Report on the Deliberation Results

June 13, 2017
Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau
Ministry of Health, Labour and Welfare

Brand Name	Spinraza Intrathecal injection 12 mg
Non-proprietary Name	Nusinersen Sodium (JAN*)
Applicant	Biogen Japan Ltd.
Date of Application	December 7, 2016

Results of Deliberation

In its meeting held on June 9, 2017, the First Committee on New Drugs concluded that the product may be approved and that this result should be reported 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.

Conditions of Approval

1. The applicant is required to develop and appropriately implement a risk management plan.
2. Due to the very limited number of patients studied in Japan, the applicant is required to conduct a use-results survey, covering all patients treated with the product, during the re-examination period, in order to obtain information on the characteristics of patients treated with the product, collect data on the safety and efficacy of the product as soon as possible, and take necessary measures to ensure proper use of the product.

**Japanese Accepted Name (modified INN)*

Review Report

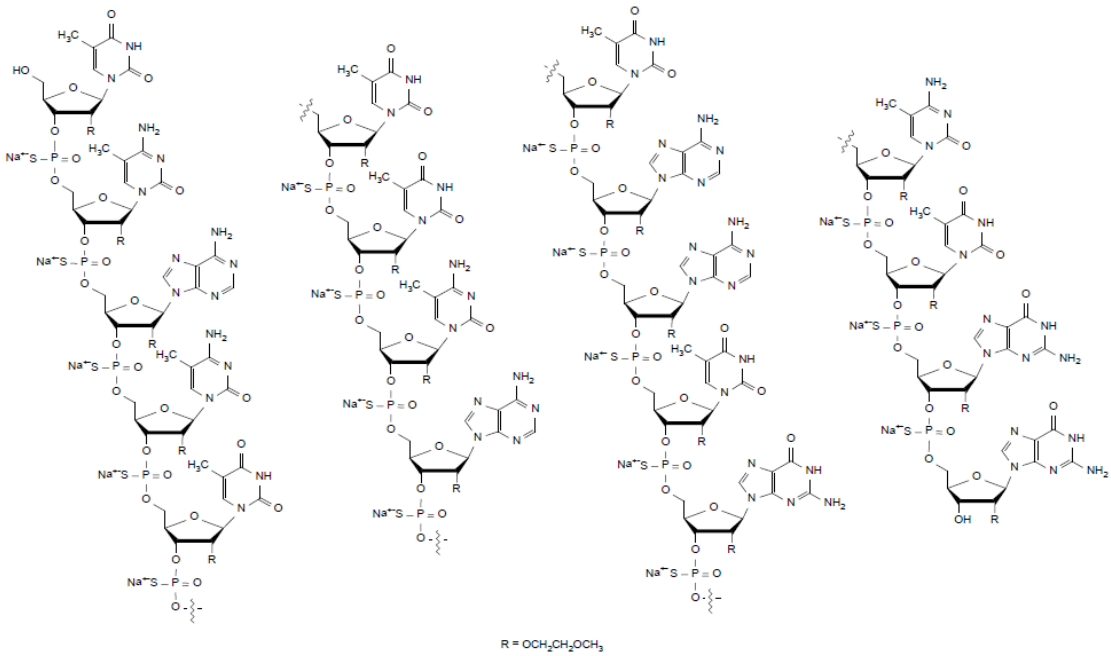
May 31, 2017

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	Spinraza Intrathecal injection 12 mg
Non-proprietary Name	Nusinersen Sodium
Applicant	Biogen Japan Ltd.
Date of Application	December 7, 2016
Dosage Form/Strength	Solution for injection: Each vial (5 mL) contains 12.63 mg of Nusinersen Sodium (equivalent to 12 mg of nusinersen).
Application Classification	Prescription drug, (1) Drug with a new active ingredient

Chemical Structure



Molecular formula: $C_{234}H_{323}N_{61}Na_{17}O_{128}P_{17}S_{17}$

Molecular weight: 7500.89

Chemical name:

Heptadeca sodium salt of *all-P-ambo*-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-*P*-thioadenylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-*P*-thioadenylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-*P*-thioadenylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-*P*-thioadenylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-*P*-thioguanylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiocytidylyl-(3'→5')-2'-*O*-(2-methoxyethyl)-5-methyl-*P*-thiouridylyl-(3'→5')-2'-*O*- (2-methoxyethyl)-*P*-thioguanylyl-(3'→5')-2'-*O*-(2-methoxyethyl)guanosine

Items Warranting Special Mention

Orphan drug (Drug Designation No. 392 of 2016 [*28 yaku*]; PSEHB/PED Notification No. 1124-6 dated November 24, 2016, by the Pharmaceutical Evaluation Division, Pharmaceutical Safety and Environmental Health Bureau, Ministry of Health, Labour and Welfare)

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 infantile spinal muscular atrophy, and that the produc 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 Infantile spinal muscular atrophy

Dosage and Administration The usual dose of nusinersen is shown in the table below. Spinraza treatment should be initiated with 4 doses at Weeks 0, 2, 4 and 9 followed by dosing every 4 months. Spinraza should be administered as an intrathecal bolus injection over 1 to 3 minutes.

Age on the day of dosing	Dose	Injection volume
0-90 days	9.6 mg	4 mL
91-180 days	10.3 mg	4.3 mL
181-365 days	10.8 mg	4.5 mL
366-730 days	11.3 mg	4.7 mL
≥731 days	12 mg	5 mL

Conditions of Approval

1. The applicant is required to develop and appropriately implement a risk management plan.
2. Due to the very limited number of patients studied in Japan, the applicant is required to conduct a use-results survey, covering all patients treated with the product, during the re-examination period, in order to obtain information on the characteristics of patients treated with the product, collect data on the safety and efficacy of the product as soon as possible, and take necessary measures to ensure proper use of the product.

Attachment

Review Report (1)

April 28, 2017

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.

Product Submitted for Approval

Brand Name Spinraza Intrathecal injection 12 mg

Non-proprietary Name Nusinersen Sodium

Applicant Biogen Japan Ltd.

Date of Application December 7, 2016

Dosage Form/Strength Solution for injection: Each vial (5 mL) contains 12.63 mg of Nusinersen Sodium (equivalent to 12 mg of nusinersen).

Proposed Indication Spinal muscular atrophy

Proposed Dosage and Administration

Spinraza treatment should be initiated as early as possible after diagnosis. The dose of nusinersen is 12 mg (5 mL) for patients aged >2 years (24 months). For infants aged ≤2 years (24 months) who have a smaller volume of cerebrospinal fluid, the dose of nusinersen should be adjusted based on their age as shown in the table below. Spinraza should be administered intrathecally by lumbar puncture.

Age	Dose (mg)	Injection volume (mL)	Loading doses (Dosing days)	Maintenance doses*
0-3 months	9.6 mg	4 mL	Days 0 (the day of the first dose), 14, 28, and 63	Every 4 months
3-6 months	10.3 mg	4.3 mL		
6-12 months	10.8 mg	4.5 mL		
12-24 months	11.3 mg	4.7 mL		
> 24 months	12 mg	5 mL		

* A maintenance dose should be started after the 4th loading dose.

Table of Contents

List of Abbreviations

2'-MOE	2'-*O*-(2-methoxyethyl)
Impurity a	[redacted] impurity
Impurity b	[redacted] impurity
ALT	Alanine Aminotransferase
ApoB	Apolipoprotein B
ASO	Antisense Oligonucleotide
AST	Aspartate Aminotransferase
AUC	Area Under Concentration-time Curve
BCRP	Breast Cancer Resistance Protein
BLAST	Basic Local Alignment Search Tool
BNC2	Basonuclin 2
BSEP	Bile Salt Export Pump
BUN	Blood Urea Nitrogen
CHOP INTEND	Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CL	Clearance
CL_{csf}	CSF clearance (transfer into plasma)
CL_p	Plasma clearance
CMAP	Compound Muscle Action Potential
C_{max}	Maximum Concentration
CRP	C-reactive Protein
CSF	Cerebrospinal Fluid
CTD	Common Technical Document
DMT	4,4'-dimethoxytrityl
DNA	Deoxyribonucleic Acid
DYNC1H1	Dynein Cytoplasmic 1 Heavy Chain 1
EC_{50}	Effective Concentration, 50%
EFCAB6	EF-hand Calcium Binding Domain 6
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
FIGLA	Folliculogenesis Specific BHLH Transcription Factor
FOXP1	Forkhead Box P1
GCCR	Glucocorticoid Receptor
GLP	Good Laboratory Practice
hERG	Human Ether-a-go-go Related Gene
HINE	Hammersmith Infant Neurological Examination
HLGT	High Level Group Term
HLT	High-Level Term
hnRNP	Heterogeneous Nuclear Ribonucleoprotein
HPLC	High Performance Liquid Chromatography
Hyb-ECL	Hybridization Electrochemiluminescence
Hyb-ELISA	Hybridization Enzyme-linked Immunosorbent Assay
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th revision
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICH Q1E guideline	"Guideline on Evaluation of Stability Data" (PMSB/ELD Notification No. 0603004 dated June 3, 2003
IGHMBP2	Immunoglobulin Mu Binding Protein 2

ITT	Intention-to-Treat
LC-MS	Liquid Chromatography Mass Spectrometry
LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
MECOM	MDS1 and EVI1 complex locus
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	Messenger Ribonucleic Acid
MSR1	Macrophage Scavenger Receptor 1
c	Compound [redacted]
d	Compound [redacted]
e-1	Compound [redacted]
e-2	Compound [redacted]
e-3	Compound [redacted]
e-4	Compound [redacted]
e-5	Compound [redacted]
e-6	Compound [redacted]
e-7	Compound [redacted]
e-8	Compound [redacted]
NMR	Nuclear Magnetic Resonance Spectrum
NZW	New Zealand White
Impurity f	[redacted] impurity
Impurity g	[redacted] impurity
Impurity h	[redacted] impurity
Impurity i	[redacted] impurity
OAT	Organic Anion Transporter
OATP	Organic Anion Transporting Polypeptide
OCT	Organic Cation Transporter
PD	Pharmacodynamics
P-gp	P-glycoprotein
PPK	Population Pharmacokinetics
PT	Preferred Term
PTEN	Phosphatase And Tensin Homolog
Q_{csf}	Inter-compartmental clearance within the CSF
Q_p	Inter-compartmental clearance within the plasma
QTc	Corrected QT
QTcF	Fridericia-corrected QT
RNA	Ribonucleic Acid
RPGRIP1L	Retinitis Pigmentosa GTPase Regulator Interacting Protein 1 Like
RSF1	Remodeling and Spacing Factor 1
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Sprague-Dawley
SMA	Spinal Muscular Atrophy
SMN	Survival Motor Neuron
SMN1	Survival Motor Neuron 1
SMN2	Survival Motor Neuron 2
SMQ	Standardized MedDRA Query

SOC	System Organ Class
STAT4	Signal Transducer And Activator of Transcription 4
$t_{1/2}$	Elimination Half-life
t_{max}	Time to Reach Maximum Concentration
TRHDE	Thyrotropin Releasing Hormone Degrading Enzyme
ULK2	Unc-51 Like Kinase 2
V_{csfc}	Volume of distribution in the central CSF compartment
V_{csfp}	Volume of distribution in the peripheral CSF compartment
V_{pc}	Volume of distribution in the central plasma compartment
V_{pp}	Volume of distribution in the peripheral plasma compartment
j	[redacted] impurity
[redacted]	[redacted] impurity
Impurity k	[redacted] impurity
Impurity l	[redacted] impurity
Impurity m	[redacted] impurity
Spinraza	Spinraza Intrathecal injection 12 mg
Nusinersen	Nusinersen Sodium

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

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by degeneration of the motor neurons in the anterior horn of the spinal cord, resulting in atrophy of the voluntary muscles of the limbs and trunk. SMA is caused by a deletion of the *SMN1* gene or loss-of-function mutations in the *SMN1* gene, etc. that lead to survival motor neuron (SMN) protein deficiency. SMA has been categorized into 5 types: Types 0, I, II, III, and IV. The severity of symptoms significantly varies depending on phenotypes. While infants with SMA die shortly after birth (Type 0), patients with SMA manifesting after 20 to 30 years of age experience mild to moderate muscle weakness, but have a normal life expectancy (Type IV) (*Lancet Neurol*. 2012; 11: 443-52, *J Child Neurol*. 2007; 22: 1027-49). Type I SMA is the most common form of SMA (approximately 58% of all SMA patients; *Eur J Hum Genet.* 2004; 12: 1015-23). Infants with Type I SMA generally have generalized muscle weakness and a decline in motor function, are unable to sit unassisted, and attain almost no motor developmental milestones after 6 months of age. It has been reported that 1.3% of Type I SMA patients without respiratory support survived beyond 24 months (*Pediatrics*. 2013; 131: e1509-14), and the major cause of death is lung disease due to neuromuscular weakness (*J Child Neurol*. 2007; 22: 1027-49). Type II SMA patients can sit independently, but are unable to walk, and Type III SMA patients are initially able to walk, but lose this ability as the disease progresses (*Arch Neurol.* 1995; 52: 518-23). Types II and III SMA represent approximately 29% and 13% of SMA cases, respectively (*Eur J Hum Genet*. 2004; 12: 1015-23). Although the incidences of Types 0 and IV are considered to be low, there are a few specific reports on the incidence. In Japan, SMA has been designated as "designated intractable disease." The estimated prevalence of SMA is 0.5 to 1.0 per 100,000 in Japan (*Journal of Tokyo Women's Medical University*. 2007; 83: E52-7). SMA patients are eligible for specific medical care for designated intractable diseases. A total of 894 specific medical care recipient certificates were issued for SMA patients in the fiscal year 2014 (http://www.nanbyou.or.jp/entry/1356).

Nusinersen Sodium (hereinafter referred to as nusinersen) is a 2'-MOE modified ASO consisting of 18 nucleotide residues discovered by ISIS Pharmaceuticals, Inc. (the US) (a predecessor of Ionis Pharmaceuticals, Inc.). It binds to the *SMN2* pre-mRNA to modulate splicing of the SMN2 gene that produces defective SMN protein, resulting in an increase in normal SMN protein expression. The development of nusinersen began overseas in [redacted] 20[redacted] and then the applicant acquired global development rights. Nusinersen was approved in December 2016 in the US. The EU application for nusinersen is under review as of March 2017.

In Japan, ISIS Pharmaceuticals, Inc. (a predecessor of Ionis Pharmaceuticals, Inc.) initiated a clinical study involving mainly Type I SMA patients in August 2014. Claiming that the efficacy and safety of nusinersen in the treatment of SMA have been demonstrated, the applicant has filed a marketing application for nusinersen. As of November 24, 2016, nusinersen was designated as an orphan drug with the intended indication of "spinal muscular atrophy" (Drug Designation No. 392 of 2016 [*28 yaku*]). Clinical studies involving mainly Types II and III SMA patients began in November 2014, and a marketing application was submitted on [redacted], 20[redacted].

In Japan, adenosine triphosphate disodium hydrate for injection has been approved for the indications of "progressive spinal muscular atrophy and its similar diseases."

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 white to yellow solid or powder, and its appearance, solubility, pH, crystalline polymorphism, melting point, hygroscopicity, dissociation constant, and partition coefficient have been determined. The drug substance is an 18-mer oligonucleotide where the 2'-hydroxy groups of the ribofuranosyl rings are replaced with methoxyethyl groups and the phosphodiester internucleotide linkages are replaced with phosphorothioate diester internucleotide linkages. The drug substance is a mixture of 2^{17} diastereoisomers because it exhibits stereoisomerism at the phosphorus atom.

The chemical structure of the drug substance has been confirmed by NMR (^{1}H-, ^{13}C-, ^{31}P-NMR), high-resolution mass spectrometry, elemental analysis, infrared spectroscopy, ultraviolet spectroscopy, and X-ray powder diffraction.

2.1.2 Manufacturing process

The drug substance is [redacted] have been defined as critical steps. [redacted] are controlled as critical intermediates.

Quality by Design (QbD) approaches were utilized to perform mainly the following studies.

- Identification of [redacted] as critical quality attributes impacted by the manufacturing process.
- Identification of critical process parameters through quality risk assessment by [redacted] followed by establishment of standard operating conditions.

2.1.3 Control of drug substance

The proposed specifications for the drug substance consist of content, appearance, identification (most abundant mass [mass spectrum], liquid chromatography, mass spectrum [LC-MS], counterion [inductively coupled plasma-optical emission spectrometry]), purity (related substances [LC-MS], residual solvents [gas chromatography], elemental impurities [inductively coupled plasma mass spectrometry]), bacterial endotoxins, microbial limits, and assay (LC-MS).

2.1.4 Stability of drug substance

Stability studies on the drug substance are shown in Table 1. Photostability data showed that the drug substance is photostable.

Table 1. Stability studies on drug substance

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term	3 commercial-scale batches	−20°C	-	double low-density polyethylene bags /high-density polyethylene container	24 months

A re-test period of 24 months was proposed for the drug substance when stored at −20 ± 5°C in double low-density polyethylene bags within a high-density polyethylene container. The long-term testing will be continued for up to 60 months.

2.2 Drug product

2.2.1 Description and composition of drug product and formulation development

The drug product is a solution for injection containing 2.53 mg/mL of Nusinersen Sodium (equivalent to 2.4 mg/mL of nusinersen) in a vial. The fill volume is 5 mL. It contains the following excipients: sodium phosphate monobasic dihydrate, sodium phosphate dibasic anhydrous, sodium chloride, potassium chloride, calcium chloride dihydrate, magnesium chloride, sodium hydroxide, hydrochloric acid, and Water for Injection.

2.2.2 Manufacturing process

The drug product is manufactured through a process composed of buffer preparation, [redacted], bioburden reduction and filtration, [redacted], [redacted], and packaging/labeling/storage/testing. [redacted], [redacted], [redacted], and [redacted] have been defined as critical steps. Process controls have been established for [redacted], [redacted], and [redacted].

2.2.3 Control of drug product

The proposed specifications for the drug product consist of strength, appearance, identification (most abundant mass [mass spectrum], liquid chromatography), purity (related substances [LC-MS]), osmolality, pH, bacterial endotoxins, extractable volume, foreign insoluble matter, insoluble particulate matter, sterility, and assay (LC-MS).

2.2.4 Stability of drug product

Stability studies on the drug product are shown in Table 2. Photostability data showed that the drug product is photostable.

Table 2. Stability studies on drug product

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term	Manufacturing Site A/3 commercial-scale batches	5°C	-	glass vial /bromobutyl rubber stopper /aluminium cap	24 months
	Manufacturing Site B/3 commercial-scale batches				3 months[a)]
Accelerated	Manufacturing Site A/3 commercial-scale batches	25°C	60% RH		6 months
	Manufacturing Site B/3 commercial-scale batches				3 months[a)]

a) Testing is currently ongoing, and 6 months of stability data will be reported in the Review Report (2).

In accordance with the ICH Q1E guideline, a shelf life of 30 months was proposed for the drug product in a glass vial closed with a bromobutyl rubber stopper and an aluminium cap, when stored at 2°C to 8°C, protected from light. The long-term testing will be continued for up to 60 months.

2.R Outline of the review conducted by PMDA

2.R.1 Drug substance manufacturing process development

PMDA asked the applicant to explain studies conducted to assure consistent quality for the development of the drug substance manufacturing process.

The applicant's explanation:

[redacted] and [redacted] were identified as critical quality attributes [redacted]. Then, [redacted] was identified, and taking account of the results of studies on [redacted] (Table 3), [redacted]. The drug substance is a huge molecule with a molecular weight of 7500.89. [redacted]

Table 3. Studies on [redacted]

[redacted]	[redacted]
j	Increased by [redacted]
o	Increased by [redacted]
n	Increased by [redacted]
[redacted]	Increased by [redacted]
Impurity h	Increased by [redacted]
Impurity f Impurity g	Increased by [redacted]
Impurity l Impurity m	[redacted]

Table 4 shows data from the analysis of drug substance batches [redacted]. Although [redacted] tended to be different slightly, nusinersen is a medicinal product for a rare disease with about 1000 patients in Japan. [redacted].

Table 4. Drug substance batch analysis data

Production batch		#1	#2	#3	#4
[redacted]	Nusinersen Sodium	+	++	++	++
Related substances	j	++	++	+	+
	Total o	++	+	+	+
	Total e-2 and e-4	+	+	+	+
	e-1	+	+	+	+
	e-3	++	+	+	+
	Total n	+	+	+	+
	Total e-6 and e-8	+	+	+	+
	e-5	+	+	+	+
	e-7	+	+	+	+
	[redacted]	+	+	+	+
	Impurity h	+	+	+	+
	Impurity f	+	+	+	+
	Impurity g	+	+	+	+
	Impurity b	+	+	+	+
	Impurity i	+	+	+	+
	Impurity a Impurity k	+	+	+	+
	Impurity l	++	+	+	+
	Impurity m	+++	+	+	++
	[redacted] impurity	+	+	+	+
	[redacted] impurity	+	+	+	+
	Total [redacted] impurities	+	++	+	+

PMDA's view:

[redacted] are acceptable.

2.R.2 Control of "n" and "o"

[redacted], the robustness of the drug substance manufacturing process cannot be determined at present. Nusinersen may hybridize with sequences with mismatches [see Section 3.R.2.1]. Given the above circumstances, [redacted] "n" and "o" potentially hybridize with off-target sequences, resulting in unintended off-target effects. On the basis of the above, PMDA asked the applicant to explain [redacted] "n" and "o" [redacted].

The applicant's explanation:

- As described in Section 2.R.1, the applicant controls [redacted]
- [redacted]
  (a) [redacted]
  (b) [redacted]

[redacted]

- [redacted]

PMDA's view:

Given that [redacted] (Table 4) show batch-to-batch variability in the impurity profile of the drug substance and that it is difficult to assure the consistency of [redacted] "n" and "o" profiles, [redacted] "n" or "o" [redacted], potentially resulting in off-target effects due to hybridization to off-target sequences in humans. Thus, there are not adequate controls for impurities in the drug substance at present. However, because (i) certain studies for control of impurities have been conducted according to the current level of technology and (ii) SMA (mainly Type I SMA) is a fatal, serious disease, the current method of control is acceptable, provided that the following measures are taken appropriately.

- [redacted]
- [redacted]
- [redacted].
  (a) [redacted] "n" and e-5, e-7 and e-6, e-8 [redacted]
  (b) [redacted] "o" and e-1, e-3 and e-2, e-4 [redacted]
  (c) [redacted]
- Collect information on the drug product lots used via post-marketing surveillance. Based on the collected information together with post-marketing safety information reported in and outside Japan, watch for any differences in safety profile between different drug substance batches, and report the results to PMDA periodically.

PMDA instructed the applicant to take the above measures, and the applicant agreed to take the measures appropriately.

2.R.3 Control of [redacted] for drug substance

PMDA asked the applicant to explain [redacted], taking into account that [redacted] and that [redacted] (*Nucleic Acids Res.* 2014; 42: 13456-68).

The applicant's explanation:

[redacted]

[redacted]

Table 5. [redacted]

[redacted]	Experiment 1	Experiment 2
[redacted]	[redacted]	[redacted]

The applicant also provided the following explanation:

[redacted].

PMDA accepted the applicant's explanation.

2.R.4 Novel excipients

The novel excipients contained in the drug product are potassium chloride, calcium chloride dihydrate, and magnesium chloride, which are new ingredients used in the development of intrathecal formulations.

PMDA's view:

All novel excipients conform to the compendial specifications in Japan, and there are therefore no particular problems with the specifications. Based on the submitted data, these excipients at the levels used in the drug product are unlikely to cause safety issues.

Moreover, the manufacturing process, specifications, storage, and shelf-life for the drug substance and the drug product have been established and controlled adequately. Six months of stability data (long-term and accelerated data) on the drug product manufactured at Manufacturing Site B is reported in the Review Report (2) [see Section 2.2.4].

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

3.1.1 Induction of SMN protein expression

The RNA from GM03813 cells[1)] transfected with nusinersen (0.3-100 nmol/L) was extracted, and the *SMN2* RNA was amplified using the RT-PCR method. As a result, *SMN2* transcripts including exon 7 were produced (EC_{50}, 1.9 nmol/L) (reference data, CTD 4.2.1.1-5).

Lysates were prepared from GM03813 cells[1)] transfected with nusinersen (100 nmol/L). SMN protein was detected in the cell lysates by Western blotting. There was a 1.9-fold increase in the amount of SMN protein produced compared to untreated cells (CTD 4.3-35, *Am J Hum Genet.* 2008; 82: 834-48).

GM03813 cells[1)] transfected with nusinersen (50 nmol/L) were stained for immunofluorescence microscopy, which revealed localization of the SMN protein to 4'-6-diamidino-2-phenylindole-stained region. The applicant discussed that the SMN protein is present in the nucleus in cells (reference data, CTD 4.2.1.1-2).

A biotinylated *SMN2* intron 7 RNA was incubated with HeLa cell nuclear extract. The biotinylated *SMN2* intron 7 RNA was pulled down using avidin-coated beads, and the bound hnRNP A1, A2, and B1[2)] were detected by Western blotting. Decreases were observed in HeLa cells transfected with nusinersen (30 nmol/L) compared to untreated HeLa cells. The applicant discussed that nusinersen inhibited the binding of hnRNP A1, A2, and B1 to *SMN2* intron 7 RNA (reference data, CTD 4.2.1.1-3).

3.1.2 Effects in mouse models of SMA[3)]

Nusinersen (0-350 μg) was administered to SMN2 mice[3)] and C/C mice[3)] as a single intracerebroventricular (ICV) bolus injection. Nusinersen increased the amount of the transcript including exon 7 expressed in the spinal cord and brain. The EC_{50} values in the spinal cord and brain were 1.6 and 5.7 μg/g, respectively, in SMN2 mice, and 1.2 and 3.7 μg/g, respectively, in C/C mice (reference data, CTD 4.2.1.1-4).

Following administration of nusinersen (0-350 μg) as a single ICV bolus injection to SMN2 mice,[3)] human SMN protein was immunohistochemical stained for microscopy. Human SMN protein expression was observed in the brain and spinal cord (reference data, CTD 4.2.1.1-4).

Nusinersen (4 μg) was administered to Δ7SMA mice[3)] (on postnatal day 0) as a single ICV bolus injection. Changes observed in the treated animals were improved morphology of neuromuscular junctions, increased

1) SMA patient fibroblasts
2) Bind to the nusinersen-target region in *SMN2* intron 7 (CTD 4.3-35, *Am J Hum Genet.* 2008; 82: 834-48).
3) Mouse models of SMA that express the human *SMN2* gene used were (1) SMN2 mice in which endogenous *Smn* has been removed and human *SMN2* has been randomly integrated into the mouse genome, (2) C/C mice, which have a chimeric gene composed of endogenous *Smn* exon 1-6 and the human *SMN2* exon 7 and 8 and the human *SMN2* gene, (3) Δ7SMA mice, which lack endogenous *Smn* and contain human *SMN2* and human *SMN2* lacking exon 7, (4) Hung 2 SMN2 copy mice in which endogenous *Smn* has been removed and 2 copies of human *SMN2* have been randomly integrated into the mouse genome.

size of myofibers, improved righting reflex[4] and grip strength,[5] and increased survival (median survival, 16 days in the untreated group, 26 days in the nusinersen group) (CTD 4.3-61, *Sci Transl Med*. 2011; 3: 72ra18).

Following administration of nusinersen (20 μg) as a single ICV injection in Hung 2 SMN2 copy mice[3] (on postnatal day 1), spinal cord tissues were taken 7 days after dosing. Dosing of nusinersen increased exon 7 inclusion (the level of *SMN2* exon 7 inclusion,[6] 13% to 14% in the vehicle control group, 83% to 86% in the nusinersen group) and SMN protein expression. Nusinersen extended survival (median survival, 10 days in the vehicle control group, 16 days in the nusinersen group) (CTD 4.3-33, *Nature*. 2011; 478: 123-6).

3.2 Secondary pharmacodynamics

3.2.1 Effect on peripheral tissue necrosis (CTD 4.3-32, *Genes Dev*. 2010; 24: 1574-9)

SMN2 mice[3] received a single ICV bolus injection of nusinersen (20 μg) on gestation day 15. Nusinersen delayed the onset of tail necrosis, which is seen in the mouse model (the onset of tail necrosis was observed 3 weeks after birth in untreated mice and 9 weeks after birth in nusinersen-treated mice).

3.2.2 *In silico* analyses to search for transcripts other than *SMN2* transcripts to which nusinersen may bind

Transcripts to which nusinersen may bind were analyzed using BLAST (*Proc Natl Acad Sci USA*. 1989; 86: 441-5) and Bowtie (*Genome Biol*. 2009; 10: R25).[7] A BLAST search found no highly homologous genes (continuous match of ≥16 bases), but identified zinc finger matrin-type 4, neuron navigator 2, and a hypothetical gene[8] as highly homologous genes (continuous match of ≥15 bases). On the other hand, a Bowtie search revealed that in addition to these 3 genes, 3 different hypothetical genes[8] were homologous (CTD 4.2.1.1-7).

3.3 Safety pharmacology

A summary of safety pharmacology studies of nusinersen is shown in Table 6.

4) A test was conducted to determine the time taken for the mouse to reposition itself onto all four paws after being placed in a supine position (maximum, 60 seconds).
5) Each mouse was placed on a wire mesh (1 cm^2 grids) and then the mesh with the mouse was inverted. The latency for the mouse to release the mesh was recorded.
6) Percentage of *SMN2* transcripts including exon 7
7) BLAST: Genome Reference Consortium Human Build 37 patch release 5 (GRCh37.p5) and Bowtie: Genome Reference Consortium Human Build 38 patch release 4 (GRCh38.p4) were used for analyses.
8) Based on BLAST and Bowtie searches, LOC643542 was a highly homologous hypothetical gene. Based on a Bowtie search only, LOC102724356, LOC105373684, and LOC105371082 were highly homologous hypothetical genes.

Table 6. Summary of safety pharmacology studies

Organ systems evaluated	Animal species	Endpoints or Method of assessment	Dose	Route of administration	Findings	CTD
CNS	Cynomolgus monkey (2-3/sex/group)	body temperature, gait, clinical observations, general sensory function, motor function, cerebral reflexes, spinal reflexes	1, 3, and 7 mg	IT	7 mg group: decreases in lower spinal reflexes, decreased body temperature	4.2.3.1-1
	Juvenile cynomolgus monkey (9-10 months of age) (6-9/sex/group)	body temperature, gait, clinical observations, general sensory function, motor function, cerebral reflexes, spinal reflexes	0.3, 1, and 3 mg/dose, 15 doses[a)]	IT	3 mg/dose group: decreases in lower spinal reflexes	4.2.3.2-1
	Juvenile cynomolgus monkey (9-11 months of age) (5-7/sex/group)	body temperature, general sensorimotor, cerebral reflexes, spinal reflexes, patellar tendon reflex, grip reflex (legs), learning ability,[c)] modified Irwin functional observational battery	0.3, 1.0, and 4.0 mg/week, 13 doses[b)]	IT	4.0 mg/dose group: decreased or absent lower spinal reflexes	4.2.3.2-2
Cardiovascular system	SD rat (8 males/group)	systolic blood pressure, diastolic blood pressure, mean arterial pressure, heart rate	0.02, 0.06, and 0.2 mg/day, 25 days	IT	No effects	4.2.1.3-1
	Cynomolgus monkey (2-3/sex/group)	Heart rate	1, 3, and 7 mg	IT	No effects	4.2.3.1-1
	Juvenile cynomolgus monkey (9-10 months of age) (6-9/sex/group)	Heart rate, ECG	0.3, 1, and 3 mg/dose, 15 doses[a)]	IT	No effects	4.2.3.2-1
	Juvenile cynomolgus monkey (9-11 months of age) (5-7/sex/group)	ECG, systolic blood pressure, diastolic blood pressure, mean arterial pressure	0.3, 1.0, and 4.0 mg/week, 13 doses[b)]	IT	No effects	4.2.3.2-2
Respiratory system	SD rat (8 males/group)	respiratory rate, tidal volume, minute volume	0.02, 0.06, and 0.2 mg/day, 25 days	IT	No effects	4.2.1.3-1
	Cynomolgus monkey (2-3/sex/group)	respiratory rate	1, 3, and 7 mg	IT	No effects	4.2.3.1-1
	Juvenile cynomolgus monkey (9-10 months of age) (6-9/sex/group)	respiratory rate	0.3, 1, and 3 mg/dose, 15 doses[a)]	IT	No effects	4.2.3.2-1
Renal/urinary system	Cynomolgus monkey (2-3/sex/group)	serum creatinine, blood urea nitrogen	1, 3, and 7 mg	IT	No effects	4.2.3.1-1
	Juvenile cynomolgus monkey (9-10 months of age) (6-9/sex/group)	urinalysis (urine volume, specific gravity, color, pH, protein, occult blood, glucose), serum creatinine, blood urea nitrogen	0.3, 1, and 3 mg/dose, 15 doses[a)]	IT	No effects	4.2.3.2-1
	Juvenile cynomolgus monkey (5-7/sex/group)	urinalysis (specific gravity, color, pH, protein, occult blood, glucose), serum creatinine, blood urea nitrogen	0.3, 1.0, and 4.0 mg/week, 13 doses[b)]	IT	No effects	4.2.3.2-2

a) Animals at 0.3 and l mg/dose received 5 weekly doses (on Days 1, 8, 15, 22, and 29) followed by biweekly dosing (on Days 43, 57, 71, 85, and 99). Animals at 3 mg/dose received weekly dosing (15 doses in total).
b) Nusinersen was administered once weekly for the first 5 doses (on Days 1, 8, 15, 22, and 29) followed by a dose every 6 weeks thereafter (on Days 71, 113, 155, 197, 239, 281, 323, and 365).
c) Assessed using the Wisconsin General Testing Apparatus.

3.R Outline of the review conducted by PMDA

3.R.1 Mechanism of action of nusinersen

PMDA asked the applicant to explain the mechanism of action of nusinersen, taking account of the mechanism of development of SMA.

The applicant's explanation:

The mechanism of development of SMA has not fully been elucidated at present, but is considered to be as follows.

