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Protocol 232SM201 was approved by:

_____ _____

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Biogen

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1. SYNOPSIS

This is a brief summary. For details, refer to the body of the protocol.

Protocol Title:	An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy
Protocol Number:	232SM201
Version Number:	9
Name of Study Treatment:	Nusinersen (also known as BIIB058, ISIS 396443, and Spinraza™)
Study Phase:	2
Study Indication:	Spinal muscular atrophy (SMA)
Study Rationale:	The rationale for this study is to investigate the feasibility of treating newborns with genetically diagnosed SMA prior to the onset of symptoms, to explore endpoints that may be appropriate in presymptomatic SMA, and to explore the efficacy and safety of nusinersen in this patient population. The study was previously extended (per Protocol Version 7 [02 October 2019]) for the collection of endpoint data in all subjects through 8 years of age in order to assess the long-term safety, efficacy, and tolerability of nusinersen.
Study Objectives and Endpoints:	The primary objective of the study is to examine the efficacy of multiple doses of nusinersen administered intrathecally (IT) in preventing or delaying the need for respiratory intervention or death in infants with genetically diagnosed and presymptomatic SMA. The primary endpoint that relates to this objective is the time to death or respiratory intervention (invasive or noninvasive ventilation for ≥6 hours/day continuously for 7 or more days OR tracheostomy). Secondary objective and endpoints are as follows: • To examine the effects of nusinersen in infants with genetically diagnosed and presymptomatic SMA on the development of clinically manifested SMA as determined by a composite of clinical features seen in subjects with

SMA; growth and function; and safety, tolerability, and pharmacokinetics (PK)

- The secondary efficacy endpoints of this study are as follows (all assessed at approximately 13 and 24 months of age, unless otherwise noted):
  - Proportion of subjects developing clinically manifested SMA as defined by any of the following:
    - Age-adjusted weight <5th percentile or decrease of ≥2 major weight growth curve percentiles (3rd, 5th, 10th, 25th, or 50th) or a percutaneous gastric tube placement for nutritional support
    - Failure to achieve the ability to sit without support
    - Failure to achieve standing with assistance
    - Failure to achieve hands-and-knees crawling
    - Failure to achieve walking with assistance by 24 months of age
    - Failure to achieve standing alone by 24 months of age
    - Failure to achieve walking alone by 24 months of age
  - Proportion of subjects alive
  - Attainment of motor milestones assessed as part of the Hammersmith Infant Neurological Examination (Section 2)
  - Attainment of motor milestones as assessed by World Health Organization criteria
  - Change from Baseline in the Children's Hospital of Philadelphia Infant Test of

Neuromuscular Disorders motor function scale

- Change from Baseline in Hammersmith Functional Motor Scale – Expanded
- Change from Baseline in growth parameters: weight for age/length, head circumference, chest circumference, head-to-chest circumference ratio, and arm circumference

- The secondary safety endpoints of this study are as follows:
  - Incidence of adverse events and/or serious adverse events
  - Change from Baseline in clinical laboratory parameters, electrocardiograms, and vital signs
  - Neurological examinations
- The secondary PK endpoint of this study is cerebrospinal fluid (CSF) and plasma nusinersen concentrations.

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

Study Design: This is a Phase 2, open-label, multicenter, multinational, single-arm study. The study will assess the efficacy, safety, tolerability, and PK of multiple doses of nusinersen in subjects with genetically diagnosed and presymptomatic SMA. The duration of the study was previously extended (per Protocol Version 7 [02 October 2019]) to assess long-term safety and tolerability and continue to explore the efficacy of IT administered nusinersen in these subjects. All subjects will receive nusinersen at 1 dose level, which will be administered as IT injections by lumbar puncture.

Study Location:	Global, multicenter
Number of Planned Subjects:	Up to 25 subjects will be treated in the study.
Study Population:	This study will be conducted in subjects ≤6 weeks of age with genetic documentation of 5q SMA homozygous gene deletion or mutation or compound heterozygous mutation, genetic documentation of 2 or 3 copies of the survival motor neuron 2 gene, ulnar CMAP ≥1 mV, and the absence of signs or symptoms of SMA. Detailed criteria are described in Section 8.
Treatment Groups:	One dose level will be evaluated, and all subjects will be dosed with 12 mg (5 mL) regardless of age. In previous versions of the protocol (Versions 1 through 5), the volume of the injection, and thus the dose, was adjusted based on the subject's age on the day of dosing, such that each subject received a 12-mg scaled equivalent dose based on CSF volume scaling. Nusinersen will be administered IT on Days 1, 15, 29, 64, 183, 302, 421, 540, 659, 778, 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1849, 1968, 2087, 2206, 2325, 2444, 2563, 2682, and 2801 (±14 days for all days).
Duration of Treatment and Follow-up:	For subjects who meet the eligibility criteria and remain enrolled in the study, total study duration will be approximately 8 years. The study will consist of a Screening Period, Treatment Period, and a post-treatment follow-up evaluation. Subjects who prematurely withdraw from the study are to complete the early termination study procedures and observations at the time of withdrawal.

2. LIST OF ABBREVIATIONS

2′-MOE	2′-*O*-(2-methoxyethyl)
[redacted]	[redacted]
[redacted]	[redacted]
AE	adverse event
ASO	antisense oligonucleotide
[redacted]	[redacted]
CHOP INTEND	Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CI	confidence interval
CMAP	compound muscle action potential
CNS	central nervous system
CRF	case report form
CRO	contract research organization
CSF	cerebrospinal fluid
[redacted]	[redacted]
[redacted]	[redacted]
DHA	Directions for Handling and Administration
DNA	deoxyribonucleic acid
ECG	electrocardiogram
FAS	Full Analysis Set
FL	full-length
GCP	Good Clinical Practice
HFMSE	Hammersmith Functional Motor Scale – Expanded
HINE	Hammersmith Infant Neurological Examination
hnRNP	heterogeneous nuclear ribonucleoproteins
[redacted]	[redacted]
ICF	informed consent form
ICH	International Council on Harmonisation
IRT	interactive response technology
IT	Intrathecal(ly)
LP	lumbar puncture
mRNA	messenger ribonucleic acid
[redacted]	[redacted]
[redacted]	[redacted]
PK	pharmacokinetic(s)
[redacted]	[redacted]
PPS	Per-Protocol Set
[redacted]	[redacted]
[redacted]	[redacted]
RNA	ribonucleic acid
SAE	serious adverse event
SMA	spinal muscular atrophy

SMN	survival motor neuron
SMN1	survival motor neuron 1
SMN2	survival motor neuron 2
[redacted]	[redacted]
snRNA	small nuclear ribonucleic acid
SUSAR	suspected unexpected serious adverse reaction
US	United States
WHO	World Health Organization

3. SPONSOR INFORMATION

Biogen MA Inc. is the Sponsor of the study in the United States (US). Biogen Idec Research Limited (Biogen Idec) is the Sponsor of the study globally. Biogen (or designee) will be responsible for managing the study globally.

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For urgent medical issues in which the study's Medical Director should be contacted, please refer to the Study Reference Guide for complete contact information.

Biogen may transfer any or all of its study-related responsibilities to a contract research organization (CRO) and other third parties; however, Biogen retains overall accountability for these activities.

4. INTRODUCTION

4.1. Overview of Spinal Muscular Atrophy

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. Despite being a rare disease, prior to the availability of therapeutic options, SMA was one of the most common genetic causes of death in infants, with a reported birth prevalence ranging from 8.5 to 10.3 per 100,000 live births [Arkblad 2009; Jedrzejowska 2010; Prior 2010; Sugarman 2012; Tassie 2013]. The natural history of SMA includes 4 major recognized phenotypes that are dependent on age of onset and achieved motor abilities. The most severe form, Type I SMA (equivalent to infantile-onset SMA), has a disease onset within the first few months of life; these children are never able to sit or walk and usually die from respiratory failure by 2 years of age. Patients with Type II SMA are able to sit but never walk unaided, with symptoms presenting between 6 and 18 months of age. Patients with Type III SMA are able to sit and walk but may become severely and increasingly disabled. Patients with Type IV or adult-onset SMA have an age of onset over 18 years and have normal life expectancies.

In 95% of patients with SMA, a deletion in the survival motor neuron 1 (*SMN1)* gene on chromosome 5q11-q13 is found, with the remaining 5% attributable to small mutations in the same gene [Helmken 2003; Lefebvre 1995]. *SMN1* lies in the telomeric portion of an inverted duplication of a region of chromosome 5. The centromeric half of the duplication contains a homologous gene named survival motor neuron 2 *(SMN2)* that differs from *SMN1* by 5 to 11 nucleotides [Lorson 1999; Monani 1999]. The open reading frames for both genes encode proteins with identical amino acid sequences. Survival motor neuron *(SMN)* gene transcripts, similar to most mammalian transcripts, undergo alternative splicing in which certain exons are either included or excluded from the mature protein coding transcripts [Keren 2010]. In particular, exon 7 of the *SMN1* gene is alternatively spliced with 90% to 95% of the mature messenger ribonucleic acid (mRNA) transcripts derived from the *SMN1* gene containing exon 7, and 5% to 10% of transcripts missing exon 7. The transcripts missing exon 7 (often referred to as Δ7) produce a truncated protein that is defective and unstable [Cho and Dreyfuss 2010]. One of the 5 to 11 nucleotide differences between *SMN1* and *SMN2* is a C to T substitution, which occurs in exon 7 of the *SMN2* gene resulting in an alternative splicing pattern that favors skipping of exon 7. The result shows that as much as 90% of the transcripts produced from *SMN2* are missing exon 7. The remainder, SMN2 transcripts containing exon 7, produces a full-length (FL) protein product identical to the SMN1 protein because the C to T substitution is silent. Humans have a variable copy number of the *SMN2* gene (0 to 8 copies) [Wirth 2006]. The number of *SMN2* copies and the resulting amount of FL-SMN protein expressed in patients with SMA (10% to 40% of normal SMN protein levels) correlate with SMA disease severity, thus, *SMN2* is a key modifier of disease phenotype [Coovert 1997; Feldkötter 2002; Lefebvre 1997; Prior 2004].

4.2. Current Therapies for Spinal Muscular Atrophy

Nusinersen, also referred to as BIIB058, ISIS 396443, or Spinraza™, has been approved in over 55 countries for the treatment of SMA, including in the US (December 2016), European Union (May 2017), and other major markets. The gene therapy, onasemnogene abeparvovec-xioi (Zolgensma®) is an AAV9 vector expressing an *SMN1* gene delivered intravenously. Zolgensma was approved in the US (May 2019) and Japan (March 2020) for the treatment of SMA Type I in patients less than 2 years of age, and in the European Union (May 2020) for the treatment of 5q SMA, with a dosing guidance by body weight up to 21 kg. Risdiplam (Evrysdi™), is an oral *SMN2*-directed splicing modifier indicated for the treatment of SMA in patients ≥ 2 months of age with a clinical diagnosis of SMA Type 1, 2, or 3, or with 1 to 4 *SMN2* copies. It was approved in the US (August 2020), European Union (March 2021) and has since received approval in at least 5 other markets.

In countries where nusinersen and/or other therapies are not approved, current medical care is limited to supportive care, focused on respiratory support, nutritional support, and management of resulting musculotendinous contractures and neuromuscular scoliosis through bracing, physical therapy, and surgery, with specific guidelines according to age of SMA onset [Finkel 2017]; [Mercuri 2018].

4.3. Nusinersen

4.3.1. Mechanism of Action

Nusinersen is a fully modified, 2′-*O*-(2-methoxyethyl) [2′-MOE] chimeric antisense oligonucleotide (ASO) drug designed to bind to a specific sequence in the intron downstream of exon 7 of the *SMN2* gene transcript. The region of the pre-mRNA targeted by nusinersen is normally occupied by heterogeneous nuclear ribonucleoproteins (hnRNP) A1/2 proteins, masking the U1 small nuclear ribonucleic acid (snRNA) binding site at the 5′-exon-intron junction of exon 7. U1 snRNA base pairs to the sequences that define the 5′-splice site, which is thought to be one of the first steps that initiate splicing of an intron. Nusinersen displaces the hnRNP A1/2 proteins from the pre-mRNA binding site, allowing U1 snRNA to bind to the exon-intron junction and promote assembly of the spliceosomal complex, thus promoting inclusion of exon 7 into the mRNA, which results in production of FL-SMN protein.

4.3.2. Chemistry

Chemically, nusinersen is a synthetic oligomer of 18 nucleotides (i.e., an 18-mer) that are connected sequentially by phosphorothioate linkages. Each of the 17 internucleotide linkages is a 3′-*O* to 5′-*O* phosphorothioate diester. The 18 sugar residues are uniformly modified with 2′-MOE. These MOE-modified nucleotides (1) increased affinity to the target mRNA [McKay 1999], (2) increased resistance to exonucleases and endonucleases (thereby increasing stability in tissue) [Geary 2003], and (3) ameliorated some of the high-dose toxicities associated with ASO containing only the phosphorothioate linkages [Henry 2000].

The sequence of nusinersen is written as follows:

5′-^{Me}U^{Me}CA^{Me}C^{Me}U^{Me}U^{Me}U^{Me}CA^{Me}UAA^{Me}UG^{Me}C^{Me}UGG-3′

where A and G are 2′-MOE nucleosides, ^{Me}C is 5-methyl-2′-MOE cytidine, and ^{Me}U designates 5-methyl-2′-MOE uridine.

4.3.3. Preclinical Experience

Nusinersen was identified after an extensive screen of >500 2′-MOE oligonucleotides in reporter gene assays, in vitro splicing assays, and SMA patient fibroblasts [Hua 2007; Hua 2008]. Data have shown that nusinersen promotes a concentration-dependent increase in FL transcripts (including exon 7) in patient fibroblast cells, achieving >90% FL-SMN2 transcripts, and forms nuclear structures called gems, known to contain SMN protein. In a mild mouse model of SMA, nusinersen promoted inclusion of exon 7 in the SMN2 transgene in a variety of peripheral tissues when dosed systemically [Hua 2008] and in central nervous system (CNS) tissue, including the spinal cord, when injected into the lateral ventricle. Nusinersen produced >90% exon 7 inclusion in the transgenic mice and increased SMN protein production in motor neurons, resulting in the appearance of gems in motor neurons. These studies were extended to a more severe mouse model of SMA (SMA Δ7) [Le 2005], where the CNS delivery of drug produced a dose-dependent effect on SMN2 exon 7 inclusion, SMN protein production, and survival. These mice treated with nusinersen demonstrated improved weight gain; improvements in muscle morphology, muscle strength, and motor coordination; and improved morphology of the motor neuron junctions [Passini 2011]. Furthermore, nusinersen was shown to distribute widely in the CNS after intrathecal (IT) administration in monkeys [Passini 2011].

The pharmacokinetics (PK) and toxicity of nusinersen were assessed after a single IT lumbar bolus injection (1 to 7 mg) in adult monkeys, after 14 weeks (with a 4-week interim sacrifice) of repeated IT lumbar bolus injections (0.3 to 3 mg/week or every other week) in juvenile monkeys, and after 53 weeks of repeated IT lumbar bolus injections in juvenile monkeys. In addition, a dedicated PK study in adult monkeys was performed to assess the half-life of nusinersen in cerebrospinal fluid (CSF), tissues, and plasma.

See the Investigator's Brochure for detailed information on nonclinical studies.

4.3.4. Clinical Experience

Nusinersen has been evaluated in a completed open-label, single-ascending-dose, Phase 1 study designed to assess the safety, tolerability, and PK of nusinersen in subjects with SMA (ISIS 396443-CS1). A single dose of nusinersen was administered by IT injection to subjects with SMA 2 to 14 years of age. Four doses (1, 3, 6, and 9 mg) were evaluated sequentially. Each dose was studied in a cohort of 6 or 10 subjects, where all subjects received nusinersen.

Nusinersen was evaluated in 8 additional completed studies, ISIS 396443-CS1, ISIS 396443-CS2, ISIS 396443-CS12, ISIS 396443-CS4, ISIS 396443-CS3A, ISIS 396443-CS3B, ISIS 396443-CS10, and 232SM202, and is currently being evaluated in 4 additional ongoing studies, ISIS 396443-CS11, 232SM404, 232SM203, and 232SM302.

The primary support for the safety and efficacy of nusinersen in the treatment of SMA is derived from the final analysis of Study ISIS 396443-CS3B, the sham-controlled study in subjects with infantile-onset SMA, and the final analysis of Study ISIS 396443-CS4, the sham-controlled study in subjects with later-onset SMA. Subjects receiving nusinersen in these 2 studies achieved statistically significant improvements in motor function compared with subjects in the control arms.

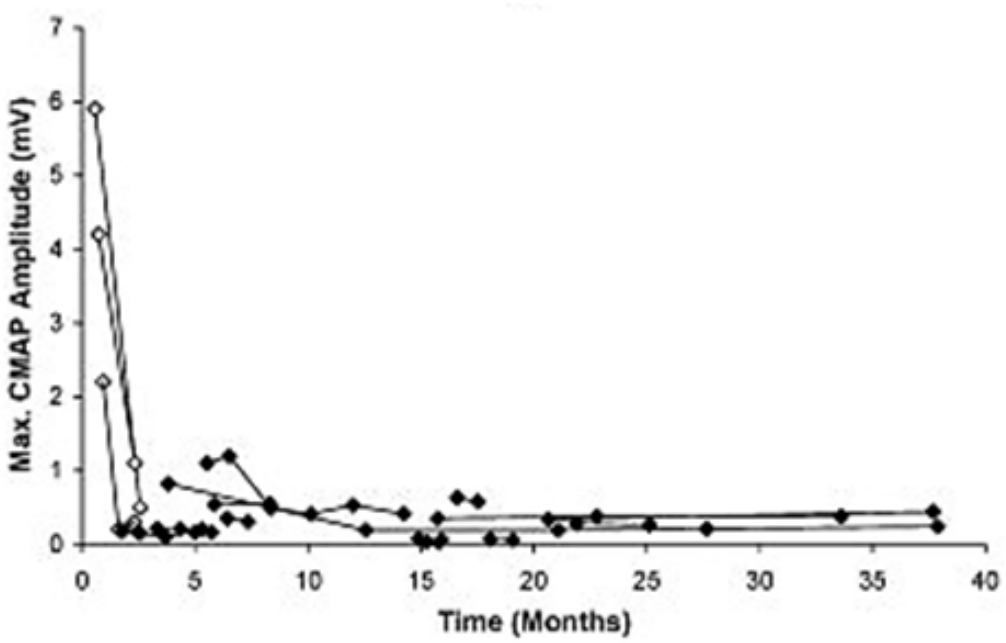
Uncontrolled studies involving subjects with infantile-onset SMA (Study ISIS 396443-CS3A) and later-onset SMA (longitudinal analyses of Studies ISIS 396443-CS2 and ISIS 396443-CS12) are highly supportive of the results of the pivotal efficacy studies (Studies ISIS 396443-CS3B and ISIS 396443-CS4) and provide evidence of the long-term benefit of treatment with nusinersen.

