manner. In addition, the foreign Study 201 employed an internal standard test method with 2 reference proteins (myosin heavy chain and α-actinin).

Table 31. Summary of Western blot in each clinical study

	Foreign Study 201	Japanese Study P1/2	
		Old method	New method
Diluent, negative control	Lysate of muscle from patients with DMD	Sample buffer	Lysate of muscle from patients with DMD
Normal control	Mixture of muscle biopsy specimens from healthy subjects aged 3, 8, 13, 14, and 32 years	Muscle biopsy specimen from a man aged 61 years	Muscle biopsy specimen from a male aged 61 years
Standard sample	[redacted]% of the normal control, prepared for each gel	Normal control	[redacted]% of the normal control, prepared for each gel
Lower limit of quantitation	1%	[redacted]%	1%
Standard curve	Curve of second degree	-	[redacted]
Major suitability criteria	Regression line: R2 > 0.95 Signal of 1% normal control is higher than that of DMD only (0%).	Band automatically detected under default setting CV of dystrophin/spectrin ratio (n = 3) falls within [redacted]%.	Regression line: R2 >0.95 Signal of 1% normal control is higher than that of negative control (0%).
Loading control	α-Actinin Myosin heavy chain	Spectrin	α-Actinin
Protein(s) for standardization	α-Actinin Myosin heavy chain	Spectrin	None

- Each measurement method was validated based on specificity, repeatability, calibration curve, lower limit of quantitation, etc. All these items of the method used in Japanese Study P1/2 met the criteria. However, by the method used in foreign Study 201, inter-analyst reproducibility [41)] and repeatability[42)] did not meet the pre-determined criteria (coefficient of variation [CV] <[redacted]). At the validation of the method in foreign Study 201, the addition of a point of <1% of normal control (0.6%) to the calibration curve was considered. However, due to the failure in meeting the pre-determined criteria, measurement was performed in the study by using a modified calibration curve without the point of <1% of normal control (0.6%). The Western blot method used in foreign Study 201 with the modified calibration curve range is currently under validation.

- While the lower limit of quantitation was 1% in either study, the calibration curve was extrapolated to the measured values of <1%, and the obtained values were included in the analysis. In the analysis, (a) baseline values of <1% were assumed as "1%," and post-dose values of <1% as "0%," (b) both baseline and post-dose values of <1% as "1%," or (c) both baseline and post-dose values of <1% as "0%." The results showed no large numerical variations, and these assumptions were considered unlikely to affect the conclusion on the efficacy. Thus, it is acceptable to obtain values by extrapolating the calibration curve to values below the lower limit of quantitation (1%) of the validated calibration curve.

PMDA's view:

- At present, there are no significant concerns with the primary endpoint of "dystrophin expression measured by Western blot" or with the measurement methods in the studies.

- In the validation of the new method in Japanese Study P1/2 and the method in foreign Study 201, the inclusion of measured values <1% of the normal control led to a failure to create an appropriate calibration curve. The calculated values falling outside the range of the calibration curve cannot be regarded as accurate measured values. However, given the difficulty in the creation of an accurate

41) Each sample was measured by 2 persons, and the variance between the measurements were assessed.
42) Each sample was measured multiple times by one person, and the variances among the measurements were assessed.

calibration curve with a value of <1% by the Western blot technique in Japanese Study P1/2 and foreign Study 201, the applicant's view that values smaller than the lower limit of quantitation (1%) do not largely change the conclusion on the efficacy is acceptable.

- Clinical significance of dystrophin expression increased by viltolarsen in clinical studies is to be further discussed in Section 7.R.3.2.

- Additional validation is ongoing for the method in foreign Study 201. The matter is to be discussed based on the validation results in the Review Report (2).

7.R.3.2 Dystrophin expression level

PMDA asked the applicant to explain the efficacy of viltolarsen based on the dystrophin expression level in Japanese Study P1/2 and foreign Study 201.

The applicant's explanation:

- Table 32 shows change in dystrophin expression measured by Western blot from baseline to Week 24 (Week 25 in foreign Study 201) in Japanese Study P1/2 and foreign Study 201. Dystrophin expression tended to increase from baseline to Week 24 of viltolarsen treatment in both studies.

- At 40 mg/kg, the mean change in dystrophin levels measured by Western blot from pre-dose to post-dose was higher in foreign Study 201 than in Japanese Study P1/2. An analysis of influential factors on dystrophin expression revealed that subjects harboring exon 45-52 or 49-52 deletion mutation tended to have a lower dystrophin expression level at the end of treatment than subjects with the other exon deletion mutations [see Section 7.R.3.3]. The difference in percentage of subjects harboring exon 45-52 deletion mutation (50% [2 of 4] of subjects in Japanese Study P1/2, 25% [2 of 8] of subjects in foreign Study 201) may have affected the result. At 80 mg/kg, the increase in dystrophin expression was comparable between the studies.

Table 32. Change in dystrophin expression measured by Western blot from baseline (Japanese Study P1/2 and foreign Study 201)

Study	Dose	Number of subjects evaluated	Dystrophin expression (percentage with respect to the normal control)			*P* value[a)]
			Baseline	Week 24	Change	
Japanese Study P1/2[b)]	40 mg/kg	4	0.459 ± 0.150	1.916 ± 1.701	1.458 ± 1.587	0.1636
	80 mg/kg	4	0.394 ± 0.167	5.208 ± 3.122	4.814 ± 3.113	0.0536
Foreign Study 201[c)]	40 mg/kg	8	0.3 ± 0.10	5.7 ± 2.37	5.4 ± 2.40	0.0004
	80 mg/kg	8	0.6 ± 0.82	5.9 ± 4.50	5.3 ± 4.48	0.0123

Mean ± SD
Values of <1%measured by Western blot, the lower limit of quantitation, were reference values, which were included in the tabulation.
a) Paired t-test
b) Results measured by Western blot (new method)
c) Values standardized with myosin heavy chain

- In addition, all 24 subjects in Japanese Study P1/2 and foreign Study 201 had increased dystrophin expression level at Week 24, and a >4% change in dystrophin expression level from baseline to Week 24 was observed in 3 of 4 subjects in the 80 mg/kg group in Japanese Study P1/2, 7 of 8 subjects in the 40 mg/kg group, and 3 of 8 subjects in the 80 mg/kg group in foreign Study 201. Furthermore, a

>10% change was observed in 1 subject in the 40 mg/kg group and 2 subjects in the 80 mg/kg group in foreign Study 201 (Table 33).

Table 33. Changes in dystrophin expression measured by Western blot for each subject

Dose[a)]	40 mg/kg/Week 12				40 mg/kg/Week 24				80 mg/kg/Week 12				80 mg/kg/Week 24			
Study P1/2	0.76	0.14	0.37	-0.34[b)]	3.23	0.23	0.01	2.36	-0.20	2.20	0.63	0.39	7.85	6.42	0.69	4.30
Dose	40 mg/kg								80 mg/kg							
Study 201[c)]	4.2	2.8	10.0	4.4	4.7	4.2	8.1	5.1	3.6	2.6	2.5	0.7	4.8	13.9	10.3	4.0

Percentage (%) with respect to the normal control
a) Dose/time of muscle biopsy
b) Additional analysis to detect bands in an expected molecular weight region
c) Standardized with myosin heavy chain

- The relationship between dystrophin expression level in skeletal muscle of patients with BMD or DMD and clinical phenotypes (age to start wheelchair) is classified into 4 clinical categories (DMD, Severe BMD, Moderate BMD, and Mild BMD) (Table 34). The pathological condition of patients with severe BMD in whom the dystrophin expression level is 3% to 10% of the normal control is more serious than typical BMD but milder than DMD (*N Engl J Med.* 1989;39:1011-7, *N Engl J Med.* 1988;318:1363-8).

Table 34. Relationship between dystrophin expression level in skeletal muscles in patients with BMD or DMD and clinical phenotype (age to start wheelchair)

Clinical category	Age to start wheelchair	Dystrophin expression level
DMD	11 years	<3% of normal
Severe BMD	13 to 20 years	3%-10%
Moderate BMD	≥20 years	≥20%
Mild BMD	≥20 years	≥20%

- According to a report on the relation between dystrophin expression level and clinical severity in 33 patients with BMD, while patients with BMD expressing dystrophin of <10% showed a serious pathological course early, patients expressing dystrophin 3% to 78% indicated no linear relation between the dystrophin expression level and muscle strength or between clinical function-related milestones, such as difficulty to climb stairs and use of walk-assistive devices, and age (*J Neurol Neurosurg Psychiatry.* 2014;85:747-53). Some patients maintained their motor functions despite low dystrophin expression (*Acta Myol.* 2011;30:182-4, *Neuromuscul Disord.* 2013;23:25-8, etc.).

PMDA accepted the above explanation and considers that clinical significance of dystrophin expression increased by viltolarsen has been explained to a certain extent. However, the effect of viltolarsen on motor functions is to be further discussed in Section 7.R.3.5.

7.R.3.3 Factors affecting dystrophin expression

PMDA asked the applicant to present results from an analysis on the dystrophin expression level in Japanese Study P1/2 and foreign Study 201 by exon deletion site, and to explain whether the dystrophin expression level varied by exon deletion site.

The applicant's explanation:
Table 35 shows changes in dystrophin from baseline to the end of treatment in Japanese Study P1/2 and foreign Study 201. In subjects harboring exon 45-52 or 49-52 deletion mutation, the dystrophin

expression level at the end of treatment tended to be low, although a clear conclusion cannot be reached due to large variations among the subjects and the limited number of subjects.

Table 35. Dystrophin expression measured by Western blot by exon deletion site (Japanese Study P1/2 and foreign Study 201)

Deletion site	Change from baseline to end of treatment[a)]					
	Japanese Study P1/2 Percentage (%) with respect to the normal control			Foreign Study 201 Percentage (%) with respect to the normal control (standardized with myosin heavy chain)		
	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)
45-52	0.202 ± 0.178 (3)	0.274 ± 0.431 (3)	0.238 ± 0.297 (6)	2.8, 4.2 (2)	2.8 ± 1.51 (5)	3.0 ± 1.34 (7)
47-52	-	-	-	10.0 (1)	-	10.0 (1)
48-52	0.136, 2.364 (2)	2.198 (1)	1.566 ± 1.241 (3)	4.4 (1)	10.3, 13.9 (2)	9.5 ± 4.80 (3)
49-52	-6.083, 0.759 (2)	-	-6.083, 0.759 (2)	4.2, 4.7 (2)	4.0 (1)	4.3 ± 0.38 (3)
50-52	3.228 (1)	7.847 (1)	3.228, 7.847 (2)	5.1, 8.1 (2)	-	5.1, 8.1 (2)
52	-	3.803 ± 2.899 (3)	3.803 ± 2.899 (3)	-	-	-

mean ± SD (Number of subjects evaluated), Individual values for n = ≤2
-, No applicable subjects
Values <1%, the lower limit of quantitation, were handled as reference values, and the tabulation was performed including the reference values.
a) Week 24 in Study 201 and Week 12 or 24 in Study P1/2

The applicant's explanation about efficacy in patients harboring exon 45-52 or 49-52 deletion mutation:

- Among 6 subjects harboring exon 45-52 deletion mutation in whom the expression level was low in Japanese Study P1/2, 4 underwent Week 12 measurement. Among 7 subjects harboring exon 45-52 deletion mutation in foreign Study 201, 4 showed lower dystrophin expression than patients harboring deletion mutation at different sites, and the remaining 3 subjects showed comparable expression level to that in those harboring deletion mutation at different sites. In both Japanese Study P1/2 and foreign Study 201, no remarkable differences were observed in exon skipping efficiency measured by RT-PCR[7)] or immunofluorescent staining between subjects harboring exon 45-52 deletion mutation and subjects harboring the other deletion mutation (Table 36). Accordingly, there was no clear trend toward attenuated efficacy in patients harboring exon 45-52 deletion mutation as compared with patients harboring the other deletion mutation. Dystrophin protein produced in patients harboring exon 45-52 deletion mutation was reported to be less stable than that in patients harboring the other deletion mutation (*Biochemistry.* 2019;58:2061-76). However, this report is based on analyses and measurements performed using a solution of the internally deleted protein alone, which was very different from the physiological condition. Under the physiological condition, dystrophin is present in the form of dystrophin-glycoprotein complex, and, therefore, the report alone does not provide sufficient grounds to conclude that dystrophin expression by viltolarsen is low in patients harboring exon 45-52 deletion mutation.