- In many patients, SMA is a result of reduced levels of the SMN protein caused by a deletion of the *SMN1* gene located on chromosome 5q or loss-of-function mutations in the *SMN1* gene (CTD 4.3-43, *Cell*. 1995; 80: 155-65; CTD 4.3-63, *Am J Med Genet Part A*. 2004; 130A: 307-10).
- According to Human Protein Atlas (http://www.proteinatlas.org/), the SMN protein is likely to be expressed in all tissues, and its expression levels are high especially in the bone marrow, bronchus, gallbladder, nasopharynx, pancreas, testis, and bladder, in addition to the brain and spinal cord. The SMN protein forms an SMN complex, and the SMN complex has been reported to be involved in the formation of small nuclear ribonucleoproteins and RNA processing (*Semin Cell Dev Biol*. 2014; 32: 22-9). However, RNA species subject to processing by the SMN complex have not been determined, and its precise function is unknown. Thus, the mechanism of pathogenesis of SMA (e.g., dysfunction and death of motor neurons in the anterior horn of the spinal cord) caused by a deficiency of SMN protein is unclear.
- In 4% to 5% of all patients with SMA, genes other than *SMN1* on chromosome 5q are responsible for the SMA phenotype (*Am J Hum Genet*. 1999; 64: 1340-56). Patients with SMA related to abnormalities in the *IGHMBP2* gene, *DYNC1H1* gene, and other abnormalities have been reported as *SMN1*-unlinked SMA patients (*Pediatr Clin North Am.* 2015; 62: 743-66).

The applicant added the following explanation on the mechanism of action of nusinersen:

- Humans carry a nearly identical copy of the *SMN1* gene called the *SMN2* gene. Both genes encode the SMN protein. A cytosine-to-thymine substitution at position 6 of exon 7 of the *SMN2* gene results in an alternative splicing pattern that favors skipping of exon 7. The majority of the transcripts produced from the *SMN2* gene are missing exon 7 and translated into a protein with a deletion of amino acids encoded by exon 7, and a minority of the transcripts are translated into SMN protein nearly identical to that expressed from the *SMN1* gene. The half-life of a protein with a deletion of amino acids encoded by exon 7 is shorter than that of full-length SMN protein translated from the normal *SMN1* gene (CTD 4.3-12, *Genes Dev.* 2010; 24: 438-42). SMA patients with more copies of the *SMN2* gene have been reported to have a less severe form of the disease (CTD 5.4-27, *Am J Hum Genet.* 2002; 70: 358-68). This suggests that protein translated from the *SMN2* gene functions as a partial alternative to full-length SMN protein in patients with SMA.
- Multiple regions of exon 7 in the *SMN2* pre-mRNA have been shown to be effective in producing SMN protein containing amino acids encoded by exon 7 when bound by an ASO (CTD 4.3-34, *PLoS Biol.* 2007; 5: e73; CTD 4.3-71, *Nucleic Acids Res*. 2007; 35: 371-89). Nusinersen is an ASO designed to bind to one of these regions, intron 7 (a site 10-27 nucleotides downstream from the intron/exon junction) (CTD 4.3-35).

- Nusinersen increased the amount of *SMN2* transcripts including exon 7 and SMN protein expression *in vitro* and in mouse models of SMA [see Sections 3.1.1 and 3.1.2]. Nusinersen also showed improved morphology of neuromuscular junctions, increased size of myofibers, and improved righting reflex and grip strength, and other improvements in a mouse model of SMA. These findings indicate that nusinersen improves the symptoms of SMA by inducing SMN protein production.
- Given that (i) the mechanism of action of nusinersen is not the induction of transcription but the reduction of skipping (reference data, CTD 4.2.1.1-1), (ii) autopsy samples from SMA infants treated with nusinersen showed 28% to 70%[9] *SMN2* exon 7 inclusion[6] in a foreign phase II study (CTD 5.3.5.2-1, Study CS3A), and (iii) SMA patients have 0 to 4 copies of *SMN2* (CTD 5.4-23), the level of increased SMN protein expression in SMA patients treated with nusinersen corresponds to up to 2.8 copies of the normal human *SMN1* gene (4 copies × 70%). However, taking into account a report on 3 copies of *SMN1* in cell lines derived from healthy non-SMA subjects (*Mol Genet Genomic Med*. 2015; 3: 248-57), increased SMN protein expression in patients treated with nusinersen is unlikely to become a major safety issue.

PMDA's view:

The mechanism of development of SMA has not fully been elucidated at present, and the function of protein translated from the *SMN2* gene is unclear. However, the mechanism of action has been discussed to a certain degree based on the currently available findings. The level of increased SMN protein expression in patients treated with nusinersen may exceed the expression level in healthy humans. No clear safety risk associated with increased SMN protein expression in nusinersen-treated patients has been suggested at present, but details of risks remain unknown. Taking account of these concerns, the applicant should collect information on the relationship between *SMN2* copy number and safety via post-marketing surveillance.

3.R.2 Safety evaluation of nusinersen

3.R.2.1 Safety related to interactions between nusinersen and genes

Nusinersen is an ASO, and (1) effects on the target gene (*SMN2*), (2) off-target effects due to hybridization to off-target sequences (hybridization-dependent off-target effects), and (3) off-target effects independent of interactions with genes (hybridization-independent off-target effects) are anticipated. PMDA asked the applicant to explain the safety of nusinersen from the standpoint of (1) and (2).

The applicant's explanation on safety related to (1) effects on the target gene (*SMN2*):

- The *SMN2* gene does not exist in common laboratory animals including mice, rats, dogs, and cynomolgus monkeys, and only humans have the *SMN2* gene. The safety of nusinersen cannot be evaluated in animal species commonly used in non-clinical studies.
- Nusinersen exposure and SMN protein expression in the CNS tissues are unlikely to become a major problem based on the findings from safety pharmacology studies [see Sections 3.R.1 and 3.R.2.2]. On the other hand, a non-clinical systemic tissue distribution study of nusinersen (Study APK02) is currently ongoing. Given that nusinersen exposure and SMN protein expression in different tissues are unclear at present [see Section 4.R.1], and that the precise function of the SMN complex, which is formed by SMN

9) Lumbar spinal cord, thoracic spinal cord, cervical spinal cord, motor cortex, and diencephalon (thalamus) tissues from 3 subjects who died in Study CS3A were analyzed.

protein, is unknown [see Section 3.R.1], safety related to (1) effects on the target gene should be determined carefully based on safety information from clinical studies.

- Serious adverse events occurred in many patients in clinical studies of nusinersen, most of which were related to disease progression. A causal relationship between the events and nusinersen was also ruled out [see Section 7.R.4.1 and Table 21 and Table 23].
- Furthermore, lumbar puncture-related adverse events, renal impairment, hepatic impairment, effects on blood coagulation system, gastrointestinal effects, and other adverse events [Sections 7.R.4.1 to 7.R.4.6] were assessed. At present, nusinersen is potentially associated with lumbar puncture-related adverse events, renal impairment, hepatic impairment, and coagulation abnormalities, but no other apparent risks associated with nusinersen have been suggested.
- As described above, because common laboratory animals lack the *SMN2* gene, safety related to (1) effects on the target gene cannot be evaluated in non-clinical studies. However, no specific risks associated with (1) effects on the target gene are anticipated at present, and (1) effects on the target gene are therefore unlikely to become a clinically relevant issue in humans.

The applicant's explanation on safety related to (2) hybridization-dependent off-target effects:

- Homologous sequences (continuous match of ≥15 bases) only were initially searched for transcripts to which nusinersen may bind (CTD 4.2.1.1-7). In the course of the regulatory review, however, PMDA pointed out that not only sequences with terminal mismatches, inserts, and gaps, but also sequences with internal mismatches, inserts, and gaps should have been searched. For this reason, analyses using Bowtie (*Genome Biol*. 2009; 10: R25) were performed again, regardless of the location of mismatches and gaps.[10)] The analyses identified 110 off-target candidate genes to which nusinersen may bind. Among these genes, 12 were selected for safety related to hybridization-dependent off-target effects.[11)] Events reported to be related to these genes and events of concern associated with their protein functions are shown in Table 7.

10) Human reference genome sequence dataset (version hg38) and comprehensive transcriptome database (Gencde v21) were used for analyses. Sequences with internal or terminal 2-base mismatches or gaps were searched in view of feasibility etc.

11) Pubmed gene (https://www.ncbi.nlm.nih.gov/gene/) and Online Mendelian Inheritance in Men (https://www.omim.org/) were searched to select genes that meet any of the 4 criteria listed below.
(1) A gene reported to be related to any disease or physiological effect
(2) A gene encoding a protein which is the target of a drug under development.
(3) A gene to be reported to be involved in decreased function induced by inhibited expression of a protein encoded by the gene.
(4) Oncogene or antioncogene

Table 7. Events reported to be related to the genes for which safety related to hybridization-dependent off-target effects should be evaluated and events of concern associated with their protein functions

Gene	Diseases reported to be related to the gene in humans, knockout mice, etc. and diseases of concern associated with its protein function
FIGLA	premature ovarian failure, male and female infertility
STAT4	systemic lupus erythematosus, infections, immune system disorder, abnormal insulin secretion, nephropathy
FOXP1	malignancies, mental retardation, malformation, bleeding, abortion, neonatal death, infections
MECOM	malignancies, radioulnar synostosis, thrombocytopenia, abortion, deafness, otitis media, infections, malformation, neonatal death, bleeding, growth failure, skin oedema
MSR1	Barrett's oesophagus, prostate cancer, arteriosclerosis, oesophageal carcinoma, Alzheimer's disease, infections
BNC2	skin discolouration, malformation
RSF1	hepatitis B, ovarian cancer, neonatal death, growth failure, metabolism and nutrition disorders, increased bone mineral content, malformation
TRDHE	metabolism and nutrition disorders
FREM2	Fraser syndrome, bleeding, neonatal death, abortion, malformation
RPGRIP1L	Joubert syndrome, Meckel-Gruber syndrome, retinal degeneration, malformation
ULK2	CNS disease
EFCAB6	male infertility, benign and malignant tumors

Information registered in Pubmed gene (https://www.ncbi.nlm.nih.gov/gene/), Online Mendelian Inheritance in Men (https://www.omim.org/), and Mouse Genome Informatics (http://www.informatics.jax.org/)

- The identified off-target candidate genes were assessed for safety concerns. Embryo-fetal developmental abnormalities including malformation in FOXP1, MECOM, BNC2, RSF1, and RPGRIP1L knockout mice (FOXP1, ventricular septal defect; MECOM, abnormal forebrain morphology; BNC2, decreased tongue size; RSF1, abnormal digit morphology; FREM2, neural tube defect; RPGRIP1L, exencephaly, etc.) have been reported. However, there should be low safety concerns about the effects of nusinersen on embryo-fetal development in humans because nusinersen does not cross the placenta [see Section 4.2.3].
- Many patients participating in clinical studies of nusinersen experienced serious adverse events, most of which were related to disease progression, and a causal relationship between the events and nusinersen was also ruled out [see Section 7.R.4.1 and Table 21 and Table 23]. Thus, hybridization-dependent off-target effects are unlikely to become a clinically relevant issue. The homology of the genes listed in Table 7 between humans and animals, and the occurrence of events etc. listed in Table 7 in clinical studies are currently being checked.

PMDA's view:

Safety related to (1) effects on the target gene (*SMN2*) has been discussed to a certain extent based on the currently available findings.

Safety related to (2) hybridization-dependent off-target effects

Whether nusinersen actually hybridizes with transcripts of the candidate genes identified by *in silico* analyses in the human body is unclear, and safety information from clinical studies is very limited. Given these points, normally, this issue should be investigated in non-clinical studies wherever possible. Gene expression analysis such as *in vitro* microarray analysis of human samples should have been performed to assess the potential for nusinersen to hybridize with transcripts of the candidate genes identified *in silico* in the human body. At present, clinical studies of nusinersen have raised no clear safety concerns, but a final conclusion on whether there is any risk anticipated from effects on the functions of the candidate genes should be drawn after reviewing the occurrence of associated adverse events. This matter will be discussed in the Review Report (2). Given that SMA (mainly Type I SMA) is a fatal, serious disease, marketing nusinersen without performing gene expression analysis of human samples is acceptable unless there have been major safety issues

in human at present. However, since 12 off-target candidate genes have been identified for nusinersen, the following measures should be taken:

- The genes listed in Table 7 include those related to teratogenicity or carcinogenicity, which are difficult to assess in clinical studies. Hence, safety should be evaluated considering the homology of the genes between humans and animal species used in non-clinical studies. If genes whose potential risk in humans cannot be ruled out are identified, information on the genes should be communicated to healthcare professionals in clinical practice.
- Gene expression analysis of human samples should be performed as soon as possible. If nusinersen affects the expression of the candidate genes in humans, the need for a relevant precaution in the package insert should be determined promptly.

A final decision will be made, taking account of comments from the Expert Discussion.

Safety related to (3) hybridization-independent off-target effects is discussed in Section 3.R.2.2.

3.R.2.2 Safety pharmacology

PMDA asked the applicant to explain safety in humans, taking account of the description in Section 3.R.2.1 and data from safety pharmacology studies of nusinersen.

The applicant's explanation:

(1) Effects on the target gene (*SMN2*) cannot be evaluated because no relevant species exists.

(2) Hybridization-dependent off-target effects are unlikely to become a relevant safety issue [see Section 3.R.2.1].

The applicant's explanation on (3) hybridization-independent off-target effects:

- Findings of CNS effects were decreased or absent lower spinal reflexes observed in cynomolgus monkey studies (CTD 4.2.3.1-1, CTD 4.2.3.2-1, CTD 4.2.3.2-2). In a 53-week, intermittent dose IT toxicity study in juvenile monkeys (CTD 4.2.3.2-2), there was an increase in the number of days required to complete the learning test[12)] in 1 male in the high dose group (7 males, 6 females) (CTD 4.2.3.2-2). This was not considered related to nusinersen [see Section 5.R.1]. Based on the above findings, the safety margin between the maximum dose used which produced no CNS effects (decreased or absent lower spinal reflexes) in cynomolgus monkeys and the recommended clinical dose (12-mg scaled equivalent dose in Table 20) in humans was 0.34- to 1.69-fold when the CSF nusinersen concentration[13)] at 4 months after the last dose in cynomolgus monkeys was compared with the CSF trough concentration[14)] in humans.

12) The learning test was performed using the Wisconsin General Testing Apparatus. In the test, monkeys learned the task (gaining a food reward for the correct choice among 3 wells) in several phases, and then had to make correct choices.

13) CSF nusinersen concentration at 4 months after a single IT dose of 3 mg of nusinersen in a single IT dose toxicity study in monkeys (CTD 4.2.3.1-1) (23 ng/mL, estimated based on the measured concentration at 8 days after the last dose and $t_{1/2}$ [102 days]); CSF nusinersen concentration at 4 months after the last dose in juvenile monkeys receiving multiple IT doses of 1 mg of nusinersen in a 14-week intermittent dose IT toxicity study (CTD 4.2.3.2-1) (5 ng/mL, estimated based on the measured concentration at 106 days after the last dose and $t_{1/2}$ [102 days]); CSF nusinersen concentration at 84 days after the last dose in juvenile monkeys receiving multiple IT doses of 1 mg of nusinersen in a 53-week intermittent dose IT toxicity study (CTD 4.2.3.2-2) (4.6 ng/mL, estimated from concentrations in the 4 mg/dose group, assuming linearity)

14) Compared with the CSF trough concentration (13.6 ng/mL) on Day 757 (126 days after the previous dose) in Cohort 2 in a foreign phase II study in SMA patients (CTD 5.3.5.2-1, Study CS3A).

- When the effects of other 2'-MOE modified ASOs [15)] on the hERG potassium channel current (cardiovascular effects) were assessed *in vitro*, there were no biologically meaningful, significant changes (CTD 4.3-39, *J Pharmacol Toxicol Methods*. 2014; 69: 49-60). Hence, an *in vitro* hERG study was considered unnecessary. When *in vivo* effects of nusinersen were assessed, there were no treatment-related effects in safety pharmacology studies of nusinersen. The safety margins based on the plasma C_{max} and $AUC_{0\text{-}24h}$[16)] between the maximum dose used which produced no cardiovascular effects in monkeys and the recommended clinical dose (12-mg scaled equivalent dose in Table 20) in humans were 1.4- to 2.8-fold and 1.2- to 2.3-fold, respectively.[17)]
- There were no treatment-related effects on the respiratory system in safety pharmacology studies of nusinersen. The safety margins based on the plasma C_{max} and $AUC_{0\text{-}24h}$[16)] between the maximum dose used which produced no respiratory effects in monkeys and the recommended clinical dose (12-mg scaled equivalent dose in Table 20)[17)] in humans were 1.4- to 2.8-fold and 1.2- to 2.3-fold, respectively.
- There were no treatment-related effects on the renal/urinary system in safety pharmacology studies of nusinersen. The safety margins based on the plasma C_{max} and $AUC_{0\text{-}24h}$[16)] between the maximum dose used which produced no renal/urinary effects in monkeys and the recommended clinical dose (12-mg scaled equivalent dose in Table 20) [17)] in humans were 1.4- to 2.8-fold and 1.2- to 2.3-fold, respectively.

PMDA's view:

No additional *in vitro* electrophysiological studies are necessary because safety pharmacology studies that assessed the effects on the cardiovascular system suggested no risks such as proarrhythmia and because QT/QTc interval prolongation and pro-arrhythmic risk in humans were evaluated and clinical studies of nusinersen showed no trend towards an increased incidence of pro-arrhythmic adverse events in the nusinersen group than in the sham-procedure control group [Section 6.R.3]. However, the method for assessment of QT/QTc interval prolongation and pro-arrhythmic effect in clinical studies should have been determined after conducting an *in vitro* electrophysiological study of nusinersen prior to the initiation of clinical studies, for the following reasons: (i) nusinersen may affect the potassium channel etc. via hybridization-independent off-target effects, (ii) administration of nusinersen results in systemic exposure in humans [see Section 6.2], (iii) the detailed function of the SMN complex is unknown, and (iv) there are limitations to predicting the risk of QT prolongation associated with nusinersen based on data from studies with other oligonucleotides.

CNS effects should be discussed in Section 5.R.1 based on the results from toxicity studies and clinical studies. Because the safety margin for effects on the respiratory and renal/urinary systems was close to 1, these effects should be discussed in Sections 7.R.4.1 and 7.R.4.3 based on the results from clinical studies.

15) ISIS 116847 (ASO targeting PTEN), ISIS 141923 (scramble ASO), ISIS 345198 (ASO targeting GCCR), ISIS 353512 (ASO targeting CRP), ISIS 379804 (ASO targeting ApoB), ISIS 420476 (ASO targeting GCCR), ISIS 487660 (ASO targeting α1-antitrypsine)

16) Plasma C_{max} (2.61 μg/mL) and $AUC_{0\text{-}24h}$ (14.6 μg·h/mL) after a single IT dose of 7 mg of nusinersen in a single IT dose toxicity study in monkeys (CTD 4.2.3.1-1); plasma C_{max} (1.97 μg/mL) and $AUC_{0\text{-}24h}$ (12.0 μg·h/mL) after the first dose in juvenile monkeys receiving multiple IT doses of 3 mg of nusinersen in a 14-week intermittent dose IT toxicity study (CTD 4.2.3.2-1); plasma C_{max} (3.74 μg/mL) and $AUC_{0\text{-}24h}$ (22.8 μg·h/mL) after the first dose in juvenile monkeys receiving multiple IT doses of 4 mg of nusinersen in a 53-week intermittent dose IT toxicity study (CTD 4.2.3.2-2)

17) Plasma C_{max} (1.36 μg/mL) and $AUC_{0\text{-}24h}$ (10.0 μg·h/mL) after the first dose in infants aged 0 to 3 months receiving multiple 12-mg scaled equivalent (Table 20) IT doses of nusinersen in a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B).

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

The applicant submitted non-clinical pharmacokinetic data including the results from absorption, distribution, metabolism, and excretion studies of nusinersen in mice, rabbits, and monkeys.

CSF and plasma concentrations of nusinersen in monkeys were determined by Hyb-ELISA (lower limit of quantitation [LLOQ], 1.5 ng/mL) or Hyb-ECL (LLOQ, 0.05 ng/mL), and tissue concentrations of nusinersen were determined by Hyb-ELISA (LLOQ, 15.0 μg/g).[18)] Tissue concentrations of nusinersen in mice and rabbits were determined by HPLC (LLOQ, 10.0 μg/g). Concentrations of nusinersen metabolites/degradation products[19)] were determined in CSF and plasma by LC-MS/MS (LLOQ, 25.0 ng/mL) and in the kidney cortex and other tissues[20)] by LC-MS (LLOQ, 10.0 μg/g). Anti-nusinersen antibodies in plasma were measured by ELISA (LLOQ, 60 ng/mL).

Unless otherwise specified, doses are expressed in terms of nusinersen and concentrations are expressed in terms of nusinersen sodium. The t_{max} is expressed as the median and other pharmacokinetic parameters are expressed as the mean ± standard deviation (SD).

4.1 Absorption

4.1.1 Single-dose study (CTD 4.2.3.1-1)

Following a single IT bolus dose of 1, 3, or 7 mg of nusinersen in male and female monkeys (3-8/sex/group), the CSF nusinersen concentrations on Day 7 (males/females) were 9.9 ± 3.5/10.5 ± 6.9, 56.6 ± 49.6/35.2 ± 44.7, and 79.9 ± 3.1/48.3 ± 45.1 ng/mL, respectively. At 3 mg, the CSF C_{max} of nusinersen was 410 ± 77/893 ± 509[21)] μg/mL, the $AUC_{0\text{-}24h}$ was 1303 ± 253/2205 ± 1058[21)] μg·h/mL, and the CL was 2.21 ± 0.53/1.63 ± 0.97 mL/h. Plasma pharmacokinetic parameters of nusinersen are shown in Table 8. There were no consistent gender-related differences.

Table 8. Plasma pharmacokinetic parameters of nusinersen in monkeys after single IT bolus dose

Dose level	C_{max} (μg/mL)		t_{max} (h)[a)]		$AUC_{0\text{-}24h}$ (μg·h/mL)	
	Males	Females	Males	Females	Males	Females
1 mg	0.16 ± 0.03 (3)	0.28 ± 0.18 (3)	6 (3)	4 (3)	1.92 ± 0.49 (3)	2.14 ± 0.37 (3)
3 mg	0.44 ± 0.17 (8)	0.48 ± 0.29 (5)[b)]	4 (8)	4 (5)[b)]	4.76 ± 1.49 (8)	4.10 ± 2.42 (5)[b)]
7 mg	1.21 ± 0.48 (3)	4.02 ± 1.77 (3)	4 (3)	2 (3)	12.0 ± 3.85 (3)	17.1 ± 3.46 (3)

Mean ± SD (No. of animals assessed)

a) Median (No. of animals assessed)

b) Due to rapid transfer of nusinersen from CSF to plasma, abnormal CSF and plasma nusinersen concentrations over time were observed in 2 animals compared to others. The C_{max} (2.95 and 2.96 μg/mL), t_{max} (1 and 1 h), and $AUC_{0\text{-}24h}$ (5.80 and 6.30 μg·h/mL) in these 2 animals were excluded from analysis.

18) Monkey plasma, CSF, or tissue (liver, brain, spinal cord) was spiked with a metabolite ("o" [3'-or 5'-deletion]) 30.0 ng/mL (plasma and CSF) or 400 ng/g (tissue), and metabolite concentrations were determined by Hyb-ELISA method used for determination of nusinersen concentrations. A similar reaction occurred with the metabolite (65.0%-97.7% of nusinersen), suggesting that this assay lacks adequate specificity. When metabolite concentrations in CSF, plasma, and tissues after administration of nusinersen (CTD 4.2.3.2-1) were measured by LC-MS/MS, the major metabolite was "o" (3'-deletion), and the relative abundance of "o" (3'-deletion) in CSF, plasma, and tissue was 0%, up to 4.1%, and up to 21.6% of the total oligonucleotides, respectively [see Section 4.3]. Thus, the applicant discussed that the effects of metabolites are insignificant at least in CSF and plasma nusinersen concentrations.

19) Deletion of nucleotides from the 3' end ("o," "p," "q," "r," "s"); and deletion of nucleotides from the 5' end ("o," "p," "q," "r")

20) The relative abundance of each metabolite in the brain, spinal cord, and liver was expressed as % total peak area of the chromatogram (including the peak area of nusinersen).

21) Due to rapid transfer of nusinersen from CSF to plasma, abnormal CSF and plasma nusinersen concentrations over time were observed in 2 animals compared to others. The C_{max} (321 and 455 μg/mL) and $AUC_{0\text{-}24h}$ (213 and 231 μg·h/mL) in these 2 animals were excluded from analysis.

4.1.2 Repeated-dose studies

Male monkeys (17 monkeys for IT administration, 3 monkeys for IV administration) received either 4 weekly IT bolus doses or 4 weekly IV bolus doses of nusinersen at 1 mg/dose. The plasma C_{max} values of nusinersen after the last dose in the IT and IV bolus injection groups were 0.154 ± 0.114 and 0.367 ± 0.044 μg/mL (n = 17 and n = 3), respectively, and the $AUC_{0\text{-}24h}$ values were 1.53 ± 0.44 and 0.72 ± 0.07 μg·h/mL (n = 17 and n = 3), respectively. The nusinersen trough concentrations in CSF on Days 8 and 22 in the IT bolus injection group were 0.116 ± 0.146 and 0.134 ± 0.088 μg/mL (n = 15 for both), respectively, indicating accumulation with multiple dosing. In addition, CSF and plasma nusinersen concentrations were measured multiple times during the 1 year after the last dose for 4 animals in the IT bolus injection group. The CSF and plasma nusinersen concentrations at 1 hour after the last dose in these animals were 118.8 ± 30.4 and 0.03 ± 0.02 μg/mL, respectively, and the $t_{1/2}$ values were 100.2 ± 57.4 days (n = 4) and 91.4/109 days (n = 2), respectively (Reference data CTD 4.2.2.2-1).

Nusinersen was administered to male and female juvenile monkeys 9 to 10 months of age (7-9 females/group, 6-8 males/group) by IT bolus injection at doses of 0.3, 1, and 3 mg for 14 weeks.[22)] The plasma C_{max} values of nusinersen on Day 99 (males/females) were 0.09 ± 0.03/0.14 ± 0.08, 0.51 ± 0.20/0.60 ± 0.03, and 1.55 ± 0.30/1.57 ± 0.25 μg/mL, respectively, and the $AUC_{0\text{-}24h}$ values were 0.85 ± 0.14/1.20 ± 0.48, 3.45 ± 0.97/3.56 ± 0.41, and 9.92 ± 2.46/12.1 ± 1.70 μg·h/mL, respectively. The $t_{1/2}$ could not be calculated for the nusinersen 0.3 and 1 mg/dose groups, and was 34.5 ± 22.4 days (n = 4) in the nusinersen 3 mg/dose group. The nusinersen trough concentrations in CSF are shown in Table 9 (CTD 4.2.3.2-1).

Table 9. Nusinersen trough concentrations in CSF in juvenile monkeys after multiple IT bolus doses

	Time point	CSF nusinersen concentration (ng/mL)			
		Cistema magna		Lumbar	
		Males	Females	Males	Females
0.3 mg/dose	Day 36	3.95 ± 3.43 (3)	5.49 ± 3.49 (3)	6.82 ± 10.4 (3)	2.97, 28.7 (2)
	Day 106	16.0 ± 5.44 (3)	15.1 ± 8.51 (3)	11.2, 12.2 (2)	7.81, 29.7 (2)
1 mg/dose	Day 36	57.4 ± 52.8 (3)	41.7 ± 46.3 (3)	0, 68.2(2)	16.1 ± 8.76 (3)
	Day 106	28.5 ± 10.6 (3)	15.9 ± 7.93 (3)	6.74, 20.6 (2)	5.21 (1)
3 mg/dose	Day 36	82.5 ± 54.4 (3)	12.1, 72.4 (2)	57.8 ± 7.88 (3)	55.4 (1)
	Day 106	108 ± 29.5 (3)	161 ± 107 (4)	25.4, 47.2 (2)	161 (1)

Mean ± SD (No. of animals assessed). Individual values are listed for n ≤2.

Nusinersen was administered to male and female juvenile monkeys 9 to 11 months of age (5-7/sex/group) by IT bolus injection at doses of 0.3, 1, and 4 mg for 53 weeks.[23)] Plasma pharmacokinetic parameters of nusinersen are shown in Table 10. On Day 1, the C_{max} and AUC tended to increase more than dose-proportionally. The nusinersen trough concentrations in CSF (males/females) were 21.1 ± 22.6/7.0 ± 2.9, 11.5 ± 4.7/15.3 ± 8.1, and 11.3 ± 6.9/25.5 ± 10.5 ng/mL, respectively, on Day 8, and 14.5 ± 15.1/9.5 ± 3.0, 17.4 ± 4.6/29.1 ± 22.8, and 42.0 ± 20.2/61.5 ± 49.6 ng/mL, respectively, on Day 372. The $t_{1/2}$ could not be calculated for the nusinersen 0.3 and 1 mg/dose groups, and was 52.9/55.8 days (n = 2) in the nusinersen 4 mg/dose group. Anti-nusinersen antibodies in plasma were detected in 6.3% (3 of 48) of animals (1 animal in the nusinersen 1 mg/dose group, 2 animals in the nusinersen 4 mg/dose group) (CTD 4.2.3.2-2).

22) Animals at 0.3 and l mg/dose received 5 weekly doses (on Study Days 1, 8, 15, 22, and 29) followed by biweekly dosing (on Days 43, 57, 71, 85, and 99). Animals at 3 mg/dose received weekly dosing (15 doses in total).

23) Nusinersen was administered once weekly for the first 5 doses (on Days 1, 8, 15, 22, and 29) followed by a dose every 6 weeks thereafter (on Days 71, 113, 155, 197, 239, 281, 323, and 365).

Table 10. Plasma pharmacokinetic parameters of nusinersen in juvenile monkeys after multiple IT bolus doses

	Time point	C_{max} (µg/mL)		t_{max} (h)[a)]		$AUC_{0\text{-}168h}$ (µg·h/mL)	
		Males	Females	Males	Females	Males	Females
0.3 mg	Day 1	0.15 ± 0.02 (5)	0.09 ± 0.02 (5)	2 (5)	4 (5)	1.24 ± 0.24 (5)	1.00 ± 0.10 (5)
	Day 365	0.11 ± 0.07 (5)	0.06 ± 0.02 (5)	1 (5)	2 (5)	0.78 ± 0.39 (5)	0.65 ± 0.06 (5)
1 mg	Day 1	0.59 ± 0.21 (5)	0.55 ± 0.22 (5)	2 (5)	2 (5)	4.61 ± 1.18 (5)	4.49 ± 1.02 (5)
	Day 365	0.49 ± 0.34 (5)	0.42 ± 0.17 (5)	4 (5)	2 (5)	5.06 ± 5.68 (5)	2.82 ± 0.43 (5)
4 mg	Day 1	4.97 ± 1.47 (7)	2.51 ± 0.86 (7)	2 (7)	4 (7)	30.1 ± 9.23 (7)	20.9 ± 4.92 (7)
	Day 365	1.61 ± 0.38 (7)	1.42 ± 0.68 (7)	2 (7)	2 (7)	12.1 ± 2.86 (7)	11.3 ± 2.54 (7)

Mean ± SD (No. of animals assessed)
a) Median

4.2 Distribution

4.2.1 Tissue distribution

Following a single IT bolus dose of 7 mg of nusinersen in male and female monkeys, the CNS tissue and liver concentrations were determined. On Day 8, the highest nusinersen concentration was measured in the liver followed by the lumbar spinal cord, thoracic spinal cord, and brain cortex (CTD 4.2.3.1-1).

Male monkeys received 4 weekly IT doses of 1 mg of nusinersen, and nusinersen concentrations in the CNS tissues, skeletal muscle, kidney cortex, and liver were determined. On Day 29, the highest nusinersen concentration was measured in the lumbar spinal cord followed by the kidney cortex, thoracic spinal cord, and cervical spinal cord (reference data, CTD 4.2.2.2-1).

Nusinersen was administered to male and female juvenile monkeys 9 to 10 months of age by IT bolus injection at a dose of 3 mg for 14 weeks,[22)] and nusinersen concentrations in the CNS tissues and liver were determined. On Day 106, the highest nusinersen concentration was measured in the lumbar spinal cord followed by the brain cortex, liver, and thoracic spinal cord (CTD 4.2.3.2-1).

Nusinersen was administered to male and female juvenile monkeys 9 to 11 months of age by IT bolus injection at doses of 0.3, 1, and 4 mg for 53 weeks.[23)] Nusinersen concentrations in different tissues on Days 372 and 554 and the estimated terminal phase elimination half-lives are shown in Table 11 (CTD 4.2.3.2-2).

Table 11. Nusinersen concentrations in different tissues on Days 372 and 554 and estimated terminal phase elimination half-lives in juvenile monkeys after multiple IT bolus doses

	Day 372 (n = 10/group)			Day 554 (n = 4)	Estimated terminal phase elimination half-life (Day)
	0.3 mg/dose	1 mg/dose	4 mg/dose	4 mg/dose	
Cerebellum	4.3 ± 1.4	20.8 ± 16.0	39.6 ± 19.7	34.5 ± 23.2	-[a)]
Brain cortex	4.2 ± 1.9	16.6 ± 6.0	71.3 ± 29.3	43.8 ± 4.4	-[a)]
Hippocampus	10.1 ± 2.8	28.9 ± 10.3	88.9 ± 18.0	46.0 ± 7.8	191[b)]
Pons	4.1 ± 3.1	8.8 ± 4.0	34.2 ± 15.5	16.6 ± 4.3	174[b)]
Cervical spinal cord	6.7 ± 2.8	15.9 ± 5.1	35.1 ± 11.0	18.4 ± 6.1	195[b)]
Thoracic spinal cord	14.0 ± 9.3	23.0 ± 10.6	59.8 ± 11.7	20.3 ± 5.6	117[b)]
Lumbar Spinal Cord	21.4 ± 9.3	53.5 ± 33.6	99.0 ± 71.5	39.7 ± 10.8	138[b)]
Kidney cortex	23.7 ± 17.6	42.8 ± 19.0	239 ± 176	6.2 ± 3.5	34.5[b)]
Liver	0.9 ± 0.4	2.8 ± 1.6	21.9 ± 9.2	0.1 ± 0.1	23.4[b)]

Mean ± SD
a) No value reported due to insufficient decrease in concentration over the evaluated time interval.
b) Half-life estimated using tissue concentrations from Day 372 to 554.