See the Investigator's Brochure for detailed information on clinical studies.

4.4. Study Rationale

This study is being conducted to investigate whether nusinersen can prevent or profoundly attenuate the severity of SMA in patients who are presymptomatic. Patients with SMA almost invariably have a "normal period" immediately after birth. Their activity, weight, and motor activities are normal during that period. Although existing data on patients with presymptomatic SMA are scant, evidence indicates that these patients have normal or near-normal motor neuron function in that "honeymoon" period. In Figure 1, the patients with presymptomatic SMA (open diamonds) all have compound muscle action potentials (CMAPs) of >2 mV. Further, when followed into the symptomatic period (closed diamonds), these patients have a profound loss in the number and activity of motor neurons. Therefore, the intent of this study is to investigate whether nusinersen can prevent the loss of motor neurons that occurs in the early postnatal period of patients with *SMN1* homozygous deletion or mutation or compound heterozygous mutation.

Figure 1: CMAP for Patients With Type I SMA



Source: [Swoboda 2005]
CMAP = compound muscle action potential; SMA = spinal muscular atrophy.

The following are several important points that support the rationale for this study:

- Genetic diagnosis of SMA is straightforward and extremely accurate using blood or dried blood spots from a heel stick. It can be done in 3 to 7 days.
- The penetrance of SMA given a genetic diagnosis is virtually 100%.
- There is reasonably high genotype-phenotype correlation such that the *SMN2* copy number can be used to predict the severity of disease (moderate or severe) with approximately 80% to 85% accuracy [Burghes and Beattie 2009; Prior 2010; Swoboda 2005].
- Experience from another genetic pediatric disease with a profound neuromuscular phenotype (Pompe disease) suggests that presymptomatic identification and treatment of infants lead to markedly improved clinical outcomes compared to treatment offered after symptomatic presentation [Chien 2009].

The study was previously extended (per Protocol Version 7 [02 October 2019]) for the collection of endpoint data in all subjects through 8 years of age in order to assess the long-term safety, efficacy, and tolerability of nusinersen.

4.5. Rationale for Dose and Schedule Selection

The nusinersen dose and dose interval for this study were selected based on nonclinical toxicology and PK observations from studies in monkeys using single-dose and repeat-dosing IT administration, consideration of the target tissue concentration anticipated for drug pharmacology, and safety data in the completed and ongoing clinical studies of nusinersen to date. Based on the pharmacology and PK results in SMA transgenic mice, it was estimated that a target spinal cord tissue concentration between 2 and 10 μg/g will produce 50% to 90% SMN2 exon 7 inclusion. Nonclinical studies in juvenile monkeys receiving IT doses of nusinersen showed a resulting gradient of distribution of nusinersen along the spinal cord, with mean lumbar spinal cord concentrations ranging from 1.6- to 2.3-fold and 2.0- to 3.5-fold higher than thoracic and cervical spinal cord levels, respectively. The dose selected for this multiple-dose clinical study (equivalent to 12 mg of nusinersen) is predicted to achieve levels at the high end of this range (approximately 10 μg/g lumbar and 3 μg/g cervical spinal cord tissue concentrations) after the first dose.

The loading dose interval (i.e., doses on Days 1, 15, 29, and 64) was selected as the dose interval based on the nonclinical PK and pharmacology data to achieve and maintain nusinersen spinal cord tissue levels that are predicted to be within the upper end of the pharmacologically active range by Day 64 (predicted to be approximately 30 μg/g lumbar and 10 μg/g cervical tissue concentrations), while at the same time considering subject safety and convenience for repeated lumbar puncture (LP) IT injections.

The maintenance dose interval (once every 4 months or 119 days) was selected based on nonclinical PK and pharmacology data and clinical PK data from subjects in ongoing and completed clinical studies, with the goal of maintaining the spinal cord tissue levels of nusinersen at a steady-state level within the estimated pharmacologically active range. The elimination half-life of nusinersen from human CSF is approximately 135 to 177 days and was estimated based on a limited number of postdose levels. Although CNS tissue half-life cannot be measured in humans, the median terminal elimination half-life was measured in the CNS tissue of adult monkeys and was found to be 116 days (approximately 4 months). Because the site of action of nusinersen is within the CNS tissues, these findings support maintenance doses administered every 4 months.

Nusinersen will be administered as an IT injection. All subjects will be dosed with 12 mg (5 mL) regardless of age. Because results from PK models showed similar concentrations and potential for higher efficacy with higher concentrations, the dosing regimen was adjusted to 12 mg (5 mL) regardless of age, which in result will lower the risk for dosing errors while still maintaining favorable safety margins. Dosing instructions and details regarding administration will be provided in the Directions for Handling and Administration (DHA). In previous versions of the protocol (Versions 1 through 5), the volume of the injection, and thus the dose, was adjusted based on the subject's age on the day of dosing, such that each subject received a 12-mg scaled equivalent dose based on CSF volume scaling. Thus, younger subjects were given a lower dose of nusinersen, achieved by injecting a smaller volume that was proportional to the estimated CSF volume for age, such that the dose volume was equivalent to 5 mL for a 2-year-old child to adult.

5. SCHEDULE OF ACTIVITIES FOR STUDY 232SM201

5.1. Schedule of Activities: Screening Through Day 779

Table 1: Schedule of Activities: Screening Through Day 779

Study Period	**Screen**	**Treatment/Follow-Up**																	
Study Day	**D -21 to D -1**	**D1**			**D2**	**D15 (±1D)**			**D16**	**D29 (±1D)**			**D30**	**D64, D183, D302, D421, D540, D659, D778 (±14D)**			**D65, D184 D303, D422, D541, D660, D779[1]**	**D365, D700 (±14D)**
		Pre-Dose	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		
Informed Consent	X																	
Inclusion/Exclusion Criteria	X	X[2]																
Medical History	X																	
Sibling SMA Data	X																	
Vital Signs and Pulse Oximetry	X	X		4X[3]	X	X		4X[3]	X	X		4X[3]	X	X		4X[3]	X	X
Weight	X	X			X	X			X	X			X	X			X	X
Growth Parameters	X													X[4]				X
Physical Examination	X	X				X				X				X				X
Ventilator Use	X	X			X	X			X	X			X	X			X	X
[redacted]																		
Neurological Examination	X	X		2X[6]	X	X		2X[6]	X	X		2X[6]	X	X		2X[6]	X	X
ECG	X				X							X						X

Study Period	Screen	Treatment/Follow-Up																
Study Day	**D -21 to D -1**	**D1**			**D2**	**D15 (±1D)**			**D16**	**D29 (±1D)**			**D30**	**D64, D183, D302, D421, D540, D659, D778 (±14D)**			**D65, D184 D303, D422, D541, D660, D779[1]**	**D365, D700 (±14D)**
		Pre-Dose	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		
Laboratory Safety Tests[7]	X					X				X				X[4]				X
Coagulation Laboratory Tests[8]	X	X				X				X				X				X
Immunogenicity		X												X[4]				X
CSF PK [redacted]		X				X				X				X				
[redacted]																		
Plasma PK[9]				X										X[4]				
[redacted]																		
Study Treatment Injection			X				X				X				X			
Inpatient Stay (24 hours)				X														
CHOP INTEND/ HFMSE[11]	X[12]													X[4]				X
HINE Motor Milestones	X[12]													X[4]				X
WHO Motor Milestones[13]	X[12]													X[4]				X
[redacted]																		

Study Period	Screen	Treatment/Follow-Up															
Study Day	D -21 to D -1	D1			D2	D15 (±1D)			D16	D29 (±1D)			D30	D64, D183, D302, D421, D540, D659, D778 (±14D)			D65, D184 D303, D422, D541, D660, D779[1]	D365, D700 (±14D)
		Pre-Dose	LP	Post-Dose		Pre-Dose	LP	Post-Dose		Pre-Dose	LP	Post-Dose		Pre-Dose	LP	Post-Dose		
[redacted]																		
Con Med and Ancillary Procedure Recording	X----------X																	
AE Collection	X----------X																	

[redacted]; AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted]; Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; DNA = deoxyribonucleic acid; ECG = electrocardiogram; HFMSE = Hammersmith Functional Motor Scale – Expanded; HINE = Hammersmith Infant Neurological Exam; LP = lumbar puncture; PK = pharmacokinetic(s); [redacted]; RNA = ribonucleic acid; SMA = spinal muscular atrophy; [redacted]; Term = termination; WHO = World Health Organization.

Note: Monitoring telephone calls will occur on a monthly (±14 days) basis starting on Day 94 and continuing to Day 897. At telephone contact, changes in concomitant medications and AEs will be recorded, as well as information on the subject's ventilator use and SMA disease status. Videotaping of the motor milestone assessments, all motor function assessments, and physical examinations will be optional.

[1] These safety monitoring visits will occur on the day after the subject receives an injection of study treatment. At the time of the implementation of Protocol Version 7, onsite safety assessments are no longer required per protocol on the day following injection of study treatment; however, a postdose follow-up email or phone call evaluation will be required within 1 to 7 days after the dosing visits on Days 64, 183, 302, 421, 540, 659, and 778.

[2] Investigator to check for signs and symptoms consistent with SMA.

[3] Vital signs will be collected at 4 timepoints postdose: 1, 2, 4, and 6 hours (±15 minutes).

[4] These assessments may be performed up to 7 days prior to dosing, if necessary.

[redacted]

[6] Neurological examinations will be conducted at 2 timepoints: 3 and 6 hours after dosing.

[7] Serum chemistry, hematology, urinalysis, and urine total protein; see Appendix A, Table 8, and Table 9 for a list of analytes and collection timepoints based on the subject's weight at Screening. For subjects ≥3 kg at Screening, once they reach a weight of 5.4 kg or the Day 183 visit, whichever happens first, blood samples for all analytes should be collected according to the above schedule. The blood sample for Cystatin C analysis will not be collected at any timepoint for subjects with a Screening weight of <3 kg. Urinalysis will be conducted according to the above schedule, independent of a subject's weight.

[8] Coagulation testing will be conducted up to 7 days prior to dosing at the time of the implementation of Protocol Version 7, and results must be reviewed prior to dosing.

[9] See Table 2 for the detailed PK sampling schedule. [redacted]

[10] A blood sample for RNA and DNA assessments will be drawn on Day 184. For subjects who have already completed Day 184 at the time of the implementation of Protocol Version 4, blood samples should be obtained at the next visit. At the time of the implementation of Protocol Version 6, a blood sample for RNA and DNA assessments will be drawn on the same day as injection of study treatment (Day 183) or at the next visit.

[11] CHOP INTEND will be assessed in subjects until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed. All subjects ≥2 years of age will be evaluated using the HFMSE. See Section 13.1.4 and Section 13.1.4.2.

[12] The CHOP INTEND, HINE, and WHO motor milestone assessments will be conducted as part of the initial screening assessments. If they are conducted within 7 days prior to dosing, they will only need to be performed once; otherwise, they will need to be repeated within 7 days prior to dosing.

[13] WHO motor milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments made by the subject's caregiver.

[redacted]

Table 2: Pharmacokinetic Sampling Schedule

Study Day	Timepoints	Blood Collection (mL)	CSF Collection (mL)[1]
D1	Predose	NA	0.5
	4 hr (±1 hr)	0.5	NA
D15	Predose	NA	0.5
D29	Predose	NA	0.5
D64	Predose	0.5	0.5
D183	Predose	0.5	0.5
D302	Predose	0.5	0.5
D421	Predose	0.5	0.5
D540	Predose	0.5	0.5
D659	Predose	0.5	0.5
D778	Predose	0.5	0.5
D897	Predose	0.5	0.5
D1016	Predose	0.5	0.5
D1135	Predose	0.5	0.5
D1254	Predose	0.5	0.5
D1373	Predose	0.5	0.5
D1492	Predose	0.5	0.5
D1611	Predose	0.5	0.5
D1730	Predose	0.5	0.5
D1849	Predose	0.5	0.5
D1968	Predose	0.5	0.5
D2087	Predose	0.5	0.5
D2206	Predose	0.5	0.5
D2325	Predose	0.5	0.5
D2444	Predose	0.5	0.5
D2563	Predose	0.5	0.5
D2682	Predose	0.5	0.5
D2801	Predose	0.5	0.5

CSF = cerebrospinal fluid; D = day; hr = hour; mRNA = messenger ribonucleic acid; NA = not applicable (no collection scheduled); PK = pharmacokinetic; SMA = spinal muscular atrophy.
Note: Details on sampling, preparation, and shipment are included in the Study Laboratory Manual.

[redacted]

[1] Four to 5 mL of CSF will be collected for analyses and for PK assessments (see Section 11.1), but only 0.5 mL will be used for the PK analysis.

5.2. Schedule of Activities: Day 897 Through Day 2891 (End of Study)

Table 3: Schedule of Activities: Day 897 Through Day 1730

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																							
	D897			D1016			D1135			D1254			D1373			D1492			D1611			D1730		
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post
Sibling SMA Data	X									X									X					
Vital Signs and Pulse Oximetry[1]	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X
Weight	X			X			X			X			X			X			X			X		
Growth Parameters[2]	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]		
Physical Examination	X			X			X			X			X			X			X			X		
Ventilator Use	X			X			X			X			X			X			X			X		
[redacted]																								
Neurological Examination[4]	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X
[redacted]																								
ECG	X[3]									X[3]									X[3]					
Laboratory Safety Tests[5]	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]		
Coagulation Laboratory Tests[6]	X			X			X			X			X			X			X			X		
Immunogenicity	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]		

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																							
	D897			D1016			D1135			D1254			D1373			D1492			D1611			D1730		
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post
CSF PK [redacted]	X			X			X			X			X			X			X			X		
[redacted]																								
Plasma PK[7]	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]		
Study Treatment Injection		X			X			X			X			X			X			X			X	
CHOP INTEND/ HFMSE[8]	X[3]						X[3]						X[3]						X[3]					
WHO Motor Milestones[9]	X[3]						X[3]						X[3]						X[3]					
[redacted]																								
Con Med and Ancillary Procedure Recording	X----------X																							
AE Collection	X----------X																							

[redacted]; AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted]; Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; DNA = deoxyribonucleic acid; ECG = electrocardiogram; EOS = End of Study; HFMSE = Hammersmith Functional Motor Scale – Expanded; HINE = Hammersmith Infant Neurological Exam; LP = lumbar puncture; [redacted]; PK = pharmacokinetic(s); [redacted]; RNA = ribonucleic acid; SMA = spinal muscular atrophy; [redacted]; Term = termination; WHO = World Health Organization.

Notes: Starting from Day 897, follow-up safety monitoring telephone calls or emails will occur 1 to 14 days postdose and every other month for the duration of the study, except for the months when in-clinic visits occur. At telephone contact or via email, changes in concomitant medications and AEs will be recorded, as well as information on the

subject's ventilator use and SMA disease status. Where possible, all visits from Day 897 through Day 1730 can be concluded in 1 day. The final visit will occur 90 days after the last study treatment injection. Videotaping of the motor milestone assessments, all motor function assessments, and physical examinations will be optional.

For growth parameters, [redacted], ECG, laboratory safety tests, CHOP INTEND/HFMSE, WHO motor milestones, [redacted], if an assessment is not performed at a visit, attempts should be made to perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

For study visits with a large number of planned assessments, assessments can be completed over 1 to 2 days and assessments for primary and secondary outcomes (e.g., motor milestones/functional assessments) should be prioritized.

[1] Vital signs will be taken predose and approximately 1 hour postdose (±15 minutes) at every onsite visit throughout the study.

[2] Growth parameters of body length and/or height (for all subjects), and head circumference, chest circumference, and arm circumference (for subjects up to 36 months of age) will be measured.

[3] These assessments may be performed up to 7 days prior to dosing, if necessary.

[4] Sections 1 and 3 of the HINE will be conducted on all subjects ≤24 months of age. For all subjects >24 months of age, standard neurological examinations, which include assessments of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be conducted. Neurological examinations are conducted within 7 days of dosing and approximately 1 hour after dosing (or when sedation has worn off if it was used).

[5] Among the laboratory measurements, quantitative urine total protein will be assessed at the local laboratory If urinary protein concentration is >0.2 g/L, repeat testing and further evaluation should be considered; quantitative urine total protein should be prioritized if there is not enough urine sample for all tests.

[6] Coagulation testing will continue to be conducted at each visit or up to 7 days prior to dosing and results should be reviewed prior to dosing. In situations where predose verification of coagulation laboratory results is not feasible or optimal (e.g., prolonged sedation of the subject would be required to allow adequate time for lab processing), the Investigator may exercise discretionary clinical judgment and proceed with the LP procedure, based on the subject's clinical and coagulation lab history.