- Both 2 patients harboring exon 49-52 deletion mutation in Japanese Study P1/2 received viltolarsen 40 mg/kg and underwent Week 12 measurement. Of these, 1 patient had a high dystrophin value of 6.390% at baseline (change, -6.08%). Thus dystrophin expression level decreased in patients harboring exon 49-52 deletion mutation in the 40 mg/kg group and in the entire subjects,[43)] resulting

[43)] The potential reason for the high baseline dystrophin value of this subject is that the measurement was performed on the revertant fiber (muscle fiber with a fluorescence intensity ratio comparable to that of normal muscle fiber in dystrophin immunostaining) and trace dystrophin (muscle fiber with dystrophin fluorescence that is slight in intensity and weaker than that of normal muscle fiber). The re-measurement was performed on another muscle block, and the baseline dystrophin value was 0.508%. In this subject, a change in exon skipping efficiency measured by RT-PCR was 14.63%, which was almost comparable to the mean at Week 12 in the 40 mg/kg group of 15.56%, and the result of immunofluorescent staining also did not largely differ from those of other subjects.

in the trend of small changes in dystrophin expression level in the patients harboring exon 49-52 deletion mutation. The trend of low exon 53 skipping efficiency[7)] in patients harboring exon 49-52 deletion mutation may be attributable to the dose of viltolarsen administered, because the 2 patients in Japanese Study P1/2 patients received viltolarsen 40 mg/kg and underwent Week 12 measurement, and 2 of 3 relevant patients in foreign Study 201 received viltolarsen 40 mg/kg.

Table 36. Percentage of dystrophin-positive muscle fibers in number in immunofluorescent staining and change in exon skipping efficiency from baseline[a)] by exon deletion site (Japanese Study P1/2 and foreign Study 201)

Deletion site	Percentage of dystrophin-positive muscle fibers in number with respect to normal control in immunofluorescent staining (%)					
	Japanese Study P1/2			Foreign Study 201		
	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)
Entire	0.1 ± 0.6 (8)	1.3 ± 2.2 (8)	0.7 ± 1.7 (16)	12.8 ± 8.05 (8)	33.0 ± 20.43 (8)	22.9 ± 18.28 (16)
45-52	-0.2 ± 0.2 (3)	0.4 ± 0.4 (3)	0.1 ± 0.4 (6)	10.66, 10.90 (2)	24.7 ± 13.50 (5)	20.8 ± 12.95 (7)
47-52	-	-	-	11.70 (1)	-	11.70 (1)
48-52	-0.0, 1.1 (2)	0.3 (1)	0.5 ± 0.6 (3)	11.90 (1)	53.33, 68.14 (2)	44.5 ± 29.15 (3)
49-52	-0.6, 0.2 (2)	-	-0.6, 0.2 (2)	4.00, 12.66 (2)	18.72 (1)	11.8 ± 7.40 (3)
50-52	0.6 (1)	6.5 (1)	0.6, 6.5 (2)	8.82, 31.50 (2)	-	8.82, 31.50 (2)
52	-	0.8 ± 1.2 (3)	0.8 ± 1.2 (3)	-	-	-
Deletion site	Exon skipping efficiency measured by RT-PCR (molar concentration ratio, %)					
	Japanese Study P1/2			Foreign Study 201		
	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)
Entire	21.77 ± 10.86 (8)	42.40 ± 11.26 (8)	32.08 ± 15.09 (16)	17.4 ± 7.17 (8)	43.9 ± 16.68 (8)	30.6 ± 18.45 (16)
45-52	28.23 ± 14.52 (3)	31.11 ± 3.70 (3)	29.67 ± 9.60 (6)	17.56, 25.72 (2)	44.3 ± 17.82 (5)	37.9 ± 18.44 (7)
47-52	-	-	-	26.62 (1)	-	26.62 (1)
48-52	18.04, 25.42 (2)	47.05 (1)	30.17 ± 15.08 (3)	15.02 (1)	52.57, 54.72 (2)	40.8 ± 22.33 (3)
49-52	7.7, 14.63 (2)	-	7.7, 14.63 (2)	7.54, 10.93 (2)	21.89 (1)	13.5 ± 7.50 (3)
50-52	23.66 (1)	61.37 (1)	23.66, 61.37 (2)	12.43, 23.41 (2)	-	12.43, 23.41 (2)
52	-	45.81 ± 5.54 (3)	45.81 ± 5.54 (3)	-	-	-

Mean ± SD (No. of subjects evaluated); Individual values when n = ≤2 ; -, No applicable subjects
a) Week 24 in foreign Study 201 and Week 12 or 24 in Japanese Study P1/2

The applicant's explanation:
Patients harboring exon 43-52 deletion mutation were not included in the clinical studies of viltolarsen. However, in light of the action mechanism of viltolarsen and the dystrophin expression observed in patients harboring exon deletion mutation other than 43-52 in the clinical studies conducted so far, and there is no clearly different trend in the efficacy of viltolarsen by exon deletion site. Therefore, viltolarsen has promising efficacy in these patients as well. The package insert will caution against no experience in the use of viltolarsen in patients harboring exon 43-52 deletion mutation.

Japanese Study P1/2 included patients irrespectively of the ability to walk. PMDA asked the applicant to explain the efficacy in patients who had lost the ability to walk owing to disease progression.

The applicant's explanation:
Table 37 shows changes in dystrophin expression measured by Western blot in ambulatory and nonambulatory subjects in Japanese Study P1/2 and foreign Study 201. Ambulatory subjects in both 40 and 80 mg/kg groups showed greater changes in dystrophin level from baseline to the end of treatment. Even so, the following observations indicate that viltolarsen is expected have efficacy in nonambulatory patients as well:

- A potential factor contributing to the trend of small change in dystrophin level in nonambulatory subjects was that 1 of 3 nonambulatory subjects in Japanese Study P1/2 had a remarkably high baseline dystrophin value of 6.390%, resulting in a significantly small change in dystrophin expression level from baseline of -6.08%. In addition, because the subject received viltolarsen 40 mg/kg and underwent Week 12 measurement, the high baseline value influenced dystrophin expression level in nonambulatory subjects in the 40 mg/kg group and all nonambulatory subjects.

- There was no large difference in change from baseline in the percentage of dystrophin-positive muscle fiber count or the exon 53 skipping efficiency[7)] between ambulatory and nonambulatory subjects (Table 38).

Table 37. Change in dystrophin expression measured by Western blot from baseline by ability to walk (Japanese Study P1/2 and foreign Study 201)

Ability to walk	Change from baseline to time of muscle biopsy[a)]					
	Japanese Study P1/2 Percentage with respect to the normal control			Foreign Study 201 Percentage with respect to the normal control (standardized with myosin heavy chain)		
	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)
Ambulatory	1.180 ± 1.299 (6)	3.084 ± 3.166 (7)	2.205 ± 2.587 (13)	5.4 ± 2.4 (8)	5.3 ± 4.48 (8)	5.4 ± 3.47 (16)
Non-ambulatory	-3.035 ± 4.311 (2)	0.687 (1)	-1.794 ± 3.730 (3)	-	-	-

Mean ± SD (No. of subjects evaluated), Values of <1%, the lower limit of quantitation, were reference values, and they were included in the tabulation.
-, No applicable subjects
a) Week 12 or 24 in Japanese Study P1/2 and Week 24 in foreign Study 201

Table 38. Percentage of dystrophin-positive muscle fiber count in immunofluorescent staining and change in exon skipping efficiency from baseline[a)] by ability to walk (Japanese Study P1/2 and foreign Study 201)

Ability to walk	Percentage of dystrophin-positive muscle fiber count with respect to normal control in immunofluorescent staining (%)					
	Japanese Study P1/2			Foreign Study 201		
	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)
Ambulatory	0.2 ± 0.6 (6)	1.2 ± 2.4 (7)	0.7 ± 1.8 (13)	12.8 ± 8.05 (8)	33.0 ± 20.43 (8)	22.9 ± 18.28 (16)
Non-ambulatory	-0.4 ± 0.3 (2)	2.1 (1)	0.4 ± 1.5 (3)	-	-	-
Ability to walk	Exon skipping efficiency measured by RT-PCR (molar concentration ratio, %)					
	Japanese Study P1/2			Foreign Study 201		
	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)	40 mg/kg (n = 8)	80 mg/kg (n = 8)	Total (n = 16)
Ambulatory	19.35 ± 7.37 (6)	41.46 ± 11.82 (7)	31.25 ± 14.97 (13)	17.4 ± 7.17 (8)	43.9 ± 16.68 (8)	30.6 ± 18.45 (16)
Non-ambulatory	29.03 ± 20.36 (2)	48.97 (1)	35.67 ± 18.43 (3)	-	-	-

Mean ± SD (No. of subjects evaluated)
-, No applicable subjects
a) Week 12 or 24 in Japanese Study P1/2 and Week 24 in foreign Study 201

The applicant's explanation:

There were only 3 nonambulatory subjects. This extremely small number of subjects precludes a clear interpretation of the clinical efficacy of viltolarsen in this patient group. However, a comparison of results from quantitative muscle strength test[44)] between baseline and Week 24 indicated that muscle strength was maintained at ≥1 measurement site in all the nonambulatory subjects; serum creatine kinase

[44)] In Study P1/2 and Study 201, quantitative muscle strength tests using different test sites and devices were performed.
Study P1/2 (micro FET dynamometer): Knee joint extension, knee joint flexion, hip joint flexion, hip joint extension, foot joint dorsiflexion, and foot joint plantar flexion
Study 201 (CINRG QMT system): Grip strength, elbow joint flexion (biceps brachii muscle), elbow joint extension (triceps brachii muscle), knee joint flexion (hamstring), knee joint flexion (quadriceps femoris)

(CK) values tended to be maintained or decreased; and muscle strength was maintained at many sites in the 80 mg/kg group as compared to the 40 mg/kg group. Given these, viltolarsen is expected to have clinical efficacy in nonambulatory patients.

PMDA's view:

- The test results suggest the possibility that the efficacy of viltolarsen is attenuated in patients harboring exon 45-52 or 49-52 deletion mutation and nonambulatory patients. This observation should be disseminated to healthcare professionals.

- There is no experience in the use of viltolarsen in patients harboring exon 43-52 deletion mutation. However, viltolarsen is expected to show efficacy to a certain extent in these patients in light of its action mechanism. There are limited medications available for these patients with this rare, serious illness, as with for those harboring the other exon deletion mutation. Therefore, viltolarsen should also be used to treat patients harboring exon 43-52 deletion mutation. The lack of use experience in these patients should be disseminated.

- Furthermore, effects of patient characteristics on the efficacy of viltolarsen and the efficacy in patients with advanced DMD should be continuously investigated in the post-marketing setting.

7.R.3.4 Efficacy on motor functions

PMDA asked the applicant to explain the efficacy of viltolarsen on motor functions.

The applicant's explanation:

Table 39 shows results on time to walk/run 10 meters, time to stand/time to stand from supine, and 6-minute walk distance evaluated in both Japanese Study P1/2 and foreign Study 201. Although the lack of a comparator precluded a definitive evaluation, in patients with DMD, which is a disease progressing with time, viltolarsen suppressed deterioration or improved time to walk/run 10 meters (velocity) in Japanese Study P1/2 and 6-minute walk distance in foreign Study 201 in the 80 mg/kg group as compared with the 40 mg/kg group.