4.2.2 Plasma protein binding

Nusinersen (0.1 or 5 μg/mL) was added to mouse and monkey plasma, and protein binding was determined using the ultrafiltration method. The plasma protein binding was 96.0% to 96.6% in mouse plasma and 97.0% to 99.4% in monkey plasma (reference data, CTD 5.3.2.1-1; reference data, CTD 5.3.2.1-3).

Nusinersen (5 or 150 μg/mL) was added to monkey CSF, and protein binding was determined using the ultrafiltration method. The protein binding in CSF was 0% to 3.0% (reference data, CTD 5.3.2.1-2; reference data, CTD 5.3.2.1-3).

4.2.3 Placental transfer

Nusinersen was administered subcutaneously at doses of 3, 10, and 25 mg/kg every other day to female mice for 2 weeks prior to mating, during the mating period, and until gestation day 15. At all dose levels, nusinersen concentrations in the fetal liver on gestation day 18 were below the LLOQ. The maternal placental tissue concentrations of nusinersen at 3 and 10 mg/kg were below the LLOQ. At 25 mg/kg, the maternal placental tissue concentration was 2.3% of the maternal liver concentration (CTD 4.2.3.5.1-1).

Rabbits were given subcutaneously 6, 12.6, or 25 mg/kg nusinersen every other day from gestation day 6 through gestation day 18. At all dose levels, nusinersen concentrations in the fetal liver on gestation days 20 and 28 were below the LLOQ. The maternal placental tissue concentration was ≤8.2% of the maternal liver concentration (CTD 4.2.3.5.2-2).

4.3 Metabolism (reference data, CTD 5.3.1.4-8)

Nusinersen was administered to juvenile monkeys 9 to 10 months of age by IT bolus injection at a dose of 3 mg once weekly for 14 weeks[22)] (CTD 4.2.3.2-1), and the relative abundance of intact nusinersen and metabolites in CSF (Day 36) and plasma (Days 1, 36, and 99) were determined by LC-MS/MS. Intact nusinersen was only detected in CSF, and no metabolites were present. In plasma, intact nusinersen and "o" (3'-deletion) were detected (relative abundance[24)] on Days 1, 36 and 99, 0% to 4.1%).

Nusinersen was administered to juvenile monkeys 9 to 10 months of age by IT bolus injection at a dose of 3 mg once weekly for 14 weeks[22)] (CTD 4.2.3.2-1), and the relative abundance of intact nusinersen and metabolites in the liver, brain cortex, and lumbar spinal cord on Day 106 were determined (by LC-MS). The predominant metabolite detected in the tissues was "o" (3'-deletion), and its relative abundance[24)] was 10.1% to 21.6%. Nusinersen was administered to juvenile monkeys 9 to 11 months of age by IT bolus injection at a dose of 4 mg for 53 weeks[23)] (CTD 4.2.3.2-2), and the relative abundance of intact nusinersen and metabolites in the kidney cortex on Day 372 was determined (by LC-MS). The predominant metabolite detected in the tissue was "o" (3' deletion), and its relative abundance[24)] was 12.3% to 15.6%.

24) "o" (3'-deletion) as a percentage of the total oligonucleotides (%)

4.4 Excretion

4.4.1 Urinary and fecal excretion

Urinary or fecal excretion of nusinersen was not evaluated. Other 2'-MOE modified ASOs having the same backbone structure as nusinersen, have been shown to be hydrolyzed by 3' and 5' exonucleases and then excreted primarily in the urine, with limited fecal excretion (*Drug Metab Dispos.* 2003; 31: 1419-28, *Expert Opin Drug Metab Toxicol.* 2009; 5: 381-91). The applicant therefore discussed that urinary excretion is likely to represent the major pathway for nusinersen as well.

4.4.2 Excretion in milk (CTD 4.2.3.5.3-1)

Pregnant mice were given subcutaneously 5, 20, or 60 mg/kg/week (1.4, 5.7, or 17.1 mg/kg/dose, respectively) of nusinersen every other day from gestation day 6 through gestation day 16 and once weekly until post-parturition day 18. On post-parturition day 13, the nusinersen concentration in milk was 0.0220% to 0.0227% of the maternal liver concentration of nusinersen.

4.R Outline of the review conducted by PMDA

4.R.1 Tissue accumulation of nusinersen and safety in humans

PMDA asked the applicant to explain safety in tissues into which nusinersen distributes in high concentrations after administration.

The applicant's explanation:

It was decided to file a marketing application for nusinersen, based on the results of an interim analysis for a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B) [see Section 7.R.3.1]. Hence, a systemic tissue distribution study of radiolabeled nusinersen in rats (Study APK02) is still ongoing.

- Following intravenous or subcutaneous administration of other 2'-MOE modified ASOs which have the same backbone structure as and a different sequence from nusinersen, all 2'-MOE modified ASOs were distributed primarily to the liver and kidney. They were also present in the spleen, lymph nodes, thyroid gland, parathyroid gland, stomach, and bone marrow.[25)] The above findings suggest that the pharmacokinetics of 2'-MOE modified ASOs are almost sequence-independent, and that nusinersen is also likely to be distributed into these tissues.
- When administered into systemic circulation, 2'-MOE modified ASOs do not cross the blood-brain-barrier. Thus, distribution from the systemic circulation to the CNS tissues is not expected (*Adv Drug Deliv Rev*. 2015; 87: 46-51). Nusinersen administered by intrathecal injection is expected to be distributed into the CNS tissues.
- Nusinersen concentrations in the CNS tissues, liver, kidney cortex, and skeletal muscle were determined in non-clinical studies of nusinersen [see Section 4.2.1]. Nusinersen were distributed into the CNS tissues, liver, and kidney cortex. Nusinersen was detected in the CNS tissues and kidney cortex up to 189 days after the last dose (Table 11). Concentrations of nusinersen in the liver increased more than dose-proportionally. Nusinersen was hardly distributed into the skeletal muscle.

25) *Drug Metab Dispos.* 2003; 31: 1419-28, *Drug Metab Dispos.* 2007; 35: 460-8, *Adv Drug Deliv Rev*. 2015; 87: 46-51, *J Pharm Sci*. 2004; 93: 48-59

The applicant further provided the following explanation on safety in these tissues, taking account of the findings from non-clinical studies and the occurrence of adverse events in Japanese and foreign clinical studies:

- **CNS tissues**

  The significant findings observed in toxicity studies in monkeys (CTD 4.2.3.2-1, CTD 4.2.3.2-2) were hippocampal neuronal vacuolation and necrotic cells. On the other hand, clinical studies showed no trend towards a higher incidence of CNS adverse events in nusinersen-treated subjects [see Section 5.R.1].

- **Liver, kidney, and stomach**

  There were no significant findings in non-clinical studies. Clinical studies did not indicate that the incidence of hepatic impairment, renal impairment, or gastrointestinal adverse events and hepatic or renal laboratory changes can become major safety issues [see Sections 7.R.4.3, 7.R.4.4, and 7.R.4.6].

- **Spleen, lymph nodes, and bone marrow**

  While non-clinical studies produced no significant findings in the bone marrow, vacuolated macrophages were present in the lymph nodes in a 13-week intermittent dose subcutaneous toxicity study in juvenile mice (CTD 4.2.3.2-3). In a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), the incidences of lymph node- and bone marrow-related adverse events[26] were 7.3% (3 of 41 subjects) in the sham-procedure control group and 3.8% (3 of 80 subjects) in the nusinersen group.and no serious adverse events were observed. Although the main adverse event was ecchymosis whose incidence was higher in the nusinersen group (0% [0 of 41 subjects] in the sham-procedure control group, 2.5% [2 of 80 subjects] in the nusinersen group), the effects of nusinersen on the coagulation system are unlikely to become a major safety issue [see Section 7.R.4.5]. In Study CS3B, no spleen[27]-related adverse events were reported.

- **Thyroid gland and parathyroid gland**

  There were no significant findings in non-clinical studies. No thyroid-related adverse events[28] or parathyroid-related adverse events[29] were reported in Study CS3B.

The applicant's explanation based on the above:

Although a systemic tissue distribution study of nusinersen is currently ongoing, nusinersen distribution was predicted, utilizing the results of tissue distribution studies of other 2'-MOE modified ASOs as well to assess safety in the tissues where nusinersen accumulates. Currently available data indicate that a clinically relevant safety issue is unlikely to arise in humans.

PMDA's view:

Information on systemic tissue distribution is important. If the regulatory submission based on the results of an interim analysis for Study CS3B was intended, the study plan should have been developed so as to obtain necessary non-clinical data before the regulatory submission. However, tissue distribution of nusinersen can be evaluated/examined with the currently available data, based on the clinical positioning of nusinersen and the following reasons: (1) systemic tissue distribution of other 2'-MOE modified ASOs has been examined, (2) nusinersen distribution to the CNS tissues, liver, kidney cortex, etc. (the major tissues where nusinersen is

26) Events coded to the MedDRA SOC "Blood and lymphatic system disorders," HLGT "Spleen, lymphatic and reticuloendothelial system disorders," and HLTs "Bone marrow and immune tissue imaging procedures" and "Bone marrow and immune tissue histopathology procedures"
27) Events coded to the MedDRA HLT "Spleen disorders"
28) Events coded to the MedDRA HLGT "Thyroid gland disorders" and HLT "Thyroid analyses"
29) Events coded to the MedDRA HLGT "Parathyroid gland disorders" and HLT "Parathyroid analyses"

expected to accumulate) has been studied, and (3) adverse events targeting the specific organs were very rare in clinical studies. The applicant should complete a systemic tissue distribution study of radiolabeled nusinersen in rats as soon as possible and submit the study results to PMDA.

In addition, the applicant should ensure that the package insert includes appropriate precautions about effects on the major tissues where nusinersen is expected to accumulate (especially, effects on the liver function, renal function, and coagulation system) and should collect information via post-marketing surveillance[see Sections 7.R.4.4, 7.R.4.3, and 7.R.4.5]. Although no clear CNS effects were observed in clinical studies, findings of toxicological significance such as hippocampal vacuolation observed in juvenile monkey toxicity studies should be listed in the package insert to provide information to healthcare professionals [see Section 5.R.1]. Effects on the spleen, thyroid gland, parathyroid gland, and stomach are unlikely to raise clinically relevant safety issues, based on the presented non-clinical and clinical data. The applicant should collect information on safety in the tissues/organs where nusinersen accumulates via post-marketing surveillance, taking also account of information on systemic tissue distribution of nusinersen that will become available.

5. Toxicity and Outline of the Review Conducted by PMDA

The applicant submitted the results from toxicity studies of nusinersen (single-dose toxicity, repeated-dose toxicity, genotoxicity, reproductive and developmental toxicity, and other toxicity studies [an impurity study, a study on hippocampal vacuolation]). Some non-GLP studies were evaluated as reference data. Unless otherwise specified, doses are expressed in terms of nusinersen.

5.1 Single-dose toxicity

5.1.1 Single IT dose toxicity study in monkeys (CTD 4.2.3.1-1)

Cynomolgus monkeys (2-3/sex/group) were given single IT bolus injections of nusinersen 0 (vehicle[30]), 1, 3, or 7 mg. There were no nusinersen-related deaths. The clinical signs observed were decreased body temperature and respiratory rate and transient deficits in spinal reflexes (cutaneous, tail, limb) in 1 male at 7 mg. On basis of the above findings, the applicant determined that the approximate lethal dose was >7 mg/dose (1.9 mg/kg in males, 2.4 mg/kg in females).

5.1.2 Acute toxicity assessment using rodents

No single-dose toxicity studies in rodents were conducted. Acute toxicity was assessed in a 13-week intermittent dose subcutaneous toxicity study in juvenile mice (CTD 4.2.3.2-3). There were no nusinersen-related effects.

5.2 Repeated-dose toxicity

A repeated subcutaneous dose toxicity study in mice (13 weeks) and repeated IT dose toxicity studies in monkeys (14 weeks, 53 weeks) were conducted. The main target organ of intrathecally administered nusinersen was the hippocampus (neuronal vacuolation and necrosis, glial cell necrosis). The safety margins between the no observed adverse effect level (NOAEL) in monkeys (0.3 mg/dose) and the

30) Artificial CSF (150 mM Na, 3.0 mM K, 1.4 mM Ca, 0.8 mM Mg, 1.0 mM P, 155 mM Cl)

recommended clinical dose (12 mg scaled equivalent dose in Table 20) in humans based on the CSF trough concentration and the cumulative dose (scaled by brain weight) were 0.10-fold[31)] and 1.1-fold,[32)] respectively.

5.2.1 Repeated-dose toxicity study in mice

5.2.1.1 Thirteen-week intermittent dose subcutaneous toxicity study in juvenile mouse (CTD 4.2.3.2-3)

Nusinersen was administered subcutaneously to juvenile mice on postnatal day (PND) 4 (CD-1, 10/sex/group) at doses of 0 (vehicle[33)]), 1, 10, and 50 mg/kg for 13 weeks.[34)] There were no nusinersen-related deaths. Histopathological examination revealed inflammation at the injection sites in the 10 and 50 mg/kg groups and Kupffer cell hypertrophy in the liver (males only), vacuolated macrophages in the lymph nodes, and basophilic granules within the proximal tubular epithelium of the kidneys in the 50 mg/kg group. On the basis of the above findings, the applicant determined that the NOAEL was 50 mg/kg because the observed findings were all related to class effects of oligonucleotides and were of low toxicological significance, as previously reported with other 2'-MOE modified ASOs.[35)]

5.2.2 Repeated-dose toxicity studies in monkeys

5.2.2.1 Fourteen-week intermittent dose IT toxicity study in juvenile monkey (CTD 4.2.3.2-1)

Nusinersen was administered to juvenile cynomolgus monkeys 9 to 10 months of age (7-9 females/group, 6-8 males/group) by IT bolus injection at doses of 0 (vehicle[30)]), 0.3, 1, and 3 mg for 14 weeks.[22)] There were no nusinersen-related deaths. Clinical signs observed were transient deficits in spinal reflexes (cutaneous, hindlimb, tail). Histopathological examination revealed hippocampal neuronal vacuolation and neuronal and glial cell necrosis at 3 mg/dose. Hippocampal neuronal vacuolation was still present following 12 weeks of recovery. On the basis of the above findings, the applicant determined that the NOAEL was 1 mg/dose.

5.2.2.2 Fifty-three-week intermittent dose IT toxicity study in juvenile monkey (CTD 4.2.3.2-2)

Nusinersen was administered to juvenile cynomolgus monkeys 9 to 11 months of age (5-7/sex/group) by IT bolus injection at doses of 0 (vehicle[30)]), 0.3, 1, and 4 mg for 53 weeks.[23)] There were no nusinersen-related deaths. Clinical signs observed were transient deficits in spinal reflexes (patellar tendon, hindlimb, anal) at 4 mg/dose. Histopathological examination revealed hippocampal neuronal vacuolation in males at ≥1 mg/dose and females at 4 mg/dose, and necrotic cells and cellular debris in the hippocampus in males at ≥1 mg/dose. Hippocampal vacuolation was still present following 26 weeks of recovery. In a learning test,[12)] nusinersen had no effects on learning and memory. On the basis of the above findings, the applicant determined that the NOAEL was 0.3 mg/dose.

---

31) The CSF trough concentration (13.6 ng/mL) on Day 757 (126 days after the previous dose) in Cohort 2 in a foreign phase II study (CTD 5.3.5.2-1, Study CS3A) was compared with the CSF nusinersen concentration 84 days after the last dose (1.4 ng/mL, estimated from concentrations in the 4 mg/dose group, assuming linearity) in juvenile monkeys receiving repeated IT doses of 0.3 mg of nusinersen in a 53-week intermittent dose IT toxicity study (CTD 4.2.3.2-2).

32) The cumulative dose of nusinersen during the first year of treatment before maintenance dosing in humans (72 mg) was compared with 20 × the yearly cumulative dose of 3.9 mg (78 mg) in a 53-week intermittent dose IT toxicity study in juvenile monkeys (CTD 4.2.3.2-2) (using the NOAEL of 0.3 mg/dose and a 20-fold difference in brain weight between species).

33) Saline

34) Weekly for the first 4 doses (on PNDs 4, 11, 18, and 25) and biweekly thereafter (on PNDs 39, 53, 67, 81, and 95).

35) Antisense Drug Technology: Toxicologic Properties of 2'-Methyoxyethyl Chimeric Antisense Inhibitors in Animal and Man. CRC Press; 2008. p.327-62

5.3 Genotoxicity

Nusinersen was tested in *in vitro* assays (bacterial reverse mutation assay [CTD 4.2.3.3.1-1], chromosomal aberration assay in Chinese hamster ovary cells [CTD 4.2.3.3.1-2]), and *in vivo* assay (mouse bone marrow micronucleus assay [CTD 4.2.3.3.2-1]), all of which produced negative results. Thus, the applicant considers that nusinersen is unlikely to be genotoxic.

5.4 Carcinogenicity

Since SMA (mainly Type I SMA) is a fatal, serious disease, no carcinogenicity data were submitted in the present application, based on the "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 the "Revision of Guidelines for Carcinogenicity Studies of Drugs" (PFSB/ELD Notification No. 1127001 dated November 27, 2008) [see Section 5.R.3].

5.5 Reproductive and developmental toxicity

A fertility and embryo-fetal development study in mice, an embryo-fetal development study in rabbits, and a mouse study for effects on pre- and postnatal development, including maternal function, were conducted. All studies showed no nusinersen-related effects on the offspring.

5.5.1 Fertility and embryo-fetal development study (CTD 4.2.3.5.1-1)

Nusinersen was administered subcutaneously every other day at doses of 0 (vehicle[33)]), 3, 10, and 25 mg/kg to mice (CD-1, 25/sex/group). Males were dosed for 4 weeks prior to mating, throughout the mating period, and until necropsy, and females were dosed for 2 weeks prior to mating, throughout the mating period, and until gestation day 15. There were no nusinersen-related deaths. Effects on parental animals noted at 25 mg/kg/dose were vacuolation of Leydig cells in the testes, and infiltration of macrophages in the epididymis, prostate gland, and seminal vesicles. There were no effects on fertility, sperm concentration, sperm motility, the number of corpora lutea, or the number of implantations. In the fetuses, there were no effects on fetal viability or body weight, nor were there any external, skeletal, or visceral findings, etc. On the basis of the above findings, the applicant determined that the NOAEL for parental general toxicity and fertility and embryo-fetal development was 25 mg/kg/dose (87.5 mg/kg/week) because the findings observed in the paternal testes have been reported with other 2'-MOE modified ASOs as findings of low toxicological significance.[35)]

5.5.2 Embryo-fetal development study (CTD 4.2.3.5.2-2)

Pregnant rabbits (NZW, 17 or 18/group) were dosed subcutaneously with 0 (vehicle[33)]), 6, 12.6, or 25 mg/kg of nusinersen every other day from gestation day 6 through gestation day 18. There were no nusinersen-related deaths. No nusinersen-related effects on maternal animals or embryo-fetal development (malformations, variations, etc.) were observed, taking account of the laboratory's historical data. On the basis of the above findings, the applicant determined that the NOAEL for maternal toxicity and embryo-fetal development was 25 mg/kg/dose (87.5 mg/kg/week).

5.5.3 Study for effects on pre- and postnatal development, including maternal function (CTD 4.2.3.5.3-1)

Pregnant mice (CD-1, 10/group) were dosed subcutaneously with 0 (vehicle[33)]), 5, 20, or 60 mg/kg/week of nusinersen every other day from gestation day 6 through gestation day 16 (1.4, 5.7, or 17.1 mg/kg/dose, respectively) and weekly until post-parturition day 18. There were no nusinersen-related deaths. No effects on maternal animals or F1 pups (physical development, spontaneous motor activity, learning/memory, sexual maturation, etc.) were observed. On the basis of the above findings, the applicant determined that the NOAEL for maternal toxicity and F1 offspring development was 60 mg/kg/week (17.1 mg/kg/dose).

5.6 Local tolerance

The local tolerance of nusinersen was evaluated in single-dose and repeated-dose toxicity studies in monkeys, which showed no evidence of irritation at the injection site.

5.7 Other toxicity studies

5.7.1 Impurity qualification study (CTD 4.2.3.7.6-1)

Different formulations of nusinersen containing 4.1% to 6.7% potential oligonucleotide impurities[36)] were used in this study. Nusinersen was administered subcutaneously at doses of 0 (vehicle[33)]), 5, and 25 mg/kg weekly for 13 weeks (14 doses in total) to mice (CD-1, 6/sex/group). Histopathological examination revealed vacuolated macrophages in the liver and basophilic granules within the proximal tubular epithelium of the kidneys in the nusinersen group and all nusinersen/impurity mixture groups. There were no toxicologically significant differences between the groups receiving nusinersen alone and those receiving nusinersen with impurities.

5.7.2 Mechanistic study on hippocampal vacuolation (reference data, CTD 4.2.3.7.7-1)

Nusinersen was administered to cynomolgus monkeys (3/sex/group) by IT bolus injection at a dose of 5 mg once weekly for 5 weeks (6 doses in total). There were no nusinersen-related deaths. Clinical signs observed were nusinersen-related transient deficits in spinal reflexes (cutaneous, hindlimb, tail). Histopathological examination revealed hippocampal vacuolation in formalin-fixed brains, and the vacuolated cells present immunostained positive for the presence of nusinersen. On the other hand, hippocampal vacuolation was absent in brain sections processed using alternative fixation methods (fixation in Carnoy's solution or modified Karnovsky's solution).

5.7.3 Immunotoxicity

The immunotoxicity of nusinersen was evaluated in a 53-week intermittent dose IT toxicity study in juvenile monkeys (CTD 4.2.3.2-2). Despite the detection of anti-nusinersen antibodies, no histopathological changes were noted in immune tissues and the T-cell dependent antibody response assay was negative. The applicant therefore considers that nusinersen is unlikely to be immunotoxic.

36) "j," [redacted], Impurity a, [redacted], Impurity b, Impurity k, Impurity f, Impurity h, Impurity g, [redacted], [redacted], Impurity l, Impurity m

5.7.4 Photosafety

No photosafety study of nusinersen was conducted. Although nusinersen absorbed light from 290 to 700 nm, the applicant considers that there are no particular concerns about the photosafety of nusinersen, because (i) systemic and skin exposure in humans following IT administration of nusinersen is considered low and (ii) no skin phototoxicity-related adverse events[37] were reported in clinical studies.

5.R Outline of the review conducted by PMDA

5.R.1 Hippocampal vacuolation

Hippocampal neuronal vacuolation and necrotic cells were observed in repeated-dose toxicity studies in juvenile monkeys (CTD 4.2.3.2-1, CTD 4.2.3.2-2). PMDA asked the applicant to explain safety in humans.

The applicant explained that findings such as neuronal vacuolation, neuronal and glial cell necrosis in the hippocampus observed in repeated-dose toxicity studies in juvenile monkeys should be of low toxicological significance.

The applicant's explanation on these findings:

- Vacuoles in the hippocampus observed in the repeated-dose toxicity studies in juvenile monkeys were morphologically similar to vacuoles in the proximal tubular epithelium of the kidneys reported in toxicity studies of other 2'-MOE modified ASOs.[38] As with vacuolation in the proximal tubule observed with other 2'-MOE modified ASOs, hippocampal vacuolation was absent in brain sections processed using fixation methods other than formalin fixation in a mechanistic study on hippocampal vacuolation with nusinersen (reference data, CTD 4.2.3.7.7-1). Hence, as reported with other 2'-MOE modified ASOs, vacuoles in the hippocampus in monkeys treated with nusinersen are considered secondary to elution of oligonucleotide present in intracellular endosomes or lysosomes during the formalin fixation/processing of the tissues,[38] and this finding is unlikely to be of toxicological concern.
- In the repeated-dose toxicity studies in juvenile monkeys, slight to minimal vacuolation and neuronal and glial cell necrosis, or cellular debris in the hippocampus were observed. All of these findings were limited to the inferior region of the hippocampus, and the severity of the toxicological findings remained unchanged after 53 weeks of dosing compared to 14 weeks of dosing.
- In a 53-week intermittent dose IT toxicity study in juvenile monkeys (CTD 4.2.3.2-2), there were no neurobehavioral changes. In a learning test,[12] there was an increase in the number of days required to complete the learning test in 1 male in the high dose group (7 males, 6 females), which was attributed to variability in response, and this finding was not observed following 26 weeks of recovery. Thus, nusinersen should have no effects on learning and memory.

The incidences of CNS adverse events[39] in humans were 31.7% (13 of 41 subjects) in the sham-procedure control group and 25.0% (20 of 80 subjects) in the nusinersen group in a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B). Main adverse events included failure to thrive (0% [0 of 41 subjects] vs. 2.5% [2 of 80 subjects]), anxiety (0% [0 of 41 subjects] vs. 2.5% [2 of 80 subjects]), nystagmus (2.4% [1 of 41 subjects]

37) Events coded to the MedDRA HLT "Photosensitivity and photodermatosis conditions"
38) *Nucleic Acid Ther*. 2016; 26: 199-209
39) Events coded to the MedDRA SOCs "Nervous system disorders" and "Psychiatric disorders"

vs. 2.5% [2 of 80 subjects]), irritability (2.4% [1 of 41 subjects] vs. 2.5% [2 of 80 subjects]), and agitation (4.9% [2 of 41 subjects] vs. 2.5% [2 of 80 subjects]). The incidences of serious CNS adverse events were 7.3% (3 of 41 subjects) in the sham-procedure control group and 3.8% (3 of 80 subjects) in the nusinersen group, showing no trend towards a higher incidence in the nusinersen group compared to the sham-procedure control group. In foreign short-term treatment studies in SMA patients aged ≥2 years,[40)] the incidence of CNS adverse events was 62.5% (35 of 56 subjects). Main adverse events included headache (35.7% [20 of 56 subjects]), post lumbar puncture syndrome (33.9% [19 of 56 subjects]), and agitation (3.6% [2 of 56 subjects]).

Hippocampal neuronal vacuolation and necrotic cells observed in the repeated-dose toxicity studies in juvenile monkeys were findings of low toxicological significance, and there have been no safety concerns about the CNS effects of nusinersen in humans. Thus, the CNS effects of nusinersen are unlikely to raise a clinically relevant issue. No particular precaution is required at present.

PMDA's view:
The applicant interpreted hippocampal neuronal vacuolation and necrotic cells observed in the repeated-dose toxicity studies in juvenile monkeys as being of low toxicological significance. However, there was an increase in the number of days required to complete the learning test[12)] in 1 of 7 males in the high dose group. Considering that no particular abnormalities were observed in this animal during the memory test conducted pre-dose, nusinersen may have affected learning and memory. Given that necrosis of the renal tubular epithelium and decreased renal function associated with vacuolation in the renal proximal tubular epithelium have been observed in toxicity studies of other 2'-MOE modified ASOs,[38)] hippocampal physiological function may have been affected by hippocampal vacuolation induced by nusinersen.

Based on the above, hippocampal neuronal vacuolation and necrotic cells observed in the repeated-dose toxicity studies in juvenile monkeys were findings of toxicological significance. The safety margin between the NOAEL in the 53-week intermittent dose IT toxicity study in juvenile monkeys (0.3 mg/dose) and the recommended clinical dose was ≤1.1-fold [see Section 5.2], which does not ensure adequate safety in humans. Thus, the applicant should list the findings observed in these studies in the package insert to provide information to healthcare professionals. Furthermore, the applicant should collect information on the occurrence of CNS adverse events and effects on memory and learning via post-marketing surveillance.

5.R.2 Chronic repeat-dose toxicity
A chronic repeat-dose toxicity study was conducted in monkeys only. PMDA asked the applicant to explain the appropriateness of non-clinical safety evaluation of long-term nusinersen treatment.

The applicant's explanation:
- In non-clinical studies of other 2'-MOE modified ASOs administered intravenously or subcutaneously, the monkey is the species closest to the human in terms of tissue distribution and cellular uptake and class effects of 2'-MOE modified ASOs (pro-inflammatory effects, effects on the kidney, liver, and platelets).[35)] Repeated dose studies can be conducted in monkeys using IT bolus injection as the intended route of

40) CTD 5.3.4.2-1, Study CS1; CTD 5.3.4.2-2, Study CS2; CTD 5.3.5.2-3, Study CS10

administration in humans. Studies in rodents can employ only intravenous and subcutaneous routes of administration, which do not allow for adequate exposure in the CNS tissues (the target tissues of nusinersen). Taking into account the above, the applicant considered that information on the long-term safety of nusinersen in humans can be obtained from repeated IT dose toxicity studies in juvenile monkeys.

- The pharmacokinetics of 2'-MOE modified ASOs are almost sequence-independent [see Section 4.R.1]. In addition, most of the toxicological findings previously reported with other 2'-MOE modified ASOs (e.g., effects on the kidney, liver, platelets), except for pro-inflammatory effects, are sequence-independent. Although the pro-inflammatory effects of 2'-MOE modified ASOs are sequence-dependent, the significance of risk assessment for pro-inflammatory effects using mice is limited because mice overpredict their pro-inflammatory effects compared to primates.[35)] The repeat-dose toxicity of other 2'-MOE modified ASOs after systemic exposure with intravenous and subcutaneous dosing in mice has already been determined and toxicological findings similar to those observed with other 2'-MOE modified ASOs were seen in a 13-week intermittent dose subcutaneous toxicity study of nusinersen in juvenile mice (CTD 4.2.3.2-3). Based on these findings, the significance of conducting a chronic repeat-dose toxicity study of nusinersen in mice was considered low.
- Published literature has reported the similarity of metabolite profiles of 2'-MOE modified ASOs between mice and monkeys.[41)] From the standpoint of metabolite safety evaluation, therefore, the applicant considered that the non-clinical safety (chronic repeat-dose toxicity) of nusinersen can be evaluated in a juvenile monkey 53-week intermittent dose IT toxicity study only (CTD 4.2.3.2-2).
- Table 12 shows the incidence of adverse events by time intervals of study treatment in a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B) and a long-term extension study (reference data, CTD 5.3.5.4-3, Study CS11 [patients who entered this study after Study CS3B]). There was no trend towards an increasing incidence of a specific adverse event with prolonged treatment with nusinersen. There was no trend towards increasing incidences of serious or severe adverse events.

41) *Drug Metab Dispos.* 2003; 31: 1419-28, *Drug Metab Dispos*. 2007; 35: 460-8

Table 12. Incidence of adverse events by time intervals of study treatment in Studies CS3B and CS11

	1-180 days		181-360 days		361-540 days		541-720 days	
Treatment group in Study CS3B	S	Nusinersen	S	Nusinersen	S	Nusinersen	S	Nusinersen
No. of evaluable subjects	41	80	26	62	16	40	10	21
All adverse events	39 (95.1)	73 (91.3)	20 (76.9)	56 (90.3)	12 (75.0)	29 (72.5)	7 (70.0)	14 (66.7)
Serious adverse events	33 (80.5)	52 (65.0)	12 (46.2)	28 (45.2)	7 (43.8)	16 (40.0)	2 (20.0)	5 (23.8)
Severe adverse events	27 (65.9)	40 (50.0)	12 (46.2)	20 (32.3)	5 (31.3)	11 (27.5)	2 (20.0)	2 (9.5)
Incidence of adverse events by major MedDRA SOC								
Infections and infestations	27 (65.9)	58 (72.5)	16 (61.5)	44 (71.0)	10 (62.5)	22 (55.0)	4 (40.0)	7 (33.3)
Respiratory, thoracic and mediastinal disorders	34 (82.9)	52 (65.0)	12 (46.2)	27 (43.5)	5 (31.3)	13 (32.5)	2 (20.0)	3 (14.3)
Gastrointestinal disorders	21 (51.2)	45 (56.3)	9 (34.6)	19 (30.6)	2 (12.5)	7 (17.5)	1 (10.0)	3 (14.3)
General disorders and administration site conditions	27 (65.9)	34 (42.5)	7 (26.9)	23 (37.1)	4 (25.0)	12 (30.0)	3 (30.0)	2 (9.5)
Skin and subcutaneous tissue disorders	11 (26.8)	16 (20.0)	3 (11.5)	12 (19.4)	7 (43.8)	2 (5.0)	1 (10.0)	1 (4.8)
Investigations	11 (26.8)	15 (18.8)	3 (11.5)	9 (14.5)	3 (18.8)	5 (12.5)	1 (10.0)	1 (4.8)
Cardiac disorders	9 (22.0)	15 (18.8)	4 (15.4)	3 (4.8)	1 (6.3)	1 (2.5)	0	0
Metabolism and nutrition disorders	9 (22.0)	11 (13.8)	2 (7.7)	2 (3.2)	4 (25.0)	3 (7.5)	1 (10.0)	1 (4.8)
Injury, poisoning and procedural complications	8 (19.5)	10 (12.5)	4 (15.4)	6 (9.7)	1 (6.3)	2 (5.0)	0	1 (4.8)
Psychiatric disorders	4 (9.8)	8 (10.0)	1 (3.8)	1 (1.6)	0	0	0	0
Nervous system disorders	2 (4.9)	7 (8.8)	1 (3.8)	1 (1.6)	0	1 (2.5)	0	1 (4.8)
Musculoskeletal and connective tissue disorders	2 (4.9)	6 (7.5)	3 (11.5)	4 (6.5)	3 (18.8)	3 (7.5)	1 (10.0)	1 (4.8)
Immune system disorders	3 (7.3)	3 (3.8)	2 (7.7)	3 (4.8)	1 (6.3)	3 (7.5)	0	1 (4.8)
Eye disorders	2 (4.9)	3 (3.8)	1 (3.8)	1 (1.6)	0	0	0	0
Reproductive system and breast disorders	0	3 (3.8)	0	0	0	0	0	0
Renal and urinary disorders	0	2 (2.5)	1 (3.8)	0	1 (6.3)	0	0	0
Vascular disorders	0	2 (2.5)	0	3 (4.8)	1 (6.3)	0	0	0

n (incidence [%])
: sham-procedure control

Since there have been no particular safety concerns about long-term treatment with nusinersen in humans at present, the significance of conducting a chronic repeat-dose toxicity study in the rodent should be low.