[7] See Table 2 for the detailed PK sampling schedule. [redacted]

[8] CHOP INTEND will be assessed in subjects until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed. All subjects ≥2 years of age will be evaluated using the HFMSE. See Section 13.1.4 and Section 13.1.4.2.

[9] WHO motor milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments made by the subject's caregiver.

Table 4: Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																											PTFU
	D1849			D1968			D2087			D2206			D2325			D2444			D2563			D2682			D2801			D2891 (EOS)/ Early Term[1]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
Sibling SMA Data				X									X									X						X
Vital Signs and Pulse Oximetry[2]	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X
Weight	X			X			X			X			X			X			X			X			X			X
Growth Parameters[3]	X[4]									X[4]									X[4]									X
Physical Examination	X			X			X			X			X			X			X			X			X			X
Ventilator Use	X			X			X			X			X			X			X			X			X			X
[redacted]																												
Neurological Examination[5]	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X
[redacted]																												
ECG	X[4]									X[4]									X[4]									X
Echocardiogram[7]	X[4]									X[4]									X[4]									X
Laboratory Safety Tests[8]	X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X
Coagulation Laboratory Tests[9]	X			X			X			X			X			X			X			X			X			X
Immunogenicity	X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X
CSF PK [redacted]	X			X			X			X			X			X			X			X			X			
[redacted]																												
Plasma PK[10]	X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																											PTFU
	D1849			D1968			D2087			D2206			D2325			D2444			D2563			D2682			D2801			D2891 (EOS)/ Early Term[1]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
[redacted]																												
Study Treatment Injection		X			X			X			X			X			X			X			X			X		
CHOP INTEND/ HFMSE[11]	X[4]									X[4]									X[4]									X
WHO Motor Milestones[12]	X[4]									X[4]									X[4]									X
[redacted]																												
Con Med and Ancillary Procedure Recording	X----------X																											
AE Collection	X----------X																											

[redacted]; AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted]; Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; [redacted]; DNA = deoxyribonucleic acid; ECG = electrocardiogram; EOS = End of Study; HFMSE = Hammersmith Functional Motor Scale – Expanded; HINE = Hammersmith Infant Neurological Exam; LP = lumbar puncture; [redacted] PK = pharmacokinetic(s); [redacted]; PTFU = Post-Treatment Follow-Up; [redacted]; RNA = ribonucleic acid; SMA = spinal muscular atrophy; [redacted] Term = termination; US = United States; WHO = World Health Organization.

Notes: Starting from Day 897, follow-up safety monitoring telephone calls or emails will occur 1 to 14 days postdose and every other month for the duration of the study, except for the months when in-clinic visits occur. At telephone contact or via email, changes in concomitant medications and AEs will be recorded, as well as information on the subject's ventilator use and SMA disease status. Where possible, all visits from Day 1849 through Day 2891 can be concluded in 1 day. The final visit will occur 90 days after the last study treatment injection. Videotaping of the motor milestone assessments, all motor function assessments, and physical examinations will be optional.

For growth parameters, [redacted] ECG, echocardiogram, laboratory safety tests, CHOP INTEND/HFMSE, WHO motor milestones, [redacted], if an assessment is not performed at a visit, attempts should be made to perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

For study visits with a large number of planned assessments, assessments can be completed over 1 to 2 days and assessments for primary and secondary outcomes (e.g., motor milestones/functional assessments) should be prioritized.

[1] At the Early Termination Visit, age-appropriate assessments should be performed.

[2] Vital signs will be taken predose and approximately 1 hour postdose (±15 minutes) at every onsite visit throughout the study.

[3] Growth parameters of body length and/or height (for all subjects), and head circumference, chest circumference, and arm circumference (for subjects up to 36 months of age) will be measured.

[4] These assessments may be performed up to 7 days prior to dosing, if necessary.

[5] Sections 1 and 3 of the HINE will be conducted on all subjects ≤24 months of age. For all subjects >24 months of age, standard neurological examinations, which include assessments of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be conducted. Neurological examinations are conducted within 7 days of dosing and approximately 1 hour after dosing (or when sedation has worn off if it was used).

[redacted]

[7] Echocardiogram will only be assessed locally at the site as per local practice.

[8] Among the laboratory measurements, quantitative urine total protein will be assessed at the local laboratory. If urinary protein concentration is >0.2 g/L, repeat testing and further evaluation should be considered; quantitative urine total protein should be prioritized if there is not enough urine sample for all tests.

[9] Coagulation testing will continue to be conducted at each visit or up to 7 days prior to dosing and results should be reviewed prior to dosing. In situations where predose verification of coagulation laboratory results is not feasible or optimal (e.g., prolonged sedation of the subject would be required to allow adequate time for lab processing), the Investigator may exercise discretionary clinical judgment and proceed with the LP procedure, based on the subject's clinical and coagulation lab history.

[10] See Table 2 for the detailed PK sampling schedule. [redacted]

[11] CHOP INTEND will be assessed in subjects until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed. All subjects ≥2 years of age will be evaluated using the HFMSE. See Section 13.1.4 and Section 13.1.4.2.

[12] WHO motor milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments made by the subject's caregiver.

[redacted]

[16] These assessments will be for US sites and for subjects 5 years of age and older at US sites who have English (US) as their primary language.

6. STUDY OBJECTIVES AND ENDPOINTS

6.1. Objectives

6.1.1. Primary Objective

The primary objective of the study is to examine the efficacy of multiple doses of nusinersen administered IT in preventing or delaying the need for respiratory intervention or death in infants with genetically diagnosed and presymptomatic SMA.

6.1.2. Secondary Objectives

Secondary objectives of this study are to examine the effects of nusinersen in infants with genetically diagnosed and presymptomatic SMA on the following:

- Development of clinically manifested SMA as determined by a composite of clinical features seen in subjects with SMA
- Growth and function
- Safety, tolerability, and PK

6.2. Endpoints

6.2.1. Primary Endpoint

The primary endpoint of the study is the time to death or respiratory intervention (invasive or noninvasive ventilation for ≥6 hours/day continuously for 7 or more days OR tracheostomy).

6.2.2. Secondary Endpoints

Efficacy

The secondary efficacy endpoints of this study are as follows (all assessed at approximately 13 and 24 months of age, unless otherwise noted):

- Proportion of subjects developing clinically manifested SMA as defined by any of the following:
    - Age-adjusted weight <5th percentile or decrease of ≥2 major weight growth curve percentiles (3rd, 5th, 10th, 25th, or 50th) or a percutaneous gastric tube placement for nutritional support
    - Failure to achieve the ability to sit without support

  - Failure to achieve standing with assistance
  - Failure to achieve hands-and-knees crawling
  - Failure to achieve walking with assistance by 24 months of age
  - Failure to achieve standing alone by 24 months of age
  - Failure to achieve walking alone by 24 months of age

- Proportion of subjects alive
- Attainment of motor milestones assessed as part of the HINE (Section 2)
- Attainment of motor milestones as assessed by World Health Organization (WHO) criteria
- Change from Baseline in the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) motor function scale
- Change from Baseline in HFMSE
- Change from Baseline in growth parameters: weight for age/length, head circumference, chest circumference, head-to-chest circumference ratio, and arm circumference

Safety

The secondary safety endpoints of this study are as follows:

- Incidence of adverse events (AEs) and/or serious adverse events (SAEs)
- Change from Baseline in clinical laboratory parameters, electrocardiograms (ECGs), and vital signs
- Neurological examinations

Pharmacokinetics

The secondary PK endpoint of this study is CSF and plasma nusinersen concentrations.

[redacted]

7. STUDY DESIGN

7.1. Study Overview

This is a Phase 2, open-label, multicenter, multinational, single-arm study. The study will assess the efficacy, safety, tolerability, and PK of multiple doses of nusinersen in subjects with genetically diagnosed and presymptomatic SMA. The duration of the study was previously extended (per Protocol Version 7 [02 October 2019]) to assess long-term safety and tolerability and continue to explore the efficacy of IT administered nusinersen in patients with genetically confirmed SMA. Up to 25 subjects are planned to be treated in the study. All subjects will receive nusinersen. One dose level will be evaluated, and all subjects will be dosed with 12 mg (5 mL) regardless of age. In previous versions of the protocol (Versions 1 through 5), the volume of the injection, and thus the dose, was adjusted based on the subject's age on the day of dosing, such that each subject received a 12-mg scaled equivalent dose based on CSF volume scaling. All subjects will have the opportunity to participate in this study for up to approximately 8 years.

7.2. Overall Study Duration and Follow-Up

The study will consist of a Screening Period, Treatment Period, and a post-treatment follow-up evaluation.

Total study duration for subjects who participate in the study will be approximately 8 years.

7.2.1. Screening

Subject eligibility for the study will be determined within 3 weeks prior to study entry (Day -21 to Day -1) and confirmed prior to dosing. If a subject initially fails any of the screening criteria, they will be allowed to be rescreened 1 time during the Screening Period at the discretion of the Investigator.

7.2.2. Treatment

Eligible subjects will be admitted to the study center on Study Day 1, undergo predose evaluations, and then receive an LP injection of study treatment. After the injection on Day 1, subjects will remain at the study center for at least 24 hours post-procedure for safety monitoring.

Subjects will return to the study center on Days 15, 29, 64, 183, 302, 421, 540, 659, and 778 for follow-up evaluations and subsequent injections, for a total of 10 injections over the study period. For injections received on Day 15 and following visits, subjects will remain at the study center for at least 6 hours post-procedure for safety monitoring. An overnight stay is required for the first injection, but is otherwise optional at the discretion of the Investigator. Safety monitoring visits will occur on the day after injections. At the time of the implementation of Protocol Version 7, onsite safety assessments are no longer required per protocol on the day following injection of study treatment; however, a postdose follow-up email or phone call

evaluation will be required within 1 to 7 days after the dosing visits on Days 64, 183, 302, 421, 540, 659, and 778. In addition, the study center will monitor the subject's condition through telephone contact on a monthly basis starting on Day 94 and continuing to Day 897. Two additional study visits will be required on Days 365 and 700 in order to collect information for the 13- and 24-month of age assessments; a study treatment injection will not occur at these visits.

A CSF sample will be taken predose on each injection day.

Subjects will return to the study site on Days 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1849, 1968, 2087, 2206, 2325, 2444, 2563, 2682, and 2801 (±14 days for all days) for subsequent injections of nusinersen and follow-up evaluations (±14 days) over a dosing period of approximately 5.5 years. Subjects will remain at the study site for at least 1 hour postdose for safety monitoring and can be discharged at the discretion of the Site Investigator and in compliance with the institutional requirements once the subjects have adequately recovered from the dosing procedure. From Day 897 through Day 2801, overnight stays are optional for all injections at the discretion of the Investigator. Study assessments can be performed over multiple days if needed. Follow-up safety monitoring telephone calls will occur 1 to 14 days postdose and every other month for the duration of the study, except for the months when in-clinic visits occur. During these calls, changes in concomitant medications, AEs, and ventilator use/SMA disease status that have occurred since the last phone call or study visit will be recorded.

7.2.3. Follow-Up

Subjects are to return to the study site for a follow-up evaluation (Final Study Visit on Day 2891) approximately 3 months after the last dose of study treatment.

Subjects who prematurely withdraw from the study are to complete the early termination study procedures and observations at the time of withdrawal.

7.3. Study Stopping Rules

There are no specific stopping rules for this study, but the Investigator should discuss concerns relating to individual subjects with the Medical Monitor and the Sponsor to ensure that it is appropriate for the subject to continue study treatment.

The Sponsor may terminate this study at any time, after informing Investigators. The Sponsor (or designee) will notify Investigators if the study is to be placed on hold or terminated or when completed.

7.4. End of Study

The end of study is last subject, last visit (either in-person visit or telephone contact).

8. SELECTION OF SUBJECTS

8.1. Inclusion Criteria

To be eligible to participate in this study, candidates must meet the following eligibility criteria at Screening or at the timepoint specified in the individual eligibility criterion listed:

1. Ability of parent(s) or guardian(s) to understand the purpose and risks of the study and provide signed and dated informed consent and authorization to use confidential health information in accordance with national and local subject privacy regulations.

2. Age ≤6 weeks at first dose.

3. Genetic documentation of 5q SMA homozygous gene deletion or mutation or compound heterozygous mutation.

4. Genetic documentation of 2 or 3 copies of *SMN2*.

5. Ulnar CMAP ≥1 mV at Baseline.

6. Receiving adequate nutrition and hydration (without gastrostomy) in the opinion of the Investigator.

7. Body weight ≥3rd percentile for age using appropriate country-specific guidelines.

8. Gestational age of 37 to 42 weeks for singleton births; gestational age of 34 to 42 weeks for twins.

9. Able to complete all study procedures, measurements, and visits and parent(s) or guardian(s)/subject has adequately supportive psychosocial circumstances in the opinion of the Investigator.

8.2. Exclusion Criteria

Candidates will be excluded from study entry if any of the following exclusion criteria exist at Screening or at the timepoint specified in the individual criterion listed:

1. Hypoxemia (oxygen saturation <96% awake or asleep without any supplemental oxygen or respiratory support, or for altitudes >1000 m, oxygen saturation of <92% awake or asleep without any supplemental oxygen or respiratory support).

2. Any clinical signs or symptoms at Screening or immediately prior to the first dosing (Day 1) that are, in the opinion of the Investigator, strongly suggestive of SMA.

3. Presence of an untreated or inadequately treated active infection requiring systemic antiviral or antimicrobial therapy at any time during the Screening Period.

4. History of brain or spinal cord disease that would interfere with the LP procedures, CSF circulation, or safety assessments.

5. Presence of an implanted shunt for the drainage of CSF or an implanted CNS catheter.

6. History of bacterial meningitis or viral encephalitis.

7. Clinically significant abnormalities in hematology or clinical chemistry parameters, as assessed by the Investigator, at Screening that would render the subject unsuitable for inclusion.

8. Treatment with an investigational drug given for the treatment of SMA (e.g., albuterol/ salbutamol, riluzole, carnitine, sodium phenylbutyrate, valproate, hydroxyurea, etc.), biological agent, or device. Any history of gene therapy, prior ASO treatment, or cell transplantation.

9. Diagnosis of neonatal Respiratory Distress Syndrome requiring surfactant replacement therapy or invasive ventilatory support.

10. The subject's parent(s) or legal guardian(s) is unable to understand the nature, scope, and possible consequences of the study or is unable to or does not agree to comply with study requirements.

11. Ongoing medical condition that, according to the Investigator, would interfere with the conduct and assessments of the study. Examples are medical disability that would interfere with the assessment of safety or would compromise the ability of the subject to undergo study procedures.

12. Other unspecified reasons that, in the opinion of the Investigator or Sponsor, make the subject unsuitable for enrollment.

9. ENROLLMENT AND REGISTRATION PROCEDURES

9.1. Screening and Enrollment

The subject's legally authorized representative(s) (e.g., parent[s] or legal guardian[s]) must provide informed consent before any study-specific screening tests are performed (see Section 17.3). The reason for which a subject was chosen for screening in this study will be captured. When a subject's parent(s)/guardian(s) signs the informed consent form (ICF), that subject is assigned a unique subject identification number through the interactive response technology (IRT) system and is considered to be enrolled in the study. Participating study sites are required to document all screened candidates initially considered for inclusion in this study. If a subject is excluded from the study, the reasons for exclusion will be documented in the subject's source documents, within the IRT system, and on the screening log.

9.2. Registration of Subjects

Subjects will be registered after all screening assessments have been completed and after the Investigator has verified that the subjects are eligible per criteria in Sections 8.1 and 8.2. No subject may begin treatment until the subject is documented as registered for the study in the IRT system. Any subject identification numbers that are assigned will not be reused even if the subject does not receive treatment.

All subjects will receive nusinersen.

Refer to the Study Reference Guide for details on registration through the IRT system.

9.3. Blinding Procedures

Not applicable.

10. DISCONTINUATION OF STUDY TREATMENT AND WITHDRAWAL OF SUBJECTS FROM THE STUDY

10.1. Discontinuation of Study Treatment

A subject *must* permanently discontinue study treatment for any of the following reasons:

- The subject's parent(s)/legal guardian(s) withdraws consent.
- The subject experiences a medical emergency that necessitates permanent discontinuation of study treatment.
- At the discretion of the Investigator for medical reasons.
- At the discretion of the Investigator or Sponsor for noncompliance.

The primary reason for discontinuation of study treatment must be recorded in the subject's case report form (CRF).

Subjects who discontinue treatment will complete an early termination visit 4 months (+14 days) after administration of the last dose of nusinersen, unless consent is withdrawn (see Section 10.2).

10.2. Withdrawal of Subjects From Study

Subjects must be withdrawn from the study for any one of the following reasons:

- The subject's parent(s)/legal guardian(s) withdraws consent.
- The subject's parent(s)/legal guardian(s) are unwilling or unable to comply with the protocol.

The primary reason for the subject's withdrawal from the study must be recorded in the subject's CRF.

Subjects who prematurely withdraw will be encouraged to complete the early termination evaluation assessments at the time of withdrawal (see Table 3).

Subjects who withdraw from the study will not be replaced.

11. STUDY TREATMENT USE

11.1. Regimen

Refer to and follow the DHA.

Each subject will receive a single IT bolus (1 to 3 minutes) LP injection of nusinersen by dedicated study personnel on Days 1, 15, 29, 64, 183, 302, 421, 540, 659, 778, 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1849, 1968, 2087, 2206, 2325, 2444, 2563, 2682, and 2801 of the study using a "spinal anesthesia" needle and a 5-mL syringe. A 22-gauge spinal anesthesia needle is recommended. The target site for needle insertion is the L3/L4 space, but may be 1 segment above or 1 to 2 segments below this level, if needed. Spinal ultrasound or fluoroscopy may be used for the LP procedure, if deemed necessary, but is not required. Local anesthesia may be used for the procedure, at the discretion of the study center.