Table 39. Motor function tests in Japanese Study P1/2 and foreign Study 201

	Study	Dose	Number of subjects evaluated	Mean ± SD		
				Baseline	Week 24	Change
Time to walk/run 10 meters (m/sec)	Japanese Study P1/2	40 mg/kg	6	1.659 ± 0.541	1.467 ± 0.625	-0.192 ± 0.101
		80 mg/kg	7	1.466 ± 0.544	1.386 ± 0.482	-0.080 ± 0.148
	Foreign Study 201	40 mg/kg	8	1.67 ± 0.385	1.88 ± 0.484	0.21 ± 0.291
		80 mg/kg	8	1.88 ± 0.357	2.12 ± 0.394	0.24 ± 0.222
Time to stand/time to stand from supine (rise/sec)[a)]	Japanese Study P1/2	40 mg/kg	5	0.2219 ± 0.0981	0.1724 ± 0.1362	-0.0496 ± 0.0408
		80 mg/kg	6	0.1525 ± 0.0888	0.1274 ± 0.0461	-0.0251 ± 0.0615
	Foreign Study 201	40 mg/kg	8	0.26 ± 0.058	0.28 ± 0.128	0.02 ± 0.093
		80 mg/kg	8	0.25 ± 0.090	0.27 ± 0.078	0.02 ± 0.060
6-minute walk distance (m)	Japanese Study P1/2	40 mg/kg	6	346.5 ± 102.7	317.3 ± 118.1	-29.2 ± 37.3
		80 mg/kg	7	316.7 ± 90.5	291.7 ± 88.7	-25.0 ± 41.1
	Foreign Study 201	40 mg/kg	8	391.4 ± 33.27	407.0 ± 38.24	15.6 ± 26.40
		80 mg/kg	8	353.4 ± 106.32	407.6 ± 120.07	44.0 ± 41.98

a) Time to stand was evaluated in Japanese Study P1/2, and time to stand from supine was evaluated in foreign Study 201.

In foreign Study 201, the comparison with the CINRG natural history population, the external comparator, showed improving trends in multiple endpoints in patients receiving viltolarsen (Table 30). The comparison of results in Study 201 with the natural history was justified as follows:

- The FDA guidance for drug development DMD issued in February 2018[40] states that an external comparator, if used, should have similarities to the treatment group in age, baseline value of the primary efficacy endpoint, dose and duration of concomitant therapy, and genotype. Accordingly, eligible subjects were selected from the CINRG natural history population based on the major inclusion and exclusion criteria for foreign Study 201.

- Table 40 shows baseline characteristics of patients in foreign Study 201 and the CINRG natural history population who met the major inclusion and exclusion criteria for foreign Study 201. Patient characteristics and baseline values in motor function tests did not tend to differ largely between patients in foreign Study 201 and patients extracted from the natural history population. Of patients included in the external comparator, 9 were eligible for exon 53 skipping therapy, and 56 not eligible. The course of disease progression in patients with DMD eligible for exon 53 skipping therapy was not different from other patients with DMD (*Hum Mutat.* 2018;39:1193-202, *PLoS One.* 2014;9:e83400, etc.) or was faster in speed (*J Neurol Neurosurg Psyhiatry.* 2016;87:149-55). If patients with DMD ineligible for exon 53 skipping therapy were included in the natural history population for comparison, it would not lead to the overestimation of the efficacy of viltolarsen. In addition, of 9 patients with DMD eligible for exon 53 skipping therapy in the external comparator, only 2 or 3 patients provided results from motor function tests, precluding a comparison with the clinical study.

Table 40. Comparison of patient characteristics between the study population in foreign Study 201 and natural history population

		Foreign Study 201			Natural history population		
		40 mg/kg	80 mg/kg	Total	Exon 53 skipping	Other than exon 53 skipping	Total
Number of subjects evaluated		8	8	16	9	56	65
Age (years)		7.5 ± 1.75	7.2 ± 2.03	7.4 ± 1.84	6.3 ± 1.07	7.2 ± 1.36	7.1 ± 1.35
Race[a]	Caucasian	8 (100)	7 (87.5)	15 (93.8)	7 (77.8)	48 (85.7)	55 (84.6)
	Black[b]	0	0	0	0	1 (1.8)	1 (1.5)
	Asian	0	1 (12.5)	1 (6.3)	1 (11.1)	3 (5.4)	4 (6.2)
	Other	0	0	0	1 (11.1)	4 (7.1)	5 (7.7)
Height (cm)		114.6 ± 6.50	112.2 ± 9.97	113.4 ± 8.22	111.3 ± 7.57	117.0 ± 10.20	116.2 ± 10.03
Body weight		23.7± 4.70	22.3 ± 6.16	23.0 ± 5.34	21.6 ± 3.99	24.4 ± 6.22	24.0 ± 6.02
BMI		17.9 ± 2.28	17.4 ± 2.02	17.7 ± 2.10	17.3 ± 1.99	17.6 ± 2.30	17.5 ± 2.25
Time to stand (rise/sec)		0.26 ± 0.058	0.25 ± 0.090	0.25 ± 0.074	0.23 ± 0.068	0.21 ± 0.092	0.22 ± 0.089
Time to walk/run 10 meters (m/sec)		1.67 ± 0.385	1.88 ± 0.357	1.77 ± 0.374	1.92 ± 0.458	1.90 ± 0.470	1.91 ± 0.465
Time to climb 4 steps (task/sec)		0.27 ± 0.081	0.32 ± 0.081	0.30 ± 0.082	0.30 ± 0.082	0.27 ± 0.105	0.28 ± 0.112
NSAA Total Score		24.8 ± 5.92	23.8 ± 5.09	24.3 ± 5.36	28.0 ± 6.68	25.2 ± 5.11	25.7 ± 5.37
6-minute walk distance (m)		391.4 ± 33.27	353.4 ± 106.32	372.4 ± 78.59	428.4 ± 63.50	403.2 ± 184.51	408.0 ± 167.16

Mean ± SD
a) Number of patients (percentage)
b) Including African American

- For foreign Study 201, time points of motor function tests, study centers, contract research organization, procedures for motor function tests, and training methods were determined so that the study would be designed to allow the comparison with the CINRG natural history population.

- The concomitant use of steroids would affect the efficacy evaluation. However, the types and dosage regimens of steroids used in subjects in foreign Study 201 were considered within standard based on survey results on the type and dosage regimen of steroids used in patients with DMD in the US (*J Neuromuscul Dis.* 2015;2:63-72, *BMC Neurol.* 2019;19:84).

Then, published natural history[45)] data, placebo group data[46)] from past clinical studies[47)] of the other products, and data of the viltolarsen group in foreign Study 201 and its extension study (foreign Study 202) were compared. An improving trend of motor functions was observed in foreign Study 201 (Tables 41 and 42), suggesting that dystrophin expression induced by viltolarsen improves motor functions in patients with DMD.

Table 41. Comparison of results from motor function tests between foreign Study 201+ 202 and natural history

	Foreign Study 201 + 202	Natural history A[a)]		Natural history B[b)]	Natural history C[c)]	
		A1	A2		C1	C2
Number of subjects evaluated	16	132	28	28	23	27
Age (years)	7.4 ± 1.84	7.96 ± 2.32	8.6 ± 1.96	8.14	6.3 ± 0.5	8.1 ± 0.5
Population	Eligible for exon 53 skipping therapy	Exon deletion	Eligible for exon 53 skipping therapy	Eligible for exon 53 skipping therapy	DMD aged 5-6.9 years	DMD aged 7-8.9 years
Time to walk/run 10 meters (m/sec)						
Baseline	1.77 ± 0.374	-	-	-	2.16 ± 0.35	1.98 ± 0.48
Change (Week 49)	0.36 ± 0.450	-	-	-	0.07 ± 0.32	-0.33 ± 0.32
Time to stand/time to stand from supine (rise/sec)						
Baseline	0.252 ± 0.074	-	-	-	0.32 ± 0.09	0.24 ± 0.12
Change (Week 49)	0.024 ± 0.100	-	-	-	-0.01 ± 0.06	-0.07 ± 0.06
6-minute walk distance (m)						
Baseline	372.4 ± 78.59	368.07 ± 71.93	344.11 ± 67.16	359.46	-	-
Change (Week 49)	6.9 ± 52.5	-14.95 ± 75.09	-34.18 ± 77.99	-28.97	-	-

Mean ± SD
-, No data available
a) Natural history data from patients with DMD in Italy (14 centers) and Belgium (1 center) (*PLoS One.* 2014;9:e83400)
b) Natural history data from patients with DMD in Italy (12 centers), Belgium (1 center), and UK (1 center) (*PLoS One.* 2019;14:e0218683)
c) Natural history data from patients with DMD in the US (3 centers) (*Muscle Nerve.* 2018;58:631-8)

45) *PLoS One*. 2014;9:e83400, *PLoS One*. 2019;14:e0218683, *Muscle Nerve*. 2018;58:631-8
46) Required patient characteristic data included age information and motor function test results at baseline and change up to Week 25 or 49.
47) *Lancet*. 2017;390:1489-98, *Neurology.* 2017;89:1811-20

Table 42. Comparison of 6-minute walk distance between the viltolarsen group data in foreign Study 201+ 202 and the placebo group data from past clinical studies of the other products

	Foreign Study 201 + 202	DP3P[b] placebo	AP3P[c] placebo	TP3P[d] placebo
Number of subjects evaluated	16	61	115	116
Age (years)	7.4 ± 1.84 7.7 (4.3, 9.8)[a]	8.0 ± 2.37	9.0 (8, 10)[a]	9.4 ± 1.76
Population	Eligible for exon 53 skipping therapy	Eligible for exon 51 skipping therapy	Nonsense mutation	Exon deletion 69 Exon duplication 20 Subexonic insertion/deletion 6 Nonsense/missense mutation 3 Others/not done 18
6-minute walk distance (m)				
Baseline	372.4 ± 78.59 380 (201, 569)[a]	348.00 ± 92.153	370.5 (314, 422)[a]	337.5 ± 51.2
Change (Week 49)	6.9 ± 52.5	-52.65 ± 10.423	-60.7 ± 9.3	-51.0 ± 9.3

Mean ± SD
a) Median (min, max)
b) Phase III study of drisapersen (DMD114044) (disclosed by GlaxoSmithKline)
c) Phase III study of ataluren (*Lancet.* 2017;390:1489-98)
d) Phase III study of tadalafil (*Neurology.* 2017;89:1811-20)

In addition, the applicant explained that a placebo-controlled, global Phase 3 study[48] with the primary endpoint of time to stand from supine, including Japanese patients, is currently ongoing, and the study results will be reported when available.

PMDA's view:

- The comparison between foreign Study 201 and the external comparator such as the CINRG natural history population is an exploratory approach, and is different from a comparison between groups that has established comparability warranted by randomization. Nevertheless, the obtained study results suggest that dystrophin expression increased by viltolarsen tended to improve motor functions. In addition, in both Japanese Study P1/2 and foreign Study 201, dystrophin expression was increased, and significance of the expression level has been explained to a certain extent [see Section 7.R.3.2]. Based on these points, the efficacy of viltolarsen on motor functions is promising.

- The efficacy of viltolarsen should be continuously investigated in the post-marketing setting.

- The final decision on the appropriateness of the above views will be made taking account of comments raised in the Expert Discussion.

7.R.3.5 Efficacy of long-term treatment

PMDA asked the applicant to explain the efficacy of long-term treatment with viltolarsen.

The applicant's explanation:

- In 16 subjects who received viltolarsen for >1 year in foreign Study 201 and its extension study, Study 202, values of all motor function test items at Week 73 were lower than the peak time but better than baseline, and the motor functions at Week 25 were more or less maintained or improved (Table 43).

[48] A placebo-controlled, double-blind, parallel-group study in ambulatory boy with DMD aged ≥4 and <8 years. Viltolarsen 80 mg/kg is intravenously administered once weekly for 48 weeks to investigate the efficacy and safety. The study is conducted at up to 35 centers in approximately 15 countries in Europe, North America (the US and Canada), Asia (Japan and Korea), South America, and Australia, etc.

- In the natural course of DMD, exercise capacity peaks around the age of 5 years before gradual decline with disease progression, and the ability to walk is lost around the age of 10 years (*Practical Guideline for Duchenne Muscular Dystrophy (DMD) 2014.* Nankodo Co., Ltd.; 2014:p2). In foreign Study 201, the majority (13 of 16) of the subjects began to receive viltolarsen at the age of >5 years, but their motor functions at Week 73 improved from baseline, and peaked after 1 year of treatment (Week 49) in >1 subject. This indicates that viltolarsen is expected to have efficacy on motor functions even in the treatment of >1 year.

- Post-marketing surveillance using a registry of patients with DMD in Japan is planned to investigate the efficacy of long-term treatment further.