PMDA's view:

There is no consensus that the repeat-dose toxicity profiles of chemically-modified ASOs are sequence- and length-independent and that such findings allow for extrapolation of the results of toxicological evaluation of other ASOs. Toxicities previously unreported with other 2'-MOE modified ASOs may occur due to the effect of a sequence specific to nusinersen. Thus, chronic repeat-dose toxicity studies should have been conducted in the rodent as well as in the monkey, essentially. Although there have been no particular safety concerns about long-term treatment with nusinersen in humans at present, it cannot be concluded that the currently available data adequately support the long-term safety of nusinersen because the number of patients treated with nusinersen was limited. Given that SMA (mainly type I SMA) is a fatal, serious disease, it is acceptable to offer nusinersen to patients based on the currently presented non-clinical and clinical data, but the applicant should collect information on the long-term safety of nusinersen in humans via post-marketing surveillance.

5.R.3 Carcinogenicity studies

Given that there was significant systemic exposure in human subjects receiving nusinersen [see Section 6.2], nusinersen needs carcinogenicity studies. PMDA asked the applicant to explain the plan to conduct carcinogenicity studies.

The applicant's explanation:

A 2-year subcutaneous carcinogenicity study in CD-1 mice, which were used in a 13-week intermittent dose subcutaneous toxicity study, is planned. No other carcinogenicity studies are required for the following reasons.

- Since the biodistribution and metabolite profiles of 2'-MOE modified ASOs have been reported to be similar across species,[41] a mouse carcinogenicity study allows for the non-clinical assessment of the carcinogenic potential of nusinersen.
- From the standpoint of the effects of nusinersen on the target gene (*SMN2*), nusinersen increases endogenous SMN protein production in SMA patients. Taking account of the biological characteristics of SMA and the mechanism of action of nusinersen, the pharmacological action of nusinersen, i.e., modulation of splicing of the SMN transcript, does not pose a carcinogenic risk. Because common laboratory animals including mice, rats, dogs, and cynomolgus monkeys does not carry the *SMN2* gene, carcinogenic risk cannot be assessed in animal species commonly used in non-clinical studies [see Section 3.R.2].
- Carcinogenic risk was assessed from the standpoint of hybridization-dependent off-target effects of nusinersen, but there has been no information suggesting carcinogenic risk at present for the reasons below.
  (a) Although *in silico* screening suggested the potential for interaction of nusinersen with some human genes including a tumor suppressor gene, FOXP1 [see Section 3.R.2.1], no information suggesting carcinogenic risk has been obtained based on the biological properties of each gene and the phenotypes of knockout mice.
  (b) Since the human genomic sequences are substantially different from the animal genomic sequences, a rodent carcinogenicity study has little significance for assessing the human carcinogenic risk of nusinersen due to hybridization-dependent off-target effects.
- Carcinogenic risk was assessed from the standpoint of hybridization-independent off-target effects of nusinersen, but no data from non-clinical studies of nusinersen have suggested the carcinogenic potential of nusinersen for the reasons below.
  (a) There were no findings considered related to carcinogenicity (preneoplastic lesions, cellular dysplasia, findings suggestive of endocrine disrupting effects or immunosuppressive effects) in nusinersen repeated-dose toxicity studies in mice and monkeys.
  (b) Nusinersen tested negative for genotoxicity.
  (c) In carcinogenicity studies of another 2'-MOE modified ASO (mipomersen sodium), there were increased incidences of hepatocellular adenomas and fibrosarcoma of the skin/subcutis in mice and increased incidences of fibrosarcoma and malignant fibrous histiocytoma of the skin/subcutis in rats.[42] All neoplastic lesions were related to pro-inflammatory effects in rodents and were reported to be of low human relevance.[43]

PMDA's view:

Nusinersen should be tested for carcinogenicity in at least 1 rodent species, though there are limitations to assessing the carcinogenic potential of nusinersen in rodents for the following reasons: (1) The carcinogenic

42) https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM323927.pdf

43) Antisense Drug Technology: Toxicologic Properties of 2'-Methyoxyethyl Chimeric Antisense Inhibitors in Animal and Man. CRC Press; 2008. p.305-26, *Circ Res*. 2013; 112: 1479-90

potential of 2'-MOE modified ASOs has already been assessed to a certain extent; (2) mice and rats lack the *SMN2* gene, the target gene of nusinersen, and (3) due to the infeasibility of conducting chronic repeat-dose studies in rodents by the IT route, the carcinogenic risk of nusinersen in the CNS tissues, which are exposed to nusinersen after administration of nusinersen, is difficult to assess in rodents. Further, SMA (mainly type I SMA) is a fatal, serious disease. Additional submission of the study results after filing marketing application is acceptable, but a 2-year subcutaneous carcinogenicity study in CD-1 mice should be conducted as soon as possible. The applicant should collect information on the occurrence of malignant tumors via post-marketing surveillance.

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

No biopharmaceutic data have been submitted.

Nusinersen concentrations in human plasma, CSF, and urine were determined by Hyb-ELISA (LLOQ, 1.0 ng/mL) or Hyb-ECL (LLOQ, 0.05 ng/mL). Tissue nusinersen concentrations were determined by Hyb-ELISA (LLOQ, 0.06 μg/g).[44)] Concentrations of nusinersen metabolites/degradation products[19)] in human plasma, CSF, and urine were determined by LC-MS/MS (LLOQ, 10.0, 25.0, and 5.0 ng/mL, respectively). SMN protein concentrations in CSF were determined by ELISA (LLOQ, 0.12 pg/mL), and anti-nusinersen antibodies in plasma were measured by ELISA (LLOQ, 50 ng/mL).

6.2 Clinical pharmacology

The applicant submitted the results from phase I studies in non-Japanese SMA patients,[45)] a multi-regional phase III study in Japanese and non-Japanese SMA patients (CTD 5.3.5.1-1, Study CS3B), a phase II study in non-Japanese SMA patients (CTD 5.3.5.2-1, Study CS3A), and a phase II study in non-Japanese subjects with a mutation in the SMN gene (CTD 5.3.5.2-2, Study 232SM201) as evaluation data. The applicant also submitted the results from *in vitro* studies using human biomaterials.[46)] Unless otherwise specified, doses are expressed in terms of nusinersen and concentrations are expressed in terms of nusinersen sodium. The t_{max} is expressed as the median and other pharmacokinetic parameters are expressed as the mean or the mean ± SD.

6.2.1 Studies using human biomaterials

Nusinersen (0.1 or 5 μg/mL) was added to human plasma, and protein binding was determined using the ultrafiltration method. The plasma protein binding of nusinersen was 94.1% to 96.1% (reference data, CTD 5.3.2.1-1, Study IS04; reference data, CTD 5.3.2.1-3, Study IS06).

---

44) Human CSF, plasma, and urine were spiked with nusinersen 30.0 ng/mL (Hyb-ELISA) or 0.15 to 7.5 ng/mL (Hyb-ECL) and then further spiked with its metabolite ("o" [3'- or 5'-deletion]) (5% to 100% of nusinersen). Then nusinersen concentrations were determined by Hyb-ELISA and Hyb-ECL. The percent difference from the theoretical concentration was -2.23% to 241%, indicating that the spiked metabolite interfered with the assay and that nusinersen concentrations were overestimated. When metabolite concentrations in plasma and CSF samples after administration of nusinersen were determined by LC-MS/MS, no metabolites were detected in CSF. A maximum value of 5.8% of "o" (relative abundance) as the primary metabolite was detected in plasma [see Section 6.2.2].

45) CTD 5.3.4.2-1, Study CS1; CTD 5.3.4.2-2, Study CS2; CTD 5.3.5.2-3, Study CS10; CTD 5.3.5.2-4, Study CS12

46) Reference data, CTD 5.3.2.1-1, Study IS04; reference data, CTD 5.3.2.1-2, Study IS05; reference data, CTD 5.3.2.1-3, Study IS06; reference data, CTD 5.3.2.2-1, Study IS12; reference data, CTD 5.3.2.2-2, Study IS13; reference data, CTD 5.3.2.2-3, Study IS14

Nusinersen (5 or 150 μg/mL) was added to human CSF, and protein binding was determined using the ultrafiltration method. The protein biding of nusinersen in CSF was 15.1% to 24.9% at 5 μg/mL and 0% at 150 μg/mL (reference data, CTD 5.3.2.1-2, Study IS05; reference data, CTD 5.3.2.1-3, Study IS06).

Human hepatocytes were added with nusinersen (1.0-100 μg/mL), and the CYP1A2, CYP2B6, and CYP3A4 induction potential of nusinersen was evaluated. Nusinersen did not induce these metabolizing enzymes (reference data, CTD 5.3.2.2-2, Study IS13).

CYP inhibition by nusinersen (0.1-100 μg/mL) in human hepatocytes was evaluated using substrates for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4.[47)] Nusinersen did not inhibit these metabolizing enzymes (reference data, CTD 5.3.2.2-3, Study IS14).

Nusinersen (100 μM) did not inhibit BCRP, P-gp, OAT1, OAT3, OCT1, OCT2, OATP1B1, OATP1B3, or BSEP (reference data, CTD 5.3.2.2-1, Study IS12).

6.2.2 Studies in SMA patients, etc.

6.2.2.1 Foreign phase II study (CTD 5.3.5.2-1, Study CS3A [Data Cutoff, [redacted] 20[redacted]])

Non-Japanese SMA patients (36-210 days of age, 20 subjects included in pharmacokinetic, pharmacodynamic, and immunogenic assessments [4 in Cohort 1, 16 in Cohort 2]) were included in the study. Patients in Cohort 1 received 6-mg scaled equivalent IT doses of nusinersen and patients in Cohort 2 received 12-mg scaled equivalent IT doses of nusinersen [see Table 20] on Days 1, 15, and 85. Then, all patients received nine 12-mg scaled equivalent IT doses of nusinersen every 126 days (on Days 253, 379, 505, 631, 757, 883, 1009, 1135, and 1261) during the maintenance phase. The plasma C_{max} values of nusinersen on Day 1 in Cohorts 1 and 2 were 396 ± 311 and 829 ± 625 ng/mL, respectively, and the $AUC_{0\text{-}24h}$ values were 2818 ± 1463 and 7040 ± 3964 ng·h/mL, respectively. Nusinersen trough concentrations in CSF are shown in Table 13. The concentrations tended to increase with multiple dosing.

Table 13. Nusinersen trough concentrations in CSF in non-Japanese SMA patients receiving multiple IT doses of nusinersen

Time point	Cohort 1		Cohort 2	
	No. of evaluable subjects	Concentration (ng/mL)	No. of evaluable subjects	Concentration (ng/mL)
Day 15	4	1.68 ± 0.87	16	3.57 ± 3.51
Day 85	4	1.11 ± 1.28	13	3.84 ± 2.99 a)
Day 253	4	1.24 ± 0.53	13	4.93 ± 3.99
Day 379	2	1.87, 3.07	13	9.29 ± 9.19
Day 505	2	2.92, 6.57	11	5.91 ± 4.26
Day 631	2	4.28, 10.63	5	10.3 ± 4.04
Day 757	2	3.90, 8.69	4	13.6 ± 10.2
Day 883	2	5.19, 17.50	-b)	-b)

Mean ± SD, Individual values are listed for n ≤2.
a) Data on a markedly high nusinersen trough concentration in CSF in 1 patient (980.6 ng/mL) were excluded from analysis.
b) Not determined.

The percent changes from baseline in SMN protein concentration in CSF on Day 253 were 27.8 ± 4.47% in Cohort 1 and 114 ± 273% in Cohort 2.

47) phenacetin for CYP1A2, bupropion for CYP2B6, diclofenac for CYP2C9, *S*-mephenytoin for CYP2C19, dextromethorphan for CYP2D6, chlorzoxazone for CYP2E1, midazolam and testosterone for CYP3A4

Although 5.0% (1 of 20) of subjects was transiently positive for plasma anti-nusinersen antibodies, there was no clear association between antibody development and nusinersen trough concentrations in CSF in this subject. There was a trend towards improvements in motor milestones, CHOP INTEND total score, and CMAP amplitude.

Three subjects who died during the study[48)] were autopsied. Nusinersen concentrations and the relative ratios of levels of *SMN2* mRNA containing exon 7[49)] in the spinal cord (lumbar spinal cord, thoracic spinal cord, cervical spinal cord), cerebral cortex (frontal cortex, temporal cortex, motor cortex), and other tissues (cerebellum, hippocampus, diencephalon, brainstem, liver, kidney) are shown in Table 14. Nusinersen concentrations tended to be high in the kidney and spinal cord. There was a trend towards higher levels of *SMN2* mRNA containing exon 7 in the spinal cord.

Table 14. Nusinersen concentrations and relative ratios of levels of *SMN2* mRNA containing exon 7 in tissues from subjects who died during the study

		Nusinersen concentration (μg/g)			Relative ratio of levels of *SMN2* mRNA containing exon 7 [a)]		
		Subject 1303	Subject 2302	Subject 2311	Subject 1303	Subject 2302	Subject 2311
Days from last dose to autopsy		12 days	79 days	36 days	12 days	79 days	36 days
Spinal cord	Lumbar spinal cord	26.6	19.5	31.8	2.6	2.2	2.9
	Thoracic spinal cord	23.7	15.9	14.2	2.5	2.2	3.2
	Cervical spinal cord	15.5	11.9	23.3	2.3	1.8	2.2
Cerebral cortex	Frontal cortex	1.5	2.6	14.7	1.9	1.8	2.8
	Temporal cortex	2.1	3.6	3.5	1.6	1.6	2.0
	Motor cortex	8.7	5.8	10.5	2.4	1.9	3.4
Cerebellum		4.2	4.4	2.8	1.3	1.1	0.5
Hippocampus		2.8	2.1	7.3	2.7	1.8	2.9
Diencephalon (thalamus)		1.3	0.2	4.0	1.6	1.0	2.2
Brainstem		13.8	3.7	8.1	1.0	1.9	1.9
Liver		12.9	1.3	2.4	- [b)]	- [b)]	- [b)]
Kidney		36.5	18.4	25.1	- [b)]	- [b)]	- [b)]

a) Ratio relative to the mean level of *SMN2* mRNA containing exon 7 in the thoracic spinal cord from 4 SMA patients untreated with nusinersen
b) Not determined.

6.2.2.2 Multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B [Data Cutoff, [redacted] 20[redacted]])

Japanese and non-Japanese patients with SMA (30-262 days of age [52-242 days of age in the nusinersen group], 121 subjects included in pharmacokinetic and immunogenic assessments) received 12-mg scaled equivalent IT doses of nusinersen (Table 20) on Days 1, 15, 29, and 64 and then two 12-mg scaled equivalent IT doses of nusinersen (Table 20) every 119 days (on Days 183 and 302) during the maintenance phase. The plasma C_{max} of nusinersen on Day 1 was 1103 ± 854 ng/mL, and the $AUC_{0\text{-}24h}$ was

48) Subject 1303 received 6-mg scaled equivalent doses of nusinersen (Table 20) on Days 1, 15, and 85 and a 12-mg scaled equivalent dose of nusinersen on Day 251 (instead of visit on Day 253) and then died 12 days after the last dose. Subject 2302 received 12-mg scaled equivalent doses of nusinersen on Days 1, 15, and 85 and then died 79 days after the last dose. Subject 2311 received 12-mg scaled equivalent doses of nusinersen on Days 1, 15, and 85 and then died 36 days after the last dose.
49) Ratio relative to the mean level of *SMN2* mRNA containing exon 7 in the thoracic spinal cord from 4 SMA patients untreated with nusinersen

10,075 ± 4833 ng·h/mL. Nusinersen trough concentrations in plasma and CSF are shown in Table 15. Nusinersen trough concentrations in CSF tended to increase with multiple dosing.

Table 15. Nusinersen trough concentrations in plasma and CSF
in Japanese and non-Japanese patients with SMA receiving multiple IT doses of nusinersen

Time point	Plasma concentration		CSF concentration	
	No. of evaluable subjects	Trough concentration (ng/mL)	No. of evaluable subjects	Trough concentration (ng/mL)
Day 15	- [a)]	- [a)]	68	3.96 ± 2.33 [c)]
Day 29	67	2.34 ± 0.96	69	5.58 ± 3.49
Day 64	55	2.33 ± 0.94 [b)]	56	6.68 ± 4.42 [c)]
Day 183	34	1.62 ± 3.14 [b)]	36	6.72 ± 2.72
Day 302	20	0.84 ± 0.33	19	11.2 ± 6.92 [c)]

Mean ± SD
a) Not determined.
b) Data on markedly high plasma nusinersen concentrations (1 subject on Day 64 [262.5 ng/mL], 1 subject on Day 183 [236.7 ng/mL]) were excluded from analysis.
c) Data on markedly high CSF nusinersen concentrations (2 subjects on Day 15 [26.3 and 179.1 ng/mL], 2 subjects on Day 64 [55.0 and 62.1 ng/mL], 1 subject on Day 302 [49.1 ng/mL]) were excluded from analysis.

Although 1.25% (1 of 80) of subjects in the nusinersen group was transiently positive for plasma anti-nusinersen antibodies, there was no clear association between antibody development and nusinersen trough concentrations in CSF in this subject. There was a trend towards improvements in motor milestones, CHOP INTEND total score, and CMAP amplitude.

6.2.2.3 Foreign phase I study (CTD 5.3.4.2-1, Study CS1)

Non-Japanese patients with SMA (2-14 years of age, 28 subjects included in pharmacokinetic and pharmacodynamic assessments) received a single IT dose of 1, 3, 6, or 9 mg of nusinersen. Plasma pharmacokinetic parameters of nusinersen are shown in Table 16. The CSF nusinersen concentrations on Day 8 were 1.06 ± 0.54, 2.18 ± 1.38, 3.70 ± 1.23, and 3.91 ± 0.82 ng/mL, respectively, and the CSF nusinersen concentration increased less than dose-proportionally up to 9 mg.

Table 16. Plasma pharmacokinetic parameters of nusinersen in non-Japanese patients with SMA following a single IT dose of nusinersen

Dose	No. of evaluable subjects	C_{max} (ng/mL)	t_{max} (h)[a)]	$AUC_{0\text{-}20h}$ (ng·h/mL)[b)]
1 mg	6	8.74 ± 6.31	2.05	90.9 ± 55.0[c)]
3 mg	6	42.7 ± 38.0	5.04	413 ± 116 [c)]
6 mg	6	49.4 ± 19.6	4.05	605 ± 234 [c)]
9 mg	10	118 ± 70.8	4.08	1022 ± 492 [d)]

Mean ± SD
a) Median
b) Calculated from subjects who had at least 1 additional blood sample collected at 6, 8, or 12 hours.
c) n = 5
d) n = 8

The percent changes from baseline in SMN protein concentration in CSF[50)] were 61.5 ± 121.5%, 38.2 ± 39.0%, 115.8 ± 58.0%, and 160.9 ± 215.7%, respectively.

The presence of anti-nusinersen antibodies in plasma was determined in the nusinersen 6 and 9 mg groups. No plasma anti-nusinersen antibodies were detected in any subject.

50) 1 mg group, percent change from baseline on Day 292 to Day 426; 3 mg group, percent change from baseline on Day 296 to Day 427; 6 mg group, percent change from baseline on Day 337 to Day 459; 9 mg group, percent change from baseline on Day 289 to Day 399

6.2.2.4 Foreign phase I study (CTD 5.3.4.2-2, Study CS2)

Non-Japanese patients with SMA (2-15 years of age, 34 subjects included in pharmacokinetic, pharmacodynamic, and immunogenic assessments [51]) received multiple IT doses of 3, 6, or 12 mg of nusinersen on Days 1, 29, and 85 or multiple IT doses of 9 mg of nusinersen on Days 1 and 85. Plasma pharmacokinetic parameters of nusinersen and nusinersen trough concentrations in CSF are shown in Table 17. In patients who entered in this study after completing Study CS1 (6 patients, all in the 1 mg group), based on comparison of CSF nusinersen concentrations on Day 8 of Study CS1 with predose CSF nusinersen concentrations in this study, the apparent $t_{1/2}$ in CSF was estimated to be 135 ± 42.8 days. In the nusinersen 12 mg/dose group, 0.008% and 0.5% of the administered dose were excreted in urine by 24 hours post-dose on Days 1 and 85, respectively.

Table 17. Plasma pharmacokinetic parameters of nusinersen and nusinersen trough concentrations in CSF in non-Japanese patients with SMA receiving multiple IT doses of nusinersen

Dose level	Time point	No. of evaluable subjects	Plasma concentration			CSF concentration
			C_{max} (ng/mL)	t_{max} (h)[b)]	$AUC_{0\text{-}6h}$ (ng·h/mL)	Trough concentration (ng/mL)
3 mg/dose	Day 1	8	51.5 ± 72.7	5.09	181 ± 225	0.065 ± 0.117[c,d)]
	Day 85	8	32.5 ± 32.3	6.08	110 ± 107	2.12 ± 0.573
6 mg/dose	Day 1	8	79.8 ± 57.2	5.93	306 ± 259	0.056 ± 0.104
	Day 85	8	52.8 ± 33.5	6.04	179 ± 135	3.76 ± 2.05
9 mg/dose	Day 1	9	141 ± 52.8	3.92	601 ± 249	-[a)]
	Day 85	9	127 ± 37.7	4.12	524 ± 185	1.50 ± 0.447
12 mg/dose	Day 1	9	208 ± 110	4.05	823 ± 442	-
	Day 85	8	132 ± 85.6	5.92	555 ± 398	3.36 ± 1.04[c,e)]

Mean ± SD
a) Not determined.
b) Median
c) Data on markedly high CSF nusinersen concentrations (1 subject in the 3 mg/dose group on Day 1 [27.9 ng/mL], 1 subject in the 12 mg/dose group on Day 85 [983 ng/mL]) were excluded from analysis.
d) n = 6
e) n = 8

Metabolite trough concentrations in plasma and CSF on Day 85 were determined by LC-MS/MS. No metabolite was detected in CSF, whereas "o" (3'-deletion) (relative abundance, up to 5.8%) was detected in plasma. Metabolites excreted in urine by 24 hours post-dose on Day 85 in the nusinersen 12 mg/dose group were measured by LC-MS/MS, and the metabolites detected were "o" and "p" (3'-deletion), which accounted for up to 50.3% and 25.0% of the total oligonucleotides, respectively.

The percent changes from baseline in SMN protein concentration in CSF on Day 85 were 23.2 ± 60.4%, 14.7 ± 47.6%, 114.7 ± 115.1%, and 22.9 ± 50.1%, respectively.

No plasma anti-nusinersen antibodies were detected in any subject.

6.2.2.5 Foreign phase I study (CTD 5.3.5.2-3, Study CS10)

Non-Japanese patients with SMA who had received 3, 6, or 9 mg of nusinersen in Study CS1 (CTD 5.3.4.2-1) (2-11 years of age, 18 subjects included in pharmacokinetic and immunogenic assessments [4 in the 6 mg group, 14 in the 9 mg group) received a single IT dose of 6 or 9 mg of nusinersen. In the 6 and 9 mg groups, the

51) Six of 34 subjects (3 in the 3 mg/dose group, 3 in the 6 mg/dose group) had received 1 mg of nusinersen in Study CS1 (CTD 5.3.4.2-1).

plasma C_{max} values of nusinersen were 73.6 ± 26.9 and 89.0 ± 58.3 ng/mL, respectively, the t_{max} values were 6.03 and 5.39 hours, respectively, the $t_{1/2}$ values were 79.9/93.2[52)] and 63.1 ± 7.97 days, respectively, and the $AUC_{0\text{-}6h}$ values were 275 ± 158 and 361 ± 266 ng·h/mL, respectively. Based on CSF nusinersen concentrations on Days 8 and 29 in Study CS1 and pre-dose CSF nusinersen concentrations in this study, the apparent $t_{1/2}$ values in CSF at doses of 3, 6, and 9 mg were estimated to be 135 ± 14.8, 163 ± 26.5, and 177 ± 41.3 days, respectively.

No plasma anti-nusinersen antibodies were detected in any subject.

6.2.2.6 Foreign phase I study (CTD 5.3.5.2-4, Study CS12)

Non-Japanese patients with SMA who had received nusinersen at 3, 6, 9, or 12 mg/dose in Study CS2 (CTD 5.3.4.2-2) and non-Japanese patients with SMA who had received nusinersen at 6 or 9 mg/dose in Study CS10 (CTD 5.3.5.2-3) (3-17 years of age, 47 subjects included in pharmacokinetic and immunogenic assessments) received multiple IT doses of 12 mg of nusinersen on Days 1, 169, 351, and 533. The plasma C_{max} of nusinersen on Day 1 was 189 ± 138 ng/mL, the t_{max} was 4.13 hours, and the $AUC_{0\text{-}6h}$ was 694 ± 473 ng·h/mL. The nusinersen trough concentration in CSF on Day 169 was 3.10 ± 1.43 ng/mL.[53)]

Although 4.3% (2 of 47) of subjects tested positive for plasma anti-nusinersen antibodies and anti-nusinersen antibodies were detected throughout the study period in 1 of the 2 subjects, there was no clear association between antibody development and nusinersen trough concentrations in CSF in either subject.

6.2.2.7 Foreign phase II study (CTD 5.3.5.2-2, Study 232SM201 [Data Cutoff, [redacted] 20[redacted]])

Non-Japanese subjects with a mutation in the SMN gene[54)] (8-42 days of age, 17 subjects included in pharmacokinetic and immunogenic assessments) received 12-mg scaled equivalent IT doses of nusinersen (Table 20) on Days 1, 15, 29, and 64 and then 6 IT doses of nusinersen every 119 days (on Days 183, 302, 421, 540, 659, and 778) during the maintenance phase. The plasma nusinersen concentration at 4 hours post-dose on Day 1 was 448.6 ± 352.3 ng/mL (13 subjects). Nusinersen trough concentrations in plasma and CSF are shown in Table 18.

Table 18. Nusinersen trough concentrations in plasma and CSF
in non-Japanese subjects receiving multiple 12-mg scaled equivalent IT doses of nusinersen

Time point	Plasma concentration		CSF concentration	
	No. of evaluable subjects	Trough concentration (ng/mL)	No. of evaluable subjects	Trough concentration (ng/mL)
Day 15	-[a)]	-[a)]	13	20.2 ± 17.1
Day 29	-[a)]	-[a)]	11	32.4 ± 26.0
Day 64	11	1.5 ± 0.4	11	34.7 ± 32.7
Day 183	9	0.8 ± 0.26	9	19.7±18.1
Day 302	1	0.8	-[a)]	-[a)]

Mean ± SD
a) Not determined.

52) As the number of evaluable subjects is 2, individual values are listed.
53) Markedly high CSF nusinersen concentrations in 2 subjects (1258 and 6930 ng/mL) were excluded from analysis.
54) Subjects with genetic documentation of 5qSMA (*SMN1* homozygous gene deletion or mutation or compound heterozygous mutation) with 2 or 3 copies of the *SMN2* gene

No plasma anti-nusinersen antibodies were detected in any subject.

6.2.3 PPK analysis (CTD 5.3.3.5-1, IS11 analysis; CTD 5.3.3.5-2, CPP-17-001-BIIB058 analysis)

Plasma and CSF nusinersen concentration data obtained from foreign clinical studies[55] (plasma concentration data collected from 75 subjects at 1255 sampling points, CSF concentration data collected from 73 subjects at 297 sampling points) were used for PPK analysis.[56] As a result, the pharmacokinetics of nusinersen were described by a 4-compartment model, with 2 compartments representing the CSF and CNS tissues and 2 compartments representing the plasma and peripheral tissues. Sex, race (Asian, Caucasian, Black), body weight, age, height, and body surface area were tested as potential covariates on the CL_p, CL_{csf}, V_{pc}, and V_{csfc}, and body weight was selected as a significant covariate on the V_{pc}, V_{csfc}, and CL_p.[57]

6.R Outline of the review conducted by PMDA

6.R.1 Ethnic differences in the pharmacokinetics of nusinersen

PMDA asked the applicant to explain if there are ethnic differences in the pharmacokinetics of nusinersen between Japanese and non-Japanese patients.

The applicant's explanation:

Nusinersen administered via IT injection is distributed to CSF and CNS tissues, absorbed into the systemic circulation via CSF turnover, and metabolized slowly via hydrolysis mediated by 3' and 5' exonucleases that are expressed ubiquitously. Ethnic differences in the pharmacokinetics of nusinersen are unlikely to be seen for the following reasons: (i) There are generally no ethnic differences in the CSF volume (*Cerebrospinal Fluid Res.* 2008; 5: 10, *Cereb Cortex*. 2001; 11: 335-42), (ii) there are no reports on ethnic differences in the transport of 2'-MOE modified ASOs from CSF to CNS tissues or across the blood-CSF barrier, and (iii) 3' and 5' exonucleases are ubiquitously expressed in the body and their genetic polymorphism or ethnic differences in their expression level have not been reported.

Although only 2 Japanese subjects were enrolled in the nusinersen group of a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), the pharmacokinetic parameters of nusinersen in plasma and CSF in these subjects were as follows: their plasma C_{max} values on Day 1 were 2068 and 2123 ng/mL, their plasma $AUC_{0\text{-}24h}$ values on Day 1 were 15,809 and 19,630 ng·h/mL, and their nusinersen trough concentrations in CSF on Day 29 were 2.662 and 5.261 ng/mL. The pharmacokinetic parameters in all subjects were as follows: the plasma C_{max} on Day 1 was 1103 ± 854 ng/mL, the plasma $AUC_{0\text{-}24h}$ on Day 1 was 10,075 ± 4833 ng·h/mL, and the nusinersen trough concentration in CSF on Day 29 was 5.58 ± 3.49 ng/mL. Although the C_{max} and $AUC_{0\text{-}24h}$ of nusinersen in plasma in the Japanese subjects were slightly higher than the mean values in all subjects, the values in the Japanese subjects were within the C_{max} and $AUC_{0\text{-}24h}$ ranges of non-Japanese subjects. Thus, there should be no major differences.

Based on the above, there should be no clear ethnic differences in the pharmacokinetics of nusinersen.

---

55) CTD 5.3.4.2-1, Study CS1; CTD 5.3.4.2-2, Study CS2; CTD 5.3.5.2-3, Study CS10; CTD 5.3.5.2-4, Study CS12; CTD 5.3.5.2-1, Study CS3A

56) NONMEM Version 7.3 was used.

57) CL_{csf} = 0.105 L/h, Q_p = 0.451 L/h, Q_{csf} = 0.068 L/h, V_{pp} = 382 L, V_{csfp} = 295 L. For body weight ≤20 kg, CL_p = 3.19 × (body weight/20) 1.17 L/h and V_{pc} = 49.1 × (body weight/20) 1.14 L. For body weight >20 kg, CL_p = 3.19 L/h and V_{pc} = 49.1 L. For body weight ≤10 kg, V_{csfc} = 0.245 × body weight/10 L. For body weight >10 kg, V_{csfc} = 0.245 L

PMDA accepted the applicant's explanation.

6.R.2 Pharmacokinetics in patients with renal impairment

Published literature has reported that urinary excretion is the primary pathway for clearance of 2'-MOE modified ASOs (*Drug Metab Dispos*. 2003; 31: 1419-28, *Expert Opin Drug Metab Toxicol.* 2009; 5: 381-91). PMDA asked the applicant to explain the pharmacokinetics of nusinersen in patients with renal impairment and the need for dose adjustment.

The applicant's explanation:

In a mass balance study in which radiolabeled 2'-MOE modified ASO was administered intravenously to rats, approximately 80% of the administered dose was excreted in urine by 90 days post-dose. Thus, renal excretion was considered to be the major pathway for clearance of intact oligonucleotide and associated metabolites. Approximately 20% of 2'-MOE modified ASO excreted in urine was the parent drug over the period of 0 to 24 hours (15% and 3% of the administered dose were excreted in urine as 2'-MOE modified ASO and the parent drug, respectively; *Drug Metab Dispos*. 2003; 31:1419-28).

Then, based on the occurrence of renal impairment-related adverse events[58)] in clinical studies in SMA patients,[59)] whether subjects had renal impairment was assessed post-hoc. No subjects had renal impairment. Thus, there are no nusinersen pharmacokinetic or safety data from patients with renal impairment. There are also no reports on the pharmacokinetics of other 2'-MOE modified ASOs in patients with renal impairment.

Given that renal excretion contributes significantly to the clearance of nusinersen, careful administration of nusinersen in patients with renal impairment will be advised in the package insert.

PMDA accepted the above explanation. However, the applicant should collect information on the safety and efficacy of nusinersen in patients with renal impairment via post-marketing surveillance.

6.R.3 QT/QTc interval prolongation

PMDA asked the applicant to explain QT/QTc interval prolongation by nusinersen.

The applicant's explanation:

Based on the following points, no risk of QT/QTc interval prolongation has been suggested at present. No particular precautions in the package insert are required.

- The results from non-clinical studies of other 2'-MOE modified ASOs indicate that nusinersen has no effect on the hERG channel. Safety pharmacology studies of nusinersen showed no effects on ECG parameters or heart rate [see Section 3.R.2.2].
- In a thorough QT study in which another 2'-MOE modified ASO was administered subcutaneously or intravenously at 200 mg/dose (16-fold the maximum dose per administration of nusinersen), no effect on

58) Events coded to the MedDRA SOC "Renal and urinary disorders" and HLGT "Renal and urinary tract investigations and urinalyses"

59) CTD 5.3.5.2-1, Study CS3A; CTD 5.3.5.1-1, Study CS3B; CTD 5.3.4.2-1, Study CS1; CTD 5.3.4.2-2, Study ISIS 396443-CS2; CTD 5.3.5.2-3, Study CS10; CTD 5.3.5.2-4, Study CS12; CTD 5.3.5.2-2, Study 232SM201

QT intervals was observed (*Eur J Clin Pharmacol*. 2016; 72: 267-75). Since nusinersen is for IT injection, it is difficult to conduct a thorough QT study in healthy adults. Thus, a thorough QT study of nusinersen was considered unnecessary.