Prior to each injection, 4 to 5 mL of CSF fluid will be collected for analyses. CSF will be used for measurement of nusinersen PK analyses. [redacted]

All subjects will be dosed with 12 mg (5 mL) regardless of age.

Note that injections should not occur within 72 hours after an immunization.

If a loading dose is delayed or missed, nusinersen should be administered as soon as possible, with at least 14 days between doses, and dosing continued at the prescribed dosing frequency. In the maintenance phase, if a planned dose is delayed or missed, nusinersen should be administered as soon as possible and dosing continued at the prescribed dosing frequency.

11.2. Modification of Dose and/or Treatment Schedule

No adjustment of dose is permitted. If dosing does not occur as per the schedule of activities, a protocol deviation should be logged. In the event of delayed or missed doses due to, for example, a concurrent illness or other circumstances (e.g., COVID-19 pandemic), the Investigator should refer to the DHA which includes recommended dosing administration in the event of delayed or missed doses.

11.3. Precautions

There are no protocol-required treatment precautions.

11.4. Compliance

Compliance with treatment dosing is to be monitored and recorded by site staff.

11.5. Concomitant Therapy and Procedures

11.5.1. Concomitant Therapy

A concomitant therapy is any drug or substance administered between Screening and the Final Study Visit/Telephone Call.

11.5.1.1. Allowed Concomitant Therapy

Any concomitant medications including SMA therapies will be captured in the CRF.

Approved concomitant therapies may be used at the discretion of the Investigator.

With implementation of Protocol Version 9, Investigators with participants newly seeking combination therapy with other SMA therapies should consult and obtain approval from the Medical Monitor. This will be permitted for country-approved SMA therapies if the total number of study participants concurrently receiving nusinersen and other SMA therapies has not yet reached 20% of all enrolled participants. This is consistent with primary and secondary objectives of this study. Any participants already receiving combination therapy with other SMA therapies at the time of Protocol Version 9 implementation may continue this combination therapy regardless of the 20% limit.

11.5.1.2. Disallowed Concomitant Therapy

As noted above in Section 11.5.1.1, with implementation of Protocol Version 9, Investigators with participants newly seeking combination therapy of nusinersen with other SMA therapies should consult the Medical Monitor. Any participants already receiving investigational drug for any other condition should discuss with the Medical Monitor.

11.5.2. Concomitant/Ancillary Procedures

A concomitant/ancillary procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed between the time the subject is enrolled in the study and the Final Study Visit/Telephone Call.

12. STUDY TREATMENT MANAGEMENT

Study treatment will be manufactured, handled, and stored in accordance with applicable Good Manufacturing Practice.

Study site staff should follow the DHA for specific instructions on the handling, preparation, administration, and disposal of the study treatment. The DHA aligns with all other references including the protocol.

Study treatment must be dispensed only by a pharmacist or appropriately qualified staff. Study treatment is to be dispensed only for subjects enrolled in this study. Once study treatment is prepared for a subject, it can only be administered to that subject. Study treatment vials are for one-time use only; any study treatment remaining in the vial should not be used for another subject.

12.1. Nusinersen

Nusinersen is formulated as an isotonic solution at 2.4 mg/mL and is provided as a 5.0-mL fill volume in a 6-mL clear glass vial. Nusinersen drug product contains the heptadecasodium salt of an 18-base residue phosphorothioate oligonucleotide.

The contents of the study treatment label will be in accordance with all applicable regulatory requirements. At a minimum, the label will include a study reference code, drug identifier, quantity of dosage units, lot number, and other pertinent information in accordance with local laws. The expiry or use-by date is stored in the IRT system, and printable assignment reports are available to site personnel. Study treatment should not be used after the expiration date.

12.1.1. Nusinersen Preparation

The individual preparing nusinersen should carefully review the instructions provided in the DHA.

If the packaging is damaged, or if there is anything unusual about the appearance or attributes of the vial(s) or drug, do not use the drug. The vial(s) in question should be saved at the study site and the problem immediately reported to the Sponsor (or designee).

12.1.2. Nusinersen Storage

Study treatment must be stored in a secure location.

Nusinersen is to be protected from light and stored long term at 2°C to 8°C in a locked refrigerator with limited access. For the most up-to-date storage requirements, follow the instructions provided in the DHA.

12.1.3. Nusinersen Handling and Disposal

The Investigator must return all used and unused vials of nusinersen as instructed by the Sponsor (or designee), unless approved for onsite destruction.

If any used nusinersen supplies are to be destroyed at the study site, the institution or appropriate site personnel must obtain prior approval from the Sponsor (or designee), by providing, in writing, the destruction policy or details of the method of destruction. After such destruction, the Sponsor (or designee) must be notified, in writing, of the details of the study treatment destroyed (e.g., lot or kit numbers, quantities), the date of destruction, and proof of destruction.

12.1.4. Nusinersen Accountability

Accountability for study treatment is the responsibility of the Investigator. The study site must maintain accurate records demonstrating dates and amount of study treatment received, to whom dispensed (subject-by-subject accounting), and accounts of any study treatment accidentally or deliberately destroyed or lost.

Unless otherwise notified, all vials, both used and unused, must be saved for study treatment accountability. By the end of the study, reconciliation must be made between the amount of nusinersen supplied, dispensed, and subsequently destroyed or returned to the Sponsor (or designee). A written explanation must be provided for any discrepancies.

13. EFFICACY, PHARMACOKINETIC, [redacted] ASSESSMENTS

See Section 5 for the timing of all assessments.

13.1. Efficacy Assessments

The following clinical assessments will be performed to evaluate the efficacy of nusinersen:

- Survival
- [redacted]
- [redacted]
- Growth parameters
- WHO motor milestones
- HINE motor milestones (Screening through Day 779 only)
- CHOP INTEND motor function scale
- HFMSE
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

Videotaping of all motor milestone and motor function assessments will be optional.

If a subject has or had a sibling with SMA and if consent is given, data for the sibling will be collected at 8 timepoints during the course of the study: at Screening (or the first visit after the implementation of Protocol Version 4), Day 897, Day 1254, Day 1611, Day 1968, Day 2325, Day 2682, and at the end of the study (or Early Termination Visit). Data to be collected from siblings with SMA will be nonbiologic and noninvasive and will include historical data for *SMN2* gene copy number, sibling treatment history, etc. (see Table 1 and Table 3 for timing of sibling data collection).

SMA is caused by loss of SMN protein due to a homozygous deletion or mutation or compound heterozygous mutation in the *SMN1* gene on chromosome 5q11-q13. Thus, patients with SMA are completely dependent on the amount of SMN protein produced by the *SMN2* gene. Genetic modifiers, such as the number of copies of the *SMN2* gene, are known to impact the eventual phenotype of SMA. The incidence and prevalence of SMA and its subtypes have been reported to vary based on country. One hypothesis for these variances is a difference in the frequency of certain genetic mutations that may be associated with different racial and ethnic groups within each country. Therefore, the race/ethnicity of subjects is collected as part of the medical history, where local regulations allow.

[redacted]

13.1.2. Growth Parameters

Growth parameters of body length and/or height (for all subjects), head circumference (for subjects up to 36 months of age), chest circumference (for subjects up to 36 months of age), and arm circumference (for subjects up to 36 months of age) will be measured.

Additional parameters of weight-for-age, weight-for-length, and head-to-chest circumference ratio will be calculated. If an assessment is not performed at a visit, attempts should be made to

perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

13.1.3. Motor Milestones

For the purposes of this protocol, ambulatory will be defined as any subject who has achieved independent walking as defined by the WHO Motor Milestones criteria (Test Item #6 – Walking Alone). Videotaping of the WHO and/or HINE motor milestone assessments will be optional. If an assessment is not performed at a visit, attempts should be made to perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

For all subjects, motor milestones will be assessed using the WHO Motor Milestones criteria [WHO Multicentre Growth Reference Study Group 2006; Wijnhoven 2004].

For subjects <2 years of age who have achieved or have not yet achieved independent walking, motor milestones will be assessed using Section 2 of the HINE, which is composed of 8 motor milestone categories as follows: voluntary grasp, ability to kick in supine position, head control, rolling, sitting, crawling, standing, and walking. Within each motor milestone category, there are 3 to 5 levels that can be achieved. All 8 motor milestones will be tested during each assessment. A subject whose results after testing all appear in the first column (no grasp, no kicking, unable to maintain head upright, and so on) has not achieved any motor milestone. Motor milestone achievement is depicted by movement from the left side of Table 5 to the right side of the table, as denoted by the Milestone Level Progression arrow in the table [Haataja 1999].

Table 5: Hammersmith Infant Neurological Examination Section 2 - Motor Milestones

Motor Milestone Category	Milestone Level Progression (Age Expected in Heathy Infants[1]) →				
Voluntary grasp	No grasp	Uses whole hand	Finger and thumb; immature grasp	Pincer grasp	
Ability to kick (in supine)	No kicking	Kicks horizontal; legs do not lift	Upward (vertically) [3 months]	Touches leg (4 to 5 months)	Touches toes (5 to 6 months)
Head control	Unable to maintain upright (<3 months)	Wobbles (4 months)	All the time upright (5 months)		
Rolling	No rolling	Rolling to side (4 months)	Prone to supine (6 months)	Supine to prone (7 months)	
Sitting	Cannot sit	Sit with support at hips (4 months)	Props (6 months)	Stable sit (7 months)	Pivots (rotates) [10 months]
Crawling	Does not lift head	On elbow (3 months)	On outstretched hand (4 to 5 months)	Crawling flat on abdomen (8 months)	On hands and knees (10 months)
Standing	Does not support weight	Supports weight (4 to 5 months)	Stands with support (8 months)	Stands unaided (12 months)	
Walking	No walking	Bouncing (6 months)	Cruising (holding on) [11 months]	Walking independently (15 months)	

[1] Values for healthy infants in [Haataja 1999].

The proportion of motor milestone responders is defined based on the 8 motor milestones categories, with the exclusion of voluntary grasp using the assessment at the later study visits, as follows:

i. Subject demonstrates at least a 2-point increase in the motor milestone category of ability to kick or achievement of the maximal score on that category (touching toes) or a 1-point increase in the motor milestones of head control, rolling, sitting, crawling, standing, or walking; AND

ii. Among the 8 motor milestone categories with the exclusion of voluntary grasp, subject demonstrates improvement (defined in [i]) in more categories than worsening.

Note: For the category of ability to kick, similar to the definition of improvement in (i) mentioned previously, worsening is defined as at least a 2-point decrease or decrease to the lowest possible score of no kicking. For the other 6 categories, worsening is defined as at least a 1-point decrease.

13.1.4. Motor Function Assessments

Motor function assessments include all assessments listed in Section 13.1.4.1, Section 13.1.4.2, and Section 13.1.4.3. The assessments that are performed at a given visit will depend on the subject's age at that visit and current motor abilities.

Videotaping of all motor function assessments will be optional.

13.1.4.1. Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease

CHOP INTEND will be assessed in subjects with infantile-onset SMA until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed.

The CHOP INTEND test was specifically designed to evaluate the motor skills of infants with significant motor weakness, including infants with SMA [Glanzman 2010]. The CHOP INTEND test captures neck, trunk, and proximal and distal limb strength in 14 elicited and 2 observational items. The CHOP INTEND has been established as a safe and reliable infant motor measure in infantile-onset SMA and has been validated [Glanzman 2011]. If an assessment is not performed at a visit, attempts should be made to perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

13.1.4.2. Hammersmith Functional Motor Scale – Expanded

All subjects ≥2 years of age will be evaluated using the HFMSE for the duration of the study. Subjects who are ≥2 years of age but have not yet achieved the maximum score of 64 with CHOP INTEND will be assessed with both until a CHOP INTEND maximum score of 64 is achieved. The HFMSE should be performed after the CHOP INTEND with an approximately 15-minute rest period in between to allow the subject to be fully engaged with both assessments.

The HFMSE is a reliable and validated tool used to assess motor function in children with SMA. The scale was originally developed with 20 scored activities and was devised for use in children with Type II and Type III SMA with limited ambulation to give objective information on motor ability and clinical progression [Main 2003]. The expanded scale includes an additional module of 13 items developed to allow for evaluation of ambulatory patients with SMA [O'Hagen 2007]. The HFMSE has been shown to be highly correlated with other clinical assessments and has shown good test-retest reliability. If an assessment is not performed at a visit, attempts should be made to perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

[redacted] [redacted]

[redacted]

[redacted]

[redacted] [redacted]

[redacted]

[redacted] [redacted]

[redacted].

[redacted] [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

13.2. Pharmacokinetic (Nusinersen Concentration) Assessments

The following parameters will be calculated to assess the PK of nusinersen:

- Plasma nusinersen concentrations
- CSF nusinersen concentrations

[redacted]

13.4. Immunogenicity Assessments

The samples for the anti-nusinersen plasma antibody will be collected in accordance to the schedule provided in Table 1, Table 3 , and Table 4 and banked. Samples may be analyzed at the Sponsor's discretion.

[redacted]

[redacted]

14. SAFETY ASSESSMENTS

See Section 5 for the timing of all safety assessments.

14.1. Clinical Safety Assessments

The following clinical assessments will be performed to evaluate the safety profile of nusinersen:

- Neurological examinations: Sections 1 and 3 of the HINE will be conducted on all subjects ≤24 months of age and will be collected predose, 3 and 6 hours after dosing from Screening through Day 779. For all subjects >24 months of age, standard neurological examinations will be conducted within 7 days of dosing and approximately 1 hour after dosing (or when sedation has worn off if it was used) from Day 897 through Day 2891
- Physical examinations and weight
- Vital sign measurements: temperature, pulse rate, resting systolic and diastolic blood pressure, and respiratory rate
- Pulse oximetry
- 12-Lead ECGs
- Echocardiograms
- Concomitant therapy recording
- AE recording

14.1.1. Neurological Examinations

Neurological examinations will be performed predose **and** approximately 3 and 6 hours after dosing from Day 1 through Day 779 and within 7 days of dosing **and** approximately 1 hour after dosing from Day 897 through Day 2891. **Please note**: If sedation was used, please allow sufficient recovery time for the subject to be fully engaged in the examination. It is important that the data collected truly reflect the subject's neurological performance.

The HINE (Sections 1 and 3) will be conducted in all subjects ≤24 months of age. This standard examination (developed by [Dubowitz and Dubowitz 1981]) is a quantitative scorable method for assessing the neurological development of infants between 2 and 24 months of age. The examination includes assessment of cranial nerve functions, posture, movements, tone, and reflexes.

For all subjects >24 months of age, standard neurological examinations, which include assessments of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be conducted.

14.1.2. Electrocardiograms

ECGs will be performed for all subjects at Screening, Day 2, Day 29 postdose, Day 365, Day 700, Day 897, Day 1254, Day 1611, Day 1849, Day 2206, Day 2563, and at the end of the study (or Early Termination Visit). If an assessment is not performed at a visit, attempts should be made to perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

After the ECG is completed, an initial local read of the ECG should occur before the ECG is sent for central read (all ECGs will be centrally read). If the subject's initial ECG results show a QTc interval of ≥500 ms, then the ECG should be repeated (prior to the subject leaving the visit). If the second ECG QTc again reads ≥500 ms, the physician should use his or her best clinical judgement to address the condition.

Additional ECGs may be performed per the judgement of the Investigator, as deemed clinically necessary.

14.1.3. Echocardiograms

At the time of the implementation of Protocol Version 7, echocardiograms will be performed for all subjects at Days 1849, 2206, 2563, and at the end of the study (or Early Termination Visit). Echocardiograms will only be assessed locally at the site as per local practice. If an assessment is not performed at a visit, attempts should be made to perform the assessment at the subsequent dosing visit(s) until completed; however, an assessment should not be performed more than once at any visit.

14.1.4. Physical Examinations

Physical examinations will be performed for all subjects at every onsite visit throughout the study. Any abnormal findings observed during physical examinations will be captured as AEs and reported according to Section 15.

Videotaping of the physical examinations will be optional.

14.1.5. Vital Signs

Vital signs include resting systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature. Additionally, pulse oximetry will be collected. From Screening through Day 779, vital signs and pulse oximetry will be collected predose and at 4 timepoints postdose: 1, 2, 4, and 6 hours. From Day 897 through Day 2891, vital signs and pulse oximetry will be taken predose and approximately 1 hour postdose (±15 minutes) at every onsite visit throughout the study.

14.2. Laboratory Safety Assessments

Laboratory measurements of serum chemistry, hematology, urinalysis, and urine total protein will be collected at Screening, Day 15, Day 29, and every 4 months thereafter (i.e., Days 64, 183,

302, 421, 540, 659, 778, 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1849, 1968, 2087, 2206, 2325, 2444, 2563, 2682, and 2801), as well as on Day 365, Day 700, and Day 2891 (End of Study)/Early Termination Visit. The laboratory analytes to be measured are shown in Appendix A. Quantitative urine total protein will be assessed at the local laboratory. For urinary protein concentration >0.2 g/L, repeat testing and further evaluation should be considered; quantitative urine total protein should be prioritized if there is not enough urine sample for all tests.

Due to blood collection volume limitations for newborns, the laboratory safety assessments described above will be performed according to the schedules listed in Table 8 and Table 9. Coagulation testing was not performed at every visit in previous versions of the protocol (Versions 1 through 5); therefore not all subjects will have coagulation results prior to all doses received during this timeframe. At the time of implementation of Protocol Version 6, coagulation testing was added, was to be conducted at each visit, and results were required to be reviewed prior to dosing. Coagulation testing will continue to be conducted at each visit or up to 7 days prior to dosing and results should be reviewed prior to dosing. In situations where predose verification of coagulation laboratory results is not feasible or optimal (e.g., prolonged sedation of the subject would be required to allow adequate time for lab processing), the Investigator may exercise discretionary clinical judgment and proceed with the LP procedure, based on the subject's clinical and coagulation lab history.