Table 43. Changes in descriptive statistic value on motor function test items (foreign Study 201+ 202[a])

Item	Group	Timepoint	Number of subjects evaluated	Measured value (mean ± SD)	Change (mean ± SD)
6-minute walk distance (m)	All	Baseline	16	372.4 ± 78.6	-
		Week 25	15	407.3 ± 83.1	28.9 ± 36.3
		Week 73	16	385.4 ± 67.1	13.0 ± 64.1
		Peak	16	433.8 ± 78.1	61.4 ± 37.8
	80 mg/kg	Baseline	8	353.4 ± 106.3	-
		Week 25	7	407.6 ± 120.1	44.0 ± 42.0
		Week 73	8	386.9 ± 85.5	33.5 ± 67.4
		Peak	8	426.4 ± 106.2	73.0 ± 34.7
Time to stand (rise/sec)	All	Baseline	16	0.2519 ± 0.0736	-
		Week 25	16	0.2764 ± 0.1025	0.0245 ± 0.0754
		Week 73	16	0.2760 ± 0.1147	0.0241 ± 0.1058
		Peak	16	0.3397 ± 0.0938	0.0878 ± 0.0744
	80 mg/kg	Baseline	8	0.2479 ± 0.0904	
		Week 25	8	0.2721 ± 0.0777	0.0242 ± 0.0601
		Week 73	8	0.2810 ± 0.1224	0.0331 ± 0.1016
		Peak	8	0.3262 ± 0.0923	0.0783 ± 0.0637
Time to climb 4 steps (task/sec)	All	Baseline	16	0.2965 ± 0.0820	
		Week 25	16	0.3282 ± 0.1219	0.0318 ± 0.0885
		Week 73	16	0.3408 ± 0.1442	0.0443 ± 0.1017
		Peak	16	0.4027 ± 0.1307	0.1062 ± 0.0917
	80 mg/kg	Baseline	8	0.3204 ± 0.0808	
		Week 25	8	0.3171 ± 0.0757	-0.0033 ± 0.0541
		Week 73	8	0.3495 ± 0.1289	0.0290 ± 0.0753
		Peak	8	0.3922 ± 0.1131	0.0718 ± 0.0571
Time to walk/run 10 meters (m/sec)	All	Baseline	16	1.773 ± 0.374	
		Week 25	16	2.000 ± 0.443	0.227 ± 0.251
		Week 73	16	2.139 ± 0.546	0.366 ± 0.439
		Peak	16	2.443 ± 0.436	0.670 ± 0.392
	80 mg/kg	Baseline	8	1.876 ± 0.357	
		Week 25	8	2.119 ± 0.394	0.242 ± 0.222
		Week 73	8	2.162 ± 0.466	0.286 ± 0.301
		Peak	8	2.547 ± 0.204	0.670 ± 0.329
NSAA (total score)	All	Baseline	16	24.3 ± 5.4	
		Week 25	16	25.1 ± 5.2	0.8 ± 2.9
		Week 73	16	24.9 ± 6.7	0.6 ± 4.2
		Peak	16	27.5 ± 5.0	3.3 ± 3.4
	80 mg/kg	Baseline	8	23.8 ± 5.1	
		Week 25	8	24.9 ± 4.5	1.1 ± 2.8
		Week 73	8	25.0 ± 6.9	1.3 ± 3.8
		Peak	8	27.3 ± 5.3	3.5 ± 2.6

a) The data cut-off at Week 73 in foreign Study 202 was to tabulate the safety data, and the efficacy data at this timepoint were not fixed. The descriptive statistic values at Week 73 and timepoint of the peak were calculated from pre-QC data.

PMDA's view:

The data submitted are the results from the open-label, uncontrolled study in patients with different characteristics and do not show clear efficacy in long-term treatment. However, viltolarsen increases dystrophin expression and slows disease progression, and thus has efficacy to a certain extent in

prolonged treatment. The efficacy of long-term treatment with viltolarsen should be continuously investigated in the post- marketing setting.

7.R.4 Safety

7.R.4.1 Adverse events related to renal dysfunction

Non-clinical safety studies in mice, rats, and cynomolgus monkeys showed dilation of renal tubule/epithelial vacuolation and deposition of basophilic substances [see Section 5.R.1]. PMDA asked the applicant to explain the occurrence of adverse events related to renal dysfunction in subjects receiving viltolarsen.

The applicant's explanation:

Table 44 shows the incidences of renal dysfunction-related adverse events[49] in Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2), foreign Phase II study (CTD 5.3.5.1-1, Study 201) and its extension study (Study 202), and Japanese Phase I study (CTD 5.3.5.2-1, Study DMT01). No serious adverse events, death, or adverse events leading to discontinuation occurred, and all adverse events were Grade ≤2.[50] Except for unresolved beta-N-acetyl-D-glucosaminidase increased in 1 subject in Study DMT01, all other adverse events related to renal dysfunction resolved without symptomatic treatment.

Table 44. Incidences of adverse events related to renal dysfunction

	Japanese Study P1/2		Foreign Study 201+ 202		Japanese Study DMT01		
	40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg	1.25 mg/kg	5 mg/kg	20 mg/kg
Number of subjects evaluated	8	8	8	8	3	3	4
Adverse events related to renal dysfunction	1 (12.5)	2 (25.0)	0	1 (12.5)	3 (100.0)	3 (100.0)	4 (100.0)
Serious adverse events	0	0	0	0	0	0	0
All adverse events							
Beta-N-acetyl-D-glucosaminidase increased[a)]	1 (12.5)	2 (25.0)	0[a)]	0[a)]	3 (100.0)	3 (100.0)	3 (75.0)
Albumin urine present	1 (12.5)	0	0	0	2 (66.7)	2 (66.7)	3 (75.0)
Blood urine present	1 (12.5)	0	0	0	1 (33.3)	0	0
Protein urine present	1 (12.5)	0	0	0	1 (33.3)	3 (100.0)	4 (100.0)
Alpha 1 microglobulin increased	1 (12.5)	0	0	0	0	0	0
Beta 2 microglobulin urine increased	0	1 (12.5)	0	0	0	0	2 (50.0)
Blood creatinine increased	0	0	0	1 (12.5)	0	0	0
Blood urea increased	0	0	0	1 (12.5)	0	0	0
Hypercalciuria	0	0	0	1 (12.5)	0	0	0
Cystatin C increased	0	0	0	0	1 (33.3)	1 (33.3)	0
Haematuria	0	0	0	0	1 (33.3)	0	0
Urine protein/creatinine ratio increased	0	0	0	0	1 (33.3)	0	0
Urine ketone body present	0	0	0	0	1 (33.3)	0	0

Number of subjects with the event (incidence, %)

a) For beta-N-acetyl-D-glucosaminidase, the normal reference value was not established in foreign Study 201.

[49] Including events falling under MedDRA System Organ Class (SOC) of "Renal and urinary disorders" and High Level Group Term (HLGT) "Renal and urinary tract investigations and urinalyses" as well as adverse events related to urinalysis (beta-N-acetyl-D-glucosaminidase increased, alpha 1 microglobulin increased, beta 2 microglobulin urine increased, and urine ketone body present)

[50] In any study, the severity of adverse events was rated by Grades 1 to 5 according to Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.03 (ver. 4.0 Japanese translation version by the Japan Clinical Oncology Group [JCOG]). In the case where it was not appropriate to rate the severity based on numerical values in the list of CTCAE items because of the nature of disease, it was assessed in accordance with the definitions of CTCAE grading shown below.

Grade 1 (mild): Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2 (moderate): Minimal, local or noninvasive intervention indicated; limiting activities of daily living except for age appropriate activities for self-care
Grade 3 (severe or medically significant but not immediately life-threatening): Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting activities of daily living for self-care.
Grade 4 (life-threatening): Life-threatening consequences; urgent intervention indicated.
Grade 5 (death due to adverse event): Death due to adverse event

As mentioned above, the clinical study results did not indicate a possible clinical concern about renal dysfunction-related adverse events following viltolarsen treatment. The effect on the kidney observed in non-clinical safety studies is considered to be associated with the distribution of viltolarsen in the kidney at high concentrations [see Section 4.2.1], and viltolarsen is mainly excreted into urine in the unchanged form [see Sections 4.3.2 and 4.4]. Patients with advanced DMD are frequently complicated by renal impairment, and ≥30% of patients with DMD aged ≥30 years had abnormal renal function (increased cystatin C) (*Clinical Neurology.* 2012;52:211-7). In light of the above, the possibility cannot be ruled out that patients with renal impairment are exposed to a higher level of viltolarsen than patients with normal renal function. The package insert will provide caution against possible delay in the excretion of viltolarsen in patients with DMD with renal impairment.

PMDA asked the applicant to explain how they would ensure appropriate renal function monitoring to determine the use of viltolarsen, continuation or discontinuation of the treatment.

The applicant's explanation:
No adverse events related to renal dysfunction leading to discontinuation, dose reduction, or interruption of viltolarsen occurred in the Japanese and foreign clinical studies. It is safe to say that renal functions may be monitored through regular examinations by physicians well-acquainted with treatment and management of DMD depending on the renal dysfunction of each patient and in accordance with the DMD clinical practice guideline in Japan, without specifying a particular method (*Practical Guideline for Duchenne Muscular Dystrophy (DMD) 2014.* Nankodo Co., Ltd.; 2014:p37).

PMDA accepted the above applicant's explanation. However, the importance of appropriate renal function monitoring should be communicated through the package insert. Furthermore, data on the occurrences of renal function-related adverse events should be further collected in the post-marketing setting.

7.R.4.2 Adverse events in the central nervous system

An adverse event of balance disorder occurred in a clinical study of eteplirsen, a drug in the same class. PMDA asked the applicant to explain the incidences of adverse events in the central nervous system associated with viltolarsen.

The applicant's explanation:
Table 45 shows the incidences of adverse events[51)] in the central nervous system in Japanese Study P1/2, foreign Study 201 and its extension study (foreign Study 202), and Japanese Study DMT01. No serious adverse events occurred, and all events were Grade ≤2. A causal relationship to viltolarsen was ruled out for all events. Most of the events resolved by the following day of the onset, and attention deficit hyperactivity disorder resolved 8 days after the onset.

[51)] Events falling under MedDRA/SOCs of "Nervous system disorders" and "Psychiatric disorders"

Table 45. Incidences of adverse events in the central nervous system

	Japanese Study P1/2		Foreign Study 201+ 202		Japanese Study DMT01		
	40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg	1.25 mg/kg	5 mg/kg	20 mg/kg
Number of subjects evaluated	8	8	8	8	3	3	4
Adverse events in the central nervous system	2 (25.0)	1 (12.5)	4 (50.0)	2 (25.0)	0	0	0
Serious adverse events	0	0	0	0	0	0	0
All adverse events							
Dizziness	0	1 (12.5)	1 (12.5)	0	0	0	0
Headache	1 (12.5)	0	1 (12.5)	1 (12.5)	0	0	0
Hypoaesthesia	1 (12.5)	0	0	0	0	0	0
Anxiety	0	0	1 (12.5)	0	0	0	0
Attention deficit hyperactivity disorder	0	0	1 (12.5)	0	0	0	0
Flat affect	0	0	0	1 (12.5)	0	0	0

Number of subjects affected (incidence, %)

As mentioned above, adverse events in the central nervous system following viltolarsen treatment are unlikely to raise a clinical problem.

PMDA accepted the above applicant's explanation. However, balance disorder occurred in a clinical study of a drug in the same class. *CNTNAP2* and *MYT1* are off-target gene candidates identified by an *in silico* analysis and their expression variations were observed in exon microarray and RT-PCR. They are known to be expressed in the nervous system [see Section 3.R.2]. Given these facts, information about adverse events in the central nervous system should be further collected in the post-marketing setting.

7.R.4.3 Adverse events related to drug hypersensitivity

An adverse event related to drug hypersensitivity occurred in a clinical study of eteplirsen, a drug in the same class. PMDA asked the applicant to explain the incidences of adverse events related to drug hypersensitivity associated with viltolarsen.

The applicant's explanation:

Table 46 shows the incidences of adverse events[52)] related to drug hypersensitivity in Japanese Study P1/2, foreign Study 201 and its extension study (foreign Study 202), and Japanese Study DMT01. There were no serious adverse events, death, or adverse events leading to discontinuation, and all adverse events were Grade ≤2. Grade 2 events were eczema, dermatitis contact, urticaria, conjunctivitis allergic, and rash, and all were resolving or resolved while the treatment continued, except for eczema in 1 subject.