- In a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), the changes from baseline in QTcF interval in the sham-procedure control and nusinersen groups were 5.3 ± 45.9 and -3.3 ± 44.0 ms, respectively, on Day 2, and 6.0 ± 37.7 and -4.8 ± 41.1 ms, respectively, on Day 29. The results of categorical analysis of QTcF interval data are shown in Table 19. More patients in the nusinersen group had an absolute QTcF value >500 ms or a change from baseline of >60 ms. In these subjects, QT/QTc interval prolongation may have been associated with the underlying disease, concomitant medications, or other adverse events associated with the underlying disease, etc. In phase I studies in non-Japanese patients with SMA,[45)] the proportions of patients who had an absolute QTcF value >500 ms or a change from baseline of >60 ms following administration of 1, 3, 6, 9, and 12 mg of nusinersen were 0% (0 of 6 subjects), 0% (0 of 14 subjects), 0% (0 of 18 subjects), 4.0% (1 of 25 subjects), and 2.1% (1 of 48 subjects), respectively. Although patients receiving nusinersen at higher doses had an absolute QTcF value >500 ms or a change from baseline of >60 ms, only a limited number of patients were assessed, and it is not appropriate to derive a conclusion on dose response.

Table 19. Results of categorical analysis of QTcF interval data

		Sham-procedure	12-mg scaled equivalent dose [a)]
No. of evaluable subjects		41	80
Maximum QTcF interval through the last time point (ms)	> 450	13 (31.7)	20 (25.0)
	> 480	2 (4.9)	8 (10.0)
	> 500	0	5 (6.3)
Change from baseline in QTcF interval (ms)	> 30	10 (24.4)	14 (17.5)
	> 60	3 (7.3)	5 (6.3)

n (%)
a) See Table 20.

- The incidences of adverse events related to QT/QTc interval prolongation and proarrhythmia[60)] were 12.2% (5 of 41 subjects) in the sham-procedure control group and 10.0% (8 of 80 subjects) in the nusinersen group in Study CS3B, and the main event was cardio-respiratory arrest (7.3% [3 of 41 subjects] in the sham-procedure control group, 6.3% [5 of 80 subjects] in the nusinersen group), showing no trend towards a higher incidence with nusinersen compared to sham procedure. There were no events potentially related to direct effects on cardiac conduction.

PMDA's view:

Given the applicant's explanation, the clinical positioning of nusinersen, and currently available data from clinical studies of nusinersen (no apparent QT/QTc interval prolongation or pro-arrhythmic adverse events possibly related to nusinersen were reported), it is acceptable that the applicant did not assess the pro-arrhythmic risk of nusinersen through thorough ECG monitoring etc. in clinical studies. On the other hand, Study CS3B was not intended to assess the risk of QT prolongation, and interpretation of the study results had limitations. However, all of 4 patients who had an absolute QTcF value >500 ms and a change from baseline of >60 ms were in the nusinersen group, and the risk of QT/QTc interval prolongation associated

60) Events in the MedDRA SMQ "Torsade de pointes/QT prolongation (narrow)" and PTs sudden death, ventricular fibrillation, ventricular flutter, syncope, and epilepsy

with nusinersen cannot be ruled out. Thus, no precautions regarding the risk of QT/QTc interval prolongation in the package insert are required at present, but the applicant should collect information on the occurrence of adverse events related to QT/QTc interval prolongation and proarrhythmia via post-marketing surveillance.

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

The applicant submitted the results from a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), foreign phase I studies,[45)] and a foreign phase II study (CTD 5.3.5.2-1, Study CS3A) in patients with SMA, and a foreign phase II study in non-Japanese subjects with a mutation in the SMN gene (CTD 5.3.5.2-2, Study 232SM201)[45)] as efficacy and safety evaluation data. The applicant also submitted data including the results from a foreign phase II study in SMA patients who were not eligible for enrollment in Study CS3B (reference data, CTD 5.3.5.4-2, Study 232SM202) as reference data. In the current application, the data from mainly Type I SMA patients only were evaluated, focusing on Studies CS3A and CS3B.

7.1 Foreign phase II study (CTD 5.3.5.2-1, Study CS3A [ongoing since May 2013 (Data Cutoff, [redacted])])

An open-label, uncontrolled study was conducted to assess the efficacy, safety, and pharmacokinetics of nusinersen in non-Japanese SMA patients ≥21 days and ≤7 months (210 days) of age at screening[61)] (target sample size, 20 subjects [4 in Cohort 1, 16 in Cohort 2]) [for pharmacokinetics, see Section 6.2].

Subjects were to receive IT doses of nusinersen on Days 1, 15, and 85 followed by maintenance doses on Days 253, 379, 505, 631, 757, 883, 1009, 1135, and 1261. The dose was determined based age and cohort. Subjects in Cohort 1 or Cohort 2 were to receive 6- or 12-mg scaled equivalent doses of nusinersen, respectively, on Days 1, 15 and 85 (Table 20). All subjects in Cohort 1 and Cohort 2 were to receive 12-mg scaled equivalent doses of nusinersen during the maintenance phase (Table 20).

Table 20. Dose by age in Study CS3A

Age		0-90 days	91-182 days[a)]	183-365 days[b)]	366-730 days	>730 days
Dose (mg)	6-mg scaled equivalent dose	4.8	5.2	5.4	5.6	6.0
	12-mg scaled equivalent dose	9.6	10.3	10.8	11.3	12.0

a) 91-180 days for Study CS3B.
b) 181-365 days for Study CS3B.

A total of 20 treated subjects were all included in the Safety Set. The Efficacy Set included 19 subjects who received scheduled doses of nusinersen on Days 1, 15, and 85 and attended the Day 92 assessment as of the data cutoff date (4 in Cohort 1, 15 in Cohort 2). There were 5 withdrawals, and the reasons for withdrawals were death (4 subjects) and consent withdrawal (1 subject).

61) Patients who met the following criteria were eligible.
· Patients who had onset of clinical signs and symptoms consistent with SMA at ≥21 days and ≤6 months (180 days) of age
· Patients with genetic documentation of 5q SMA (*SMN1* homozygous gene deletion or mutation)

The efficacy endpoint of the proportion of subjects who achieved improvement in motor milestones as evaluated by Section 2[62] (8 items) of the HINE at the last visit through the data cutoff date[63] were 25.0% (1 of 4 subjects) in Cohort 1 and 80.0% (12 of 15 subjects) in Cohort 2.

Adverse events (including laboratory abnormalities) occurred in all subjects (20 subjects). There were 4 deaths (respiratory failure [2 subjects]; cardio-respiratory arrest; and lower respiratory tract infection viral, 1 subject each), and a causal relationship to nusinersen could not be ruled out for 1 case of respiratory failure (Cohort 2). Other serious adverse events reported are shown in Table 21.

Table 21. Occurrence of non-fatal serious adverse events

Cohort 1	pneumonia pseudomonal, respiratory distress, and respiratory tract infection; acute respiratory failure* and parainfluenzae virus infection (a total of 2 subjects)
Cohort 2	pneumonia, respiratory distress, and respiratory tract infection viral; pneumonia*; synovitis and viral infection*; cardiac arrest and metapneumovirus infection*; apnoea, aspiration, atelectasis, bradycardia, cardio-respiratory arrest, cyanosis, enterovirus infection, hyponatraemia, metapneumovirus infection, pneumonia aspiration, respiratory failure, and rhinovirus infection; acute respiratory failure, apnoea, bronchiolitis, corona virus infection, pneumomediastinum, pneumonia viral, pneumopericardium, respiratory failure, seizure,* and vomiting; pneumonia aspiration; pneumonia, pneumonia bacterial, pneumonia viral, respiratory distress, and respiratory failure; bronchiolitis, respiratory distress, rhinovirus infection, and viral infection; respiratory distress; atelectasis, bronchiolitis, pneumonia, respiratory distress, respiratory syncytial virus bronchiolitis, rhinovirus infection, upper respiratory tract infection, and viral upper respiratory tract infection; acute respiratory failure, hypoventilation, and hypoxia; acute respiratory failure, failure to thrive, and rhinovirus infection (a total of 13 subjects)

* Events for which a causal relationship to nusinersen could not be ruled out

The incidences of adverse events (including laboratory abnormalities) for which a causal relationship to nusinersen could not be ruled out were 25.0% (1 of 4 subjects) in Cohort 1 and 37.5% (6 of 16 subjects) in Cohort 2. The observed events were acute respiratory failure, lymphadenopathy, bradycardia, rash, and headache in Cohort 1 and viral infection, pyrexia, cough, and synovitis; pneumonia and respiratory failure; convulsion; metapneumovirus infection; neutropenia; and vomiting (1 subject each) in Cohort 2.

Adverse events related to vital signs (body temperature, pulse rate, blood pressure, respiratory rate, oxygen saturation) were summarized. Pyrexia (14 subjects), hypoxia (4 subjects), bradycardia (3 subjects), tachycardia (2 subjects), oxygen saturation decreased (2 subjects), and essential hypertension, heart rate decreased, heart rate increased, hypertension, tachypnoea, and ventricular tachycardia (1 subject each) were reported as adverse events.

Although ECG abnormalities suggestive of ventricular hypertrophy were observed in 3 subjects, all of these 3 subjects had normal or almost normal echocardiogram. No subjects had a QTc value >450 ms, 3 subjects had an increase from baseline of >30 ms, and 1 subject had an increase from baseline of >60 ms.

62) Neurological examination to assess infants from 2 to 24 months of age. The assessment consists of 37 items, divided into 3 sections: Section 1, neurologic examination (cranial nerve functions, posture, movements, tone, reflexes, reactions); Section 2, motor developmental milestones; and Section 3, behavioral assessment. Section 2 includes 8 items (voluntary grasp, ability to kick, head control, rolling, sitting, crawling, standing, walking) scored on a 3- to 5-point scale according to the level of development.

63) Attainment of motor milestones was scored at each time point, and then the scores at baseline were compared with the scores at the last visit through the data cutoff date. Improvement was defined as a ≥1-point increase (a ≥2-point increase or achievement of maximal score for voluntary grasp and ability to kick) in ≥1 motor milestone category.

The applicant's explanation:
Based on the above, there should be no major safety problems with 6- or 12-mg scaled equivalent doses of nusinersen (Table 20) in non-Japanese patients with SMA, and its efficacy is also expected.

7.2 Multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B [ongoing since August 2014 (Data Cutoff, [redacted] 20[redacted])])

A randomized, double-blind, sham-procedure-controlled, parallel-group study was conducted in 14 countries or regions[64] to assess the efficacy, safety, and pharmacokinetics of nusinersen in SMA patients ≤7 months (210 days) of age at screening[65] (target sample size, 111 subjects [37 in the sham-procedure control group[66] and 74 in the nusinersen group]) [for pharmacokinetics, see Section 6.2]. Based on the outcome of an interim analysis,[67] early termination of the study was decided because of early demonstration of efficacy.

Subjects were to undergo a sham procedure or receive 12-mg scaled equivalent IT doses of nusinersen based on age (Table 20) on Days 1, 15, 29, and 64, followed by maintenance doses on Days 183 and 302. Patients who completed the study and wished to continue treatment with nusinersen and patients receiving study drug at the time of the decision to stop the study early for efficacy were allowed to participate in an extension study (reference data, CTD 5.3.5.4-3, Study CS11).

Of 122 randomized subjects (41 in the sham-procedure control group, 81 in the nusinersen group), 121 subjects (41 in the sham-procedure control group, 80 in the nusinersen group) were included in the ITT population and in the Safety Set, and 1 subject untreated with study drug was excluded from analysis. The Interim Efficacy Set included 78 subjects who had the opportunity to be assessed at the Day 183 visit as of the data cutoff date (27 in the sham-procedure control group, 51 in the nusinersen group). There were 26 withdrawals (13 in the sham-procedure control group, 13 in the nusinersen group), and the reasons for withdrawals were adverse events (12 in the sham-procedure control group, 12 in the nusinersen group) and consent withdrawal (1 in the sham-procedure control group, 1 in the nusinersen group).

The primary endpoint[68] of the study was the proportion of subjects who achieved improvement in motor milestones as evaluated by Section 2 (7 items[69]) of the HINE. The interim analysis included data at the Day 183, 302, or 394 visit assessment[70] (whichever was latest). The results of the interim analysis are

64) The US, Spain, Germany, Italy, France, Canada, Turkey, the UK, Sweden, Australia, Belgium, South Korea, Taiwan, and Japan
65) Patients who met the following criteria were eligible for enrollment in the study.
· Patients who had onset of clinical signs and symptoms consistent with SMA at ≤6 months (180 days) of age
· Patients with genetic documentation of 5q SMA (*SMN1* homozygous gene deletion or mutation)
· Patients with 2 copies of the *SMN2* gene
66) The same study drug administration procedure as in the nusinersen group was to be performed. The lumbar puncture needle broke the skin but no IT injection of drug solution was to occur.
67) An interim analysis was to be conducted, once approximately 80 subjects (including deaths and withdrawals) had the opportunity to be assessed at the Day 183 visit. A final analysis was to be conducted when the last surviving subject completed the Day 394 assessment or the study was stopped due to early demonstration of efficacy.
68) Initially, the primary endpoint for this study was "the time to death or permanent ventilation," which was changed to "the proportion of subjects who achieve improvement in motor milestones as evaluated by Section 2 (7 items[69]) of the HINE[70]" at the interim analysis [see Section 7.R.3.1].
69) 7 items of the HINE Section 2: ability to kick, head control, rolling, sitting, crawling, standing, and walking, with the exclusion of voluntary grasp
70) Attainment of motor milestones was scored at each time point, and then the scores at baseline were compared with the scores at the last visit through the data cutoff date. Improvement was defined as a ≥1-point increase (a ≥2-point increase in ability to kick or achievement of maximal score) and worsening was defined as a ≥1-point decrease (a ≥2-point decrease in ability to kick or decrease to the lowest possible score). Subjects with an

shown in Table 22. There was a statistically significant difference between the nusinersen and sham-procedure control groups ($P < 0.0001$,[71] Fisher's exact test).

Table 22. Proportion of HINE motor milestone responders (Interim Efficacy Set)

Treatment group	No. of evaluable subjects	Proportion of HINE motor milestone responders (%)	Treatment difference [95% CI][a]	*P*-value[b]
Sham-procedure control	27	0	41.2 [18.2, 61.2]	< 0.0001
Nusinersen	51	41.2 (21)		

Proportion (n)
a) Exact confidence interval for the difference in proportions
b) Fisher's exact test (significance level of 0.032 at interim analysis)

The incidences of adverse events (including laboratory abnormalities) were 92.7% (38 of 41 subjects) in the sham-procedure control group and 90.0% (72 of 80 subjects) in the nusinersen group. There were 13 deaths in the sham-procedure control group (respiratory failure [7 subjects]; cardio-respiratory arrest and respiratory distress [2 subjects each]; acute respiratory failure and unknown cause [1 subject each]) and 12 deaths in the nusinersen group (respiratory failure [4 subjects]; cardio-respiratory arrest [2 subjects]; acute respiratory failure, respiratory arrest, respiratory distress, brain injury, hypoxic-ischaemic encephalopathy, and unknown cause [1 subject each]), and a causal relationship to study drug could not be ruled out for 2 cases of cardio-respiratory arrest in the sham-procedure control group and 1 case of cardio-respiratory arrest in the nusinersen group. The occurrence of other serious adverse events is shown in Table 23.

improvement were defined as more categories with improvement than worsening. Subjects with a worsening were defined as more categories with worsening than improvement.

71) The Lan-DeMets linear α spending function was to be employed for the adjustment of significance level for the interim analysis. At the interim analysis, a significance level of 0.032 was to be used.

Table 23. Occurrence of non-fatal serious adverse events

Sham-procedure control	viral upper respiratory tract infection (2 subjects); acute respiratory failure (2 subjects); acute respiratory failure, corona virus infection, respiratory syncytial virus bronchiolitis, and viral upper respiratory tract infection; apnoea, bronchial secretion retention, cardiac arrest, cyanosis, oxygen saturation decreased, respiratory failure, and viral upper respiratory tract infection; cardiac arrest, respiratory distress, atelectasis, pneumonia bacterial, respiratory arrest, respiratory failure, respiratory syncytial virus bronchiolitis, and viral infection; apparent life threatening event, bronchiolitis, and pneumonia; respiratory arrest, respiratory distress, respiratory syncytial virus bronchiolitis, and rhinovirus infection; dyspnoea*, respiratory arrest*, respiratory failure*, and weight gain poor; pneumonia aspiration; femur fracture, pneumonia parainfluenzae viral, respiratory distress, respiratory failure, and respirovirus test positive; hypoxia, pneumonia, respiratory failure, and vomiting; bronchial secretion retention*, pneumonia aspiration, respiratory arrest*, and respiratory distress*; acute respiratory failure, cardio-respiratory arrest, pneumonia, pneumonia viral, respiratory distress, and viral upper respiratory tract infection; pneumonia aspiration and respiratory distress; pneumonia*; head injury; respiratory failure and respiratory tract infection viral; feeding disorder of infancy or early childhood and respiratory failure; acute respiratory failure*, agitation*, apnoea*, dyspnoea*, feeding disorder of infancy or early childhood*, gastrointestinal haemorrhage*, and respiratory failure*; respiratory distress; salivary hypersecretion; pneumonia pseudomonal; respiratory distress, respiratory failure, respiratory tract congestion, rhinovirus infection, and weight gain poor; and atelectasis and pneumonia aspiration (1 subject each) (a total of 27 subjects)
Nusinersen	respiratory distress[a)] (3 subjects); upper respiratory tract infection (2 subjects); pneumonia (2 subjects); acute respiratory failure, atelectasis, pneumonia, and rhinovirus infection; acute respiratory failure and atelectasis*; dysphagia, hypercapnia, hypoxia, and respiratory syncytial virus bronchiolitis; atelectasis, bronchiolitis, nosocomial infection, pneumonia bacterial, respiratory distress, respiratory failure, and rhinovirus infection; acute respiratory failure, atelectasis, respiratory distress, and viral upper respiratory tract infection; respiratory distress and respiratory failure; pneumonia, pneumonia aspiration, and respiratory failure; acute respiratory failure, atelectasis, and respiratory failure; cardio-respiratory arrest, chronic respiratory failure, gastric haemorrhage, hypoxia, pneumonia, and shock; acute respiratory distress syndrome, acute respiratory failure*, feeding intolerance, respiratory distress, and stoma site abscess; cardiac arrest, respiratory distress, and upper respiratory tract infection*; dysphagia* and weight gain poor*; aspiration, cyanosis, dehydration, feeding disorder of infancy or early childhood, pneumonia aspiration, and respiratory distress; acute respiratory failure* and pneumonia bacterial; dermatitis and vomiting; bronchitis viral, respiratory failure, and upper respiratory tract infection; acute respiratory failure, pneumonia, pneumonia aspiration, and respiratory distress; acute respiratory failure, cardio-respiratory arrest, pneumonia, and viral upper respiratory tract infection; aspiration; aspiration, hypoventilation, pneumonia, pneumonia aspiration, respiratory distress, viral upper respiratory tract infection, and wound dehiscence; acute respiratory failure, pneumonia viral, respiratory failure, respiratory syncytial virus bronchiolitis, and staphylococcal sepsis; acute respiratory failure, bronchiolitis, hypoxia, pneumonia moraxella, and pneumonia viral; acute respiratory failure, bronchiolitis, pneumonia viral, respiratory distress, respiratory failure, and rhinovirus infection; pneumonia influenzal; pneumonia aspiration*, pneumonia bacterial*, pneumonia viral, respiratory arrest*, and respiratory failure*; pyrexia and respiratory distress; atelectasis, dyspnoea, respiratory distress, respiratory failure, and rhinovirus infection; atelectasis, delayed recovery from anaesthesia, increased bronchial secretion, lower respiratory tract infection, respiratory arrest, respiratory distress, and viral infection; bronchiolitis, dyspnoea, lower respiratory tract infection, lower respiratory tract infection viral, pneumonia aspiration, respiratory arrest, respiratory distress, and rhinovirus infection; respiratory syncytial virus bronchiolitis; respiratory failure*; atelectasis* and ear infection; pneumonia, pyrexia*, respiratory disorder, and viral infection; respiratory disorder; bronchitis, pneumonia, and respiratory failure; atelectasis, bronchitis, pneumonia, and respiratory tract infection; respiratory tract infection; obstructive airways disorder and pneumonia respiratory syncytial viral; lower respiratory tract infection, pneumonia, and pneumonia parainfluenzae viral*; dyspnoea; apnoea, bronchitis, bronchitis viral, pneumonia viral, and pyrexia; apnoea, bronchial secretion retention, cardiac arrest, and rhinovirus infection; feeding intolerance, medical observation, pneumonia, respiratory failure, respiratory tract infection, vaccination complication, and vomiting; atelectasis, cardio-respiratory arrest, respiratory distress, respiratory failure, respiratory tract infection, respiratory tract infection viral, viral infection, and weight gain poor; atelectasis, feeding disorder of infancy or early childhood, gastroenteritis rotavirus, lung disorder, oxygen saturation decreased, pneumonia, respiratory distress, viral infection, and vomiting; weight gain poor; nasopharyngitis*; and dyspnoea*, lung infection*, and ventricular tachycardia* (1 subject each) (a total of 54 subjects)

* Events for which a causal relationship to study drug could not be ruled out
a) A causal relationship could not be ruled out for 1 of the 3 cases.

The incidences of adverse events (including laboratory abnormalities) for which a causal relationship to study drug could not be ruled out were 36.6% (15 of 41 subjects) in the sham-procedure control group and 37.5% (30 of 80 subjects) in the nusinersen group. The main events were pyrexia (6 subjects, 5 subjects), oxygen saturation decreased (1 subject, 3 subjects), and rash (1 subject, 3 subjects).

There were no clinically relevant changes in vital signs (body temperature, blood pressure, pulse rate, respiratory rate, oxygen saturation). ECG findings were a QTcF value >500 ms (0 subject, 5 subjects) and an increase from baseline in QTcF interval of >60 msec (3 subjects, 5 subjects).

The applicant's explanation:
The above findings has demonstrated the efficacy of nusinersen in SMA patients. Although a certain number of serious adverse events occurred, there were no major differences in the nature or incidence of adverse events between the nusinersen and sham-procedure control groups. Thus, there should be no major safety issues.

7.R Outline of the review conducted by PMDA

7.R.1 Clinical positioning of nusinersen

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

The applicant's explanation:
As the standard of care in SMA, the International Expert Consensus[72)] released in 2007 and the clinical practice manual in Japan (Clinical Practice Manual for Spinal Muscular Atrophy. Kinpodo; 2012) list respiratory support, nutritional support, and rehabilitation for the care of Type I SMA patients, but not drug therapies that alter disease progression. Although adenosine triphosphate disodium hydrate for injection has been approved for the indications of "improvement of the symptoms of progressive spinal muscular atrophy and its similar diseases" in Japan, the above Expert Consensus does not recommend its use. Medical experts in Japan also commented that adenosine triphosphate disodium hydrate for injection is not actively used for the treatment of SMA in clinical practice. At present, no effective treatment for SMA has been established worldwide.

Although there are no Japanese or foreign guidelines mentioning the clinical positioning of nusinersen at present, nusinersen will offer a new treatment option for SMA patients, for the following reasons: (i) nusinersen is considered to increase the amount of SMN protein produced by the *SMN2* gene by modulating the *SMN2* mRNA splicing pattern, thus preventing degeneration of the motor neurons; and (ii) in a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), patients treated with nusinersen achieved improved motor function.

PMDA accepted the applicant's explanation. Nusinersen will offer a new treatment option for SMA patients.

7.R.2 Evaluation based on multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B)

7.R.2.1 Intrinsic and extrinsic ethnic factors

PMDA asked the applicant to explain how intrinsic and extrinsic ethnic factors that influence the efficacy and safety of nusinersen were taken into consideration when conducting a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B).

The applicant's explanation:

- The pharmacokinetics of nusinersen in Japanese patients were unknown at the time of planning Study CS3B. However, ethnic differences in the pharmacokinetics of nusinersen are unlikely to be seen because (i) there are no ethnic differences in the activities of 3' and 5' exonucleases, the major metabolizing enzymes

72) *J Child Neurol.* 2007; 22: 1027-49

for nusinersen, and (ii) nusinersen is not a substrate for CYPs etc. and is therefore not affected by ethnic differences in metabolizing enzyme activities.

- There are no racial or ethnic differences in the genetic cause of SMA (*Genet Test Mol Biomarkers*. 2012; 16: 123-9), and Japanese SMA patients are similar to SMA patients in other countries, from a pathological point of view (CTD 5.4-123, *Brain Dev.* 2011; 33: 321-31). There are no reports that there are racial, ethnic, or regional differences in the incidence or prevalence of SMA.
- Diagnosis and standard of care of SMA have been based on the Expert Consensus[72)] released in Japan and globally in 2007.
- The use of invasive and non-invasive respiratory care differs from country to country. Physicians from Commonwealth countries were less likely to recommend tracheostomy/ventilation than U.S. physicians (7% versus 25%; *Pediatr Pulmonol*. 2012; 47: 606-11). While the proportion of patients ventilated invasively was high in Mexico, Argentina, Poland, and Italy (29.5% to 100%), 16.7% of patients were ventilated invasively in the US. None of patients were ventilated invasively in Bulgaria, Macedonia, Romania, or Serbia (*J Neurol*. 2014; 261: 152-63). Hence, a guide for the standard of respiratory care was included in the protocol appendix so that no major differences in the type of respiratory care used and the timing of initiation of respiratory care among the participating countries would occur.
- Investigators were trained to allow a unified evaluation of the HINE motor milestones, the primary endpoint for Study CS3B. Each subject was required to be assessed by the same physician throughout the study period, as a rule.

Based on the above, because the impacts of intrinsic and extrinsic ethnic factors on the efficacy and safety evaluation of nusinersen were considered small, the applicant decided to conduct a phase III study as a multi-regional study and to allow the participation of Japanese subjects in the pivotal study of nusinersen (Study CS3B). Such decision was considered appropriate. In addition, appropriate measures were taken to allow a unified efficacy evaluation.

7.R.2.2 Enrollment of Japanese patients and efficacy and safety evaluation

PMDA asked the applicant to explain the basis for sample size determination of Japanese patients for Study CS3B and the reasons why only 3 Japanese patients were enrolled in the study before the interim analysis (1 in the sham-procedure control group, 2 in the nusinersen group) and only 1 Japanese patient included in the Interim Efficacy Set (the sham-procedure control group).

The applicant's explanation:

Based on the status of enrollment in the SMA patient registry of the SMART (Spinal Muscular Atrophy Research & Treatment) consortium run by the coordinating investigator in Japan for Study CS3B, 8 to 12 candidate patients per year would exist in Japan. A high consent rate was assumed due to lack of adequately effective therapy for SMA. Thus, a target sample size of 8 Japanese patients was chosen for Study CS3B.

However, patient enrollment was very difficult in Japan for the following reasons, among others: (a) efforts were made to enroll patients, utilizing the above patient registry, but it was difficult to identify patients due to a low prevalence, (b) SMA is not well known in Japan, and there were patients who did not satisfy the eligibility

criteria due to disease progression when they had a diagnosis of SMA made by specialists, (c) there were patients who had an early diagnosis of SMA but could not be screened due to worsening of the primary disease, and (d) patients' parents did not wish to let their child participate in the clinical study. Publicity activities for the relevant academic society, the patient group, and the general public were carried out, and the coordinating investigator sent ≥400 letters to pediatricians across the nation in order to ask them to refer their patients to the study. Despite all these efforts, patient enrollment was not accelerated.

Because only 1 Japanese patient in the sham-procedure control group was included in the Interim Efficacy Set for efficacy evaluation at the interim analysis, it was considered difficult to assess differences in the efficacy of nusinersen between Japanese and non-Japanese patients based on the results of the interim analysis. Thus, efficacy data from the 2 Japanese patients in the nusinersen group not included in the Interim Efficacy Set will also be presented wherever possible for efficacy evaluation. Also for safety evaluation, the additional data from Japanese patients at the Data Cutoff as of [redacted] 20[redacted] will be submitted and discussed.

7.R.2.3 Differences in efficacy and safety among countries/regions in multi-regional phase III study

PMDA asked the applicant to explain interregional differences in efficacy and safety in Study CS3B.

The applicant's explanation:

In the Safety Set of Study CS3B, patient characteristics were compared among regions (North America/Europe/East Asia/others). Patients in East Asia tended to have longer disease duration, and there were no clinically significant differences in other patient characteristics among the regions.

The proportion of patients who achieved improvement in more milestones as evaluated by Section 2 (7 items[69]) of the HINE[70] by region is shown in Table 24. No definitive trend was observed in East Asia or other regions due to the small number of subjects, whereas similar efficacy was observed in North America and Europe.

Table 24. Proportion of HINE motor milestone responders by region (Interim Efficacy Set)

	Treatment group	N	Proportion of motor milestone responders (%)	Treatment difference [95% CI][a)]
North America[b)]	Sham-procedure control	18	0	40.0 [10.6, 64.6]
	Nusinersen	30	40.0 (12)	
Europe[c)]	Sham-procedure control	7	0	42.1 [-1.6, 76.4]
	Nusinersen	19	42.1 (8)	
East Asia[d)]	Sham-procedure control	1	0	
	Nusinersen	0	0	
Others[e)]	Sham-procedure control	1	0	
	Nusinersen	2	50.0 (1)	

Proportion (n)
a) Exact confidence interval for the difference in proportions
b) US, Canada
c) Spain, Germany, Italy, France, Sweden, Belgium
d) Japan, South Korea, Taiwan,
e) Turkey, Australia

Efficacy was assessed in Japanese patients. One Japanese patient randomized to the sham-procedure control group in Study CS3B was not a motor milestone responder,[70] and 1 of 2 Japanese patients randomized to receive nusinersen was a motor milestone responder at the final analysis. The proportion of motor milestone responders in Japanese patients was by no means inferior to that in the overall population (0% [0 of 27 subjects] in the sham-procedure control group, 41% [21 of 51 subjects] in the nusinersen group), though the number of Japanese patients was limited. Similar efficacy can be expected also in Japanese patients.

The summary of adverse events by region in the Japanese subgroup (Data Cutoff, [redacted] 20[redacted]) and the overall population (Data Cutoff, [redacted] 20[redacted]) is shown in Table 25. No definitive trend was observed in East Asia or other regions due to the small number of subjects, whereas no major differences in the nature of adverse events were observed between North America and Europe. All of adverse events occurring in 2 nusinersen-treated Japanese subjects were observed also in non-Japanese subjects, and adverse events for which a causal relationship to study drug could not be ruled out occurred in 1 Japanese subject in the sham-procedure control group (strabismus) and 1 nusinersen-treated Japanese subject (conjunctivitis, eczema infected, nasopharyngitis, eczema, rash). No major safety concerns were suggested in the Japanese subgroup.

Table 25. Summary of adverse events by region (Study CS3B, Safety Set)

	North America		Europe		East Asia		Others		Japan	
	S group	Nusinersen	S group	Nusinersen	S group	Nusinersen	S group	Nusinersen	S group	Nusinersen
N	22	38	17	30	1	3	1	9	1	2
All adverse events	22 (100)	35 (92.1)	14 (82.4)	26 (86.7)	1 (100)	3 (100)	1 (100)	8 (88.9)	1 (100)	2 (100)
Serious adverse events	19 (86.4)	29 (76.3)	13 (76.5)	22 (73.3)	0	2 (66.7)	1 (100)	3 (33.3)	0	2 (100)
Main adverse events										
Pyrexia	12 (54.5)	20 (52.6)	9 (52.9)	16 (53.3)	1 (100)	1 (33.3)	0	2 (22.2)	1 (100)	0
Upper respiratory tract infection	5 (22.7)	17 (44.7)	4 (23.5)	1 (3.3)	0	0	0	2 (22.2)	0	0
Constipation	5 (22.7)	16 (42.1)	4 (23.5)	6 (20.0)	0	0	0	2 (22.2)	0	0
Respiratory distress	10 (45.5)	11 (28.9)	4 (23.5)	8 (26.7)	0	0	0	0	0	0
Acute respiratory failure	7 (31.8)	11 (28.9)	1 (5.9)	0	0	0	0	0	0	0
Viral upper respiratory tract infection	8 (36.4)	10 (26.3)	0	0	0	0	0	0	0	0
Respiratory failure	7 (31.8)	10 (26.3)	6 (35.3)	7 (23.3)	0	0	1 (100)	0	0	0
Dysphagia	7 (31.8)	9 (23.7)	1 (5.9)	0	0	0	0	0	0	0
Atelectasis	8 (36.4)	8 (21.1)	1 (5.9)	7 (23.3)	0	0	0	0	0	0
Nasal congestion	6 (27.3)	8 (21.1)	0	0	0	0	0	0	0	0
Pneumonia	4 (18.2)	8 (21.1)	2 (11.8)	7 (23.3)	0	0	0	2 (22.2)	0	0
Teething	2 (9.1)	8 (21.1)	1 (5.9)	1 (3.3)	0	0	0	2 (22.2)	0	0
Vomiting	6 (27.3)	7 (18.4)	2 (11.8)	4 (13.3)	0	1 (33.3)	0	0	0	1 (50.0)
Rash	3 (13.6)	7 (18.4)	1 (5.9)	0	0	1 (33.3)	0	0	0	1 (50.0)
Oxygen saturation decreased	7 (31.8)	6 (15.8)	2 (11.8)	1 (3.3)	0	1 (33.3)	0	0	0	0
Diarrhoea	6 (27.3)	6 (15.8)	1 (5.9)	2 (6.7)	0	0	0	0	0	0
Pneumonia aspiration	4 (18.2)	6 (15.8)	2 (11.8)	1 (3.3)	0	0	0	0	0	0
Nasopharyngitis	4 (18.2)	6 (15.8)	0	6 (20.0)	0	2 (66.7)	0	0	0	2 (100)
Gastrooesophageal reflux disease	3 (13.6)	6 (15.8)	3 (17.6)	3 (10.0)	0	0	0	0	1 (100)	0
Hypoxia	2 (9.1)	6 (15.8)	0	0	0	0	0	0	0	0
Rhinovirus infection	4 (18.2)	5 (13.2)	1 (5.9)	3 (10.0)	0	0	0	0	0	0
Dermatitis diaper	2 (9.1)	5 (13.2)	0	1 (3.3)	0	0	0	0	0	0
Pneumonia viral	1 (4.5)	5 (13.2)	0	1 (3.3)	0	0	0	0	0	0
Cough	7 (31.8)	4 (10.5)	1 (5.9)	2 (6.7)	0	0	0	0	0	0
Tachycardia	4 (18.2)	4 (10.5)	1 (5.9)	2 (6.7)	0	0	0	0	0	0
Bronchiolitis	2 (9.1)	4 (10.5)	1 (5.9)	2 (6.7)	0	0	0	2 (22.2)	0	0
Aspiration	1 (4.5)	4 (10.5)	0	0	0	0	0	0	0	0
Oral candidiasis	1 (4.5)	4 (10.5)	2 (11.8)	2 (6.7)	0	0	0	0	0	0
Upper respiratory tract congestion	1 (4.5)	4 (10.5)	0	1 (3.3)	0	0	0	0	0	0
Dyspnoea	5 (22.7)	2 (5.3)	1 (5.9)	2 (6.7)	0	1 (33.3)	0	2 (22.2)	0	0
Viral infection	2 (9.1)	2 (5.3)	0	5 (16.7)	0	0	0	0	0	0
Respiratory tract infection	1 (4.5)	1 (2.6)	1 (5.9)	6 (20.0)	0	0	0	0	0	0
Weight gain poor	1 (4.5)	1 (2.6)	1 (5.9)	4 (13.3)	0	0	0	0	0	0
Bronchitis	0	1 (2.6)	0	4 (13.3)	0	0	0	0	0	0
Weight decreased	0	0	1 (5.9)	3 (10.0)	0	0	0	0	0	0

n (incidence [%])
S group: sham-procedure control group

Based on the above, the conduct of this study as a multi-regional study was appropriate, because Study CS3B showed no major interregional differences in patient characteristics, efficacy, or safety.