Table 8: Laboratory Safety Blood Collection Schedule for Subjects with a Screening Weight of ≥3 kg

Panel/Test	Screening[1]	Day 1	Day 15	Day 29	Day 64	Days 183, 302, 421, 540, 659, 778 (±14D)	Days 365, 700, 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1849, 1968, 2087, 2206, 2325, 2444, 2563, 2682, 2801, 2891 (±14D), and Early Term
Hematology	X		X	X	X	X	X
Serum Chemistry	X		X	X	X	X	X
Cystatin C	X				X[2]	X[2]	X
Coagulation	X	X	X	X	X	X	X

D = day(s); Term = termination.

[1] Screening laboratory tests will be collected over a two or more day period.

[2] At Day 183 or when the subject's weight is ≥5.4 kg, whichever comes first, the blood collection schedule will revert to that outlined in Section 5.

Table 9: Laboratory Safety Blood Collection Schedule for Subjects with a Screening Weight of <3 kg

Panel/Test	Screening[1]	Day 1	Day 15	Day 29	Day 64	Days 183, 302, 421, 540, 659, 778 (±14D)	Days 365, 700, 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1849, 1968, 2087, 2206, 2325, 2444, 2563, 2682, 2801, 2891 (±14D), and Early Term
Hematology	X		X	X	X	X	X
Serum Chemistry	X		X	X	X	X	X
Coagulation	X	X	X	X	X	X	X

D = day(s); Term = termination.
[1] Screening laboratory tests will be collected over a two or more day period.

15. SAFETY DEFINITIONS, RECORDING, REPORTING, AND RESPONSIBILITIES

Throughout the course of the study, every effort must be made to remain alert to possible AEs. If an AE occurs, the first concern should be for the safety of the subject. If necessary, appropriate medical intervention should be provided.

At the signing of the ICF, each subject's legally authorized representative(s) and/or main caregiver(s) must be given the names and telephone numbers of study site staff for reporting AEs and medical emergencies.

15.1. Definitions

15.1.1. Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product.

Determination of whether an abnormal laboratory value, vital sign result, and/or ECG result meets the definition of an AE will be made by the Investigator. Abnormal results are not considered AEs unless 1 or more of the following criteria are met:

- The result meets the criteria for an SAE
- The result requires the subject to receive specific corrective therapy
- The result is considered by the Investigator to be clinically significant

15.1.2. Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- In the view of the Investigator, places the subject at immediate risk of death (a life-threatening event); however, this does not include an event that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect

- Is a medically important event

A medically important event is an AE that, in the opinion of the Investigator, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. (Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or convulsions occurring at home that do not require an inpatient hospitalization.)

15.1.3. Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the subject is hospitalized; the study site must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the subject's parent(s)/legal guardian(s) consent to participate in the study.
- The condition requiring the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the subject's parent(s)/legal guardian(s) consent to participate in the study and the time of the procedure or treatment.
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission.
    - If a subject is hospitalized due to local requirements for administration of the study treatment, the hospitalization should not be considered an SAE unless one of the requirements in Section 15.1.2 is met.

15.2. Safety Classifications

15.2.1. Investigator Assessment of Events

All events must be assessed to determine the following:

- If the event meets the criteria for an SAE as defined in Section 15.1.2
- The relationship of the event to study treatment and the LP procedure as defined in Section 15.2.2
- The severity of the event as defined in Section 15.2.3

15.2.2. Relationship of Events to Study Treatment or Lumbar Puncture Procedure

The following definitions should be considered when evaluating the relationship of AEs and SAEs to the study treatment:

Relationship of Event to Study Treatment	
Related	There is clear evidence that the event is related to the use of the investigational drug (e.g., confirmation by positive re-challenge test).
Possible	The event cannot be explained by the subject's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and administration of the investigational drug.
Unlikely/ Remote	An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to investigational drug administration and/or exposure suggests that a causal relationship is unlikely. (For reporting purposes, Unlikely/Remote will be grouped together with Not Related.)
Not Related	The event can be readily explained by the subject's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and the investigational drug.

The following definitions should be considered when evaluating the relationship of AEs and SAEs to the LP procedure:

Relationship of Event to LP Procedure	
Related	There is clear evidence that the event is related to the LP procedure (e.g., bleeding from the puncture site).
Possible	The event cannot be explained by the subject's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and the LP procedure.
Unlikely/ Remote	An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to the LP procedure suggests that a causal relationship is unlikely. (For reporting purposes, Unlikely/Remote will be grouped together with Not Related.)
Not Related	The event can be readily explained by the subject's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and the LP procedure.

15.2.3. Severity of Events

The following definitions should be considered when evaluating the severity of AEs and SAEs:

Severity of Event	
Mild	The event is easily tolerated by the subject and does not affect the subject's usual daily activities.
Moderate	The event causes the subject more discomfort and interrupts the subject's usual daily activities.
Severe	The event is incapacitating and causes considerable interference with the subject's usual daily activities.

15.2.4. Expectedness of Events

Expectedness of all AEs will be determined by the Sponsor (or designee) according to the Investigator's Brochure.

15.3. Monitoring and Recording Events

15.3.1. Adverse Events

For subjects who receive study treatment, any AE experienced between the time of signing the ICF and Final Study Visit/Telephone Call is to be recorded on the CRF, regardless of the severity of the event or its relationship to study treatment. At each study visit, the Investigator will assess the subject for AEs and will record any new AEs or updates to previously reported AEs on the CRF. Note: Any new or worsened findings (including signs and symptoms of SMA) that start after the informed consent date must be recorded as AEs and should not be listed as medical history.

For subjects who never receive study treatment, no AEs need to be recorded on the applicable CRF.

An AE that is ongoing when the subject completes or discontinues the study should be followed by the Investigator, if possible, until the event has resolved or stabilized.

15.3.2. Serious Adverse Events

For subjects who receive study treatment, any SAE experienced between the time of signing the ICF and Final Study Visit/Telephone Call is to be recorded on an SAE form and on the applicable CRF, regardless of the severity of the event or its relationship to study treatment. For subjects who never receive study treatment, any SAE occurring between the time of signing the ICF and Final Study Visit/Telephone Call must be recorded on an SAE form, regardless of the severity of the event or its relationship to study treatment; however, the SAE does not need to be recorded on the applicable CRF.

SAEs must be reported to the safety vendor listed in the Study Reference Guide within 24 hours (as described in Section 15.3.3) or according to national law). Follow-up information regarding an SAE also must be reported within 24 hours.

Subjects will be followed for all SAEs until their Final Study Visit/Telephone Call. Thereafter, the event should be reported as described in the Study Reference Guide only if the Investigator considers the SAE to be related to study treatment.

Any SAE that is ongoing when the subject completes or discontinues the study will be followed by the Investigator until the event has resolved, stabilized, or returned to baseline status.

15.3.3. Immediate Reporting of Serious Adverse Events

In order to adhere to all applicable laws and regulations for reporting an SAE, the study site must formally notify the safety contact as described in the Study Reference Guide within 24 hours of the study site staff becoming aware of the SAE or according to national law. It is the Investigator's responsibility to ensure that the SAE reporting information and procedures are used and followed appropriately.

Reporting Information for SAEs

Any SAE that occurs between the time that the subject's parent(s)/legal guardian(s) has signed the ICF and Final Study Visit/Telephone Call must be reported as described in the Study Reference Guide within 24 hours of the study site staff becoming aware of the event.

A report ***must be submitted*** as described in the Study Reference Guide regardless of the following:

- Whether or not the subject has undergone study-related procedures
- Whether or not subject has received study treatment
- The severity of the event
- The relationship of the event to study treatment

To report initial or follow-up information on an SAE, fax or email a completed SAE form as described in the Study Reference Guide.

15.3.3.1. Deaths

Death is an outcome of an event. The event that resulted in death should be recorded on the appropriate CRF. All causes of death must be reported as SAEs within 24 hours of the site becoming aware of the event or according to national law. The Investigator should make every effort to obtain and send death certificates and autopsy reports as described in the Study

Reference Guide. The term death should be reported as an SAE only if the cause of death is not known and cannot be determined.

15.3.4. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are SAEs that are unexpected and judged by the Investigator or Sponsor (or designee) to be related to the study treatment administered.

The Sponsor (or designee) will report SUSARs to the appropriate regulatory authorities and Investigators as required, according to local law.

15.4. Procedures for Handling Special Situations

15.4.1. Overdose

All dosing errors (including but not limited to route of administration, wrong dose, etc.) must be reported as protocol deviations. A brief description should be provided in the deviation, including whether the subject was symptomatic or not. Dosing details should be captured on the Dosing CRF.

A dosing error will be considered an overdose when any of the following conditions are met:

- A dose given exceeds the dose level described in the protocol and Drug Handling Guidelines
- Dosing frequency exceeds 4 doses in a 2-month period (and, thus, results in a higher than acceptable cumulative dose)
- Any time study treatment is administered less than 2 weeks from the previous dose

Overdoses are not considered AEs and should not be recorded as an AE on the CRF unless an AE or an SAE occurs. All overdoses (regardless of whether or not they result in an AE) must be recorded on an overdose form and faxed to the Sponsor (or designee) within 24 hours of the site becoming aware of the overdose. If an overdose results in an SAE, both the SAE and overdose forms must be completed and faxed to the Sponsor (or designee). Should an overdose occur, the Investigator or designee must contact the Medical Monitor within 24 hours; refer to the Study Reference Guide for the complete contact information.

15.4.2. Medical Emergency

In a medical emergency requiring immediate attention, study site staff will apply appropriate medical intervention, according to current standards of care. The Investigator (or designee) should contact the study's Medical Director. Refer to the Study Reference Guide's official study contact list for complete contact information.

15.5. Safety Responsibilities

15.5.1. The Investigator

The Investigator's responsibilities include the following:

- Monitor and record all AEs, including SAEs, on the CRF regardless of the severity or relationship to study treatment.
- Determine the seriousness, relationship, and severity of each event.
- Determine the onset and resolution dates of each event.
- Complete an SAE form for each SAE and fax it as described in the Study Reference Guide within 24 hours of the study site staff becoming aware of the event or according to national law.
- Pursue SAE follow-up information actively and persistently. Follow-up information must be reported as described in the Study Reference Guide within 24 hours of the study site staff becoming aware of new information or according to national law.
- Ensure all AE and SAE reports are supported by documentation in the subjects' medical records.
- Pursue AE follow-up information, if possible, until the event has resolved or become stable. Record AE follow-up information, including resolution, on the CRF, as applicable.
- Report SAEs to local ethics committees, as required by local law.

15.5.2. The Sponsor

The Sponsor's responsibilities include the following:

- Before study site activation and subject enrollment, the Clinical Monitor (or designee) is responsible for reviewing with study site staff the definitions of AE and SAE, as well as the instructions for monitoring, recording, and reporting AEs and SAEs.
- The Sponsor (or designee) is to notify all appropriate regulatory authorities, central ethics committees, and Investigators of SAEs, as required by local law, within required time frames.

16. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

In general, continuous variables will be summarized by descriptive statistics, including number, mean, median, standard deviation, minimum, and maximum. Categorical variables will be presented with the number and percentage in each category.

The Full Analysis Set (FAS) is defined as all subjects who receive at least 1 dose of nusinersen.

The Per-Protocol Set (PPS) will include the subset of the FAS who complete at least the initial 4 doses of study treatment, have a baseline and at least the Day 183 efficacy assessments, and who have no significant protocol deviations that would be expected to affect efficacy assessments.

A number of the endpoints are age-specific and, therefore, over the course of the study as subjects get older, different scales will be utilized. Three baselines will be defined. The first baseline will be defined as the closest available assessment on or prior to Day 1 predose when the child is <6 weeks of age. The second baseline will be defined as on or prior to the Day 700 visit when the child is approximately 2 years of age. The third baseline will be defined as on or prior to Day 897 predose visit when the child is approximately 2.5 years of age.

The objectives of the study and the endpoints to be analyzed are listed in Section 6.

16.1. Efficacy

16.1.1. Analysis Population

The primary analysis of efficacy will be performed on subjects in the FAS with 2 *SMN2* copies. Analyses will be repeated in the FAS and PPS, as well as in the PPS with 2 *SMN2* copies. For the longitudinal analyses of certain endpoints, it may be necessary to define additional efficacy sets.

16.1.2. Methods of Analysis

16.1.2.1. Analysis of the Primary Endpoint

The primary endpoint is the time to respiratory intervention or death. An event of respiratory intervention will be defined as either invasive or noninvasive ventilation for ≥6 hours/day continuously for 7 or more consecutive days OR tracheostomy. The time will be the age of the subject at the first occurrence of either a respiratory intervention or death.

A Kaplan-Meier survival curve of the time to respiratory intervention or death will be presented and used to estimate the median time to event and corresponding 95% confidence interval (CI).

16.1.2.2. Analysis of the Secondary Endpoints

Age: <6 weeks to 2 years

During this period, the 13- and 24-Month Visits are of most interest. The visit at Day 365 will be used for the assessment of the endpoint at 13 months and the visit at Day 700 will be used for the assessment of the endpoint at 24 months. The proportion of subjects meeting the criteria for the following at the 13- and 24-Month Visits will be presented with a corresponding 95% CI:

- Clinically manifested SMA defined by any one of the following:
    - If at the 13- or 24-Month Visit, the subject's weight has dropped below the 5th percentile according to WHO criteria [WHO 2014]; or if compared to Baseline, a subject has decreased ≥2 major weight growth curve percentiles (3rd, 5th, 10th, 25th, or 50th) according to WHO criteria; or if a percutaneous gastric tube has been required at any point up to and including the 13- or 24-Month Visit, as documented in the concomitant procedures in the CRF
    - Failure to demonstrate the following as assessed by the WHO motor milestones:
        - At 13 and 24 months of age: ability to sit without support, standing with assistance, hands-and-knees crawling
        - Additionally, at 24 months of age: walking with assistance, standing alone, and/or walking alone
    - Subjects who discontinue or die on or before the 13- or 24-Month Visit
- Achievement of each individual motor milestone as assessed by the HINE (Section 2) and the WHO motor milestones. Subjects who discontinue prior to the 13- or 24-Month Visit will be counted in the denominator.

Age: 2 to 8 years

During this period, the 2.5, 3, 4, 5, 6, 7, and 8 years of age visits are of the most interest. The Day 897 visit will be used for 2.5 years, Day 1135 will be used for 3 years, Day 1492 will be used for 4 years, Day 1849 will be used for 5 years, Day 2206 will be used for 6 years, Day 2563 will be used for 7 years, and Day 2891 will be used for 8 years.

The proportion of subjects who have the ability to perform WHO motor milestones at Baseline and maintain this ability up to 8 years of age will be summarized by visit.

The HFMSE total score and change from Baseline will be summarized by visit using descriptive statistics.

Age: <6 weeks to 8 years

A Kaplan-Meier survival curve will be presented and used to estimate the proportion of subjects alive at 13 months and 2, 3, 4, 5, 6, 7, and 8 years of age. Corresponding 95% CIs will also be presented.

- Change from Baseline to each visit will be summarized using descriptive statistics for the following growth parameters: weight for age/length, head circumference, chest circumference, head-to-chest circumference ratio, and arm circumference.

- Change from Baseline to each visit for the CHOP INTEND motor function will be summarized using descriptive statistics. The maintenance of the ability to achieve a threshold of total score of ≥50 over time will also be presented.

- The actual value and change from Baseline in CSF survival protein concentration will be summarized.

[redacted]

16.2. Pharmacokinetics

16.2.1. Analysis Population

The PK analysis will be performed on all subjects for which there is at least 1 evaluable PK sample after the first IT injection.

16.2.2. Methods of Analysis

Results of CSF and plasma nusinersen concentrations will be summarized by sampling time. A noncompartmental analysis will be conducted to estimate the plasma PK parameters of nusinersen, if applicable, and summary statistics will be performed.

[redacted]

16.4. Immunogenicity

16.4.1. Analysis Population

The analysis population for immunogenicity will include all subjects with available immunogenicity data.

16.4.2. Methods of Analysis

If samples are analyzed, descriptive statistics of the incidence of anti-nusinersen plasma antibody concentrations will be presented.

[redacted]

16.6. Safety

All AEs, laboratory abnormalities, ECGs, and vital signs will be evaluated for safety.

16.6.1. Analysis Population

The analysis of safety will be performed on the FAS.

16.6.2. Methods of Analysis

16.6.2.1. Adverse Events

The Medical Dictionary for Regulatory Activities will be used to classify AEs.

All AEs will be analyzed based on the principle of treatment emergence. An AE will be regarded as treatment emergent if it was present prior to the first dose of study treatment and subsequently worsened, or was not present prior to the first dose of study treatment but subsequently appeared. The incidence of treatment-emergent AEs will be summarized overall, by severity, and by relationship to study treatment. A subject having the same AE more than once will be counted only once in the incidence for that event. The occurrence of the AE with the greatest severity will be used in the calculation of incidence by severity; the occurrence of the AE with the strongest relationship to study treatment will be used in the calculation of incidence by relationship to study treatment.

16.6.2.2. Clinical Laboratory Results

Clinical laboratory evaluations including hematology, blood chemistry, and urinalysis will be summarized using shift tables, presenting changes relative to each parameter's normal range. Summary statistics for actual values and changes from Baseline will also be presented.

16.6.2.3. Vital Signs

The analysis of vital signs will focus on clinically relevant abnormalities. The number and percentage of subjects with clinically relevant postbaseline abnormalities will be presented. Summary statistics for actual values and change from Baseline will also be presented.

16.6.2.4. ECGs

The number and percentage of subjects with shifts from Baseline normal to each of the categorical values denoting normal, abnormal, and abnormal (not AE) will be summarized.

16.6.2.5. Echocardiograms

The number and percentage of subjects with clinical changes in echocardiogram readings from the first assessment will be summarized.

16.7. Interim Analyses

Interim data analyses may be performed after approximately 10 subjects have been enrolled. Further details about these analyses will be described in the Statistical Analysis Plan.