52) Events falling into MedDRA SMQ of "Hypersensitivity" (narrow)

Table 46. Incidences of adverse events related to drug hypersensitivity

	Japanese Study P1/2		Foreign Study 201+ 202		Japanese Study DMT01		
	40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg	1.25 mg/kg	5 mg/kg	20 mg/kg
Number of subjects evaluated	8	8	8	8	3	3	4
Adverse events related to drug hypersensitivity	2 (25.0)	5 (62.5)	4 (50.0)	5 (62.5)	1 (33.3)	0	1 (25.0)
Serious adverse events	0	0	0	0	0	0	0
All adverse events							
Urticaria	0	2 (25.0)	0	0	0	0	0
Rash	1 (12.5)	1 (12.5)	3 (37.5)	3 (37.5)	0	0	0
Eczema	1 (12.5)	1 (12.5)	0	0	1 (33.3)	0	1 (25.0)
Dermatitis contact	1 (12.5)	0	0	1 (12.5)	0	0	0
Dermatitis	0	0	0	1 (12.5)	0	0	0
Drug hypersensitivity	0	0	1 (12.5)	0	0	0	0
Conjunctivitis allergic	0	1 (12.5)	0	0	0	0	0
Blood immunoglobulin E increased	0	0	0	0	0	0	1 (25.0)

Number of subjects with the event (incidence, %)

The clinical study results did not indicate that adverse events of viltolarsen related to drug hypersensitivity that may pose a clinical problem, but such events might lead to a serious outcome. The package insert, therefore, will note that viltolarsen is contraindicated in patients with a history of hypersensitivity to ingredients of Viltepso. Urticaria, eczema, and rash, for which a causal relationship to viltolarsen could not be ruled out, are to be mentioned in the "Adverse Reactions" section.

PMDA accepted the above applicant's explanation. However, information about drug hypersensitivity-related adverse events should be further collected in the post-marketing setting.

7.R.4.4 Adverse events related to injection site reaction

PMDA asked the applicant to explain the occurrence of adverse events related to injection site reaction associated with viltolarsen.

The applicant's explanation:

Table 47 shows the incidences of adverse events[53)] related to injection site reaction in Japanese Study P1/2, foreign Study 201 and its extension study (foreign Study 202), and Japanese Study DMT01. There were no serious adverse events, death, or adverse events leading to discontinuation. All events were Grade 1 in severity and resolved within 2 days. Time to onset of adverse events related to injection site reaction did not show any specific trend, and adverse events and the incidences of individual events did not differ among age brackets.

[53)] Events falling into MedDRA High-Level Terms (HLT)s of "Administration site reactions NEC," "Infusion site reactions," "Injection site reactions," and "Application and instillation site reactions"

Table 47. Incidences of adverse events related to injection site reaction

	Japanese Study P1/2		Foreign Study 201 + 202		Japanese Study DMT01		
	40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg	1.25 mg/kg	5 mg/kg	20 mg/kg
Number of subjects evaluated	8	8	8	8	3	3	4
Adverse events related to injection site reaction	0	3 (37.5)	3 (37.5)	2 (25.0)	0	0	0
Serious adverse events	0	0	0	0	0	0	0
All adverse events							
Injection site pain	0	1 (12.5)	1 (12.5)	0	0	0	0
Injection site erythema	0	1 (12.5)	0	0	0	0	0
Injection site swelling	0	1 (12.5)	0	0	0	0	0
Infusion site discomfort	0	0	1 (12.5)	0	0	0	0
Injection site bruising	0	0	0	1 (12.5)	0	0	0
Injection site reaction	0	0	0	1 (12.5)	0	0	0
Infusion site pain	0	0	1 (12.5)	0	0	0	0
Injection site extravasation	0	0	0	1 (12.5)	0	0	0

Number of subjects affected (incidence, %)

As shown above, adverse events related to injection site reaction following viltolarsen treatment are unlikely to raise a clinical problem.

PMDA accepted the above applicant's explanation. However, data on the occurrence of adverse events related to injection site reaction should be collected in the post-marketing setting.

7.R.4.5 Adverse events in gastrointestinal system

An adverse event of vomiting occurred in a clinical study of eteplirsen, a drug in the same class. PMDA asked the applicant to explain the occurrence of adverse events in the gastrointestinal system associated with viltolarsen.

The applicant's explanation:

Table 48 shows the incidences of adverse events[54)] in the gastrointestinal system in Japanese Study P1/2, foreign Study 201 and its extension study (foreign Study 202), and Japanese Study DMT01. There were no serious adverse events, death, or adverse events leading to discontinuation. All the events were Grade ≤2. A causal relationship to viltolarsen was not ruled out for Grade 2 abdominal pain and diarrhoea, but these events resolved while viltolarsen treatment continued.

54) Events falling under MedDRA/SOC of "Gastrointestinal disorders"

Table 48. Incidences of adverse events related to gastrointestinal system

	Japanese Study P1/2		Foreign Study 201 + 202		Japanese Study DMT01		
	40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg	1.25 mg/kg	5 mg/kg	20 mg/kg
Number of subjects evaluated	8	8	8	8	3	3	4
Adverse events related to gastrointestinal system	2 (25.0)	2 (25.0)	5 (62.5)	3 (37.5)	0	0	1 (25.0)
Serious adverse events	0	0	0	0	0	0	0
All adverse events							
Abdominal pain	0	1 (12.5)	0	0	0	0	0
Diarrhoea	0	1 (12.5)	2 (25.0)	2 (25.0)	0	0	0
Abdominal pain upper	0	1 (12.5)	1 (12.5)	0	0	0	0
Dental caries	1 (12.5)	0	0	0	0	0	0
Vomiting	1 (12.5)	0	2 (25.0)	2 (25.0)	0	0	0
Constipation	0	0	1 (12.5)	1 (12.5)	0	0	1 (25.0)
Haematochezia	0	0	1 (12.5)	1 (12.5)	0	0	0
Nausea	0	0	2 (25.0)	0	0	0	0
Toothache	0	0	1 (12.5)	0	0	0	0

Number of subjects affected (incidence, %)

As shown above, adverse events in the gastrointestinal system following viltolarsen treatment are unlikely to raise a clinical problem.

PMDA accepted the above applicant's explanation.

7.R.4.6 Adverse events related to blood disorder

PMDA asked the applicant to explain the occurrence of adverse events related to blood disorder following viltolarsen treatment.

The applicant's explanation:

Table 49 shows the incidences of adverse events[55)] related to blood disorder in Japanese Study P1/2, foreign Study 201 and its extension study (foreign Study 202), and Japanese Study DMT01. There were no serious adverse events, death, or adverse events leading to discontinuation. All events were Grade 1. Anaemia frequently occurred only in Japanese Study DMT01, but the event was considered attributable to the large volume of blood collection for this study.[56)] In Japanese Study P1/2 and foreign Study 201+ 202, thus, the volume of blood collection was reduced, resulting in no occurrence of anaemia in either study.

55) Events falling under MedDRA/SOCs of "Vascular disorders" and "Blood and lymphatic system disorders" and adverse events related to anaemia (haematocrit decreased, haemoglobin decreased, serum ferritin decreased)

56) During Study DMT01, blood was collected 36 times, and the total volume of 594.8 mL was collected

Table 49. Incidences of adverse events related to blood disorder

	Japanese Study P1/2		Foreign Study 201 + 202		Japanese Study DMT01		
	40 mg/kg	80 mg/kg	40 mg/kg	80 mg/kg	1.25 mg/kg	5 mg/kg	20 mg/kg
Number of subjects evaluated	8	8	8	8	3	3	4
Adverse events related to blood disorder	1 (12.5)	0	0	1 (12.5)	1 (33.3)	3 (100.0)	4 (100.0)
Serious adverse events	0	0	0	0	0	0	0
All adverse events							
Haematocrit decreased	1 (12.5)	0	0	0	0	0	0
Haemoglobin decreased	1 (12.5)	0	0	0	0	0	0
Serum ferritin decreased	1 (12.5)	0	0	0	0	0	0
Peripheral artery occlusion	0	0	0	1 (12.5)	0	0	0
Anaemia	0	0	0	0	1 (33.3)	3 (100.0)	3 (75.0)
Polycythaemia	0	0	0	0	0	0	1 (25.0)

Number of subjects affected (incidence, %)

As shown above, adverse events related to blood disorder in subjects receiving viltolarsen were considered unlikely to raise a clinical problem.

PMDA accepted the above applicant's explanation.

7.R.4.7 Adverse events related to cardiac functions

PMDA asked the applicant to explain the effect of viltolarsen on cardiac functions.

The applicant's explanation:

Table 50 shows percentages of subjects who experienced clinically relevant changes in blood pressure or pulse rate in Japanese Study P1/2. In foreign Study 201 and its extension study (foreign Study 202), and Japanese Study DMT01, no clinically relevant changes occurred in blood pressure or pulse rate.

Table 50. Percentage of patients in whom abnormal changes occurred in blood pressure or pulse rate after viltolarsen treatment in Japanese Study P1/2

		40 mg/kg	80 mg/kg
Number of subjects evaluated		8	8
Systolic blood pressure	High value[a)]	1 (12.5)	1 (12.5)
	Low value[b)]	4 (50.0)	6 (75.0)
Diastolic blood pressure	High value[c)]	4 (50.0)	3 (37.5)
Pulse rate	High value[d)]	6 (75.0)	5 (62.5)
	Low value[e)]	2 (25.0)	0 (0.0)

Number of subjects with the event (incidence, %)

a) ≥140 mmHg and ≥ baseline + 20 mmHg
b) <100 mmHg and ≤ baseline - 20 mmHg
c) ≥90 mmHg and ≥ baseline + 20 mmHg
d) ≥100 beats/min and ≥ baseline + 15 beats/min
e) <60 beats/min and ≤ baseline - 15 beats/min

The only cardiac function-related adverse event[57)] in Japanese Study P1/2, foreign Study 201 and its extension study (foreign Study 202), and Japanese Study DMT01 was sinus arrhythmia in 1 subject in the 40 mg/kg group in Study 202. This event of Grade 1 occurred on Day 630 of viltolarsen treatment and resolved within the day while viltolarsen treatment was being continued. A causal relationship to viltolarsen was ruled out.

[57)] Events falling into MedDRA/SOC of "Cardiac disorders"

As shown above, adverse events related to cardiac functions following viltolarsen treatment are unlikely to raise a clinical problem.

PMDA accepted the above applicant's explanation. At the same time, cardiac dysfunction develops with disease progression in patients with DMD, and thus information about the effect of viltolarsen on cardiac functions should be further collected in the post-marketing setting.

7.R.4.8 Effect on growth

PMDA asked the applicant to explain the effect of viltolarsen on growth.

The applicant's explanation:

Table 51 shows changes in the mean percentiles of height and body weight in Japanese Study P1/2, foreign Study 201 and its extension study (foreign Study 202) obtained by comparing the height and body weight of each patient with the mean height and body weight of steroid-naive patients with DMD by age (*J Pediatr.* 2013;163:1759-63).

Table 51. Changes in growth parameters in Japanese Study P1/2 and foreign Study 201 + 202 in comparison with published literature data on growth of patients with DMD

Evaluation timepoint	Japanese Study P1/2				Foreign Study 201 + 202			
	Height		Body weight		Height		Body weight	
	40 mg/kg (n = 8)	80 mg/kg (n = 7)	40 mg/kg (n = 8)	80 mg/kg (n = 7)	40 mg/kg (n = 8)	80 mg/kg (n = 8)	40 mg/kg (n = 8)	80 mg/kg (n = 8)
Baseline	23.2 ± 25.5	31.5 ± 25.4	35.9 ± 26.2	50.8 ± 28.7	28.0 ± 20.6	21.7 ± 24.0	50.0 ± 24.6	39.2 ± 26.3
Week 13	24.0 ± 23.9	29.3 ± 28.6	39.8 ± 24.0	55.4 ± 26.8	24.4 ± 17.1	24.6 ± 30.8	50.1 ± 25.6	37.9 ± 30.4
Week 21	22.4 ± 22.3	29.5 ± 27.9	38.0 ± 27.4	54.7 ± 31.4	18.4 ± 12.1	22.3 ± 25.0	49.1 ± 25.0	35.6 ± 27.3
Week 49					11.2 ± 10.9[a)]	18.8 ± 22.2	45.5 ± 25.5[a)]	38.1 ± 26.7
Week 73					18.8 ± 12.2	15.2 ± 19.1	46.6 ± 25.7	39.7 ± 25.0

Mean ± SD of percentile
a) n = 7

In either study, subjects tended to be shorter in baseline height than steroid-naïve patients with DMD, and the percentile of body weight tended to be high as compared with height. In foreign Study 201, the percentile of height tended to be decreased. The reasons for these trends are as follows:

- Pediatric patients with DMD tended to be shorter in height with a higher body mass index (BMI) than pediatric patients without DMD (*J Pediatr.* 2013;163:1759-63). The use of steroid further enhances the tendency toward shorter height and higher BMI (*J Pediatr.* 2016;173:207-13). In both Japanese Study P1/2 and foreign Study 201, the concomitant use of steroid was allowed. Because 14 of 16 subjects in Japanese Study P1/2 and all in foreign Study 201 used steroids, their baseline height tended to be short with a high percentile of body weight as compared with that of height, which is considered attributable to the steroid use.