PMDA's view:

Given that there should be no major differences in intrinsic ethnic factors associated with SMA and that the clinical study was designed so that no major extrinsic ethnic differences would occur (respiratory care was unified wherever possible), there were no major problems with the conduct of Study CS3B as a multi-regional study. Essentially, the applicant should have given full consideration to the conduct of a clinical study from the feasibility standpoint. Based on the consideration, the applicant should have made efforts to prepare the system to facilitate the clinical study and increase disease awareness, and other activities so as to achieve the target enrollment for Japanese patients. However, in light of the rarity and seriousness of SMA, it is unavoidable to assess efficacy and safety in Japanese patients and any ethnic differences between Japanese and non-Japanese patients based on the currently available data. Although consistency between the overall population and the Japanese subgroup cannot be examined due to the very limited number of Japanese patients, Study CS3B did not suggest the possibility of major differences in efficacy and safety between the overall population and the Japanese subgroup. Taking account of the study results and the applicant's explanation, it is acceptable to evaluate the efficacy and safety of nusinersen based on Study CS3B conducted as a multi-regional study. Nusinersen is expected to be effective in Japanese patients, based on the study results. Because the efficacy and safety of nusinersen in Japanese patients have not been determined at present, the applicant should collect information on the safety and efficacy of nusinersen in Japanese patients via post-marketing surveillance. A final decision will be made, taking account of comments from the Expert Discussion.

7.R.3 Efficacy of nusinersen

7.R.3.1 Appropriateness of primary endpoint

PMDA asked the applicant to explain the rationale for the primary endpoint for a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B).

The applicant's explanation:

Because the endpoint had been used in clinical studies in SMA patients (*Clin Genet*. 2009; 76: 168-78) at the time of initiating Study CS3B, the time to death or permanent ventilation was chosen as the primary endpoint. Taking also into account that the use of invasive and non-invasive respiratory care was different among the participating countries [see Section 7.R.2.1], the definition of permanent ventilation was discussed thoroughly. As a result, permanent ventilation was defined as "at least 16 hours per day of ventilatory support used continuously for >21 days in the absence of an acute reversible event," from the following points of view.

- In typical Type I SMA patients, several hours of bilevel positive airway pressure per day at home or in an outpatient setting is used as the first ventilatory support. Thus, it was considered that "ventilatory support" should include not only tracheostomy and endotracheal intubation, but also other positive pressure ventilations. Supplemental oxygen without pressure support (nasal cannula or facial mask) was not to be treated as ventilatory support because it is just passive oxygen supply.
- A patient was considered to have received permanent ventilation if a patient required ventilation support for at least 14 days in a natural history study of SMA (*Neurology*. 2014; 83: 810-7). On the other hand, the duration was 2 to 4 weeks according to the European Neuromuscular Centre Workshop group (*Neuromuscul Disord*. 2005; 15: 802-16). Thus, a duration of 21 days was selected. At least 16 hours per

day of ventilatory support was chosen based on many previous reports (*Neurology*. 2007; 69: 1931-6, *Neurology*. 2014; 83: 810-7, etc.).

New motor milestones were achieved in a foreign phase II study in mainly Type I SMA patients (CTD 5.3.5.2-1, Study CS3A), and the efficacy of nusinersen was reliably expected. Hence, in [redacted] 20[redacted], the plan was revised so as to additionally perform an interim analysis for stopping the study due to early efficacy during the study. For the interim analysis, the efficacy endpoint was reconsidered. As a result, it was thought that motor milestones are widely used to assess infant development and can detect changes in symptoms earlier than "the time to death or permanent ventilation." Thus, it was considered that the former secondary endpoint in the original protocol, the proportion of patients who achieve improvement in motor milestones as evaluated by Section 2 (7 items[69)]) of the HINE[70)] should be positioned as the primary endpoint. The former primary endpoint in the original protocol, "the time to death or permanent ventilation" was positioned as the secondary endpoint.

PMDA asked the applicant to explain the appropriateness of selecting "the proportion of patients who achieve improvement in motor milestones as evaluated by Section 2 (7 items) of the HINE as the primary endpoint for Study CS3B.

The applicant's explanation:

- The HINE is a neurological examination designed to assess the development of infants from 2 to 24 months of age.[63)] Section 2 assesses the development of motor function, and it is a simple method for evaluating infants. A study on optimality score for 12- and 18-month-old healthy infants has also been conducted (*J Pediatr*. 1999; 135: 153-6).
- Patients with Type I SMA have hypotonia, do not achieve head control, exhibit reduced motor activity, etc. as the major clinical symptoms, and are known to rarely achieve major milestones in Section 2 of the HINE (*Neuronuscle Disord*. 2016; 26: 754-9). Thus, it is considered possible to assess the treatment effect of nusinersen and explain its clinical significance based on acquisition of motor milestones.
- Typical Type I SMA patients are unable to move against gravity, but may still have the ability to perform activities that do not require movements against gravity. Hence, improvement was defined as a 2-point increase or achievement of maximal score for the category of ability to kick.[73)] Voluntary grasp was excluded from the analysis for the same reason.

PMDA accepts the above, and considers as follows:
There is no major problem with selecting "the proportion of patients who achieve improvement in motor milestones as evaluated by Section 2 (7 items) of the HINE as the primary endpoint for Study CS3B. However, as most of the causes of death in patients with Type I SMA are related to respiratory disorder (*J Child Neurol.* 2007; 22: 1027-49), and Section 2 of the HINE does not include respiratory assessment, the impact of treatment on the final prognosis will continue to be discussed in Section 7.R.3.2. SMA (mainly Type I SMA) is a fatal, serious disease, and there was little information on the efficacy of nusinersen at the

73) Scored on a 5-point scale: 0 (No kicking), 1 (Kicks horizontally but legs do not lift), 2 (Upward [vertically]), 3 (Touches leg), 4 (Touches toes).

time of initiating Study CS3B. The change of the endpoint may have been unavoidable, but it is inappropriate to change the primary endpoint or add an interim analysis after the initiation of the study.

7.R.3.2 Long-term efficacy

PMDA asked the applicant to explain the long-term efficacy of nusinersen in achieving more motor milestones.

The applicant's explanation:

Nusinersen long-term data include data from 16 patients in the nusinersen group had received nusinersen for >1 year at the interim analysis in Study CS3B. While 9 patients maintained maximal motor milestones achieved during the study period at the last visit, 2 patients showed worsening of the symptoms[70)] and the number of categories with improvement (a ≥1-point increase) was equal to the number of categories with worsening (a ≥1-point decrease) in 2 patients.[70)] The CHOP INTEND total score at the last assessment was worse than the maximal score in 9 of the 16 patients.

The results of efficacy assessment at the last visit in 20 patients who were alive at the interim analysis and completed the last study visit after the interim analysis (1 in the sham-procedure control group, 19 in the nusinersen group) in Study CS3B showed a decrease in the motor milestone score in 3 of the 19 nusinersen-treated patients. Furthermore, patient characteristics were examined for subjects with or without a decrease in the score, though in a limited number of patients. As a result, subjects with a decrease in the score tended to have onset of clinical signs and symptoms at later age and a longer disease duration, and more frequently tended to be male than female.

Taking into account that most of the causes of death in patients with Type I SMA are related to respiratory disorder, PMDA asked the applicant to explain the impact of nusinersen treatment on the long-term prognosis.

The applicant's explanation:

At the interim analysis of Study CS3B, death or permanent ventilation was counted as an event and the results of analysis of the secondary endpoint of the time to death or permanent ventilation are presented (Table 26 and Figure 1). Though some subjects were observed for a limited duration, the results suggested that the time to death or permanent ventilation tended to be prolonged in patients treated with nusinersen.

Table 26. Assessment of time to event (death or permanent ventilation) at interim analysis (ITT Set)

Treatment group	No. of evaluable subjects	No. of subjects with an event (Proportion %)	Time to event (weeks) Median [95% CI][a)]	Proportion of subjects with an event by 13 months (%)[a)]	Hazard ratio[b)]
Sham-procedure control	41	20 (48.8)	25.4 [13.6, －]	65.1	0.712
Nusinersen	80	27 (33.8)	－ [30.6, －]	48.7	

a) Estimated using the Kaplan-Meier method.
b) Calculated based on the Cox regression model adjusted for disease duration.

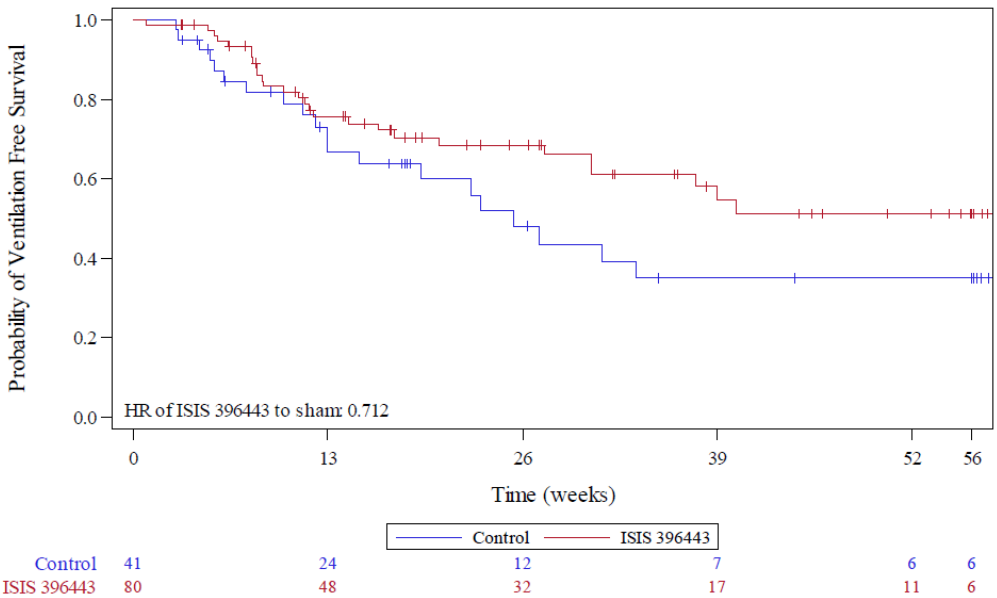


Figure 1. Kaplan-Meier cures for time to event (death or permanent ventilation) at interim analysis (ITT Set)
(Control, sham-procedure control group; ISIS 396443, nusinersen group)

PMDA accepts the above and considers as follows:

Complete remission of SMA with nusinersen cannot be expected, but nusinersen slows down the progression of SMA symptoms and is expected to improve the prognosis to a certain extent. The data submitted to date suggest that patients treated with nusinersen can acquire motor milestones but subsequently lose them with the progression of SMA symptoms. Thus, information on the efficacy of long-term treatment with nusinersen and its impact on survival prognosis should be collected via post-marketing surveillance.

7.R.3.3 Factors affecting the efficacy of nusinersen

PMDA asked the applicant to explain the factors affecting the efficacy of nusinersen.

The applicant presented the results of subgroup analyses of the proportion of subjects who achieved improvement in motor milestones as evaluated by Section 2 (7 items[69]) of the HINE[70] and the proportion of subjects who died or required permanent ventilation in Study CS3B according to patient characteristics (Table 27) and explained that while the proportion of motor milestone responders was higher in the nusinersen group than in the sham-procedure control group across all subgroups, there were differences in efficacy between some of the subgroups.

Table 27. Proportion of subjects who achieved improvement in motor milestones as evaluated by Section 2 (7 items) of the HINE and proportion of subjects who died or required permanent ventilation by patient characteristics in Study CS3B (Interim Efficacy Set)

			Motor milestones		Death or permanent ventilation	
		Treatment group	Proportion of responders	Treatment difference [95% CI][a)]	Proportion of subjects with an event	Treatment difference [95% CI][a)]
Gender	Male	Sham-procedure control	0 (0/11)	25.0 [-11.4, 57.6]	35.3 (6/17)	5.3 [-23.6, 33.0]
		Nusinersen	25.0 (6/24)		40.5 (15/37)	
	Female	Sham-procedure control	0 (0/16)	55.6 [26.5, 78.0]	58.3 (14/24)	-30.4 [-52.9, -5.5]
		Nusinersen	55.6 (15/27)		27.9 (12/43)	
Age at screening	≤ 166 days	Sham-procedure control	0 (0/10)	48.3 [12.0, 76.6]	47.1 (8/17)	-24.3 [-50.5, 3.7]
		Nusinersen	48.3 (14/29)		22.7 (10/44)	
	> 166 days	Sham-procedure control	0 (0/17)	31.8 [0.4, 59.2]	50.0 (12/24)	-2.8 [-28.7, 23.4]
		Nusinersen	31.8 (7/22)		47.2 (17/36)	
Age of onset	≤ 8 weeks	Sham-procedure control	0 (0/16)	36.4 [7.4, 61.9]	54.2 (13/24)	-18.3 [-41.4, 6.1]
		Nusinersen	36.4 (12/33)		35.8 (19/53)	
	> 8 weeks	Sham-procedure control	0 (0/11)	50.0 [12.6, 77.9]	41.2 (7/17)	-11.6 [-40.6, 18.7]
		Nusinersen	50.0 (9/18)		29.6 (8/27)	
Disease duration	≤ 13.1 weeks	Sham-procedure control	0 (0/14)	53.9 [22.5, 78.2]	47.6 (10/21)	-33.0 [-56.9, -7.6]
		Nusinersen	53.8 (14/26)		14.6 (6/41)	
	> 13.1 weeks	Sham-procedure control	0 (0/13)	28.0 [-6.2, 57.5]	50.0 (10/20)	3.9 [-22.9, 30.5]
		Nusinersen	28.0 (7/25)		53.8 (21/39)	
Baseline score for Section 2 of the HINE	≤ 1	Sham-procedure control	0 (0/18)	37.5 [8.7, 62.4]	53.8 (14/26)	-18.6 [-41.2, 5.1]
		Nusinersen	37.5 (12/32)		35.3 (18/51)	
	> 1	Sham-procedure control	0 (0/9)	47.4 [9.7, 78.8]	40.0 (6/15)	-9.0 [-39.8, 21.0]
		Nusinersen	47.4 (9/19)		31.0 (9/29)	
Respiratory symptoms	Yes	Sham-procedure control	0 (0/6)	32.0 [-11.8, 71.3]	55.6 (5/9)	5.2 [-32.7, 42.6]
		Nusinersen	32.0 (8/25)		60.7 (17/28)	
	No	Sham-procedure control	0 (0/21)	50.0 [22.3, 71.4]	46.9 (15/32)	-27.6 [-47.8, -5.6]
		Nusinersen	50.0 (13/26)		19.2 (10/52)	
Swallowing or feeding difficulties	Yes	Sham-procedure control	0 (0/10)	34.4 [-1.4, 66.3]	58.3 (7/12)	-7.1 [-38.3, 25.2]
		Nusinersen	34.4 (11/32)		51.2 (21/41)	
	No	Sham-procedure control	0 (0/17)	52.6 [21.3, 76.1]	44.8 (13/29)	-29.4 [-50.6, -5.3]
		Nusinersen	52.6 (10/19)		15.4 (6/39)	

Proportion (%) (n/N)
a) Exact confidence interval for the difference in proportions

The applicant's explanation on differences in efficacy observed between the subgroups:

- The proportion of motor milestone responders in the nusinersen group was lower among male subjects compared to female subjects, and the proportion of subjects who died or required permanent ventilation was similar between the nusinersen and sham-procedure control groups among male subjects. Although more male subjects had respiratory symptoms and swallowing or feeding difficulties at baseline, there were no major differences in other patient characteristics between male and female subjects. In Cohort 2 of Study CS3A, the proportions of motor milestone responders were 66.7% (6 of 9 subjects) among male subjects and 85.7% (6 of 7 subjects) among female subjects, showing a similar trend as that of Study CS3B. There are a number of reports on gender-related differences in the onset, prognosis, etc. of SMA (*Am J Med Genet*. 1994; 51 70-6, *J Child Neurol*. 2012; 27: 471-7, etc.). Recently, the possibility that the actin binding protein plastin 3 acts as a female-specific modifier of the SMA phenotype has been reported (*Science*. 2008; 320: 524-7), while other reports deny its action. A definitive conclusion has not been reached yet. At present,

the observed gender-related differences in the efficacy of nusinersen may have been due to differences in other patient characteristics, but the details are unknown.

- In the subgroup of subjects with a longer disease duration and the subgroup of subjects with respiratory symptoms, the proportion of motor milestone responders in the nusinersen group was lower, and the proportion of subjects who died or required permanent ventilation was similar between the nusinersen and sham-procedure control groups. Since SMA is a progressive disease and nusinersen cannot regenerate the degenerated or lost neurons based on its mechanism of action, the therapeutic effects of nusinersen may have been lost in patients with particularly advanced disease.

PMDA accepts the above, but considers as follows:
Because Study CS3B suggested that the efficacy of nusinersen may be reduced in male patients and patients with advanced disease, the results of subgroup analyses should be provided to healthcare professionals in clinical practice. The applicant should collect information on the impact of patient characteristics on the efficacy of nusinersen and efficacy in patients with advanced disease via post-marketing surveillance.

7.R.4 Safety of nusinersen

7.R.4.1 Safety profile of nusinersen

Taking into account that many patients experienced serious adverse events in a foreign phase II study (CTD 5.3.5.2-1, Study CS3A) and a multi-regional phase III study (CTD 5.3.5.1.1, Study CS3B), PMDA asked the applicant to explain the safety profile of nusinersen.

The applicant's explanation:
A summary of adverse events reported in Studies CS3A and CS3B is shown in Table 28. There was no trend towards higher incidences of serious adverse events, severe adverse events, etc. in the nusinersen group than in the sham-procedure control group.

Table 28. Adverse events in Studies CS3A and CS3B

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
All adverse events	4 (100)	16 (100)	38 (92.7)	72 (90.0)
Fatal adverse events	1 (25.0)	3 (18.8)	13 (31.7)	12 (15.0)
Serious adverse events	3 (75.0)	13 (81.3)	33 (80.5)	56 (70.0)
Severe adverse events	2 (50.0)	7 (43.8)	27 (65.9)	44 (55.0)
Adverse events leading to treatment discontinuation	0	3 (18.8)	12 (29.3)	12 (15.0)

n (Incidence [%])

The occurrence of serious adverse events in Studies CS3A and CS3B is shown in Table 29. Most of the reported serious adverse events were respiratory distress, respiratory failure, respiratory infection, etc. in both studies, and these events are expected to occur with the progression of SMA. In Study CS3B, respiratory distress and respiratory failure occurred at similar frequencies between the loading (Days 1, 15, 29, and 64) and maintenance dose periods in the sham-procedure control group, whereas these events occurred more frequently during the loading dose period (Days 1, 15, 29, and 64) in the nusinersen group. The incidence throughout the study period was similar, which does not deny the therapeutic effects of nusinersen.

Table 29. Main serious adverse events in Studies CS3A and CS3B

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
Main serious adverse events				
Respiratory distress	1 (25.0)	5 (31.3)	10 (24.4)	19 (23.8)
Respiratory failure	0	5 (31.3)	14 (34.1)	17 (21.3)
Pneumonia	0	4 (25.0)	4 (9.8)	14 (17.5)
Acute respiratory failure	1 (25.0)	3 (18.8)	7 (17.1)	11 (13.8)
Atelectasis	0	2 (12.5)	2 (4.9)	11 (13.8)
Pneumonia aspiration	0	2 (12.5)	4 (9.8)	6 (7.5)
Rhinovirus infection	0	4 (25.0)	2 (4.9)	6 (7.5)
Cardio-respiratory arrest	0	1 (6.3)	3 (7.3)	5 (6.3)
Pneumonia viral	0	2 (12.5)	1 (2.4)	5 (6.3)
Dyspnoea	0	0	2 (4.9)	4 (5.0)
Bronchiolitis	0	3 (18.8)	1 (2.4)	4 (5.0)
Viral infection	0	2 (12.5)	1 (2.4)	4 (5.0)
Upper respiratory tract infection	0	1 (6.3)	0	4 (5.0)
Respiratory tract infection	1 (25.0)	0	0	4 (5.0)
Viral upper respiratory tract infection	0	1 (6.3)	6 (14.6)	3 (3.8)
Respiratory arrest	0	0	4 (9.8)	3 (3.8)
Respiratory syncytial virus bronchiolitis	0	1 (6.3)	3 (7.3)	3 (3.8)
Hypoxia	0	1 (6.3)	1 (2.4)	3 (3.8)
Apnoea	0	2 (12.5)	2 (4.9)	2 (2.5)
Metapneumovirus infection	0	2 (12.5)	0	0

n (Incidence [%])

Based on the above, treatment with nusinersen has raised no apparent safety concerns.

PMDA accepts the above, but will continue to discuss the safety of nusinersen and the need for precautions in the package insert in Section 7.R.4, taking into account that pro-inflammatory effects and effects on the kidney, liver, and platelets are known to be class effects of 2'-MOE modified ASOs.[35)]

7.R.4.2 Lumbar puncture-related adverse events

PMDA asked the applicant to explain the occurrence of administration site reactions in relation to pro-inflammatory effects of 2'-MOE modified ASOs.

The applicant's explanation:

Concerning administration site reactions in Studies CS3A and CS3B, the occurrence of lumbar puncture-related adverse events[74)] is shown in Table 30. There was no trend towards a higher incidence in the nusinersen group than in the sham-procedure control group. Most events were mild or moderate in severity, and serious adverse events were very rare. There were no events leading to treatment discontinuation.

74) Events coded to the following MedDRA PTs:
back pain, cerebrospinal fluid leakage, epidural haemorrhage, extradural haematoma, headache, injection site haematoma, injection site haemorrhage, injection site pain, nausea, post lumbar puncture syndrome, post procedural complication, post procedural contusion, post procedural discomfort, post procedural swelling, procedural complication, procedural headache, procedural nausea, procedural pain, procedural site reaction, puncture site pain, spinal cord haematoma, spinal subarachnoid haemorrhage, subdural haematoma, vomiting, procedural dizziness, brain herniation, lumbar puncture abnormal, traumatic lumbar puncture

Table 30. Lumbar puncture-related adverse events in Studies CS3A and CS3B

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
Lumbar puncture-related adverse events	1 (75.0)	6 (37.5)	9 (22.0)	15 (18.8)
Vomiting	2 (50.0)	6 (37.5)	8 (19.5)	12 (15.0)
Procedural pain	1 (25.0)	1 (6.3)	0	2 (2.5)
Post procedural swelling	0	0	0	1 (1.3)
Procedural complication	0	0	0	1 (1.3)
Procedural site reaction	0	0	0	1 (1.3)
Post procedural contusion	0	0	1 (2.4)	0
Back pain	0	0	0	1 (1.3)
Cerebrospinal fluid leakage	0	1 (6.3)	0	0
Headache	1 (25.0)	0	0	0
Injection site haematoma	0	0	1 (2.4)	0

n (Incidence [%])

The main lumbar puncture-related adverse events observed in foreign open-label, uncontrolled studies in SMA patients ≥2 years of age[40] were headache (35.7% [20 of 56 subjects]), post lumbar puncture syndrome (33.9% [19 of 56 subjects]), vomiting (17.9% [10 of 56 subjects), etc., and the most commonly reported event was headache. Since patients who participated in Studies CS3A and CS3B had not developed adequate verbal communication skills, they were possibly unable to verbally report some of the typical symptoms of post lumbar puncture syndrome such as headache. There should be no substantial age-related differences in the safety of nusinersen. Based on the above, a precaution about lumbar puncture-related adverse events associated with nusinersen will be provided in the package insert.

PMDA accepts the above explanation. The applicant should collect information on the occurrence of lumbar puncture-related adverse events via post-marketing surveillance.

7.R.4.3 Renal impairment

PMDA asked the applicant to explain renal impairment associated with nusinersen.

The applicant's explanation:

Abnormal changes in laboratory values for renal function (urine protein, BUN, creatinine, cystatin C) observed in Studies CS3A and CS3B were assessed. As a result, 0% (0 of 4) of subjects in Cohort 1 and 12.5% (2 of 16) of subjects in Cohort 2 in Study CS3A and 12.2% (5 of 41) of sham-control subjects and 21.3% (17 of 80) of nusinersen-treated subjects in Study CS3B had abnormally high/positive urine protein after study drug administration, and there were no abnormal changes in other laboratory values for renal function.

The occurrence of renal impairment-related adverse events[59] in Studies CS3A and CS3B is shown in Table 31. In Study CS3B, the incidence of overall events was higher in the nusinersen group than in the sham-procedure control group due to more frequently reported urinary tract infection in the nusinersen group. The observed events were all mild or moderate in severity, and no nusinersen-related renal impairment occurred.

Table 31. Renal impairment-related adverse events in Studies CS3A and CS3B

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
Renal impairment-related adverse events	0	0	1 (2.4)	5 (6.3)
Urinary tract infection	0	0	0	3 (3.8)
Urinary retention	0	0	0	1 (1.3)
Hydronephrosis	0	0	0	1 (1.3)
Sugar urinary	0	0	1 (2.4)	0

n (Incidence [%])

Based on the above, though nusinersen-related renal impairment did not occur apparently, renal impairment has been reported with other 2'-MOE modified ASOs (*Toxicol Pathol*. 2015; 43: 78-89, *Mol Ther*. 2016; 24: 1771-82), and the US labeling also states that urine protein testing is required at baseline and prior to each dose of nusinersen. Taking account of these points, the package insert will state that renal impairment may occur following administration of nusinersen and that regular urine protein testing is recommended.

PMDA's view:

Given that more patients in the nusinersen group compared to the sham-procedure control group had abnormally high/positive urine protein in Study CS3B and that other 2'-MOE modified ASOs are associated with the risk of renal impairment,[35)] the possibility that renal impairment occurs following administration of nusinersen cannot be ruled out. Thus, precautions in the package insert are required. The package insert should also advise regular renal function testing during treatment with nusinersen. The applicant should collect information on the occurrence of renal impairment via post-marketing surveillance.

7.R.4.4 Hepatic impairment

PMDA asked the applicant to explain hepatic impairment associated with nusinersen.

The applicant's explanation:

The proportions of patients who had abnormally high ALT after study drug administration were 25.0% (1 of 4 subjects) in Cohort 1 and 6.3% (1 of 16 subjects) in Cohort 2 in Study CS3A and 9.8% (4 of 41 subjects) in the sham-procedure control group and 7.5% (6 of 80 subjects) in the nusinersen group in Study CS3B. The proportions of patients who had abnormally high AST were 0% (0 of 4 subjects) in Cohort 1 and 12.5% (2 of 16 subjects) in Cohort 2 in Study CS3A and 2.4% (1 of 41 subjects) in the sham-procedure control group and 3.8% (3 of 80 subjects) in the nusinersen group in Study CS3B. Nusinersen had no effects on hepatic function.

The occurrence of hepatic impairment-related adverse events[75)] in Studies CS3A and CS3B is shown in Table 32. There was no trend towards a higher incidence in the nusinersen group than in the sham-procedure control group, and most events were transient and did not lead to treatment discontinuation.

75) Events in the MedDRA SMQ "Drug related hepatic disorders - comprehensive search"

Table 32. Hepatic impairment-related adverse events in Japanese and foreign clinical studies

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
Hepatic impairment-related adverse events	0	0	1 (2.4)	3 (3.8)
ALT increased	0	0	0	1 (1.3)
AST increased	0	0	0	1 (1.3)
Liver function test abnormal	0	0	0	1 (1.3)
Hepatomegaly	0	0	0	1 (1.3)
Transaminases increased	0	0	1 (2.4)	0

n (Incidence [%])

Although persistent increases in ALT and AST have been reported with another 2'-MOE modified ASO (mipomersen sodium) (*Eur Heart J*. 2012; 33: 1142-9), since mipomersen sodium is involved in the degradation of the transcript of the gene encoding apolipoprotein B-100 in the liver and has a different mechanism of action from nusinersen, hepatic impairment is unlikely to occur with nusinersen. Thus, a precaution about hepatic impairment in the package insert is not required.

PMDA's view:

Following administration of nusinersen, no apparent increases in liver function tests were observed, and there was also no trend towards an increase in the incidence of hepatic impairment-related adverse events. However, the number of patients treated with nusinersen in clinical studies was limited, hepatic impairment is a class effect of 2'-MOE modified ASOs,[35)] and hepatic impairment may occur as a sequence-nonspecific effect of 2'-MOE modified ASOs. Taking account of the above, the package insert should state that hepatic impairment may occur and advise regular liver function testing. The applicant should collect information on the occurrence of hepatic impairment via post-marketing surveillance.

7.R.4.5 Effect on blood coagulation system

PMDA asked the applicant to explain the effect of nusinersen on blood coagulation system.

The applicant's explanation:

The proportions of patients who had abnormal changes in platelet count after study drug administration in Studies CS3A and CS3B are shown in Table 33. An abnormally low platelet count was observed in nusinersen-treated subjects only.

Table 33. Proportions of patients who had abnormal changes in platelet count after nusinersen administration in Studies CS3A and CS3B

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
Abnormally high	4 (100.0)	13 (81.3)	20 (48.8)	33 (41.3)
Abnormally low	0	2 (12.5)	0	5 (6.3)

n (Incidence [%])

The occurrence of coagulation-related adverse events[76)] in Studies CS3A and CS3B is shown in Table 34. The incidence of overall events was similar between the sham-procedure control and nusinersen groups. While

76) Events in the MedDRA SMQ "Haemorrhages" and "Embolic and thrombotic events"

vessel puncture site or injection site haemorrhage, etc. occurred frequently in the sham-procedure control group, ecchymosis, bloody stool, gastrointestinal haemorrhage, etc. were observed in the nusinersen group. Most of the observed events were mild in severity, and there were no fatal events or adverse events leading to treatment discontinuation. One subject had cerebral infarction in Study CS3A, but its causal relationship to nusinersen was denied. No apparent safety concerns have been suggested.

Table 34. Coagulation-related adverse events in Japanese and foreign clinical studies

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
Coagulation-related adverse events	1 (25.0)	5 (31.3)	6 (14.6)	5 (6.3)
Ecchymosis	0	1 (6.3)	0	2 (2.5)
Haematochezia	0	0	0	2 (2.5)
Contusion	0	1 (6.3)	0	1 (1.3)
Upper gastrointestinal haemorrhage	0	0	0	1 (1.3)
Gastric haemorrhage	0	0	0	1 (1.3)
Vessel puncture site contusion	0	1 (6.3)	2 (4.9)	0
Petechiae	0	1 (6.3)	0	0
Cerebral infarction	0	1 (6.3)	0	0
Infusion site contusion	0	0	1 (2.4)	0
Haematemesis	1 (25.0)	0	0	0
Injection site haematoma	0	0	1 (2.4)	0
Post procedural contusion	0	0	1 (2.4)	0
Diarrhoea haemorrhagic	0	0	1 (2.4)	0
Gastrointestinal haemorrhage	0	0	1 (2.4)	0
Stoma site haemorrhage	0	0	1 (2.4)	0

n (Incidence [%])

Based on the above, the package insert will state that a decreased platelet count and coagulation abnormalities may occur and that regular platelet count and coagulation laboratory testing is recommended.

PMDA's view:

Given that an abnormally low platelet count was observed in the nusinersen group only and ecchymosis, bloody stool, gastrointestinal haemorrhage, etc. occurred frequently in the nusinersen group in Study CS3B, the package insert should state that a decreased platelet count and coagulation abnormalities may occur following administration of nusinersen. The package insert should also advise regular platelet count and coagulation laboratory testing during treatment with nusinersen. The applicant should collect information on the occurrence of coagulation-related adverse events (including abnormal laboratory changes) via post-marketing surveillance.

7.R.4.6 Gastrointestinal effects

PMDA asked the applicant to explain the occurrence of gastrointestinal adverse events associated with nusinersen.

The applicant's explanation:

The occurrence of gastrointestinal adverse events[77)] in Studies CS3A and CS3B is shown in Table 35. As there was no trend towards a higher incidence in the nusinersen group compared to the sham-procedure control group, no particular risk has been suggested at present.