16.8. Sample Size Considerations

There are no formal sample size calculations for this study. Sample size is based upon feasibility.

17. ETHICAL REQUIREMENTS

The Sponsor and the Investigator must comply with all instructions, regulations, and agreements in this protocol and applicable International Council on Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines and conduct the study according to local regulations.

The Investigator may delegate responsibilities for study-related tasks where appropriate to individuals sufficiently qualified by education, training, and experience, in accordance with applicable ICH and GCP guidelines. The Investigator should maintain a list of the appropriately qualified persons to whom significant study-related duties have been delegated. The Investigator is responsible for supervising those individuals and for implementing procedures to ensure the integrity of the tasks performed and any data generated.

17.1. Declaration of Helsinki

This study will be performed in alignment with the ethical principles outlined in the Declaration of Helsinki.

17.2. Ethics Committee

The Investigator must obtain ethics committee approval of the protocol, ICF, and other required study documents prior to starting the study. The Sponsor (or designee) will submit documents on behalf of the investigational sites in countries other than the US.

If the Investigator makes any changes to the ICF, the Sponsor (or designee) must approve the changes before the ICF is submitted to the ethics committee. A copy of the approved ICF must be provided to the Sponsor (or designee). After approval, the ICF must not be altered without the agreement of the relevant ethics committee and the Sponsor (or designee).

It is the responsibility of the Investigator(s) to ensure that all aspects of institutional review are conducted in accordance with current applicable regulations.

The Sponsor (or designee) must receive a letter documenting ethics committee approval, which specifically identifies the protocol, protocol number, and ICF, prior to the initiation of the study. Protocol amendments will be subject to the same requirements as the original protocol.

A progress report must be submitted to the ethics committee at required intervals and not less than annually.

At the completion or termination of the study, the investigational site must submit a close-out letter to the ethics committee and the Sponsor (or designee).

17.3. Subject Information and Consent

Prior to performing any study-related activities under this protocol, including screening tests and assessments, written informed consent with the approved ICF must be obtained from the

subject's legally authorized representative(s) (e.g., parent[s] or legal guardian[s]) in accordance with local practice and regulations.

The background of the proposed study, the procedures, the benefits and risks of the study, and that study participation is voluntary for the subject must be explained to the subject's legally authorized representative(s). The subject's legally authorized representative(s) must be given sufficient time to consider whether the subject will participate in the study.

A copy of the signed and dated ICF must be given to the subject's legally authorized representative(s). The original signed and dated ICF will be retained with the study records. Local regulations must be complied with in respect to the final disposition of the original (wet signature) and copies of the signed and dated ICFs.

Confirmation of informed consent must also be documented in the subject's medical record.

17.4. Subject Data Protection

Prior to any testing under this protocol, including screening tests and assessments, the subject's legally authorized representative(s) must also provide all authorizations required by local law (e.g., Protected Health Information authorization in North America).

During the study, participants' race and ethnicity and full date of birth will be collected (unless the collection is not permitted by applicable law or not approved by the governing ethics committee). These data will be used in the analysis of the safety and/or PK profile of the study treatment.

SMA is caused by the loss of SMN protein due to a homozygous deletion or mutation or a compound heterozygous mutation in the *SMN1* gene on chromosome 5q11-q13. Thus, patients with SMA are completely dependent on the amount of SMN protein produced by the *SMN2* gene. Genetic modifiers, such as the number of copies of the *SMN2* gene, are known to impact the eventual phenotype of SMA. The incidence and prevalence of SMA and its subtypes have been reported to vary based on country. One hypothesis for these variances is a difference in the frequency of certain genetic mutations that may be associated with different racial and ethnic groups within each country. Therefore, the race/ethnicity of participants will be collected as part of the medical history, where local regulations allow. The full date of birth is needed in order to precisely calculate the age at achievement of motor milestones and the weight-for-age percentiles.

Study reports will be used for research purposes only. The subject will not be identified by name in the CRFs, study-related forms, study reports, or any related publications. The Sponsor, its partners and designees, ethics committees, and various government health agencies may inspect the records of this study. Every effort will be made to keep the subject's personal medical data confidential.

17.5. Compensation for Injury

The Sponsor (or designee) maintains appropriate insurance coverage for clinical studies and will follow applicable local compensation laws.

17.6. Conflict of Interest

The Investigators should address any potential conflicts of interest (e.g., financial interest in the Sponsor or its partners) with the subject's legally authorized representative(s) before the subject's legally authorized representative(s) makes a decision for the subject to participate in the study.

17.7. Registration of Study and Disclosure of Study Results

The Sponsor (or designee) will register the study and post-study results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations.

18. ADMINISTRATIVE PROCEDURES

18.1. Study Site Initiation

The Investigator must not screen any subjects prior to completion of a study initiation visit conducted by the Sponsor (or designee). This initiation visit will include a detailed review of the protocol and study procedures.

18.2. Quality Control and Quality Assurance

Quality control procedures will be implemented at each stage of data handling to ensure that all data are reliable and have been processed correctly. Data anomalies will be communicated to the sites for clarification and resolution, as appropriate.

During and/or after completion of the study, quality assurance officers named by the Sponsor or the regulatory authorities may wish to perform onsite audits or inspections. The Investigator will be expected to cooperate with any audit and to provide assistance and documentation (including source data) as requested.

18.3. Monitoring of the Study

The Investigator must permit study-related monitoring by providing direct access to source data and to the subjects' medical histories. Source data must be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data must be traceable, not obscure the original entry, and be explained if necessary (e.g., with an audit trail). The Investigator should maintain a record of the location(s) of essential documents.

The Clinical Monitor(s) will visit the Investigator(s) at regular intervals during the study and after the study has completed, as appropriate. A clinical site monitoring plan will detail who performs the monitoring, how often it is performed, and the extent of review. It will also provide the monitoring strategy, with emphasis on subject safety, data integrity, and critical data and processes.

During these visits, CRFs and supporting documentation related to the study will be reviewed and any discrepancies or omissions will be resolved.

Monitoring visits must be conducted according to the applicable ICH and GCP guidelines to ensure the protection of subject rights and well-being, protocol adherence, quality of data (accurate, complete, and verifiable), study treatment accountability, compliance with regulatory requirements, and continued adequacy of the investigational site and its facilities.

18.4. Study Funding

Biogen is funding the study. Biogen is the Sponsor of the study globally. Biogen (or designee) will be responsible for managing the study globally. All financial details are provided in the separate contract(s) between the institution, Investigator, and the Sponsor.

18.5. Publications

Details are included in the clinical trial agreement for this study.

19. FURTHER REQUIREMENTS AND GENERAL INFORMATION

19.1. External Contract Organizations

The Sponsor may transfer any or all of its study-related responsibilities to a contract research organization (CRO) and other third parties; however, the Sponsor retains overall accountability for these activities.

19.1.1. Contract Research Organization

A CRO will be responsible for administrative aspects of the study including, but not limited to, study initiation, monitoring, and management of SAE reports and data management. Before subjects are screened at each study site, the CRO will review study responsibilities with the Investigators and other study site staff, as appropriate.

19.1.2. Interactive Response Technology

IRT will be used in this study. Before subjects are screened or enrolled, the IRT vendor will provide each study site with the necessary training, a user manual, and access rights to the system.

19.1.3. Electronic Data Capture

Subject information will be captured and managed by study sites on electronic CRFs by a Web-based electronic data capture tool that is supported by a vendor and configured by the Sponsor or CRO.

19.1.4. Central Laboratories for Laboratory Assessments

A central laboratory will be selected by the Sponsor to analyze all hematology, blood chemistry, urine, and CSF samples collected at all study sites. Local laboratories may be used to accommodate acute or urgent needs as discussed in in the Study Reference Guide.

During the Screening Period, a blood sample will be collected for *SMN2* copy number analysis by the central laboratory only from those subjects without genetic documentation of *SMN2* copy number. For all other subjects, a blood sample will be collected at any time during the study for analysis of *SMN2* copy number by the central laboratory.

19.2. Study Committees

Safety data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor. The Sponsor and Medical Monitor will review safety, tolerability, and efficacy (as needed) data collected on nusinersen during this study.

19.3. Changes to Final Study Protocol

All protocol amendments must be submitted to the ethics committee and regulatory authorities if required by local law. Protocol modifications that affect subject safety, the scope of the investigation, or the scientific quality of the study must be approved by the ethics committee before implementation of such modifications to the conduct of the study. If required by local law, such modifications must also be approved by the appropriate regulatory agency prior to implementation.

However, the Sponsor (or designee) may, at any time, amend this protocol to eliminate an apparent immediate hazard to a subject. In this case, the appropriate regulatory authorities will be notified subsequent to the modification.

In the event of a protocol modification, the ICF may require similar modifications (see Sections 17.2 and 17.3).

19.4. Ethics Committee Notification of Study Completion or Termination

Where required, the regulatory authorities and ethics committees must be notified of completion or termination of this study, and sent a copy of the study synopsis in accordance with necessary timelines.

19.5. Retention of Study Data

The minimum retention time for study records will meet the strictest standard applicable to that site, as dictated by any institutional requirements or local laws or regulations. Prior to proceeding with destruction of records, the Investigator must notify the Sponsor in writing and receive written authorization from the Sponsor to destroy study records. In addition, the Investigator must notify the Sponsor of any changes in the archival arrangements including but not limited to archival at an offsite facility or transfer of ownership if the Investigator leaves the site.

19.6. Study Report Signatory

The Sponsor will follow all applicable local regulations pertaining to study report signatories.

20. REFERENCES

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21. APPENDICES

APPENDIX A. LABORATORY ANALYTES

Clinical Safety Assessments (minimum requirements)		Other Assessments
Clinical Chemistry	Urinalysis	PK
Sodium	Specific gravity	CSF and plasma nusinersen levels
Potassium	pH	
Chloride	Protein	[redacted]
Total protein	Glucose	[redacted]
Albumin	Ketones	
Calcium	Bilirubin	[redacted]
Phosphorus	Blood	[redacted]
Bicarbonate	Red blood cells	[redacted]
Glucose	WBCs	[redacted]
BUN	Epithelial cells	
Creatinine	Bacteria	Immunogenicity Evaluation
Cystatin C (for subjects with Screening weight of ≥3 kg)	Casts	Plasma anti-nusinersen Abs
	Crystals	
Total serum bilirubin (direct and indirect)	Leukocyte esterase	The following are to be assessed by local laboratory only
Alkaline phosphatase	Hematology	Urine total protein[1]
AST (SGOT)	Red blood cells	Coagulation (aPTT, PT, and INR)
ALT (SGPT)	Hemoglobin	
CPK	Hematocrit	
GGT	Platelets	
	WBCs	
	WBC differential (% and absolute)	
	• Neutrophils	
	• Eosinophils	
	• Basophils	
	• Lymphocytes	
	• Monocytes	

ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CPK = creatine phosphokinase; CSF = cerebrospinal fluid; DNA = deoxyribonucleic acid; GGT = gamma-glutamyl transferase; INR = international normalized ratio; PK = pharmacokinetic(s); PT = prothrombin time; RNA = ribonucleic acid; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase; [redacted]; WBC = white blood cell.

[1] Quantitative urine total protein should be prioritized if there is not enough urine sample for all tests. For urinary protein concentration >0.2 g/L, repeat testing and further evaluation should be considered.

22. SIGNED AGREEMENT OF THE STUDY PROTOCOL

I have read the foregoing protocol, "An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy," and agree to conduct the study according to the protocol and the applicable ICH guidelines and GCP regulations, and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

_____

Investigator's Signature Date

_____

Investigator's Name (Print)

_____

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AMENDMENT SUMMARY

Biogen Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 9

Date: 17 October 2021

EUDRA CT Number: 2014-002098-12

Version 9 of the protocol has been prepared for this amendment, which supersedes Version 8.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 232SM201 is to limit the number of participants who are receiving nusinersen concomitantly with other SMA therapies to 20% (n = 5) of the total population.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 11.5.1.1, Allowed Concomitant Therapy

Now reads:

~~Concomitant therapies may be used at the discretion of the Investigator.~~

Any concomitant medications including SMA therapies will be captured in the CRF.

Approved concomitant therapies may be used at the discretion of the Investigator.

With implementation of Protocol Version 9, Investigators with participants newly seeking combination therapy with other SMA therapies should consult and obtain approval from the Medical Monitor. This will be permitted for country-approved SMA therapies if the total number of study participants concurrently receiving nusinersen and other SMA therapies has not yet reached 20% of all enrolled participants. This is consistent with primary and secondary objectives of this study. Any participants already receiving combination therapy with other SMA therapies at the time of Protocol Version 9 implementation may continue this combination therapy regardless of the 20% limit.

Rationale: The primary objective of this study is to examine the efficacy of multiple doses of nusinersen administered IT in preventing or delaying the need for respiratory intervention or death in infants with genetically diagnosed and presymptomatic SMA. The secondary objectives of this study are to examine the effects of nusinersen in infants with genetically diagnosed and presymptomatic SMA on the development of clinically manifested SMA as determined by a composite of clinical features seen in subjects with SMA; growth and function; and safety, tolerability, and PK. Limiting the total number of participants who are being treated concurrently with nusinersen and other SMA therapies is consistent with evaluating the primary and secondary objectives of the study in subjects receiving only nusinersen. In addition, limiting the total number of participants who are being treated concurrently with nusinersen and other SMA therapies will maintain the intent of the EU postauthorization commitment to evaluate the long-term safety and efficacy of nusinersen.

This change also affects Section 11.5.1.2 (Disallowed Concomitant Therapy).

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 1.1, Synopsis

The synopsis was revised to reflect changes made throughout the protocol.

Section 5.2, Table 3, Schedule of Activities: Day 897 Through Day 1730; and Table 4, Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Change: Footnotes at the bottom of Table 3 and Table 4 were revised to allow for safety monitoring phone calls or emails after Day 897 to occur 1 to 14 days post dose as opposed to 1 to 7 days postdose.

Now reads (both footnotes):

Starting from Day 897, follow-up safety monitoring telephone calls or emails will occur 1 to ~~7~~ **14** days postdose and every other month for the duration of the study, except for the months when in-clinic visits occur.

Rationale: The window for safety monitoring telephone calls or emails was revised to 14 days to allow for additional flexibility in follow-up and for consistency with other nusinersen protocols.

This change also affects Section 7.2.2 (Treatment).

Section 5.2, Table 3, Schedule of Activities: Day 897 Through Day 1730; and Table 4, Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Change: Footnotes at the bottom of Table 3 and Table 4 were revised to clarify that if an assessment was not performed as scheduled at a visit, then the assessment should be collected at subsequent visit(s) until the assessment is completed (but should not more than once at any visit).

Now reads (both footnotes):

For growth parameters, [redacted] ECG, laboratory safety tests, CHOP INTEND/HFSME, WHO motor milestones, [redacted], if an assessment was not performed at a visit, ~~it~~ **attempts** should be **made to** perform~~ed~~ **the assessment** at the ~~next~~ **subsequent** dosing visit**(s)**

until completed; however, an assessment should not be performed more than once at any visit.

Rationale: These changes were made to clarify that the Investigator should continue to attempt to complete the missed assessment at subsequent visits until completed. This change also affects [redacted] 13.1.2 (Growth Parameters), 13.1.3 (Motor Milestones), 13.1.4.1 (Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease), 13.1.4.2 (Hammersmith Functional Motor Score – Expanded), [redacted] 14.1.2 (Electrocardiograms), and 14.1.3 (Echocardiograms).

Section 5.2, Table 3, Schedule of Activities: Day 897 Through Day 1730; and Table 4, Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Change: Footnotes were added at the bottom of Table 3 and Table 4 to clarify the prioritization of assessments on visit days when a substantial number of assessments are planned.

Now reads (both footnotes):

For study visits with a large number of planned assessments, assessments can be completed over 1 to 2 days and assessments for primary and secondary outcomes (e.g., motor milestones/functional assessments) should be prioritized.

Rationale: In the event that all assessments cannot be done at 1 visit (e.g., due to limited time, patient fatigue), completion of all assessments can be done over 2 days instead of 1 day. Prioritization of assessments is provided.

Section 5.2, Table 3, Schedule of Activities: Day 897 Through Day 1730; and Table 4, Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Change: Footnote 4 of Table 3 and footnote 5 of Table 4 were revised to change the window for completion of the neurological examination to within 7 days of dosing.

Now reads (both footnotes):

Neurological examinations are conducted ~~predose~~ **within 7 days of dosing** and approximately 1 hour after dosing (or when sedation has worn off if it was used).

Rationale: This change clarifies that the neurological examinations can be done along with other predose assessments.

This change also affects Sections 14.1 (Clinical Safety Assessments) and 14.1.1 (Neurological Examinations).

Section 5.2, Table 3, Schedule of Activities: Day 897 Through Day 1730; and Table 4, Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Change: Footnote 5 of Table 3 and footnote 7 (now footnote 8) of Table 4 were revised to provide details on prioritization of urinalysis tests in the event there is not enough sample for all tests.

Now reads (both footnotes):

Among the laboratory measurements, quantitative urine total protein will be assessed at the local laboratory. If urinary protein concentration is >0.2 g/L, repeat testing and further evaluation should be considered; **quantitative urine total protein should be prioritized if there is not enough urine sample for all tests.**

Rationale: The new footnote text is currently included as a footnote to Appendix A (Laboratory Analytes) and is added to the schedule of activity tables for clarity.

This change also affects Section 14.2 (Laboratory Safety Assessments).

Section 5.2, Table 3, Schedule of Activities: Day 897 Through Day 1730; and Table 4, Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Change: Footnote 6 of Table 3 and footnote 8 (now footnote 9) of Table 4 were revised to clarify the requirement that coagulation testing results must be reviewed prior to dosing.