- The dose of steroid in foreign Study 201 was compared with that in Japanese Phase 1/2 study. The daily dose (mean ± SD) of prednisolone, an approved steroid in Japan, was 0.571 ± 0.031 mg/kg in foreign Study 201 and 0.362 ± 0.153 mg/kg in Japanese Study P1/2. In light of the high trend of steroid dose in foreign Study 201 and no dose-dependent trend in decreasing height percentile in foreign Study 202, the concomitant steroid might have affected height.

- Based on the above, viltolarsen is unlikely to affect growth.

PMDA accepted the above applicant's explanation. However, information about the effect of long-term treatment with viltolarsen on growth should be further collected in the post-marketing setting.

7.R.4.9 Anti-dystrophin antibody

Patients with DMD congenitally lack dystrophin, and viltolarsen facilitates the production of mutant dystrophin protein, which potentially induces immune reactions. Anti-viltolarsen antibody was observed in some of the toxicity studies. PMDA therefore asked the applicant to explain the presence or absence of anti-dystrophin antibody production and its effect on the efficacy and safety.

The applicant's explanation:

- No anti-dystrophin antibody was detected in Japanese Study DMT01, Japanese Study P1/2, or the extension study from foreign Study 201 (foreign Study 202). In foreign Study 201, anti-dystrophin antibody was observed only in 1 subject (6.3%) in the 80 mg/kg group. The subject was tested positive for anti-dystrophin antibody at Weeks 13 and 24, but the change in dystrophin expression from baseline was 4.79% (standardized with myosin heavy chain), which did not tend to be largely different from those in the other subjects, and all adverse events were Grade 1 and resolved while viltolarsen treatment continued.

- Based on the above, long-term treatment with viltolarsen is unlikely to raise an immunogenicity problem due to anti-dystrophin antibody or anti-viltolarsen antibody. In the currently ongoing global Phase III study,[48)] anti-dystrophin antibody development is to be investigated further.

PMDA accepted the above applicant's explanation.

7.R.5 Indication and eligible patients

In patients harboring the target mutation in the dystrophin gene, viltolarsen binds to the exon 53 region of the dystrophin mRNA precursor so that exon 53 is skipped, leading to the expression of functional dystrophin protein, though, which is shorter-chained than the normal protein [see Section 3.R.1]. Viltolarsen actually increased dystrophin protein in the clinical studies [see Section 7.R.3.2], suggesting its certain level of efficacy on motor functions [see Section 7.R.3.4]. Given these, PMDA considers that the proposed indication of viltolarsen is of no significant concern.

While there are extremely rare cases of DMD in females reported, there is no experience in the use of viltolarsen in female patients. PMDA asked the applicant to explain the necessity of cautionary advice on use in female patients.

The applicant's explanation:

- DMD is an X-linked recessive genetic disorder induced by mutation in the dystrophin gene on the X-chromosome in males. Females affected by the mutation usually retain 1 normal X-chromosome and thus do not completely lack dystrophin and are recognized as carriers. They may be asymptomatic or present symptoms of various severity up to difficulty in walking, depending on the

degree of the inactivation of the X-chromosome with gene mutation. In extremely rare cases, however, females may also have a deficiency of dystrophin protein that results in DMD as with males, due to the inactivation of the other X chromosome, X-autosomal chromosomal translocation leading to the disruption of dystrophin gene, X-chromosome monosomy (Turner's syndrome), etc. (*Neuromuscul Disord.* 2017;27:569-73, *Brain Dev.* 1986;8:619-23).

- Viltolarsen, if administered to female carriers with a single X-chromosome harboring a deletion mutation in the dystrophin gene amenable to viltolarsen, may also act on dystrophin gene mRNA precursor transcripts from the other normal X-chromosome and induce exon 53 skipping. This may interfere the production of normal dystrophin protein. For this reason, viltolarsen should not be administered to female carriers with a normal X-chromosome. Nevertheless, female carriers are not regarded as patients with DMD and thus not included in the target population of viltolarsen. Cautionary advice on women in the package insert is therefore unnecessary.

- Viltolarsen has been developed targeting male patients with DMD. All non-clinical studies used only male animals, and the clinical studies included only boys. The efficacy and safety of viltolarsen remain unclear in patients other than males with DMD. Thus, females with DMD harboring a deletion in the dystrophin gene amenable to viltolarsen are not eligible for viltolarsen, and some advice should be given on the use of viltolarsen in women. The package insert will highlight that viltolarsen has never been administered to women.

PMDA's view:

- There is no problem with the applicant's points that female carriers with a normal X-chromosome fall outside of the target of viltolarsen and that cautionary advice on these population is unnecessary in the package insert. However, viltolarsen, if administered to female carriers with a normal X-chromosome, may affect normal dystrophin expression. Viltolarsen should not be used in female carriers, of which healthcare professionals should be well-informed through written materials.

- While extremely rare cases of female patients with DMD are reported, the non-clinical studies of viltolarsen used only male animals, and viltolarsen has never been administered to female patients. Therefore, the package insert should give a caution against the use of viltolarsen in women.

- The above conclusions and the specific description of caution will be finalized taking account of comments raised in the Expert Discussion.

7.R.6 Dosage and administration

PMDA asked the applicant to explain the justification of the proposed dosage and administration, "The usual dosage is 80 mg/kg of viltolarsen injected intravenously once weekly over 1 hour."

The applicant's explanation:

- In Japanese Phase I study (CTD 5.3.5.2-1, Study DMT01), 1.25, 5, and 20 mg/kg was intravenously administered once weekly for 12 weeks. With favorable tolerability, the high dose of 20 mg/kg induced high dystrophin expression in 1 subject (Table 27). With the expectation of achieving the

efficacy at a higher dose, a dose of 80 mg/kg once weekly was selected based on its exposure comparable to that at the tolerable dose in toxicity studies.[58)] In addition, a dose of 40 mg/kg once weekly was selected as the low dose, which was half the high dose and twice the maximum dose (20 mg/kg) in Japanese Study DMT01.

- In Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2) and foreign Phase II study (CTD 5.3.5.1-1, Study 201), viltolarsen increased Western blot-based dystrophin expression. In Japanese Study P1/2, the dystrophin expression tended to be higher at 80 mg/kg than at 40 mg/kg (Table 28). In foreign Study 201, Western blot-based dystrophin expression did not differ between 40 mg/kg and 80 mg/kg (Table 29), but immunofluorescent staining revealed greater trend of changes in dystrophin expression at 80 mg/kg than at 40 mg/kg. RT-PCR also showed greater trend of exon 53 skipping efficiency[7)] at 80 mg/kg than at 40 mg/kg (Table 36).

- Based on the above, the dose of viltolarsen was determined as 80 mg/kg to be intravenously administered once weekly.

- The administration time of ≥1 hour was specified for the following reasons: A clinical study of eteplirsen, another morpholino nucleic acid medicine with the same backbone (*Ann Neurol.* 2013;74:637-47), showed that intravenous infusion time of 1 hour posed no safety problems. Accordingly, a plasma concentration of intravenous viltolarsen was simulated over 1 hour, and the dose of viltolarsen was specified based on the simulation. In the subsequent clinical study, the intravenous infusion time of 1 hour was employed as in Japanese Study DMT01, and the efficacy of viltolarsen was demonstrated without safety concerns. Consequently, the intravenous infusion time was specified as 1 hour in the dosage regimen of viltolarsen.

- Japanese Study DMT01 used lower doses and a limited volume of undiluted viltolarsen solution. The undiluted viltolarsen was, therefore, diluted with physiological saline to make the total infusion volume of 100 mL so that infusion was readily completed in 1 hour. In accordance with the preparation method in Japanese Study DMT01, the drug solution was diluted with physiological saline to prepare 100 to 300 mL-solution in Japanese Study P1/2, and when <100 mL of study drug was available, it was diluted with physiological saline to 100 mL in foreign Study 201. These preparation procedures were specified in the study drug preparation manual, etc. Because the drug solution was diluted to ≥100 mL in the clinical studies, the dilation volume was to be specified as 100 mL in the package insert. Nevertheless, for infants weighing <10 kg, the dilation volume may as well be adjusted to reduce burden on them.

Japanese Study P1/2 included patients aged 5 to 12 years, and foreign Study 201 included patients aged 4 to 9 years. Given this, PMDA asked the applicant to explain the efficacy and safety as well as the appropriateness of the dosage regimen in patients younger than patients participated in these clinical studies.

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58) The dose of human providing exposure comparable to that ($AUC_{0\text{-}t}$, 420.6 μg/hr/mL following the last dose) at the tolerable dose of 240 mg/kg in the 12-week repeated intravenous dose toxicity study in mice (CTD 4.2.3.2-2) was estimated by linear regression analysis (Power model analysis).

The applicant's explanation:

- Japanese Study P1/2 and foreign Study 201 included 3 and 6 pediatric patients with DMD aged 4 to 6 years, respectively. In these patients, dystrophin expression and exon 53 skipping was observed without safety problems. Infants and pediatric patients aged <4 years have never been included in the clinical studies in the past. Meanwhile, increased CK values were observed in infants with DMD (*Eur J Hum Genet.* 2013;21:1049-53, *J Tokyo Wom Med Univ.* 1992;62:1175-84); and muscle breakdown and regeneration due to defective dystrophin protein are considered to occur even in infants. Viltolarsen is expected have efficacy in infants as well based on its mechanism of action.

- Viltolarsen is excreted into urine. Human renal function matures to an adult level by the age of 1 to 2 years (*Japanese Journal of Pediatric Nephrology.* 2013;26:70-5). Given, this, in patients aged 1 to 6 years, a risk of adverse events due to remarkably increased exposure is considered low. Furthermore, the non-clinical studies showed that NOAEL in juvenile mice was 60 mg/kg, which was not different from that in adult animals, and toxicological findings in juvenile mice were similar to those in adult mice. Therefore, a safety risk will be low in treating patients younger than the clinical study participants using the dosage regimen of viltolarsen intended for patients aged ≥4 years.

- Viltolarsen has never been administered to patients aged <4 years. The package insert will give a caution against the treatment in patients of this age group.

Japanese Study P1/2, foreign Study 201 and Japanese Study DMT01 excluded adult patients and patients with declined cardiopulmonary functions due to disease progression. PMDA asked the applicant to explain the efficacy and safety as well as the appropriateness of the dosage regimen for these patient populations.

The applicant's explanation:

- Given its action mechanism, viltolarsen induces dystrophin expression in the diaphragm and other muscles involved in respiration, and is expected to slow the progression of respiratory dysfunction and delay the start of use of a ventilator (*Mol Ther.* 2011;19:345-54). In addition, in patients on a ventilator whose upper limbs or fingers remain functioning, viltolarsen may contribute to the maintenance of the function. Because viltolarsen 80 mg/kg did not cause safety problems in Japanese Study P1/2 or foreign Study 201, there will be no safety concerns raised in these patient populations.

- Patients with advanced DMD aged >30 years have renal impairment (*Clinical Neurology.* 2012;52:211-7). In this patient population, the excretion of viltolarsen delays, potentially increasing the exposure. Therefore, careful administration is important for patients with renal impairment.

- Based on the above, viltolarsen 80 mg/kg is expected to have efficacy without safety concerns in adult patients and patients with cardiopulmonary impairment owing to the progression of underlying disease as well. The package insert will give appropriate caution against the use of viltolarsen for patients with renal impairment, in light of its possible delay in excretion [see Section 6.R.2].

Meanwhile, a clinical study [59] in [redacted] is being planned targeting [redacted] to [redacted].

PMDA's view:

- The proposed dosage and administration is not considered to have a problem.

- It is appropriate to highlight the lack of experience in the use of viltolarsen in infants and children aged <4 years.

- There is no problem with treating adult patients and patients with advanced disease with viltolarsen according to the dosage regimen intended for the other patients. However, the lack of experience in the use of viltolarsen in these patients should be communicated via the package insert.