77) Events coded to the MedDRA SOC "Gastrointestinal disorders"

Table 35. Gastrointestinal adverse events in Studies CS3A and CS3B

	Study CS3A		Study CS3B	
	Cohort 1	Cohort 2	Sham-procedure control	Nusinersen
N	4	16	41	80
Gastrointestinal adverse events	4 (100)	15 (93.8)	25 (61.0)	48 (60.0)
Main adverse events				
Constipation	1 (25.0)	8 (50.0)	9 (22.0)	24 (30.0)
Vomiting	2 (50.0)	6 (37.5)	8 (19.5)	12 (15.0)
Teething	2 (50.0)	2 (12.5)	3 (7.3)	11 (13.8)
Gastrooesophageal reflux disease	0	6 (37.5)	6 (14.6)	9 (11.3)
Dysphagia	0	0	8 (19.5)	9 (11.3)
Diarrhoea	1 (25.0)	4 (25.0)	7 (17.1)	8 (10.0)
Salivary hypersecretion	0	2 (12.5)	2 (4.9)	3 (3.8)
Flatus	1 (25.0)	1 (6.3)	1 (2.4)	3 (3.8)
Abdominal distension	1 (25.0)	2 (12.5)	0	1 (1.3)
Enterovirus infection	0	2 (12.5)	0	0
Hypophagia	1 (25.0)	1 (6.3)	0	0
Abdominal pain	1 (25.0)	0	0	0
Gastrointestinal hypomotility	1 (25.0)	0	0	0
Haematemesis	1 (25.0)	0	0	0

n (Incidence [%])

PMDA accepted the above explanation.

7.R.4.7 Effect on growth

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

The applicant's explanation:

Weight-for-age percentiles based on World Health Organization (WHO) Child Growth Standards[78)] at baseline in Studies CS3A and CS3B were 3.1% to 89.9% (median, 37.1%) and 0.6% to 97.8% (median, 14.2%), respectively, which were lower than those in healthy infants. Head circumference-for-age, height-for-age, and weight-for age percentiles based on WHO Child Growth Standards over time in Study CS3B are shown in Table 36, and there was a trend towards increasing percentiles in the sham-procedure control group than in the nusinersen group. In Study CS3A, head circumference-for-age percentiles based on WHO Child Growth Standards (mean ± SD [No. of evaluable subjects]) at baseline and on Day 379 were 56.10 ± 25.84 (20 subjects) and 55.37 ± 21.59 (15 subjects), respectively, height-for-age percentiles were 60.11 ± 31.05 (20 subjects) and 34.52 ± 38.67 (15 subjects), respectively, and weight-for-age percentiles were 39.98 ± 28.20 (20 subjects) and 24.61 ± 23.80 (15 subjects), respectively. As in Study CS3B, increases in height-for-age and weight-for age percentiles tended to be reduced with continued treatment with nusinersen.

Table 36. Growth parameters over time in Study CS3B

Time point	Head circumference		Height		Body weight	
	Sham-procedure control	Nusinersen	Sham-procedure control	Nusinersen	Sham-procedure control	Nusinersen
Baseline	66.3 ± 29.0 (27)	54.0 ± 27.6 (51)	45.8 ± 33.4 (27)	47.3 ± 34.0 (51)	24.3 ± 28.2 (27)	25.3 ± 25.5 (51)
Day 29	63.0 ± 27.8 (23)	51.6 ± 28.3 (48)	52.2 ± 36.5 (23)	48.3 ± 32.8 (48)	27.3 ± 26.7 (24)	25.3 ± 28.8 (49)
Day 64	62.9 ± 27.2 (21)	52.6 ± 26.9 (45)	50.1 ± 34.6 (21)	51.5 ± 33.5 (45)	27.4 ± 24.1 (21)	28.2 ± 30.6 (45)
Day 183	65.6 ± 31.5 (18)	53.5 ± 28.9 (40)	63.5 ± 32.1 (18)	42.3 ± 36.5 (40)	35.9 ± 28.5 (18)	31.9 ± 33.6 (40)
Day 302	72.1 ± 23.1 (12)	53.1 ± 31.0 (24)	66.1 ± 36.9 (12)	33.6 ± 33.6 (24)	48.9 ± 36.9 (12)	36.8 ± 31.6 (25)
Day 394	77.7 ± 27.1 (7)	58.3 ± 21.9 (16)	81.9 ± 22.4 (7)	31.4 ± 35.6 (16)	60.8 ± 33.2 (7)	28.5 ± 30.3 (16)

Mean ± SD for percentile (No. of evaluable subjects)

78) WHO Child Growth Standards. World Health Organization; 2006. http://www.who.int/childgrowth/en/

More subjects in the sham-procedure control group compared to the nusinersen group were receiving enteral tube feeding (12.2% [5 of 41 subjects] in the sham-procedure control group and 8.8% [7 of 80 subjects] in the nusinersen group at baseline). Thus, greater increases in head circumference, height, and body weight were observed in the sham-procedure control group possibly because more subjects were overfed, but the details are unknown. It has been reported that bulbar dysfunction, dysphagia, and impaired gastrointestinal motility result in low body weight in patients with Type I SMA, and there is a report that approximately 30% of patients with Type I SMA had a weight for age of less than the 3rd percentile and a height for age of less than the 3rd percentile (*Neuromuscul Disord*. 2012; 22: 966-73). Taking account of these findings, since there was a trend towards slight improvement for body weight and baseline values were maintained for head circumference and height until Day 183 (a certain number of subjects were assessed until Day 183) in the nusinersen group of Study CS3B, any reduced growth associated with nusinersen is not clinically relevant.

PMDA's view:

In Study CS3B, the withdrawal rate was higher in the sham-procedure control group (31.7% [13 of 41 subjects]) than in the nusinersen group (16.3% [13 of 80 subjects]), and subjects with worsening of symptoms were withdrawn from the study, which may have resulted in improvement in apparent percentiles in the sham-procedure control group, etc. Rigorous comparison between the treatment groups is difficult, and taking also account of the applicant's discussion, it is difficult to conclude based on these study results that nusinersen causes a reduction in growth. On the other hand, since Studies CS3A and CS3B suggested that increases in height-for-age and weight-for age percentiles may be reduced with continued treatment with nusinersen, the effect of nusinersen on growth cannot be ruled out at present. Thus, the applicant should appropriately inform healthcare professionals in clinical practice of the effect of nusinersen on growth observed in clinical studies and then collect information via post-marketing surveillance.

7.R.5 Anti-nusinersen antibodies

PMDA asked the applicant to explain the incidence of anti-nusinersen antibodies following administration of nusinersen and the pharmacokinetics, efficacy, and safety of nusinersen in patients with anti-nusinersen antibodies, and then the need for anti-nusinersen antibody measurement.

The applicant's explanation:

- In clinical studies of nusinersen, 1.25% (1 of 80) of subjects in the nusinersen group in a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), 5.0% (1 of 20) of subjects in a foreign phase II study (CTD 5.3.5.2-1, Study CS3A), and 4.3% (2 of 47) of subjects in a foreign phase I study (CTD 5.3.5.2-4, Study CS12) only tested positive for plasma anti-nusinersen antibodies, and no patients tested positive for anti-nusinersen antibodies in other clinical studies. In Study CS3B, 1 patient had a positive antibody response before study drug administration, but its reason was unclear.
- CSF and plasma nusinersen concentrations in patients positive for anti-nusinersen antibodies were within the range of those in antibody-negative patients, and decreased nusinersen exposure was not observed.

- Regarding efficacy, there was a trend towards improvement in the HINE motor milestones and CHOP INTEND total score for both patients positive for anti-nusinersen antibodies in Studies CS3A and CS3B. Thus, the presence of antibody should have no major impact on the efficacy of nusinersen.
- Regarding safety, serious adverse events observed in patients positive for anti-nusinersen antibodies in Studies CS3A and CS3B were pneumonia pseudomonal, respiratory distress, respiratory tract infection, and atelectasis (1 patient); and delayed recovery from anaesthesia, increased bronchial secretion, lower respiratory tract infection, respiratory arrest, respiratory distress, and viral infection (1 patient). Since there was no trend towards a substantial difference in the nature of adverse events between antibody-positive and antibody-negative patients, the presence of antibody should have no major impact on the safety of nusinersen.
- In the course of regulatory review in the US, FDA instructed the applicant to conduct a study to assess for the presence of anti-double-stranded DNA antibodies among patients treated with nusinersen, as a post-marketing requirement. Although a similar study has been requested also for another 2'-MOE modified ASO (mipomersen sodium), as there is no publication etc. investigating the impact of anti-double-stranded DNA antibodies on the efficacy and safety of a 2'-MOE modified ASO, the applicant also has not understood the significance of anti-double-stranded DNA antibody measurement.
- Based on the above, since the incidence of anti-nusinersen antibodies in nusinersen-treated patients was low and there has been no apparent impact of anti-nusinersen antibodies on the pharmacokinetics, efficacy, and safety of nusinersen at present, anti-nusinersen antibody measurement is not necessary after the market launch of nusinersen.

PMDA accepts the above explanation, and considers that there is no major problem with not measuring anti-nusinersen antibodies and anti-double-stranded DNA antibodies during treatment with nusinersen at present.

7.R.6 Indication and intended population

7.R.6.1 Appropriateness of proposed indication

Given that a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B) demonstrated the efficacy and safety of nusinersen in SMA patients ≤7 months (210 days) of age at screening,[65)] PMDA asked the applicant to explain the appropriateness of the proposed indication for nusinersen.

The applicant's explanation on the reason/background for the proposed indication of "spinal muscular atrophy":

- Patients are diagnosed with SMA based on their clinical symptoms.[79)] Historically, classification into 4 SMA types (I, II, III, IV) has retrospectively been based on the age of onset and the maximal achieved motor function (Table 37). In addition, Type 0 SMA is a rare type in which neonates are born with weakness or clinical signs of death.[80)]

79) (1) Lower motor neuron signs, (2) no upper motor neuron signs, (3) the disease course is progressive, and (4) the criteria for clinical laboratory findings (CK levels, electromyogram, motor nerve conduction velocity) are met, and all of the diseases in the differential diagnosis are ruled out, or gene mutation confirmed by genetic testing and all of the diseases in the differential diagnosis are ruled out.

80) *Pediatr Clin North Am*. 2015; 62: 743-66, *Nat Rev Neurol*. 2015; 11: 351-9, *Neuromuscul Disord*. 2015; 25: 593-602, *Semin Spine Surg*. 2012; 24:164-8, *Eur J Hum Genet.* 2004; 12: 1015-23, *Neuromuscul Disord*. 2015; 25: 979-89

Table 37. Subtypes of SMA

SMA type		Age at onset (diagnosis)	Life expectancy	Highest motor milestone achieved	*SMN2* copy number	% of SMA cases
0		Fetal (at birth)	Approximately 1 week	None	1	-
I	I A	Fetal (2 weeks)	Approximately 1-2 weeks	Milestones rarely achieved	1 or 2	58%
	I B	Infancy (3 months)	<2 years of age without respiratory support	Head control never achieved	2 or 3	
	I C	Infancy (3-6 months)		Head control achieved, but never rolls or sits independently		
II		Infancy (6-18 months)	>2 years; 70% alive at 25 years	Attain independent sitting when placed. May stand, but unable to walk	3 in most patients	29%
III	III A	Early childhood (18-36 months)	Normal	Able to sit independently, stand, and walk with difficulties. Majority lose ambulation before or around puberty	3 or 4	13%
	III B	Late childhood to school-age/adolescence (3-10 years, generally ≤18 years)				
IV		Adulthood (>35 years)		Normal. Increased motor impairment after onset.	≥ 4	-

- In order to assess the efficacy and safety of nusinersen in mainly Type I SMA patients in Study CS3B, eligible patients had to have the onset of symptoms at ≤6 months (180 days) of age and 2 copies of the *SMN2* gene. As a result, the study demonstrated the efficacy and safety of nusinersen [see 7.2]. Hence, it was considered that nusinersen may be indicated for mainly Type I SMA patients.
- In addition, at the time of regulatory submission, a confirmatory study in mainly Type II or III SMA patients (Study CS4) was ongoing, but open-label, uncontrolled studies involving this patient population[45)] had been conducted and there was clinical experience with nusinersen in this population, though in a limited number of patients. Given the mechanism of action of nusinersen, it was thought that the efficacy of nusinersen is expected also in patients with other types of SMA caused by genetic defects in the *SMN1* gene. SMA is a rare disease. Taking account of these points, it was considered that the broader indication of "spinal muscular atrophy" can be claimed.

7.R.6.2 Consideration of indication after regulatory submission

As shown in Table 38, the dosing regimen used was different between Study CS3B involving mainly Type I SMA patients and Study CS4 involving mainly Type II or III SMA patients. Given the dosing rationale for nusinersen [see Section 7.R.7.1], nusinersen was administered more frequently in patients with Type I SMA, the most severe phenotype, but the need for the same dosing frequency in other patient populations is unclear. Study CS4 was ongoing at the time of regulatory submission, and the efficacy and safety of nusinersen administered according to the dosing regimen selected for a patient population other than Type I SMA patients had not been determined. Thus, PMDA concluded that it is difficult at present to establish the dosing regimen for Type II, III, or IV SMA patients and propose the indication of Type I, II, III, or IV SMA, and asked the applicant to reconsider the indication.

Table 38. Study population and dosing regimen

	Study population	Dosing regimen
Study CS3B	mainly Type I	12-mg scaled equivalent doses of nusinersen on Days 1, 15, 29, and 64 followed by dosing every 4 months
Study CS4 (ongoing)	mainly Types II and III	12-mg scaled equivalent doses of nusinersen on Days 1, 29, and 85 followed by dosing every 6 months

The applicant's explanation:

Based on the main patient population for Study CS3B, nusinersen should be indicated for Type I SMA patients. However, if the indication is "Type I spinal muscular atrophy," initiation of treatment with nusinersen has to be delayed based on the following points. Thus, this was not considered appropriate.

- Patients are ultimately categorized into Type I SMA based on their clinical presentation of failure to achieve the ability to sit independently.
- Even non-SMA infants normally achieve the ability to sit independently at around 8 months of age.
- Thus, patients have to wait until the age of around 8 months to accurately be classified into Type I SMA and differentiated from those with other types of SMA.
- However, as the symptoms of Type I SMA become severe by around 8 months of age, earlier treatment initiation is desirable. Study CS3B enrolled SMA patients ≤7 months (210 days) of age at screening, and the efficacy of nusinersen initiated in Type I SMA patients ≥8 months of age has not been determined.

Thus, the applicant gave the following considerations to the indication statement to prospectively define the main patient population for Study CS3B.

- If the indication is "spinal muscular atrophy with onset at ≤6 months of age," the intended population will be defined, but SMA is not well known by physicians or the general public in Japan, and it is envisaged that there are patients without records of symptoms and onset before 6 months of age. Consequently, patients may loose a therapeutic opportunity, and this indication statement is not appropriate.
- If the indication is "infantile spinal muscular atrophy" based on ICD-10, specialists will understand that this refers to the main patient population for Study CS3B. However, since the age of onset is not explicitly indicated, non-specialists may not understand the intended population correctly.
- If the indication is "spinal muscular atrophy (only in patients with onset during infancy)," it explicitly refers to patients with onset during infancy (normally, ≥4 weeks and <1 year of age). Thus, non-specialists also will understand the main patient population for Study CS3B correctly.

Based on the above, the indication will be changed to "spinal muscular atrophy (only in patients with onset during infancy)."

PMDA's view:

Given the history of development of nusinersen, the indication should be established based on the patient population for Study CS3B. According to the applicant's explanation, if the indication is "Type I spinal muscular atrophy," initiation of treatment with nusinersen will be delayed. Thus, the indication statement that allows for prospective diagnosis and classification is more appropriate.

"Spinal muscular atrophy (only in patients with onset during infancy)" proposed by the applicant is not appropriate because Type II SMA patients with onset at 6 to 12 months of age are included. The package insert will advise that nusinersen should be used by physicians familiar with the diagnosis and treatment of SMA [see Section 7.R.8], use of nusinersen in infant patients should be acceptable in view of the seriousness of the disease and the mechanism of action of nusinersen [see Section 7.R.7], and the wording of the indication should be based on the international classification of diseases, etc. Thus, "infantile spinal muscular atrophy" is more appropriate. Although the primary endpoint for Study CS3B was related to motor function, it is

unnecessary to add "improvement of motor function" etc. to the indication statement since nusinersen slows down the progression of SMA symptoms and is expected to improve the prognosis to a certain extent [see Section 7.R.3.2]. A final decision on the indication will be made, taking account of comments from the Expert Discussion.

7.R.6.3 Genetic diagnosis in SMA patients

Taking account of the mechanism of action of nusinersen, PMDA asked the applicant to explain the need for genetic diagnosis prior to the initiation of nusinersen treatment.

The applicant's explanation:

Based on the mechanism of action of nusinersen, the efficacy of nusinersen is expected in patients with (1) SMA caused by genetic defects in the *SMN1* gene and (2) ≥1 copy of the *SMN2* gene, but the efficacy of nusinersen cannot be expected at all unless patients meet both criteria. Since it has been reported that patients who do not meet the criterion (1) account for around 4% to 5% of all patients with SMA (*Am J Hum Genet.* 1999; 64: 1340-56), a differential diagnosis should be made prior to the initiation of nusinersen treatment. Moreover, *SMN2* copy number is still determined widely because it predicts survival prognosis. Though there are no reports on SMA patients with 0 copies of the *SMN2* gene who do not meet the criterion (2) (*BMC Musculoskelet Disord*. 2015; 16: 11), as it is inferred that these patients are very short lived or die before birth, there is little need for differential diagnosis prior to administration.

Implementation of genetic diagnosis in SMA patients was surveyed among pediatricians in Japan in [redacted] 20[redacted]. As a result, 81.5% (88 of 108) of physicians who had experience in the treatment of Type I SMA made a genetic diagnosis of SMA. Although there are currently no clinical laboratory companies that provide a genetic diagnosis service for SMA patients, genetic testing of samples collected in or outside the medical institution is conducted at some medical institutions. Considerations are underway so that a clinical laboratory company can provide a genetic diagnosis service for SMA patients, and it is planned to prepare the system for providing a genetic diagnosis service after appropriately performing validation.

If a genetic diagnosis is made, normally, testing itself takes about 1 week. If a clinical laboratory company provides a genetic diagnosis service, the company needs to test a certain number of samples at a time in order to maintain profitability under the current fee-for-healthcare service system. Based on the number of SMA patients, it is estimated that it takes 2 to 4 weeks to report the test results. Though it should be rare to administer nusinersen at the first visit, if treatment with nusinersen is not initiated until the genetic diagnosis results are obtained, patients with Type I SMA might experience disease progression. Thus, initiation of treatment with nusinersen before genetic diagnosis should be allowed, and a genetic diagnosis should be made as soon as possible. If it is found that the efficacy of nusinersen is not expected in the patient, nusinersen treatment should be discontinued. These precautions should be included in the package insert.

PMDA's view:

Based on the applicant's explanation, the efficacy of nusinersen cannot be expected at all in 4% to 5% of all patients with SMA. Hence, the use of nusinersen should be limited to patients with (1) SMA caused by genetic defects in the *SMN1* gene and (2) ≥1 copy of the *SMN2* gene. Though the seriousness of SMA is understood, ≥80% of physicians made a genetic diagnosis of SMA as of [redacted] 20[redacted], it seems that the type of genetic defect causing SMA and *SMN2* copy number were determined in many cases, and it takes about 1 week to complete genetic testing itself. Taking account of these points, the package insert should advise that nusinersen should be used in patients who meet the above criteria (1) and (2). A final decision on the need for genetic diagnosis and the appropriateness of precautions will be made, taking account of comments from the Expert Discussion.

7.R.6.4 Patients with *SMN2* copy number other than 2

While patients with 2 copies of *SMN2* only were included in Study CS3B, patients with Type I SMA have 1 to 3 copies of *SMN2* (Table 37). PMDA asked the applicant to explain the appropriateness of the use of nusinersen in patients with *SMN2* copy number other than 2.

The applicant's explanation:

Given the mechanism of action of nusinersen, a certain level of efficacy of nusinersen is expected in patients with ≥1 copy of *SMN2*. Treatment with nusinersen results in increased SMN protein expression, and especially patients with ≥3 copies of *SMN2* may have higher expression levels compared with patients with 2 copies of *SMN2*, but increased SMN protein expression is unlikely to become a major safety issue [see Section 3.R.1]. Based on the above, nusinersen may be indicated for patients with *SMN2* copy number other than 2.

PMDA's view:

Although no safety risk in humans has been suggested at present, nusinersen-treated patients may have higher SMN protein expression levels than healthy humans. The effect of increased SMN protein expression on safety has not been determined yet [see Section 3.R.1]. Considering that SMA (mainly Type I SMA) is a fatal, serious, rare disease, it is acceptable not to limit the use of nusinersen to patients with 2 copies of *SMN2*. However, reduced efficacy is potentially observed in patients with 1 copy of *SMN2* and safety issues may arise in patients with ≥3 copies of *SMN2*. The package insert should advise that patients should be monitored carefully. The applicant should collect information on the relationship between *SMN2* copy number and efficacy/safety via post-marketing surveillance.

7.R.7 Dosage and administration

7.R.7.1 Dosing rationale for multi-regional phase III study

PMDA asked the applicant to explain the dosing rationale for Study CS3B, taking account of non-clinical and clinical data available before the conduct of a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B).

The applicant's explanation:

- A pharmacology study in SMN2 mice[3)] (reference data, CTD 4.2.1.1-4) indicated that nusinersen concentrations of 1 to 10 μg/g in spinal cord tissue are needed to produce pharmacologic effects of nusinersen.
- Since there were no sufficient human pharmacokinetic data, a 2-compartment model was developed using toxicokinetic data from a 14-week intermittent dose IT toxicity study in juvenile monkeys (CTD 4.2.3.2-1), and then nusinersen concentrations in spinal cord tissue over time were simulated using CSF half-life values in a phase I study in mainly Type II or III SMA patients (≥2 years of age) (CTD 5.3.4.2-2, Study CS2).[81)]
- The results predicted that the mean tissue concentrations of nusinersen in the lumbar, thoracic, and cervical spinal cord regions would be all >5 μg/g in children ≥2 years of age receiving 3 doses of 12 mg of nusinersen (on Days 1, 29, and 85) followed by maintenance doses every 6 months.
- Furthermore, more frequent dosing was considered necessary for mainly Type I SMA patients who were eligible for Study CS3B because Type I SMA was the most severe phenotype. Dosing conditions were examined to include the patient population. As a result, it was predicted that 4 doses of 12 mg of nusinersen (on Days 1, 15, 29, and 64) followed by maintenance dosing every 4 months can achieve higher steady-state exposures faster in more patients.
- Since age-related volumetric changes of CSF in humans have been reported (*Cereb Cortex*. 2001; 11:335-42), based on the dose (12 mg/dose) and CSF volume (150 mL) in children ≥2 years of age, the dose was adjusted based on the subject's age as shown in Table 20 so that the CSF nusinersen concentration would be equivalent across all age groups.

Based on the above, 12-mg scaled equivalent doses of nusinersen (Table 20) on Days 1, 15, 29, and 64 followed by dosing every 4 months for Study CS3B was considered appropriate.

7.R.7.2 Proposed dosage and administration

PMDA asked the applicant to explain the appropriateness of the proposed dosage and administration based on the results from Study CS3B, etc.

The applicant's explanation:

In a foreign phase II study in which 12-mg scaled equivalent doses of nusinersen (Table 20) were administered as in Study CS3B (CTD 5.3.5.2-1, Study CS3A), 3 subjects who died were autopsied and nusinersen concentrations in the CNS tissues were determined. Nusinersen tissue concentrations in the lumbar, thoracic, and cervical spinal cord regions were ≥10 μg/g, and ≥5 μg/g of nusinersen concentrations were observed in many other tissues (Table 14).

In Study CS3B, the plasma pharmacokinetic parameters of nusinersen and nusinersen trough concentrations in CSF after the first dose by age group (0-3 months, 3-6 months, 6-12 months) on Day 1 are shown in Table 39, and there were no major differences in exposure according to age group. In Study CS3A, nusinersen trough concentrations in plasma on Day 379 in patients aged 6 to 12 months and 12 to 24 months on Day 253 were

81) Doses were converted to human equivalent doses (× 10) based on the nominal volume difference in CSF between monkeys (15 mL, *Radiology*. 1985; 157: 373-7) and humans ≥2 years of age (150 mL, *Cereb Cortex*. 2001; 11: 335-42), and then simulations were conducted.

0.53 ± 0.14 and 0.56 ± 0.24 ng/mL, respectively, and nusinersen trough concentrations in CSF were 7.90 ± 5.54 and 9.90 ± 10.67 ng/mL, respectively. The dosing regimen used in Study CS3B was considered to provide comparable CNS tissue exposure and systemic exposure across all age groups.

Table 39. Plasma pharmacokinetic parameters of nusinersen and nusinersen trough concentrations in CSF after the first dose by age group in Study CS3B

	Plasma concentration					CSF concentration		
	Time point	No. of evaluable subjects	C_{max} (ng/mL)	t_{max} (h)[a)]	$AUC_{0\text{-}24h}$ (ng·h/mL)	Time point	No. of evaluable subjects	Concentration (ng/mL)
0-3 months	Day 1	6	1357 ± 957	2.0	11,052 ± 6324[b)]	Day 15	5	5.51 ± 2.69
3-6 months	Day 1	41	1003 ± 653	2.0	9849 ± 4985[c)]	Day 15	40	4.08 ± 2.29
6-12 months	Day 1	29	1192 ± 1071	2.0	10,204 ± 4519	Day 15	23	3.42 ± 2.24

Mean ± SD
a) Median, b) n = 5, c) n = 38

In Study CS3B, the proportions of patients who achieved improvement in motor milestones as evaluated by Section 2 (7 items[69)]) of the HINE [70)] in different age groups on Day 1 (0-3 months, 3-6 months, 6-12 months) were 0% (0 of 2 patients), 54% (15 of 28 patients), and 29% (6 of 21 patients), respectively, in the nusinersen group. Although the efficacy of nusinersen in patients 0 to 3 months of age was not determined due to the limited number of patients, the efficacy of nusinersen in patients ≥3 months of age was suggested. Given that 50% (1 of 2) of the patients aged 0 to 3 months had a ≥4-point increase in the CHOP INTEND total score, the efficacy of nusinersen was not defined. The occurrence of adverse events by age group (0-3 months, 3-6 months, 6-12 months) in Study CS3B is shown in Table 40, and there was no trend towards a higher incidence of adverse events in the nusinersen group than in the sham-procedure control group in any age group.

Table 40. Occurrence of adverse events by age group in Study CS3B

	0-3 months		3-6 months		6-12 months	
	Sham-procedure control	Nusinersen	Sham-procedure control	Nusinersen	Sham-procedure control	Nusinersen
No. of evaluable subjects	2	6	14	43	25	31
All adverse events	2 (100)	5 (83.3)	12 (85.7)	39 (90.7)	24 (96.0)	28 (90.3)
Fatal adverse events	1 (50.0)	1 (16.7)	4 (28.6)	4 (9.3)	8 (32.0)	7 (22.6)
Serious adverse events	2 (100)	3 (50.0)	10 (71.4)	31 (72.1)	21 (84.0)	22 (71.0)
Severe adverse events	2 (100)	2 (33.3)	10 (71.4)	25 (58.1)	15 (60.0)	17 (54.8)
Adverse events leading to treatment discontinuation	1 (50.0)	1 (16.7)	4 (28.6)	4 (9.3)	7 (28.0)	7 (22.6)

7.R.7.3 Dosage and administration for age groups of children who were not enrolled in Study CS3B

Taking into account that patients actually enrolled in Study CS3B were aged 52 to 242 days at the start of treatment, PMDA asked the applicant to explain the pharmacokinetics, efficacy, and safety of nusinersen in age groups of children who were not enrolled in Study CS3B, and then the appropriateness of the proposed dosage and administration.

The applicant's explanation on older patients compared to patients treated in Study CS3B:

- The pharmacokinetics of nusinersen in patients ≥6 months of age was simulated. The simulated C_{max} and $AUC_{0\text{-}\infty}$ in CSF after a single 12-mg scaled equivalent IT dose of nusinersen (Table 20) in different age groups[82)] are shown in Table 41 The distribution of the pharmacokinetic parameters across different

82) Based on parameters obtained from PPK analysis (CTD 5.3.3.5-2, CPP-17-001-BIIB058 analysis), the C_{max} and $AUC_{0\text{-}\infty}$ in CSF following a single 12-mg scaled equivalent IT dose of nusinersen (Table 20) in each age group were simulated (n = 100).

age groups overlapped. Thus, the efficacy of nusinersen is unlikely to differ substantially according to age group.

Table 41. Simulated pharmacokinetic parameters of nusinersen in CSF following a single 12-mg scaled equivalent IT dose of nusinersen (Table 20) in different age groups

Age	0-3 months	3-6 months	6-12 months	12-24 months	2-6 years	6-12 years	12-18 years
C_{max} (μg/mL)	95.3 (134)	72.5 (148)	60.9 (163)	48.6 (158)	60.1 (163)	56.0 (195)	58.1 (164)
$AUC_{0-\infty}$ (μg·h/mL)	90.4 (49.2)	94.3 (41.2)	94.0 (45.9)	94.5 (39.0)	105.0 (48.5)	96.3 (45.9)	101.0 (37.9)

Geometric mean (coefficient of variation)

- The C_{max} and AUC of nusinersen in plasma tended to be lower in patients ≥2 years of age (Table 17) compared to patients ≤6 months of age (Table 39). Since body weight increases with growth, the dose per body weight and systemic exposure are lower in older patients compared to younger patients. Thus, no clear safety concerns have been suggested.

The applicant's explanation on younger patients compared to patients treated in Study CS3B:

- In a phase II study in non-Japanese subjects with a mutation in the SMN gene (CTD 5.3.5.2-2, Study 232SM201), subjects were stratified by the median age on Day 1 (<19 days, ≥19 days). Nusinersen trough concentrations in CSF on Day 15 were 27.6 ± 21.3 and 13.9 ± 10.4 ng/mL, respectively, and plasma nusinersen concentrations at 4 hours after the first dose were 250 ± 156 and 573 ± 391 ng/mL, respectively, which were higher than those in Study CS3B.
- No systematic data on efficacy and safety in younger patients are available. Given that the pathology of Type I SMA is unlikely to differ substantially according to age group and that CSF nusinersen concentrations were higher than those in Study CS3B, a clinically relevant efficacy issue is unlikely to arise. Since no increases in the incidence or severity of specific adverse events were observed in Study 232SM201, a clinically relevant safety issue is unlikely to arise, provided that nusinersen is administered while carefully monitoring the patient's condition.

Based on the above considerations, the applicant decided not to limit the age of candidate patients and proposed dosage and administration as shown below.

[Proposed dosage and administration]

Spinraza treatment should be initiated as early as possible after diagnosis. The dose of nusinersen is 12 mg (5 mL) for patients aged >2 years (24 months). For infants aged ≤2 years (24 months) who have a smaller volume of cerebrospinal fluid, the dose of nusinersen should be adjusted based on their age and according to the table below. Spinraza should be administered intrathecally by lumbar puncture.

Age	Dose (mg)	Injection volume (mL)	Loading doses (Dosing days)	Maintenance doses*
0-3 months	9.6 mg	4 mL	Days 0 (the day of the first dose), 14, 28, and 63	Every 4 months
3-6 months	10.3 mg	4.3 mL		
6-12 months	10.8 mg	4.5 mL		
12-24 months	11.3 mg	4.7 mL		
> 24 months	12 mg	5 mL		

* A maintenance dose should be started after the 4th loading dose.

7.R.7.4 Applicant's consideration of dosage and administration after regulatory submission

The applicant's explanation:

The distributions of simulated C_{max} and $AUC_{0-\infty}$ values in CSF[83)] following a single 12-mg scaled equivalent IT dose (Table 20) or a single 12 mg IT dose of nusinersen in different age groups of patients are shown in Figure 2 and Figure 3, respectively. Although the C_{max} and $AUC_{0-\infty}$ following administration of 12 mg of nusinersen tended to be higher especially in the youngest patients, there was substantial overlap. In Study 232SM201 in infants 8 to 42 days of age (17 evaluable subjects), while the nusinersen trough concentration in CSF on Day 15 was 5- to 6-fold those in Studies CS3A and CS3B [see Sections 6.2.2.2 and 6.2.3], there was no trend towards an increase in the incidence of adverse events. Thus, there are no safety concerns about increased nusinersen exposure.

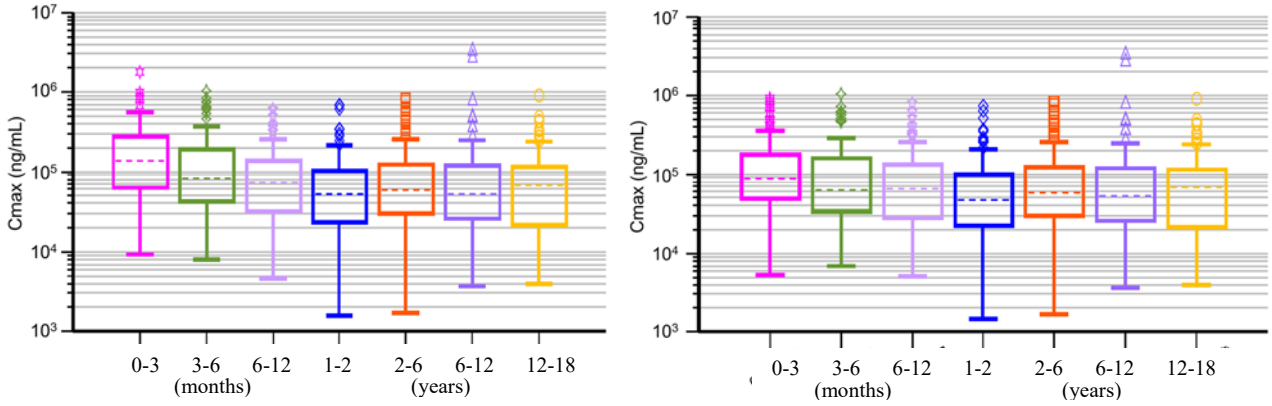


Figure 2. Distribution of simulated C_{max} values in CSF following a single 12 mg IT dose (left figure) or a single 12-mg scaled equivalent IT dose of nusinersen (Table 20) (right figure) in different age groups

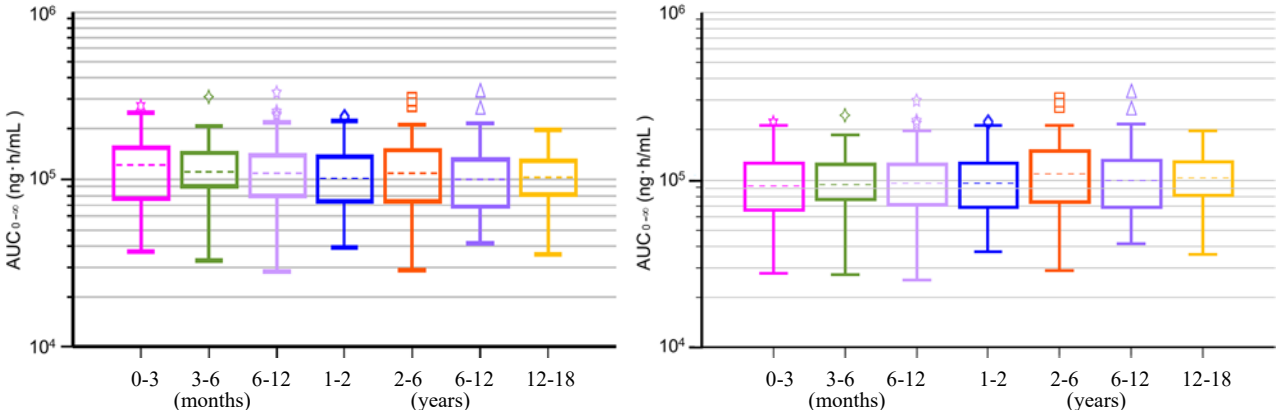


Figure 3. Distribution of simulated $AUC_{0-\infty}$ values in CSF following a single 12 mg IT dose (left figure) or a single 12-mg scaled equivalent IT dose of nusinersen (Table 20) (right figure) in different age groups

After reconsidering dosage and administration for nusinersen, the applicant hopes to change the dosing regimen from age-based dosing to fixed dosing as shown below, for the following reason: Age-based dosing of nusinersen is not particularly inconvenient in clinical practice in Japan, but its necessity is not high.