Now reads (both footnotes):

~~Coagulation testing will be conducted up to 7 days prior to dosing at the time of the implementation of Protocol Version 7, and results must be reviewed prior to dosing~~.
Coagulation testing will continue to be conducted at each visit or up to 7 days prior to dosing and results should be reviewed prior to dosing. In situations where predose verification of coagulation laboratory results is not feasible or optimal (e.g., prolonged sedation of the subject would be required to allow adequate time for lab processing), the Investigator may exercise discretionary clinical judgment and proceed with the LP procedure, based on the subject's clinical and coagulation lab history.

Rationale: While coagulation results should be reviewed prior to dosing, there may be situations where this is not feasible and the Investigator may proceed with LP based on clinical judgment.

This change also affects Section 14.2 (Laboratory Safety Assessments).

Section 5.2, Table 4, Schedule of Activities: Day 897 Through Day 2891 (End of Study)

Change: A footnote was added to Table 4 to clarify that echocardiograms will only be assessed locally at the site.

Now reads:

[7] Echocardiogram will only be assessed locally at the site as per local practice.

Rationale: Clarification regarding echocardiogram collection with what is currently being done at the sites.

This change also affects Section 14.1.3 (Echocardiograms).

[redacted]

Section 10.1, Discontinuation of Study Treatment

Change: Text was revised to indicate that all subjects who discontinue study treatment will complete an early termination visit approximately 4 months after their last dose of nusinersen.

Now reads:

Subjects who discontinue treatment will ~~continue follow-up (i.e., regular study visits, but with no postdose safety monitoring visits)~~ **complete an early termination visit 4 months (+ 14 days) after administration of the last dose of nusinersen,** unless consent is withdrawn (see Section 10.2). ~~If subjects or their parent(s)/caregiver(s) are unable to come for study visits, the minimum requirement for follow-up (i.e., site visits or telephone calls to the subject's parent or caregiver) should be documented in the source document.~~

Rationale: These changes are consistent with the rationale of this study to assess long-term safety, efficacy, and tolerability of nusinersen. Additional follow-up beyond 4 months for collection of safety assessments in subjects who discontinued nusinersen is no longer required.

Section 11.2, Modification of Dose and/or Treatment Schedule

Change: Text was revised to indicate that in case of a delayed or missed dose, an adjustment in dosing schedule was allowed in the event of unique circumstances, including those related to the COVID-19 pandemic, and details regarding recommended dosing administration are provided in the DHA.

Now reads:

No adjustment of dose is permitted. **If dosing does not occur as per the schedule of activities, a protocol deviation must be logged.** In the event of **delayed or missed doses due to, for example,** a concurrent illness **or other circumstance (e.g., COVID-19 pandemic), the Investigator should refer to the DHA which includes recommended dosing administration in the event of delayed or missed doses.** ~~that would prevent the dosing procedure from being performed safely, an adjustment in the dose schedule may be permitted but must be approved by the Medical Monitor.~~

Rationale: Text was revised to broaden the circumstances in which delayed or missed doses may have occurred and to allow adjustment to the dose schedule.

Section 11.6, Continuation of Treatment

Change: Text describing an extension study was deleted.

Now reads:

~~**11.6 Continuation of Treatment**~~

~~Subjects who continue in the study until Day 2891 will be offered the option to enter an extension study (under a separate protocol) if they meet all of the inclusion and exclusion~~

~~criteria. Subjects who do not enter the extension study are encouraged to complete all post-treatment visits.~~

Rationale: Per Protocol Version 7, the study was extended; therefore, no extension study is planned.

[redacted]

Section 17.4, Subject Data Protection

Change: Text added to include language regarding the collection of a subject's race, ethnicity, and full date of birth.

Now reads:

Prior to any testing under this protocol, including screening tests and assessments, the subject's legally authorized representative(s) must also provide all authorizations required by local law (e.g., Protected Health Information authorization in North America).

During the study, participants' race and ethnicity and full date of birth will be collected (unless the collection is not permitted by applicable law or not approved by the governing ethics committee). These data will be used in the analysis of the safety and/or PK profile of the study treatment.

SMA is caused by the loss of SMN protein due to a homozygous deletion or mutation or a compound heterozygous mutation in the *SMN1* gene on chromosome 5q11-q13. Thus, patients with SMA are completely dependent on the amount of SMN protein produced by the *SMN2* gene. Genetic modifiers, such as the number of copies of the *SMN2* gene, are known to impact the eventual phenotype of SMA. The incidence and prevalence of SMA and its subtypes have been reported to vary based on country. One hypothesis for these variances is a difference in the frequency of certain genetic mutations that may be associated with different racial and ethnic groups within each country. Therefore, the

race/ethnicity of participants will be collected as part of the medical history, where local regulations allow. The full date of birth is needed in order to precisely calculate the age at achievement of motor milestones and the weight-for-age percentiles.

Rationale: Race and ethnicity data can be collected, provided it is noted in the protocol; therefore, this text has been added for indicate that these parameters will be collected to be used in the analysis of the safety and/or PK profile of nusinersen.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- The Biogen Development Unit Head signatory was updated.
- Text added during Protocol Version 7 regarding extending the study was revised to past tense.
- Minor text clarifications were made to the synopsis (Study Design and Duration of Treatment and Follow-up).
- Background information on SMA, current therapies for SMA, and the clinical experience with nusinersen were updated.
- In Section 12, the statement "The DHA supersedes all other references (e.g., protocol)" was updated to, "The DHA aligns with all other references, including the protocol," per updates to the protocol template.
- In Section 15.3, Monitoring and Recording Events and Section 15.5, Safety Responsibilities, language regarding reporting requirements for SAEs was updated to include "or according to national law," in line with the latest protocol template update by the Sponsor to accommodate countries that may have different reporting requirements.
- Footnote numbering was updated where applicable.
- References were updated where applicable.

LIST OF ABBREVIATIONS

[redacted]	[redacted]
CHOP INTEND	Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CMAP	compound muscle action potential
CRF	case report form
DHA	Directions for Handling and Administration
EU	European Union
HFSME	Hammersmith Functional Motor Scale – Expanded
IT	intrathecally
LP	lumbar puncture
[redacted]	[redacted]
[redacted]	[redacted]
PK	pharmacokinetics
SMA	spinal muscular atrophy
[redacted]	[redacted]

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AMENDMENT SUMMARY

Biogen Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 8

Date: 12 November 2019

EUDRA CT Number: 2014-002098-12

Version 8 of the protocol has been prepared for this amendment, which supersedes Version 7 dated 02 October 2019.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 232SM201 is to modify the schedule of activities with regard to the collection of cerebrospinal fluid (CSF) samples for pharmacokinetics (PK) and survival motor neuron (SMN) protein analysis and plasma samples for PK analysis.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 5.2, Schedule of Activities: Day 897 Through Day 2891 (End of Study)

Change: The collection of CSF samples taken predose for PK and SMN protein analysis and plasma samples for PK analysis was added at Days 1849, 1968, 2206, 2325, 2563, and 2682. In addition, the footnote for plasma samples for PK analysis was updated to specify that plasma will be used to assess [redacted].

Now reads:

Table 4: Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																											PTFU
	D1849			D1968			D2087			D2206			D2325			D2444			D2563			D2682			D2801			D2891 (EOS)/ Early Term[1]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
Sibling SMA Data				X									X									X						X
Vital Signs and Pulse Oximetry[2]	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X
Weight	X			X			X			X			X			X			X			X			X			X
Growth Parameters[3]	X[4]									X[4]									X[4]									X
Physical Examination	X			X			X			X			X			X			X			X			X			X
Ventilator Use	X			X			X			X			X			X			X			X			X			X
[redacted]																												
Neurological Examination[5]	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X
[redacted]																												
ECG	X[4]									X[4]									X[4]									X
Echocardiogram	X[4]									X[4]									X[4]									X
Laboratory Safety Tests[7]	X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X
Coagulation Laboratory Tests[8]	X			X			X			X			X			X			X			X			X			X
Immunogenicity	X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X
CSF PK [redacted]	**X**			**X**			X			**X**			**X**			X			**X**			**X**			X			
[redacted]																												
Plasma PK[9]	**X[4]**			**X[4]**			X[4]			**X[4]**			**X[4]**			X[4]			**X[4]**			**X[4]**			X[4]			

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																											PTFU
	D1849			D1968			D2087			D2206			D2325			D2444			D2563			D2682			D2801			D2891 (EOS)/ Early Term[1]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
[redacted]																												
Study Treatment Injection		X			X			X			X			X			X			X			X			X		
CHOP INTEND/ HFMSE[10]	X[4]									X[4]									X[4]									X
WHO Motor Milestones[11]	X[4]									X[4]									X[4]									X
[redacted]																												
Con Med and Ancillary Procedure Recording	X----------X																											
AE Collection	X----------X																											

[redacted] AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted]; Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; [redacted]; DNA = deoxyribonucleic acid; ECG = electrocardiogram; EOS = End of Study; HFMSE = Hammersmith Functional Motor Scale – Expanded; HINE = Hammersmith Infant Neurological Exam; LP = lumbar puncture; [redacted] PK = pharmacokinetic(s); [redacted] PTFU = Post-Treatment Follow-Up; [redacted] RNA = ribonucleic acid; SMA = spinal muscular atrophy; [redacted]; Term = termination; US = United States; WHO = World Health Organization.

Notes: Starting from Day 897, follow-up safety monitoring telephone calls or emails will occur 1 to 7 days postdose and every other month for the duration of the study, except for the months when in-clinic visits occur. At telephone contact or via email, changes in concomitant medications and AEs will be recorded, as well as information on the subject's ventilator use and SMA disease status. Where possible, all visits from Day 1849 through Day 2891 can be concluded in 1 day. The final visit will occur 90 days after the last study treatment injection. Videotaping of the motor milestone assessments, all motor function assessments, and physical examinations will be optional.

For growth parameters, [redacted], ECG, echocardiogram, laboratory safety tests, CHOP INTEND/HFMSE, WHO motor milestones, [redacted], if an assessment is not performed at a visit, it should be performed at the next dosing visit.

[1] At the Early Termination Visit, age-appropriate assessments should be performed.

[2] Vital signs will be taken predose and approximately 1 hour postdose (±15 minutes) at every onsite visit throughout the study.

[3] Growth parameters of body length and/or height (for all subjects), and head circumference, chest circumference, and arm circumference (for subjects up to 36 months of age) will be measured.

[4] These assessments may be performed up to 7 days prior to dosing, if necessary.

[5] Sections 1 and 3 of the HINE will be conducted on all subjects ≤24 months of age. For all subjects >24 months of age, standard neurological examinations, which include assessments of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be conducted. Neurological examinations are conducted predose and approximately 1 hour after dosing (or when sedation has worn off if it was used).

[redacted]

[7] Among the laboratory measurements, quantitative urine total protein will be assessed at the local laboratory. If urinary protein concentration is >0.2 g/L, repeat testing and further evaluation should be considered.

[8] Coagulation testing will be conducted up to 7 days prior to dosing at the time of the implementation of Protocol Version 7, and results must be reviewed prior to dosing.

[9] See Table 2 for the detailed PK sampling schedule. [redacted]

[10] CHOP INTEND will be assessed in subjects until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed. All subjects ≥2 years of age will be evaluated using the HFMSE. See Section 13.1.4 and Section 13.1.4.2.

[11] WHO motor milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments made by the subject's caregiver.

[redacted]

Rationale: The schedule of activities was aligned with the planned sampling schedule at every visit.

The change to the collection of CSF samples for PK [redacted] and plasma samples for PK analysis also affected Table 2: Pharmacokinetic Sampling Schedule. The change to the footnote also affected Table 1: Schedule of Activities: Screening Through Day 779 and Table 3: Schedule of Activities: Day 897 Through Day 1730.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

[redacted]

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- References to “this amendment” were updated.
- Typographical errors and formatting were corrected.

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AMENDMENT SUMMARY

Biogen Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 7

Date: 02 October 2019

EUDRA CT Number: 2014-002098-12

Version 7 of the protocol has been prepared for this amendment, which supersedes Version 6 dated 20 March 2017.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 232SM201 is to extend the duration of the study to enable longer-term evaluation (through 8 years of age) of the safety and efficacy of nusinersen in subjects with spinal muscular atrophy (SMA) who initiated treatment with nusinersen prior to the onset of clinical symptoms. [redacted]

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 4.4, Study Rationale

Now reads:

The study is being extended for the collection of endpoint data in all subjects through ~~5~~**8** years of age in order to assess the long-term safety, efficacy, and tolerability of nusinersen.

Rationale: The extension of Study 232SM201 will provide subjects with presymptomatic SMA who participated in and completed Screening through Day 1730 of Study 232SM201 with the opportunity to receive nusinersen until subjects are up to 8 years of age (96 months). The extension of the treatment duration will allow the Sponsor to monitor the effect of nusinersen on subjects who initiated treatment prior to the onset of clinical symptoms for a longer period of time.

This change also affected Section 5.1, Schedule of Activities: Screening Through Day 779; Section 5.2, Schedule of Activities: Day 897 Through Day 2891 (End of Study); Section 7.1, Study Overview; Section 7.2, Overall Study Duration and Follow-Up; Section 7.2.2, Treatment; Section 7.2.3, Follow-Up; Section 11.1, Regimen; Section 11.6, Continuation of Treatment; Section 13.1, Efficacy Assessments; [redacted] Section 14.1, Clinical Safety Assessments; Section 14.1.1, Neurological Examinations; Section 14.1.2, Electrocardiograms; Section 14.1.4, Vital Signs; Section 14.2, Laboratory Safety Assessments; Section 16.1.2.2, Analysis of the Secondary Endpoints; [redacted]

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

Section 5.2, Schedule of Activities: Day 897 Through Day 2891 (End of Study)

Change: The scheduled visit period was extended up to 8 years after the first injection of nusinersen. Table 3 was revised to present the schedule of activities from Day 897 through Day 1730. Table 4 was added to present the schedule of activities from Day 1849 through Day 2891 (end of study).

Now reads:

5.2 Schedule of Activities: Day 897 Through Day ~~1820~~**2891** (End of Study)

Table 3: Schedule of Activities: Day 897 Through Day ~~1820 (End of Study)~~**1730**

Study Day	Treatment/Follow-Up (Each visit can be ±~~7~~ **14**days)																								~~Post-Treatment Follow-Up~~
	D897			D1016			D1135			D1254			D1373			D1492			D1611			D1730			~~D1820 (EOS)/ Early Term~~ [1]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
Sibling SMA Data	X									X									X						~~X~~
Vital Signs and Pulse Oximetry~~[2]~~[1]	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	~~X~~
Weight	X			X			X			X			X			X			X			X			~~X~~
Growth Parameters[2]	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			~~X~~
Physical Examination	X			X			X			X			X			X			X			X			~~X~~
Ventilator Use	X			X			X			X			X			X			X			X			~~X~~
[redacted]																									
Neurological Examination[4]	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X	X[3]		X	~~X~~
[redacted]																									
ECG	X[3]									X[3]									X[3]						~~X~~
Laboratory Safety Tests[5]	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			~~X~~
Coagulation	X			X			X			X			X			X			X			X			~~X~~

Study Day	Treatment/Follow-Up (Each visit can be ±~~7~~ **14**days)																								~~Post-Treatment Follow-Up~~
	D897			D1016			D1135			D1254			D1373			D1492			D1611			D1730			~~D1820 (EOS)/ Early Term~~ [+]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
Laboratory Tests[6]																									
Immunogenicity	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			~~X~~
CSF PK [redacted]	X			X			X			X			X			X			X			X			
[redacted]																									
Plasma PK[5][7]	X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			X[3]			
[redacted]																									
Study Treatment Injection		X			X			X			X			X			X			X			X		
CHOP INTEND/ HFMSE[6][8]	X[3]						X[3]						X[3]						X[3]						~~X~~
WHO Motor Milestones[7][9]	X[3]						X[3]						X[3]						X[3]						~~X~~
[redacted]																									

Study Day	Treatment/Follow-Up (Each visit can be ±~~7~~ **14**days)																								~~Post-Treatment Follow-Up~~
	D897			D1016			D1135			D1254			D1373			D1492			D1611			D1730			~~D1820 (EOS)/ Early Term~~ [+]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
[redacted]																									
Con Med and Ancillary Procedure Recording	X----------																								----------X
AE Collection	X----------																								----------X

[redacted] AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted] Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; [redacted] DNA = deoxyribonucleic acid; ECG = electrocardiogram; EOS = End of Study; HFMSE = Hammersmith Functional Motor Scale – Expanded; HINE = Hammersmith Infant Neurological Exam; LP = lumbar puncture; [redacted] PK = pharmacokinetic(s); RNA = ribonucleic acid; SMA = spinal muscular atrophy; [redacted] Term = termination; WHO = World Health Organization.

Notes: ~~Monitoring~~**Starting from Day 897, follow-up safety monitoring** telephone calls **or emails** will occur ~~on a monthly (±~~**1 to** 7 days~~) basis starting on Day 94~~ **postdose** and ~~continuing to~~ **every other month for** the ~~end~~**duration** of the study**, except for the months when in-clinic visits occur.** At telephone contact **or via email**, changes in concomitant medications and AEs will be recorded, as well as information on the subject's ventilator use and SMA disease status. Where possible, all visits from Day 897 through Day ~~1820~~**1730** can be concluded in 1 day. The final visit will occur 90 days after the last study treatment injection. Videotaping of the motor milestone assessments, all motor function assessments, and physical examinations will be optional.

[+] ~~At the Early Termination Visit, age-appropriate assessments should be performed.~~

For growth parameters, [redacted], ECG, laboratory safety tests, CHOP INTEND/HFMSE, WHO motor milestones, [redacted], if an assessment is not performed at a visit, it should be performed at the next dosing visit.

[1] Vital signs will be taken predose and approximately 1 hour postdose **(±15 minutes)** at every onsite visit throughout the study.

[2] **Growth parameters of body length and/or height (for all subjects), and head circumference, chest circumference, and arm circumference (for subjects up to 36 months of age) will be measured.**

[3] These assessments may be performed up to 7 days prior to dosing, if necessary.