- The efficacy and safety in patients aged <4 years, adult patients, and patients with advanced disease should be further investigated in the post-marketing setting.

7.R.7 Proper use

The action of viltolarsen may affect normal dystrophin gene expression, and this raises a safety concern in its use for patients who are ineligible for the treatment. The clinical studies on viltolarsen were conducted at extremely limited sites (5 centers in Japan and 6 centers in overseas [US and Canada]). It is important that viltolarsen be used at medical facilities where appropriate diagnosis, medication, and patient control are available. Accordingly, PMDA asked the applicant to explain measures for the proper use of viltolarsen.

The applicant's explanation:

The decision to start or continue viltolarsen treatment and on efficacy outcome must be made appropriately at medical centers specialized in DMD where dystrophin gene diagnosis is available. Therefore, discussion is underway on the following steps to be taken before supplying viltolarsen, to qualify and control medical centers dealing with viltolarsen treatment:

(a) The applicant identifies medical centers specialized in DMD providing dystrophin gene diagnosis, etc. for the confirmation of eligibility for viltolarsen, and registers these centers as potential supply destinations. A drug wholesaler, upon receiving an order for viltolarsen from a medical center, makes written inquiry to the applicant about "whether viltolarsen can be supplied to the medical center." In response, the applicant checks the medical center under inquiry against the register and informs the drug wholesaler of its decision.

(b) When a patient with confirmed eligibility for viltolarsen intends to receive the treatment at a medical center that is not the one inquired in (a), viltolarsen is supplied to the medical center only when the availability of genetic diagnosis is confirmed by the applicant.

[59] [redacted] study targeting [redacted], which is planned to [redacted].

(c) The applicant, when declining to supply viltolarsen to the medical center, gives the medial center an explanation for the decision.

Further, the applicant noted that, to promote the proper use of viltolarsen, medical centers receiving supply of viltolarsen will be provided with written materials for healthcare professionals and patients in addition to information about the product and safety measures.

The appropriateness of these actions will be finalized, taking account of comments raised in the Expert Discussion.

7.R.8 Post-marketing investigations

Based on the submitted data from the non-clinical and clinical studies, PMDA considers it necessary to collect information through post-marketing surveillance about the effect of viltolarsen on renal function, occurrence of drug hypersensitivity, effects of anti-viltolarsen antibody and anti-dystrophin antibody on the safety and efficacy, safety and efficacy of long-term treatment with viltolarsen, safety and efficacy in patients with renal impairment, effect of patient characteristics on the efficacy of viltolarsen, and efficacy in patients in an advanced pathological condition.

The applicant explained that the post-marketing surveillance of viltolarsen is planned in the forms of a drug use-results survey covering all patients treated with viltolarsen over an observable period in clinical use (up to 9 years after the start of administration of viltolarsen) and a registry-based research. The applicant noted that the placebo-controlled, global Phase III study was currently ongoing [see Section 7.R.3.4], and the results would be reported after the marketing approval.

The appropriateness of these actions will be finalized, taking account of comments raised in the Expert Discussion.

8. Results of Compliance Assessment Concerning the New Drug Application Data and Conclusion Reached by PMDA

8.1 PMDA's conclusion concerning the results of document-based GLP/GCP inspections and data integrity assessment

The new drug application data were subjected to a document-based compliance inspection and a data integrity assessment in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. On the basis of the inspection and assessment, PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

8.2 PMDA's conclusion concerning the results of the on-site GCP inspection

The new drug application data (CTD 5.3.5.1-2 and CTD 5.3.5.2-1) were subjected to an on-site GCP inspection, in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics. As a result, PMDA concluded that the clinical studies as a whole were conducted in accordance with GCP, and thus PMDA concluded that there were no obstacles to

conducting its review based on the application documents submitted. Although the findings did not significantly affect the overall evaluation of the study, the inspection revealed the following finding at some the medical institutions, and it was notified to the heads of the concerned medical institutions to seek improvement.

Finding requiring corrective action

Medical institutions

The heads of the medical institutions did not seek a comment of the Institutional Review Board on the appropriateness of the conduct of clinical study at their sites in terms of some matters mentioned in the audit report from the auditor.

9. Overall Evaluation during Preparation of the Review Report (1)

On the basis of the data submitted, PMDA has concluded that viltolarsen has efficacy in the treatment of DMD with a deletion in the dystrophin gene amenable to exon 53 skipping therapy, and that viltolarsen has acceptable safety in view of its benefits. PMDA concluded that viltolarsen provides a new treatment option to patients with DMD and thus is considered to have clinical significance. The efficacy of viltolarsen, indication, measures for proper use, and post-marketing investigations should be further discussed at the Expert Discussion.

PMDA has concluded that viltolarsen may be approved if viltolarsen is not considered to have any particular problems based on comments from the Expert Discussion.

Review Report (2)

February 17, 2020

Product Submitted for Approval

Brand Name	Viltepso Intravenous Infusion 250 mg
Non-proprietary Name	Viltolarsen
Applicant	Nippon Shinyaku Co., Ltd.
Date of Application	September 26, 2019

List of Abbreviations
See Appendix.

1. Content of the Review

Comments made during the Expert Discussion and the subsequent review conducted by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below. The expert advisors present during the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for marketing approval, in accordance with the provisions of the Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

At the Expert Discussion, the expert advisors supported PMDA's conclusion and actions in Section "7.R.7 Proper use" in the Review Report (1).

PMDA further discussed the following points and took actions as necessary.

1.1 Efficacy

PMDA's view:
Although the data comparison between the foreign Phase II study (CTD 5.3.5.1-1, Study 201) and the external comparator such as the CINRG natural history population, unlike an inter-group comparison that has established comparability warranted by randomization, is only an exploratory approach. However, the obtained study results suggest that dystrophin expression increased by viltolarsen tended to improve motor functions [see Section 7.R.3.4 of the Review Report (1)]. Based on its action that increases dystrophin expression and thereby slows disease progression, viltolarsen is expected to have a certain extent of efficacy. At the same time, the efficacy of viltolarsen in long-term treatment should be further investigated in the post-marketing setting [see Section 7.R.3.5 of the Review Report (1)]. The expert advisors supported PMDA's these conclusions.

The expert advisor commented that the post-marketing efficacy evaluation should take a quantitative approach by using time to stand, time to walk 10 meters, etc., and it is important that the evaluation in the registry survey, a part of the post-marketing surveillance, also be performed in a standardized manner.

Based on the above, PMDA instructed the applicant to ensure appropriate conduct of the ongoing global Phase III study[48)] (48-week placebo-controlled, double-blind, parallel-group study with the primary endpoint of time to stand from supine) and the survey using the Japanese registry planned as a part of the post-marketing surveillance, and submit the study data and analysis results. The applicant responded that they would take actions appropriately.

1.2 Carcinogenicity evaluation

The applicant's explanation:

The development mechanism of transitional cell carcinoma of urothelium observed in rasH2 mice was being investigated at the time of preparation of the Review Report (1) [see Section 5.R.2 of the Review Report (1)]. Granular or needle crystalline substances were observed in the proliferative lesion in the affected animals. The substances were considered viltolarsen-derived, and inflammatory lesions were also observed around them. This was explained by insolubilized viltolarsen in the urinary tract, which continuously stimulated the transitional epithelium cells of the ureteric wall. For the following reasons, the development mechanism is unlikely to be relevant to humans, and thus the administration of viltolarsen to humans is acceptable:

- The estimated maximum urine concentration of viltolarsen in rodents at the dose (50 mg/kg) in the carcinogenicity study was higher than that in humans at the clinical dose (80 mg/kg) (167.5 mg/mL in rasH2 mice, 40.9 mg/mL in humans).[60)]

- The higher-order structure formation of guanidine-rich oligonucleotides is affected by potassium ion (*Biochemistry.* 1999;38:6981-6). The urine potassium concentrations in rodents (372-397 mM) were higher than those in humans and cynomolgus monkeys (12.5-100 mM in humans [*Kanai's Manual of Clinical Laboratory Medicine* 32nd edition], 58.3-63.4 mM in cynomolgus monkeys), and thus viltolarsen is unlikely to be deposited in human urine.

- The urinary duct diameter in humans (approximately 3.4 mm in internal diameter) was greater than that in rodents (approximately 0.3 mm in external diameter) (*Biomicrofluidics.* 2019;13:014101 1-15, *Boorman's pathology of the rat*. Second edition. Academic Press; 2018:167-80), and thus the urinary system in humans is less likely to be physically stimulated by urinary deposits than that in rodents.

- To urine specimens from various animal species/strains (non-drug-treated animals), ^{14}C-viltolarsen was added and mixed until the spiked urine became visually clear, then filtered through a filter with

60) Calculated from urine drug amount per unit time (1 minute) / urine volume per unit time. The urine drug amount per unit time was estimated from the following simulation: Based on the assumption that the whole amount of the drug eliminated from the body is excreted into urine, the pharmacokinetic parameters estimated from the 26-week carcinogenicity study in rasH2 mice and Japanese Phase I/II study (CTD 5.3.5.1-2, Study P1/2) were applied to estimate the concentration of viltolarsen administered to rasH2 mice at 50 mg/kg and to humans at 80 mg/kg. The urine volume per unit time was calculated using the daily urine volumes, i.e., 1 mL for rasH2 mice (*Pharmacokinetic Research Guide from Drug Discovery to Clinical Development*. LIFE SCIENCE INFORMATION CENTER. 2003:126-7); and 920.125 mL for humans based on data from 24-hour urine collection in Study P1/2.

a pore size of 0.2 μm. The *in vitro* solubility of viltolarsen in urine was investigated by calculating the percentage of the remaining radioactivity on the filter with respect to the total radioactivity in the spiked urine. Table 52 shows the investigation results, indicating that viltolarsen is less insolubilized in human urine than in urine from C57BL/6 mice and BALB/c mice, the background strain of rasH2 mice.

- Transitional cell carcinoma is expected to occur after an urothelial disorder. Viltolarsen, however, is not genotoxic and thus is unlikely to exert carcinogenetic effect in the short term. Careful and regular monitoring for abnormal urinary sediment, urinary occult blood, and subjective symptoms will prevent progression to transitional cell carcinoma.

Table 52. *In vitro* solubility of viltolarsen in urine specimens from various animal species/strain

	Radioactivity on filter (%)[a)]					
Viltolarsen concentration (mg/mL)	Distilled water	C57BL/6 male mouse (pool urine from 10 mice)	BALB/c male mouse (pool urine from 10 mice)	CD-1 male mouse (pool urine from 10 mice)	Male cynomolgus monkey (pool urine from 3 monkeys)	Human (pool urine from 3 volunteers)
25	0.99 (1.01, 0.96)	1.79 (1.34, 2.23)	4.42 (4.88, 3.96)	0.72 (0.65, 0.79)	1.17 (1.44, 0.89)	-
	1.36 (1.25, 1.46)	-	-	-	-	1.68 (1.83, 1.53)
50	1.55 (1.37, 1.73)	5.21 (2.54, 7.87)	9.28 (7.75, 10.81)	0.68 (0.70, 0.65)	1.06 (0.98, 1.14)	-
	1.62 (1.41, 1.82)	-	-	-	-	1.48 (1.41, 1.55)
100	0.80 (0.83, 0.77)	8.13 (11.12, 5.14)	10.1 (8.43, 11.77)	0.81 (0.88, 0.74)	1.04 (0.97, 1.11)	
	1.64 (1.38, 1.90)	-	-	-	-	1.48 (1.66, 1.29)

Mean of 2 measured values
a) Value relative to pre-filtration radioactivity (100)

PMDA's view:
At present, there is no evidence suggesting that viltolarsen is likely to be carcinogenic in humans, based on the above applicant's explanation and the absence of obvious impact related to renal dysfunction including that on the urinary system in the clinical studies [see Section 7.R.4.1 of the Review Report (1)]. The disease to be treated with viltolarsen is a rare and serious illness with extremely limited therapeutic drugs available, and therefore the clinical use of viltolarsen is acceptable as long as patients are well-informed and regularly monitored by urinary sediment test, urine cytology, and ultrasonic abdominal diagnosis on the renal urinary system during the treatment. In addition, the mechanism of development of transitional cell carcinoma of urothelium and whether the mechanism is extrapolable to humans should be investigated again when results from the 2-year carcinogenicity study in rats become available.