83) Assuming that patients in each age group (n = 100) receive a single 12 mg IT dose or a single 12-mg scaled equivalent IT dose of nusinersen (Table 20), C_{max} and $AUC_{0-\infty}$ values in CSF were simulated based on parameters obtained from PPK analysis (CTD 5.3.3.5-2, CPP-17-001-BIIB058 analysis).

[Dosage and administration (Amendment proposed by the applicant)]
Spinraza treatment should be initiated as early as possible after diagnosis. The dose of nusinersen is 12 mg (5 mL). Spinraza should be administered intrathecally by lumbar puncture. Doses on Days 0, 14, 28, and 63 are followed by maintenance doses every 4 months.

The applicant's explanation:
In the US, the applicant's claim was accepted and evaluated as shown below. As a result, a fixed dose of 12 mg for all patients regardless of age was approved.

- A fixed dose of 12 mg nusinersen for all patients regardless of age group may reduce the potential for any dosing errors.
- After fixed dosing of 12 mg nusinersen compared to age-based dosing, the C_{max} and $AUC_{0-\infty}$ will be up to 25% higher. Considering the variability in CSF nusinersen concentrations, the majority of patients will have exposures in the range of those observed in clinical studies.
- Given that nusinersen was well tolerated and there was no evidence for any serious adverse events related to exposure in clinical studies, increases in the C_{max} and $AUC_{0-\infty}$ raise no new safety concerns.

PMDA's view:
The dosing regimen for Study CS3B was based on the assumptions that the PD of nusinersen are similar between rats and humans and that the pharmacokinetics of nusinersen are similar between monkeys and humans. The appropriateness of such assumptions is unclear at present. However, taking account of the intended population for nusinersen, it was unavoidable to examine the dosing regimen based on the limited data available at the time of initiating Study CS3B. Given that studies of autopsy samples from subjects in Study CS3A confirmed that target spinal cord tissue concentrations can be achieved with a dosing regimen similar to the proposed dosing regimen and that Study CS3B demonstrated the efficacy and safety of age-based dosing of nusinersen (Table 20) and showed no marked differences in the pharmacokinetics, efficacy, or safety of nusinersen among age groups, there are no major problems with the dose and dosage regimen presented in the proposed package insert. Although the nusinersen trough concentration in CSF after administration of nusinersen in Study 232SM201 in subjects 8 to 42 days of age was approximately 5 times higher than that in Study CS3B in subjects 52 to 242 days of age [see Sections 6.2.2.2 and 6.2.3], no major safety concerns have been suggested to date. SMA (mainly Type I SMA) is a fatal, serious disease. Taking account of these points, setting no age limits for the use of nusinersen is acceptable, provided that the package insert will advise that the patient's condition should be carefully observed prior to the use of nusinersen and that the efficacy and safety of nusinersen will carefully be assessed in patients younger or older than those enrolled in Study CS3B via post-marketing surveillance.

However, the proposed amendment to the dosage and administration presented by the applicant after regulatory submission is not appropriate because there is no definite need for changing the dosing regimen from the one selected for the confirmatory study, in light of the following points.

- Dosing errors for age-based doses of nusinersen can be reduced by providing sufficient information using informative materials etc.

- The efficacy and safety of the fixed dosing regimen of nusinersen in humans have not been studied systematically, and the benefits/risks of higher doses of nusinersen than those used in Study CS3B and the significance of increased doses are unknown.
- Although cumulative dose-dependent adverse events have been reported with therapeutic oligonucleotides (*Nucleic Acid Ther*. 2016; 26: 199-209), there are limited clinical study data that allow for assessment of the cumulative dose of nusinersen and the occurrence of these adverse events.

Dosage and administration for nusinersen will be finalized, taking account of comments from the Expert Discussion.

7.R.8 Nusinersen injection procedure and post-marketing proper use

Nusinersen is administered as IT injections by lumbar puncture to infants. During the development of nusinersen, the applicant explained that the conduct of an expanded access study in Japan would be difficult because nusinersen injection procedure requires considerable skill. Taking account of these points, PMDA asked the applicant to explain the details of nusinersen injection procedure and measures to ensure post-marketing proper use.

The applicant's explanation:

For a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), 2 medical institutions that were capable of performing lumbar punctures safely in infants were selected in Japan, and training for injection procedure was not provided to these medical institutions. However, these medical institutions were asked to appropriately administer nusinersen based on the guidance for intrathecal injection developed by the sponsor. The guidance contained the following instructions, among others: the target site for needle insertion should be the L3/L4 space, as a rule; ultrasound guidance may be used for needle insertion; and approximately 5.0 mL of CSF should be removed prior to administration.

Based on discussion with the medical expert, the applicant provided the following explanation:

To ensure appropriate administration of nusinersen, the package insert should advise that nusinersen should be administered by "physicians who are familiar with the diagnosis and treatment of spinal muscular atrophy and experienced in performing lumbar punctures," and the following system should be established after the market launch.

- Provide training for nusinersen administration (e.g., hands-on training, video training, simulations using medical models) to treating physicians, prior to the use of nusinersen.
- Issue a certificate to a physician who has completed the above training and is capable of performing lumbar punctures appropriately and safely, and deliver nusinersen only to the medical institutions to which a physician with a certificate belongs.
- Ensure that specialists in lumbar punctures give physicians first-hand guidance as needed. Set up a call center to provide assistance for appropriate administration.

PMDA considers that it is important to prepare the system of education and training for nusinersen injection procedure. Given that the intended population is infants and that there are many patients with scoliosis due to

disease progression, PMDA asked the applicant to explain if the package insert should include any special precautions for administration.

The applicant's explanation:

- Site for needle insertion

  Because the position of the cauda equina is different in infants, the puncture site is different between infants and adults. However, it is a common technique for intrathecal administration to infants. No precautions in the package insert are required.
- Ultrasound-guided administration

  In Study CS3B, while almost all subjects treated with nusinersen (79 of 80 subjects) received at least 1 (median, 4) IT injection placed using ultrasound guidance via lumbar puncture, ultrasound guidance was not used for all administrations, nor was required by the protocol. It is enough to advise that ultrasound guidance may be used for administration as needed in younger patients, patients with scoliosis, etc. and ultrasound-guided lumbar puncture is not mandatory. Thus, the package insert will advise that ultrasound etc. should be considered to guide intrathecal administration in these patients.
- CSF removal prior to administration

  Since CSF was removed in all subjects prior to administration of nusinersen in clinical studies, the safety of administration of nusinersen without removal of CSF is undefined. However, as there is no safety risk without removal of CSF prior to the instillation of intrathecal contrast media for myelography (*Handb Clin Neurol.* 2016; 135: 193-208), the package insert will not state that removal of CSF is essential, but just recommend it.

PMDA's view:

Since physicians need to be skilled at performing lumbar punctures for nusinersen administration, there is no major problem with the applicant's explanation (the package insert will advise that nusinersen should be administered by "physicians familiar with the diagnosis and treatment of spinal muscular atrophy and experienced in performing lumbar punctures"; training for injection procedure will be provided; and the system will be prepared so that nusinersen administration is allowed only at medical institutions to which a physician with a certificate belongs). On the other hand, the particularly important elements of the nusinersen injection procedure should be described in the package insert. Given the injection procedures employed in Study CS3B, the package insert should advise the use of ultrasound guidance etc. for administration and the removal of CSF prior to administration. A final decision on the appropriateness of the above conclusions, the content of education and training, and precautions regarding injection procedure will be made, taking account of comments from the Expert Discussion.

7.R.9 Post-marketing investigations

PMDA's view:

Given the presented non-clinical and clinical data, the applicant should collect the following information via post-marketing surveillance: the occurrence of CNS adverse events, malignancy, QT/QTc interval prolongation and pro-arrhythmic adverse events, lumbar puncture-related adverse events, and renal

impairment-, hepatic impairment-, and coagulation-related adverse events, effects on memory and learning, effect on growth, the effect of drug substance batch on safety, the relationship between *SMN2* copy number and efficacy/safety, safety in tissues/organs where nusinersen accumulates, the long-term safety of nusinersen, safety and efficacy in patients with renal impairment, the long-term efficacy of nusinersen and its impact on survival prognosis, the impact of patient characteristics on the efficacy of nusinersen, and efficacy in patients with advanced disease. After reviewing the results of analysis of adverse events related to the candidate genes, the risk associated with hybridization-dependent off-target effects will be discussed in the Review Report (2).

The applicant's explanation:
Taking into account that there is limited clinical experience with nusinersen in Japanese patients with SMA, a use-results survey, covering all patients treated with nusinersen, is planned as post-marketing surveillance of nusinersen (observation lasts until the registered patient dies, treatment with nusinersen ends, or the survey period (8 years) completes, whichever comes first).

A final decision on the appropriateness of these actions will be made, taking account of comments from 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. 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 5.3.5.1-1) 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. PMDA concluded that there were no obstacles to conducting its review based on the application documents submitted.

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

On the basis of the data submitted, PMDA has concluded that nusinersen has efficacy in the treatment of infantile SMA and that nusinersen has acceptable safety in view of its benefits. Nusinersen is clinically meaningful because it offers a new treatment option for patients with SMA. The appropriateness of the indication, the intended population, and dosage and administration, the need for genetic diagnosis, the distribution system to be implemented after the market launch, the appropriateness of the content of education and training, and the risk associated with hybridization-dependent off-target effects need further discussion at the Expert Discussion.

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

Review Report (2)

May 31, 2017

Product Submitted for Approval

Brand Name	Spinraza Intrathecal injection 12 mg
Non-proprietary Name	Nusinersen Sodium
Applicant	Biogen Japan Ltd.
Date of Application	December 7, 2016

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 in the following. The expert advisors present during the Expert Discussion were nominated based on their declarations etc. concerning the product, 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 conclusions presented in the Review Report (1).

PMDA also discussed the following points and took action as necessary.

1.1 Indication

1.1.1 Indication statement

The expert advisors supported PMDA's view (the appropriate indication is infantile spinal muscular atrophy [see Section 7.R.6 in the Review Report (1)]). Some expert advisors asked PMDA to reconsider the indication statement in the package insert in order to make it clear that the intended population is patients with onset at ≤6 months of age. Accordingly, PMDA gave the following considerations:

- Although the appropriateness of stating "spinal muscular atrophy with onset at ≤6 months of age" in the indication section was considered again, PMDA concluded that the appropriate indication is "infantile spinal muscular atrophy" because the applicant's explanation (if the age of onset is explicitly stated in the indication section, patients may loose a therapeutic opportunity [see Section 7.R.6.2 in the Review Report (1)]) was understandable.
- PMDA considered the inclusion of the following statement in the precautions for indication section etc. of the package insert: "nusinersen should be used in patients with onset ≤6 months of age" or a similar statement. However, the diagnostic criteria should not be described specifically in the precautions for indication section etc., and nusinersen is administered by physicians familiar with the diagnosis and treatment of spinal muscular atrophy [see Section 7.R.8 in the Review Report (1)]. There is little need for including this statement in the package insert. Thus, PMDA concluded that the diagnostic criteria should be described specifically in the informative material for healthcare professionals.

The expert advisors supported the above responses. PMDA instructed the applicant to modify the indication statement as shown below, and the applicant responded appropriately.

Indication
Infantile spinal muscular atrophy

1.1.2 Genetic testing

The expert advisors supported PMDA's view (the genetic test results are needed prior to the initiation of nusinersen treatment because nusinersen shows efficacy only in patients with a deletion or mutation of the Survival Motor Neuron 1 (*SMN1*) gene and ≥1 copy of the Survival Motor Neuron 2 (*SMN2*) gene [see Section 7.R.6.3 in the Review Report (1)]). At the Expert Discussion, the expert advisors commented that given that infantile spinal muscular atrophy is a serious disease and requires early treatment, it is essential to build a testing system under which the genetic test results can be available rapidly. Based on the above, PMDA asked the applicant to explain an update on the building of a genetic testing system for nusinersen administration.

The applicant's explanation:

- Consultation with 2 clinical laboratory companies is underway. At least one of the companies is expected to complete validation and be ready for offering a testing service by the date of market launch of nusinersen. It will take 2 to 3 weeks for the clinical laboratory company to report the test results.
- The genetic test results should be obtained as soon as possible so that treatment can be initiated early in patients with infantile spinal muscular atrophy. To investigate the availability of genetic testing service, the applicant held a consultation with the coordinating investigator etc. for a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B). The medical institution to which the coordinating investigator belongs, etc., still provides a genetic testing service for samples collected in or outside the medical institution, and even an academic medical institution alone can return the genetic test results for all patients with new-onset spinal muscular atrophy within about a week.
- Thus, the applicant will develop a testing system operated by a clinical laboratory company, but will also offer a genetic testing system available at academic medical institutions for patients with suspected infantile spinal muscular atrophy who require a rapid genetic diagnosis, for the time being.

Based on the above, PMDA instructed the applicant to include the following statement in the precautions for indication section of the package insert, and the applicant responded appropriately. PMDA considers that it is essential to build a genetic testing system for the use of nusinersen in clinical practice and that efforts should be continued so that a clinical laboratory company can provide an appropriate genetic testing service if academic medical institutions' cooperation is no longer an option.

Precautions for Indication
Nusinersen should be used in patients with a deletion or mutation of the *SMN1* gene and ≥1 copy of the *SMN2* gene confirmed by genetic testing.

1.2 Dosage and administration

At the Expert Discussion, the expert advisors supported PMDA's view (since a multi-regional phase III study [CTD 5.3.5.1-1, Study CS3B] demonstrated the efficacy and safety of age-based dosing of nusinersen [Table 20] and showed no marked differences in the pharmacokinetics, efficacy, or safety of nusinersen among age groups, there are no major problems with the dose and dosage regimen presented in the proposed package insert [see Section 7.R.7 in the Review Report (1)]). Regarding the proposed amendment to the dosage and administration presented by the applicant [see Section 7.R.7.4 in the Review Report (1)], the expert advisors commented that adjusting the dose based on age is not expected to be difficult in clinical practice in Japan and that there is no reason for approving a dosing regimen different from the one used in Study CS3B.

Based on the above, PMDA instructed the applicant to modify the dosage and administration statement as follows, and the applicant responded appropriately.

Dosage and administration

The usual dose of nusinersen is shown in the table below. Spinraza treatment should be initiated with 4 doses at Weeks 0, 2, 4 and 9 followed by dosing every 4 months. Spinraza should be administered as an intrathecal bolus injection over 1 to 3 minutes.

Age on the day of dosing	Dose	Injection volume
0-90 days	9.6 mg	4 mL
91-180 days	10.3 mg	4.3 mL
181-365 days	10.8 mg	4.5 mL
366-730 days	11.3 mg	4.7 mL
≥731 days	12 mg	5 mL

1.3 Safety related to interactions between nusinersen and genes

The applicant's explanation:

Although the homology of the genes listed in Table 7 between humans and animals was being checked during the preparation of the Review Report (1), the results have become available. The sequences of these genes of any animal are not identical to the sequences of the human genes. With respect to the genes for which safety related to hybridization-dependent off-target effects should be evaluated,[11)] the occurrence of events reported to be related to these genes and events of concern associated with their protein functions (Table 7) in a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B)[84)] is described below. There should be a low safety concern about all those events at present.

84) The occurrence of events that cannot be assessed based on a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B) (reproductive toxicity [infertility, abortion] and developmental toxicity [malformation, neonatal death, Fraser syndrome, Joubert syndrome, Meckel-Gruber syndrome]) and events described in the Review Report (1) (renal impairment-related adverse events [see Section 7.R.4.3 in the Review Report (1)], coagulation-related adverse events [see Section 7.R.4.5 in the Review Report (1)], CNS adverse events [see Section 5.R.1 in the Review Report (1)] is omitted.

- Premature ovarian failure,[85] systemic lupus erythematosus,[86] radioulnar synostosis,[87] malignancy,[88] mental retardation,[89] barrett's oesophagus,[90] arteriosclerosis,[91] Alzheimer's disease,[92] hepatitis B,[93] retinal degeneration,[94] or skin discolouration[95] did not occur. In a long-term extension study (Reference data CTD 5.3.5.4-3, Study CS11 [patients transferred from Study CS3B]), the incidence of skin discolouration was 1.2% (1 of 81 subjects), but its causal relationship to nusinersen was denied.
- The incidences of infection- and immune system disorder-related events[96] were 73.2% (30 of 41 subjects) in the sham-procedure control group and 73.8% (59 of 80 subjects) in the nusinersen group. The main adverse events were upper respiratory tract infection (22.0% [9 of 41 subjects] vs 25.0% [20 of 80 subjects]), pneumonia (14.6% [6 of 41 subjects] vs. 21.3% [17 of 80 subjects]), nasopharyngitis (9.8% [4 of 41 subjects] vs. 17.5% [14 of 80 subjects]), oxygen saturation decreased (22.0% [9 of 41 subjects] vs. 12.5% [10 of 80 subjects]), and viral upper respiratory tract infection (19.5% [8 of 41 subjects] vs. 12.5% [10 of 80 subjects]). The incidence of respiratory infections was higher in the nusinersen group than in the sham-procedure control group, which may have been due to variability in patient's condition at baseline or underlying disease. There was no trend towards a higher incidence of oxygen saturation decreased in the nusinersen group than in the sham-procedure control group.
- The incidences of abnormal insulin secretion-related events[97] were 7.3% (3 of 41subjects) in the sham-procedure control group and 0% (0 of 80 subjects) in the nusinersen group. No abnormal insulin secretion-related events occurred in the nusinersen group.
- The incidences of deafness- and otitis media-related events[98] were 2.4% (1 of 41 subjects) in the sham-procedure control group and 2.5% (2 of 80 subjects) in the nusinersen group. The observed events were ear haemorrhage (2.4% [1 of 41 subjects] vs. 0% [0 of 80 subjects]), ear disorder (0% [0 of 41 subjects] vs. 1.3% [1 of 80 subjects]), and ear pain (0% [0 of 41 subjects] vs. 1.3% [1 of 80 subjects]). There was no trend towards a higher incidence with nusinersen.
- The incidences of failure to thrive-related events[99] were 0% (0 of 41 subjects) in the sham-procedure control group and 2.6% (2 of 80 subjects) in the nusinersen group. The observed events were failure to

85) Events coded to the MedDRA PT Premature menopause
86) Events coded to the MedDRA PT Systemic lupus erythematosus
87) Events coded to the MedDRA PT Radioulnar synostosis
88) Events in the MedDRA SMQ "Malignancies (SMQ)"
89) Events coded to the MedDRA HLT "Mental retardation"
90) Events coded to the MedDRA PT Barrett's oesophagus
91) Events coded to the MedDRA PT Arteriosclerosis
92) Events coded to the MedDRA HLT "Alzheimer's disease (incl subtypes)"
93) Events coded to the following MedDRA PTs
Hepatitis B, Congenital hepatitis B infection, Acute hepatitis B, Chronic hepatitis B, Hepatitis B core antibody, Hepatitis B antibody abnormal, Hepatitis B antibody negative, Hepatitis B antibody normal, Hepatitis B antibody positive, Hepatitis B surface antibody positive, Hepatitis B surface antigen, Hepatitis B surface antigen negative, Hepatitis B e antigen positive, Hepatitis B core antigen, Hepatitis B e antigen negative, Hepatitis B core antibody positive, Hepatitis B e antigen positive, Hepatitis B DNA assay, Hepatitis B DNA assay positive, Hepatitis B DNA assay negative, Hepatitis B surface antibody, Hepatitis B antigen, Hepatitis B test negative, Hepatitis B DNA increased, Hepatitis B DNA decreased, Hepatitis B virus test, Hepatitis B virus test positive, Hepatitis B core antibody, Hepatitis B e antibody, Hepatitis B antibody positive, Hepatitis B antibody negative, Hepatitis B surface antibody positive, Hepatitis B surface antibody negative, Hepatitis B e antibody positive, and Hepatitis B e antibody negative
94) Events coded to MedDRA PT Retinal degeneration
95) Events coded to the MedDRA HLGT "Pigmentation disorders" and PT Skin discolouration
96) Events coded to the MedDRA SOCs "Infections and infestations," "Investigations," and "Immune system disorders"
97) Events coded to the MedDRA HLGT "Glucose metabolism disorders (incl diabetes mellitus)"
98) Events coded to the MedDRA SOC "Ear and labyrinth disorders"
99) Events coded to the MedDRA PTs postnatal growth retardation and frailty

thrive (2 subjects), which were mild in severity. Thus, failure to thrive-related events are unlikely to become a clinically relevant issue, and no precautions are required at present.

- The incidences of skin oedema-related events[100)] were 7.3% (3 of 41 subjects) in the sham-procedure control group and 1.3% (1 of 80 subjects) in the nusinersen group, and the observed events were oedema (3 subjects in the sham-procedure control group) and skin oedema (1 subject in the nusinersen group). There was no trend towards a higher incidence in the nusinersen group than in the sham-procedure control group.
- The incidences of metabolism and nutrition disorder-related events[101)] were 24.4% (10 of 41 subjects) in the sham-procedure control group and 16.3% (13 of 80 subjects) in the nusinersen group, and the main adverse events were weight gain poor (4.9% [2 of 41 subjects] vs. 6.3% [5 of 80 subjects]), feeding disorder of infancy or early childhood (4.9% [2 of 41 subjects] vs. 3.4% [3 of 80 subjects]), feeding intolerance (0% [0 of 41 subjects] vs. 3.4% [3 of 80 subjects]), and hypokalaemia (7.3% [3 of 41 subjects] vs, 0% [0 of 80 subjects]). There was no trend towards a higher incidence of adverse events in the nusinersen group than in the sham-procedure control group.
- The incidences of increased bone mineral content-related events[102)] were 12.2% (5 of 41 subjects) in the sham-procedure control group and 13.8% (11 of 80 subjects) in the nusinersen group, and the main events were scoliosis (4.9% [2 of 40 subjects] vs. 5.0% [4 of 80 subjects]), joint contracture (4.9% [2 of 40 subjects] vs. 3.8% [3 of 80 subjects]), and positional plagiocephaly (2.4% [1 of 41 subjects] vs. 2.5% [2 of 80 subjects]). There was no trend towards a higher incidence of adverse events in the nusinersen group than in the sham-procedure control group.

PMDA accepts the above explanation. With respect to 12 genes for which safety related to hybridization-dependent off-target effects should be evaluated,[11)] the data submitted has not suggested that adverse events reported to be related to these genes and adverse events of concern associated with their protein functions become a major safety issue in humans.

At the Expert Discussion, the expert advisors supported the above conclusion by PMDA, and commented that no precautions regarding hybridization with specific genes in the package insert are required at present. At the Expert Discussion, the expert advisors supported PMDA's view (gene expression analysis of human samples should be performed as soon as possible to assess the effects of nusinersen on the expression of the candidate genes in humans [see Section 3.R.2.1 in the Review Report (1)]).

Based on the above, PMDA instructed the applicant to perform gene expression analysis of human samples as soon as possible and then report the results to PMDA. The applicant explained that they will conduct the following additional studies and report the results to PMDA once the results of either quantitative reverse transcription polymerase chain reaction or Western Blot method become available.

- Appropriate cells derived from humans will be treated with nusinersen, and the expression levels of the genes and their proteins will be analyzed using quantitative reverse transcription polymerase chain reaction and Western Blot method.

100) Events coded to the MedDRA PTs containing "oedema"
101) Events coded to the MedDRA SOC "Metabolism and nutrition disorders"
102) Events coded to the MedDRA HLGT "Musculoskeletal and soft tissue investigations (excl enzyme tests)"

- If the expression level of only one of either the gene or its protein is affected, whether its effect is caused by nusinersen binding to the gene or secondary to increased SMN protein will be determined.

1.4 Education and training and precautions regarding nusinersen injection procedure

1.4.1 Education and training system

The applicant had proposed the following: provision of training to treating physicians prior to the use of nusinersen with issuance of a certificate and the development of the system under which nusinersen administration is allowed only at the medical institutions to which a physician with a certificate belong [see Section7.R.8 in the Review Report (1)]. At the Expert Discussion, the applicant's proposals were discussed. The expert advisors commented that a lumbar puncture is a very basic technique for pediatricians, and that pediatricians can appropriately administer nusinersen without training etc. At the Expert Discussion, it was concluded that since there are some points to be noted (candidate patients may have scoliosis), informative materials concerning nusinersen injection procedure should be developed and distributed to all medical institutions to which nusinersen is delivered, and the applicant's representatives should provide pediatricians with adequate information including precautions regarding nusinersen injection procedure at the time of the first delivery of the product.

Based on the above, PMDA instructed the applicant to respond to the above appropriately, and the applicant agreed to take such action.

1.4.2 Precautions

Among precautions regarding nusinersen injection procedure, the applicant gave the following supplementary explanation on ultrasound-guided administration.

- Although the applicant explained that almost all subjects treated with nusinersen (79 of 80 subjects) received at least 1 (median, 4) IT injection placed using ultrasound guidance via lumbar puncture [see Section 7.R.8 in the Review Report (1)], it was found that there were errors in the results of analysis by the applicant. The correct results were as follows: only 6.3% (5 of 80) of subjects treated with nusinersen received at least 1 IT injection placed using ultrasound guidance via lumbar puncture.
- Given that actually, ultrasound guidance was not used for administration in most patients, the applicant considers that there is no need for advising ultrasound-guided lumbar puncture in the package insert.

At the Expert Discussion, the expert advisors commented that since a lumbar puncture is a very basic technique for pediatricians, the need for the use of ultrasound etc. is not high unless under special situations, e.g. patients with scoliosis.

Accordingly, PMDA reconsidered the need for the use of ultrasound etc. to guide lumbar puncture, and then concluded that the package insert should advise that the use of ultrasound etc. should be considered for patients with severe spinal deformity. The expert advisors supported this conclusion.

At the Expert Discussion, the expert advisors also supported PMDA's view (the package insert should advise the removal of cerebrospinal fluid [CSF] prior to administration).

Based on the above, PMDA instructed the applicant to modify precautions in the package insert, and the applicant responded appropriately.

1.5 Tissue distribution of nusinersen

The applicant's explanation:

A systemic tissue distribution study of radiolabeled nusinersen in rats (Study APK02) was ongoing during the preparation of the Review Report (1). An interim report on this study has become available, which showed distribution of nusinersen into the nasal region (the turbinate and nasal mucosa), in addition to the tissues where nusinersen is expected to accumulate [see Section 4.R.1 in the Review Report (1); CNS tissues, liver, kidney cortex, etc.] based on non-clinical studies of nusinersen [see Section 4.2.1 in the Review Report (1)] and tissue distribution studies of other 2'-*O*-(2-methoxyethyl) modified antisense oligonucleotides (2'-MOE modified ASOs).[25)]

In a multi-regional phase III study (CTD 5.3.5.1-1, Study CS3B), the incidences of nasal adverse events[103)] were 19.5% (8 of 41 subjects) in the sham-procedure control group and 11.3% (9 of 80 subjects) in the nusinersen group. There was no trend towards a higher incidence of nasal adverse events in the nusinersen group.

Based on the above, safety in the nasal region is unlikely to become a clinically relevant issue at present, but relevant information will be collected via post-marketing surveillance.

PMDA accepted the applicant's explanation.

1.6 Safety of nusinersen

At the Expert Discussion, the expert advisors supported PMDA's views (precautions regarding effects on the liver function, renal function, and coagulation system, hippocampal vacuolation, and effect on learning in the package insert are required; no precautions regarding the risk of QT/QTc interval prolongation in the package insert are required at present) [see Sections 5.R.1, 6.R.3, 7.R.4.3, 7.R.4.4, and 7.R.4.5 in the Review Report (1)]. PMDA further assessed lumbar puncture-related adverse events. As a result, PMDA concluded that individual adverse drug reactions should be listed in the Other adverse reactions" section of the package insert and that no further precautions are required.

Based on the above, PMDA instructed the applicant to modify the package insert, and the applicant responded appropriately.

1.7 Risk management plan (draft)

103) Events coded to the MedDRA HLTs "Nasal disorders NEC," "Nasal congestion and inflammations," "Olfactory nerve disorders," and "Paranasal sinus and nasal cavity neoplasms malignant and unspecified"

In view of the considerations presented in Section 7.R.9 in the Review Report (1) and comments from the expert advisors at the Expert Discussion, PMDA has concluded that the risk management plan (draft) for nusinersen should include the safety and efficacy specifications presented in Table 42, and that the applicant should conduct additional pharmacovigilance activities and risk minimization activities presented in Table 43.

Table 42. Safety and efficacy specifications in the risk management plan (draft)

Safety specification		
Important identified risks	Important potential risks	Important missing information
· None	· Coagulation abnormalities · Renal impairment · Hepatic impairment · CNS effects, and effects on memory/learning · Hypersensitivity · Use in patients ≤2 months of age · QT prolongation · Use in patients with ≥3 copies of the *SMN2* gene · Use in patients with renal impairment	· Safety profile of long-term treatment · Effect of drug substance batch-to-batch differences in impurity profile on safety · Adverse events associated with interactions with the genes other than the *SMN2* gene (infections, CNS disease, etc.) · Safety in tissues/organs where nusinersen accumulates · Carcinogenicity
Efficacy specification		
·Long-term efficacy of nusinersen and its impact on survival prognosis · Efficacy of nusinersen in patients with *SMN2* copy number other than 2 · Efficacy of nusinersen in patients with advanced disease · Efficacy of nusinersen in patients on permanent ventilation		

Table 43. Summary of additional pharmacovigilance activities and risk minimization activities included under the risk management plan (draft)

Additional pharmacovigilance activities	Additional risk minimization activities
· Early post-marketing phase vigilance · Use-results survey (all-case surveillance) · Post-marketing clinical study · Gene expression analysis of human samples for hybridization-dependent off-target effects · A systemic tissue distribution study in rats (Study APK02) · A 2-year subcutaneous carcinogenicity study in mice	· Early post-marketing phase vigilance · Development and distribution of informative materials for healthcare professionals.

Based on the above, PMDA instructed the applicant to conduct post-marketing surveillance to address the above issues.

The applicant explained that they will conduct a use-results survey of patients with infantile spinal muscular atrophy shown in Table 44.

Table 44. Outline of use-results survey (draft)

Objective	To ascertain the safety and efficacy of nusinersen in routine clinical settings.
Survey method	All-case surveillance
Population	All patients treated with nusinersen after the market launch
Observation period	Until death or the end of nusinersen treatment, whichever comes first (up to 8 years).
Planned sample size	Not specified.
Main survey items	· Patient characteristics (gender, age, body weight, height, age of onset, *SMN2* copy number, motor function at baseline, use of respiratory care, etc.) · Administration of nusinersen (dose per administration, number of doses, the reason for changing dose, drug product lot number, etc.) · Previous medications, concomitant medications, concomitant therapies · Time to death or permanent ventilation, motor milestones based on Section 2 of the HINE · Occurrence of adverse events, clinical laboratory values, ECG · Verbal communication

PMDA accepts the above. The applicant should promptly provide the survey results to healthcare professionals in clinical practice.

1.8 Shelf-life for drug product

Long-term and accelerated stability studies on the drug product manufactured at Manufacturing Site B were ongoing during the preparation of the Review Report (1). The applicant submitted 6 months of stability data.

The applicant's explanation:

Based on the above long-term and accelerated stability data, there were no major differences between the drug products from Manufacturing Sites A and B. Thus, as with the drug product manufactured at Manufacturing Site A, a shelf-life of 30 months may be proposed also for the drug product manufactured at Manufacturing Site B in a glass vial closed with a bromobutyl rubber stopper and an aluminum cap, when stored at 2°C to 8°C, protected from light.

PMDA accepted the applicant's explanation.

2. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved after modifying the proposed indication and dosage and administration as shown below, with the following conditions. Since the product is an orphan drug, its 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

Infantile spinal muscular atrophy

Dosage and Administration

The usual dose of nusinersen is shown in the table below. Spinraza treatment should be initiated with 4 doses at Weeks 0, 2, 4 and 9 followed by dosing every 4 months. Spinraza should be administered as an intrathecal bolus injection over 1 to 3 minutes.

Age on the day of dosing	Dose	Injection volume
0-90 days	9.6 mg	4 mL
91-180 days	10.3 mg	4.3 mL
181-365 days	10.8 mg	4.5 mL
366-730 days	11.3 mg	4.7 mL
≥731 days	12 mg	5 mL

Conditions of Approval

1. The applicant is required to develop and appropriately implement a risk management plan.
2. Due to the very limited number of patients studied in Japan, the applicant is required to conduct a use-results survey, covering all patients treated with the product, during the re-examination period, in order to obtain information on the characteristics of patients treated with the product, collect data on the safety and efficacy of the product as soon as possible, and take necessary measures to ensure proper use of the product.