The expert advisors supported PMDA's conclusions above. Accordingly, PMDA reminded the applicant to ensure that patients be appropriately informed and monitored. The applicant responded that they would take actions appropriately.

1.3 Indication and eligible patients

PMDA previously expressed their view on the indication and patient eligibility, i.e., viltolarsen is not indicated for the treatment of female carriers with a normal X-chromosome; the non-clinical studies of viltolarsen used only male animals; and viltolarsen has never been administered to female patients, and given these, the package insert should give cautions against the use of viltolarsen in women [see Section 7.R.5 of the Review Report (1)]. Supporting the view, the expert advisors further commented as follows:

the administration of viltolarsen in women with dystrophinopathy who have a normal X-chromosome may decrease normal dystrophin expression by exon 53 skipping; and some women with dystrophinopathy who have a normal X-chromosome present with symptoms similar to DMD, owing to the inactivation of the normal X-chromosome resulting from marked skewed inactivation, etc. Therefore, it is important that the package insert gives cautions against the use of viltolarsen in this patient population. Accordingly, PMDA instructed the applicant to advise via the package insert not to use viltolarsen in women with dystrophinopathy who have a normal X-chromosome and to disseminate adequate information about the use of viltolarsen in women through written materials. The applicant responded that they would take actions appropriately.

1.4 Submission of results from additional validation of Western blot

The applicant submitted a report on additional validation [see Section 7.R.3.1 of the Review Report (1)] for the measurement method within the Western blot calibration curve ([redacted]%, [redacted]%, [redacted]%, [redacted]%, and [redacted]% of the normal control) in the foreign Phase II study (CTD 5.3.5.1-1, Study 201). The results obtained by the internal standard method using myosin heavy chain as the reference protein met all the criteria, but results with α-actinin used as the reference protein did not meet 2 of 9 items in the criteria (the quantitation limit should not exceed [redacted]% of CV, and the difference in standardized data between analysts should not exceed [redacted]%).

PMDA's conclusion:

Based on the above results, the evaluation of the Western blot-based measurements in the foreign Study 201 is acceptable as long as it focuses on the results measured by the internal standard method using myosin heavy chain as the reference protein, which met the predefined validation criteria.

1.5 Risk management plan (draft)

In view of the discussion presented in Section "7.R.8 Post-marketing investigations" in the Review Report (1) and the comments from expert advisors at the Expert Discussion, PMDA has concluded that the risk management plan (draft) for viltolarsen should include the safety and efficacy specifications presented in Table 53, and that the applicant should conduct additional pharmacovigilance activities, surveys and studies for the efficacy, and risk minimization activities presented in Tables 54 and 55. In addition, the registry survey is to be conducted using Remudy,[61)] a Japanese disease registry for patients with muscular dystrophy.

[61)] A disease registry constructed in a project under Clinical Research/Trial Promotion Research Program by Japan Agency for Medical Research and Development (AMED), "Construction of disease registration system (patient registry) to promote the Clinical Innovation Network initiative in the drug development for intractable/rare diseases"

Table 53. Safety and efficacy specifications in the risk management plan (draft)

Safety specification		
Important identified risks	Important potential risks	Important missing information
None	• Hypersensitivity • Renal impairment • Transitional epithelial carcinoma in the urinary duct, effects on the urinary system	• Safety profiles of patients on long-term treatment and patients with advanced underlying disease • Safety profiles of patients aged <4 years • Safety profiles of patients with renal impairment
Efficacy specification		
• Efficacy on motor functions		

Table 54. Summary of additional pharmacovigilance activities, surveys and studies for the efficacy, and additional risk minimization activities included under the risk management plan (draft)

Additional pharmacovigilance activities	Surveys and studies for the efficacy	Additional risk minimization activities
• Early post-marketing phase vigilance • Drug use-results survey (all-case surveillance) • Registry-based survey • Post-marketing clinical studies[a)] • Carcinogenicity study in rats	• Registry-based survey • Post-marketing clinical studies[a)]	• Disseminate data gathered during early post-marketing phase vigilance • Prepare and disseminate written materials for healthcare professionals (proper use guide) • Prepare and disseminate Written materials for patients

a) The (ongoing) global Phase III study[48)] will be reclassified as a post-marketing clinical study after approval.

Table 55. Outline of use-results survey (draft)

Objective	To investigate the safety in clinical use of viltolarsen
Survey method	All-case surveillance
Population	All the patients who have received viltolarsen since market launch
Observation period	Up to 9 years
Planned sample size	All patients during re-examination period (no planned sample size specified)
Main survey items	• Patient characteristics (sex, date of birth or age, height, body weight, inpatient/outpatient, diagnostic term, date of diagnosis, medical history, complications, independent ambulatory status [full ambulatory/non-ambulatory], use of systemic corticosteroids, dystrophin genetic test [exon deletion site], use of ventilator at baseline, and use of wheelchair at baseline) • Viltolarsen treatment (daily dose, treatment period, and reason for dose change), concomitant drugs (name of drug, route of administration, daily dose, treatment period, and reason for use), concomitant therapy, and rehabilitation • Clinical course (laboratory test [if any abnormal change] [name of test item, unit, institutional reference value, date of testing, and test value], renal function test, urinary sediment test, urine cytology, and ultrasonic examination on the renal urinary system) • Adverse events (presence/ absence of event, term of event, date of onset, seriousness, action on viltolarsen, etc.)

Based on the above, PMDA instructed the applicant to investigate the post-marketing safety and efficacy of viltolarsen appropriately. The applicant agreed.

2. Overall Evaluation

As a result of its review, PMDA concludes that the product may be approved for the indication and the dosage and administration as shown below, with the following conditions. Because the product is an orphan drug, the re-examination period is 10 years. The product is not classified as a biological product or a specified biological product. Neither the drug product nor its drug substance is classified as a poisonous drug or a powerful drug.

Indication

Duchenne muscular dystrophy with a deletion in the dystrophin gene amenable to exon 53 skipping therapy

Dosage and Administration

The usual dosage is 80 mg/kg of viltolarsen injected intravenously once weekly over 1 hour.

Approval Conditions

1. The applicant is required to develop and appropriately implement a risk management plan.

2. Because of a limited number of Japanese patients participated in clinical studies of the product, the applicant is required to conduct a drug use-results survey involving all Japanese patients treated with the product over the re-examination period to identify the characteristics of patients using the product, and to promptly collect safety and efficacy data so that necessary measures are taken to ensure the proper use of the product.

3. The applicant is required to conduct clinical studies and a Japanese registry-based survey aiming to evaluate the efficacy and safety of the product, and to submit the study data and analysis results promptly upon their completion.

Appendix

List of Abbreviations

[redacted]	[redacted]
ALDH1A2	Aldehyde dehydrogenase 1 family member A2
AMED	Japan Agency for Medical Research and Development
APCDD1	APC down-regulated 1
AST	Aspartate transaminase
AUC	Area Under Concentration-time Curve
BCRP	Breast Cancer Resistance Protein
BMD	Becker Muscular Dystrophy
BMI	Body Mass Index
BSEP	Bile Salt Export Pump
BUN	Blood Urea Nitrogen
C3	Complement 3
[redacted]	[redacted]
CAMKK2	Calcium/calmodulin-dependent protein kinase kinase 2
[redacted]	[redacted]
CGH	Comparative Genomic Hybridization
CHO	Chinese Hamster Ovary
CINRG	Cooperative International Neuromuscular Research Group
CK	Creatine Kinase
CK-MM	Skeletal muscle type creatine kinase
CL_{tot}	total Clearance
C_{max}	Maximum Concentration
C_{5min}	Plasma Concentration at 5 minutes
CNTNAP2	Contactin-associated protein-like 2
COL18A1	collagen type XVIII alpha 1 chain
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
CTCAE	Common Terminology Criteria for Adverse Events
CTD	Common Technical Document
CYP	Cytochrome P450
CV	Coefficient of variation
DMD	Duchenne Muscular Dystrophy
EC_{50}	50% Effective concentration
EEF2K	Eukaryotic elongation factor 2 kinase
FAS	Full Analysis Set
FDA	Food and Drug Administration
FOB	Functional Observation Battery
FSHR	Follicle-stimulating hormone receptor
FUT1	Fucossyltransferase 1 (H blood group)
[redacted]	[redacted]
GC	Gas Chromatography
GRIA1	Glutamate ionotropic receptor AMPA type subunit 1
GRIN2A	Glutamate ionotropic receptor NDMA type subunit 2A
HEK	Human Embryonic Kidney
hERG	Human Ether-a-go-go Related Gene
HLGT	High Level Group Term
HLT	High-Level Terms
HPLC	High Performance Liquid Chromatography

IC_{50}	50% Inhibition Concentration
ICH Q1E guideline	"Guideline on Evaluation of Stability Data" (PFSB/ELD Notification No. 0603004 dated June 3, 2003)
IL-6	Interleukin-6
IMD	Intermediate Muscular Dystrophy
IPRP HPLC	Ion Pair Reverse Phase High Performance Liquid Chromatography
IQSEC1	IQ motif and sec7 domain ArfGEF 1
IR	Infrared Absorption Spectrum
ITO-II cells	Human Testicular Tumor Cell
Ki	Inhibition Constant
LC/MS	High Performance Liquid Chromatography-Mass Spectrometry
LC-MS/MS	High Performance Liquid Chromatography-tandem Mass Spectrometry
LMTK2	Lemur tyrosine kinase 2
LRIG1	Leucine-rich repeats and immunoglobulin-like domains 1
MATE	Multidrug And Toxin Extrusion
MCP-1	Monocyte chemoattractant protein-1
MDCTN	Muscular Dystrophy Clinical Trial Network
Mdx mouse	X chromosome-linked muscular dystrophy mouse
mITT	modified Intention-to-treat
MLPA	Multiplex Ligation-dependent Probe Amplification
MMRM	Mixed Model for Repeated Measures
mRNA	Messenger Ribonucleic Acid
MS	Mass Spectrum
6MWT	6 minutes walk test
MYT1	Myelin transcription factor 1
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NMR	Nuclear Magnetic Resonance Spectroscopy
n+m mer	Compound in which m nucleotides are attached to viltolarsen (21 mer)
n-m mer	Compound in which m nucleotides are removed from viltolarsen (21 mer)
NSAA	North Star Ambulatory Assessment
OAT	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
PCDH15	Protocadherin related 15
PCR	Polymerase Chain Reaction
P-gp	P-glycoprotein
PMDA	Pharmaceuticals and Medical Devices Agency
pre-mRNA	Precursor Messenger Ribonucleic Acid
PRKCH	Protein kinase C eta type
QbD	Quality by Design
QTc	Corrected QT
QTcF interval	Fridericia-corrected QT Interval
RD	Human Rhabdomyosarcoma
RT-PCR	Reverse Transcriptase-polymerase Chain Reaction
SD	Sprague-Dawley
SLC22A10	Solute carrier family 22 member 10
SLC24A2	Solute carrier family 24 member 2
SLC25A18	Solute carrier family 25 member 18
SLIT3	Slit guidance ligand 3
SMQ	Standardized MedDRA Query
SOC	System Organ Class
Study 201	Study NS-065/NCNP-01-201 (CTD 5.3.5.1-1)
Study 202	Study NS-065/NCNP-01-202 (CTD 5.3.5.3-1)
Study DMT01	Study NCNP/DMT01 (CTD 5.3.5.2-1)

Study P1/2	Study NS065/NCNP01-P1/2 (CTD 5.3.5.1-2)
SYCP2L	synaptonemal complex protein 2 like
[redacted]	[redacted]
[redacted]	[redacted]
TIAM1	T-cell lymphoma invasion and metastasis-inducing protein 1
t_{max}	Time to Reach Maximum Concentration
TNF-α	Tumor necrosis factor alpha
$t_{1/2}$	Elimination Half-life
[redacted]	[redacted]
UGT	Uridine Diphosphate Glucuronosyltransferase
UV	Ultraviolet Spectrum
UV/VIS	Ultraviolet-visible Spectrum
Vd_{ss}	Volume of distribution at steady state
Viltepso	Viltepso Intravenous Infusion 250 mg
Viltolarsen	Viltolarsen
WDR20	WD repeat domain 20
WRN	Werner syndrome RecQ like helicase
ZMIZ1-AS1	Zinc finger MIZ-type containing 1 antisense RNA 1
ZNF557	Zinc Finger Protein 557