[4] Sections 1 and 3 of the HINE will be conducted on all subjects ≤24 months of age. For all subjects >24 months of age, standard neurological examinations, which include assessments of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be conducted. Neurological examinations are conducted predose and approximately 1 hour after dosing (or when sedation has worn off if it was used).

[5] **Among the laboratory measurements, quantitative urine total protein will be assessed at the local laboratory. If urinary protein concentration is >0.2 g/L, repeat testing and further evaluation should be considered.**

[6] **Coagulation testing will be conducted up to 7 days prior to dosing at the time of the implementation of Protocol Version 7, and results must be reviewed prior to dosing.**

[57] See Table 2 for the detailed PK sampling schedule.
[68] CHOP INTEND will be assessed in subjects until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed. All subjects ≥2 years of age will be evaluated using the HFMSE. See Section 13.1.4 and Section 13.4.2.
[79] WHO motor milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments made by the subject's caregiver.

[redacted]

Table 4: Schedule of Activities: Day 1849 Through Day 2891 (End of Study)

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																											PTFU
	D1849			D1968			D2087			D2206			D2325			D2444			D2563			D2682			D2801			D2891 (EOS)/ Early Term[1]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
Sibling SMA Data				X									X									X						X
Vital Signs and Pulse Oximetry[2]	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X
Weight	X			X			X			X			X			X			X			X			X			X
Growth Parameters[3]	X[4]									X[4]									X[4]										X
Physical Examination	X			X			X			X			X			X			X			X			X			X
Ventilator Use	X			X			X			X			X			X			X			X			X			X
[redacted]																												
Neurological Examination[5]	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X[4]		X	X
[redacted]																												
ECG	X[4]									X[4]									X[4]									X
Echocardiogram	X[4]									X[4]									X[4]									X
Laboratory Safety Tests[7]	X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X
Coagulation Laboratory Tests[8]	X			X			X			X			X			X			X			X			X			X
Immunogenicity	X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X[4]			X
CSF PK [redacted]							X									X									X			
[redacted]																												

Study Day	Treatment/Follow-Up (Each visit can be ±14 days)																											PTFU
	D1849			D1968			D2087			D2206			D2325			D2444			D2563			D2682			D2801			D2891 (EOS)/ Early Term[1]
	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	Pre	LP	Post	
[redacted]																												
Plasma PK[9]							X[4]									X[4]									X[4]			
[redacted]																												
Study Treatment Injection		X			X			X			X			X			X			X			X			X		
CHOP INTEND/ HFMSE[10]	X[4]									X[4]									X[4]									X
WHO Motor Milestones[11]	X[4]									X[4]									X[4]									X
[redacted]																												
Con Med and Ancillary Procedure Recording	X----------X																											
AE Collection	X----------X																											

[redacted] AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted] Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; [redacted] DNA = deoxyribonucleic acid; ECG = electrocardiogram; EOS = End of Study; HFMSE = Hammersmith Functional Motor Scale – Expanded; HINE = Hammersmith Infant Neurological

Exam; LP = lumbar puncture; [redacted] PK = pharmacokinetic(s); PTFU = Post-Treatment Follow-Up; [redacted] RNA = ribonucleic acid; SMA = spinal muscular atrophy; [redacted] Term = termination; US = United States; WHO = World Health Organization.

Notes: Starting from Day 897, follow-up safety monitoring telephone calls or emails will occur 1 to 7 days postdose and every other month for the duration of the study, except for the months when in-clinic visits occur. At telephone contact or via email, changes in concomitant medications and AEs will be recorded, as well as information on the subject's ventilator use and SMA disease status. Where possible, all visits from Day 1849 through Day 2891 can be concluded in 1 day. The final visit will occur 90 days after the last study treatment injection. Videotaping of the motor milestone assessments, all motor function assessments, and physical examinations will be optional.

For growth parameters, [redacted], ECG, echocardiogram, laboratory safety tests, CHOP INTEND/HFMSE, WHO motor milestones, [redacted], if an assessment is not performed at a visit, it should be performed at the next dosing visit.

[1] At the Early Termination Visit, age-appropriate assessments should be performed.

[2] Vital signs will be taken predose and approximately 1 hour postdose (±15 minutes) at every onsite visit throughout the study.

[3] Growth parameters of body length and/or height (for all subjects), and head circumference, chest circumference, and arm circumference (for subjects up to 36 months of age) will be measured.

[4] These assessments may be performed up to 7 days prior to dosing, if necessary.

[5] Sections 1 and 3 of the HINE will be conducted on all subjects ≤24 months of age. For all subjects >24 months of age, standard neurological examinations, which include assessments of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be conducted. Neurological examinations are conducted predose and approximately 1 hour after dosing (or when sedation has worn off if it was used).

[redacted]

[7] Among the laboratory measurements, quantitative urine total protein will be assessed at the local laboratory. If urinary protein concentration is >0.2 g/L, repeat testing and further evaluation should be considered.

[8] Coagulation testing will be conducted up to 7 days prior to dosing at the time of the implementation of Protocol Version 7, and results must be reviewed prior to dosing.

[9] See Table 2 for the detailed PK sampling schedule.

[10] CHOP INTEND will be assessed in subjects until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed. All subjects ≥2 years of age will be evaluated using the HFMSE. See Section 13.1.4 and Section 13.1.4.2.

[11] WHO motor milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments made by the subject's caregiver.

[redacted]

Rationale: This section was updated to reflect the extension of the study for up to approximately 8 years, with the schedule of activities for different visit periods clarified in 2 tables (Table 3 and Table 4). Of note, the window for assessment of coagulation parameters was extended to up to 7 days prior to dosing to avoid any unnecessary delay in dosing due to time required for laboratory processing. No meaningful changes in coagulation parameters are anticipated during this 7-day period. Flexibility was also introduced across efficacy assessments to optimize data collection in cases where assessments are missed.

Follow-up phone calls/emails after certain dosing visits, validity of coagulation results up to 7 days prior to dosing, and optimization of data collection for missed assessments were also applied to Section 5.1, Schedule of Activities: Screening Through Day 779, Table 1: Schedule of Activities: Screening Through Day 779. Additional collection timepoints up to 8 years for pharmacokinetic sampling was also applied to Section 5.1, Schedule of Activities: Screening Through Day 779, Table 2: Pharmacokinetic Sampling Schedule. Validity of coagulation results up to 7 days prior to dosing was also applied to Section 14.2, Laboratory Safety Assessments. [redacted]

[redacted]

Section 11.5.1, Concomitant Therapy

Change: Allowed concomitant therapy was edited, and disallowed concomitant therapy was removed.

Now reads:

11.5.1.1. Allowed Concomitant Therapy

~~Throughout the study, Investigators or designated licensed physicians involved in the study may prescribe concomitant medications or treatments deemed necessary for AEs or to provide adequate supportive care~~**Concomitant therapies may be used at the discretion of the Investigator.**

Any concomitant medications including SMA therapies will be captured in the CRF.

11.5.1.2. Disallowed Concomitant Therapy

~~Subjects are prohibited from receiving other experimental agents, including gene therapy, during the study. This includes marketed agents at experimental doses that are being tested for the treatment of SMA (albuterol/salbutamol, riluzole, carnitine, sodium phenylbutyrate, valproate, hydroxyurea, etc.).~~ **None**.

Rationale: In light of the robust safety and efficacy dataset generated for nusinersen to date, disallowed concomitant therapies were removed, and concomitant therapies can be used at the discretion of the Investigator in an effort to create a study environment more representative of real-world practice.

[redacted]

…

[redacted]

[redacted]

[redacted]

Section 14.1.3, Echocardiograms

Change: A new assessment was added.

Now reads:

Section 14.1.3, Echocardiograms

At the time of the implementation of Protocol Version 7 (this amendment), echocardiograms will be performed for all subjects at Days 1849, 2206, 2563, and at the end of the study (or Early Termination Visit). If an assessment is not performed at a visit, it should be performed at the next dosing visit.

Rationale: Echocardiogram assessments have been added to further evaluate the impact of nusinersen on potential cardiac manifestations in subjects with SMA.

This change was also applied to Section 5.2, Schedule of Activities: Day 897 Through Day 2891 (End of Study), Table 4: Schedule of Activities: Day 1849 Through Day 2891 (End of Study); Section 14.1, Clinical Safety Assessments; and Section 16.6.2.5, Echocardiograms.

Section 15.3.1, Adverse Events

Change: Clarification on the adverse events was provided.

Now reads:

For subjects who receive study treatment, any AE experienced between the time of signing the ICF and Final Study Visit/Telephone Call is to be recorded on the CRF, regardless of the severity of the event or its relationship to study treatment. At each study visit, the Investigator will assess the subject for AEs and will record any new AEs or updates to previously reported AEs on the CRF. **Note: Any new or worsened findings (including signs and symptoms of SMA) that start after the informed consent date must be recorded as AEs and should not be listed as medical history.**

Rationale: This section was updated to clarify the difference between an AE and a medical history.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- On the Sponsor Signature Page, the signatory was revised.
- Section 2, List of Abbreviations was updated.
- Section 4.2, Current Therapies for Spinal Muscular Atrophy, was revised to include Zolgensma®, a gene therapy approved in the United States for treatment of pediatric patients.
- Section 4.3.4, Clinical Experience, was updated to indicate the completed and ongoing studies.
- In Section 7.2.2, Treatment, text related to contraceptive methods was deleted. In addition, new text for postdose follow-up emails or phone calls after the dosing visits on Days 64, 183, 302, 421, 540, 659, and 778 was provided.
- In Section 9.1, Screening and Enrollment, a sentence was added to indicate that the reason for which a subject was chosen for screening will be captured.
- [redacted]; Section 13.1.2, Growth Parameters; Section 13.1.3, Motor Milestones; Section 13.1.4.1, Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease; Section 13.1.4.2, Hammersmith Functional Motor Scale – Expanded; Section 13.1.4.3, [redacted] Section 14.1.2, Electrocardiograms; and relevant footnotes in Table 1: Schedule of Activities: Screening Through Day 779, a sentence was added to clarify that if an assessment is not performed at a visit, it should be performed at the next dosing visit.
- In Section 13.1.3, Motor Milestones, a sentence related to the subject's age and motor abilities at a visit was removed. In addition, text was revised to clarify that assessment of the Hammersmith Infant Neurological Exam Section 2 is for all subjects.
- [redacted]

- Section 13.4, Immunogenicity Assessments, was updated to reflect the analysis plan for immunogenicity assessments. This change also affected Section 16.4.2, Methods of Analysis.
- [redacted]
- Section 14.1.4, Vital Signs, was updated to note that a window of ±15 minutes for the 1 hour postdose timepoint is allowed for vital signs and pulse oximetry assessments. This change also affected Table 1: Schedule of Activities: Screening Through Day 779 and Table 3: Schedule of Activities: Day 897 Through Day 1730.
- Section 14.2, Laboratory Safety Assessments, was updated indicating that assessment of quantitative urine total protein will be assessed at the local laboratory. This change was also applied to Table 3: Schedule of Activities: Day 897 Through Day 1730.
- [redacted]
- Typographical errors and formatting were corrected.

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AMENDMENT SUMMARY

Biogen Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 6

Date: 20 March 2017

EUDRA CT Number: 2014-002098-12

Version 6 of the protocol has been prepared for this amendment, which supersedes Version 5 dated 15 December 2016.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 232SM201 is to provide subjects with presymptomatic spinal muscular atrophy (SMA) with the opportunity to receive open-label nusinersen until subjects are 5 years of age (60 months).

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 4.4, Study Rationale

Now reads:

The study is being extended for the collection of endpoint data in all subjects through 5 years of age in order to assess the long-term safety, efficacy, and tolerability of nusinersen.

Rationale: The extension of Study 232SM201 will provide subjects with presymptomatic SMA who participated in and completed Screening through Day 779 of Study 232SM201 with the opportunity to receive nusinersen until subjects are up to 5 years of age (60 months). The extension of the treatment duration will allow the Sponsor to monitor the effect of nusinersen on subjects with presymptomatic SMA for a longer period of time.

This change also affects Section 5.1, Schedule of Activities: Screening Through Day 779; Section 7.1, Study Overview; Section 7.2, Overall Study Duration and Follow-Up; Section 7.2.2, Treatment; Section 7.2.3, Follow-up; Section 11.1, Regimen; Section 11.6, Continuation of Treatment; and led to the creation of Section 5.2, Schedule of Activities: Day 897 Through Day 1820 (End of Study).

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 1, Synopsis

The Synopsis was revised to reflect the changes made throughout the protocol.

Section 3, Sponsor Information

Change: The Sponsor of Study 232SM201 was changed from Ionis (Isis) Pharmaceuticals, Inc. to Biogen MA Inc., and the Medical Monitor information was revised. In addition to listing Biogen as the Sponsor, this amendment adds safety and administrative language, making this protocol consistent with Biogen's protocol template.

Now reads:

~~Isis Pharmaceuticals, Inc. (Isis)~~ **Biogen MA Inc**. is the Sponsor of the study in the United States. Biogen Idec Research Limited (Biogen Idec) is the Sponsor of the study ~~in the Rest of World~~ **globally**. Biogen (or designee) will be responsible for managing the study globally.

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Biogen may transfer any or all of its study-related responsibilities to a contract research organization (CRO) and other third parties; however, Biogen retains overall accountability for these activities.

Rationale: Biogen assumed global sponsorship of Study 232SM201.

This change also affects Section 12.1, Nusinersen; Section 15.4.2, Medical Emergency; Section 18.4, Study Funding; and Section 22, Signed Agreement of the Study Protocol.

Section 4, Introduction

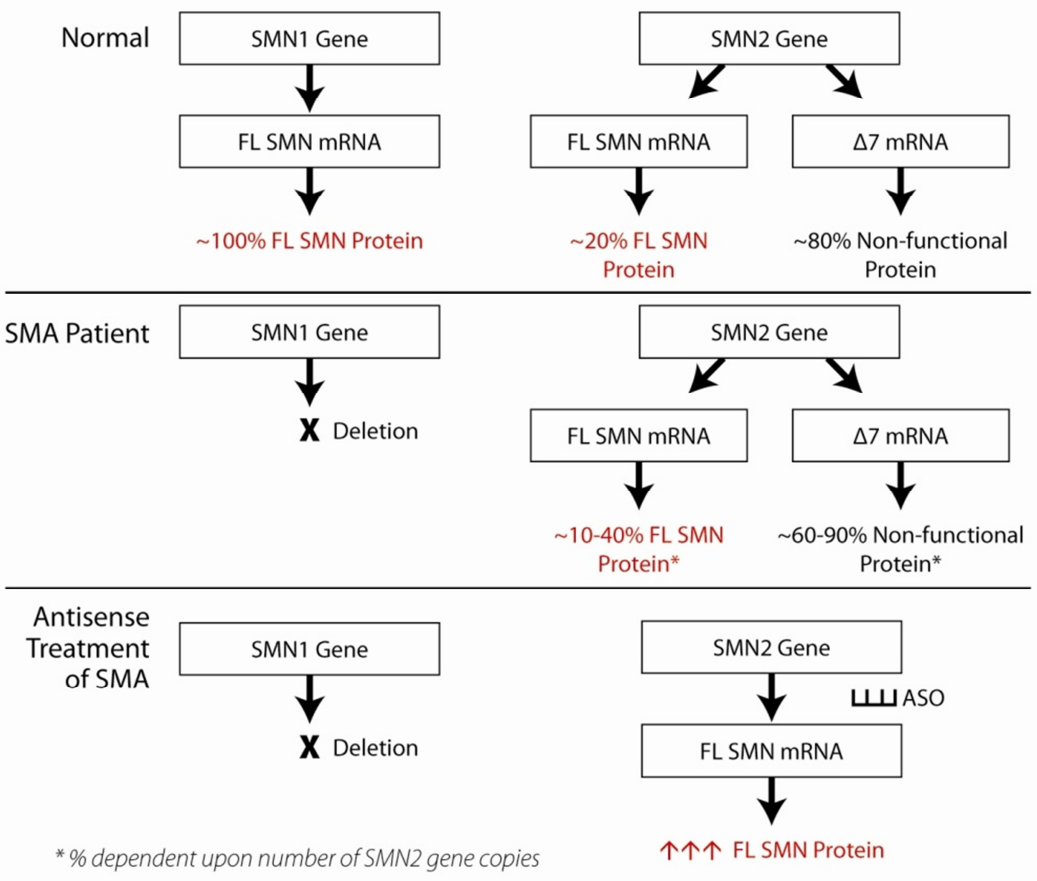
Change: The introduction was updated to provide the most current background information for SMA and the ongoing studies in which nusinersen is being investigated.

Now reads:

~~ISIS 396443 is a uniformly modified, 2′-*O*-(2-methoxyethyl) (2′-MOE), antisense oligonucleotide (ASO) drug in development for the treatment of spinal muscular atrophy (SMA) due to genetic defects in the survival motor neuron 1 (*SMN1*) gene.~~

~~ASOs are short synthetic strings of nucleotides designed to alter the expression of a targeted protein by selectively binding to the ribonucleic acid (RNA) that encodes the targeted protein. In patients with SMA, the number of survival motor neuron 2 (*SMN2*) gene copies and the resulting amount of survival motor neuron (SMN) protein are correlated with disease onset and severity. A therapeutic approach predicted to benefit patients with SMA is to increase the levels of full-length SMN2 pre-messenger ribonucleic acid (mRNA) by restoring the splicing pattern that gives rise to full-length SMN2 mRNA, thus increasing full-length SMN protein levels and SMN protein activity. ISIS 396443 is designed to bind to intron 7 of the SMN2 pre-mRNA, restoring the splicing pattern that gives rise to full-length SMN2 mRNA and thus increases full-length SMN protein levels (Figure 1).~~

~~Figure 1: ASO Therapeutic Approach for Treatment of SMA~~



~~ASO = antisense oligonucleotide; FL = full-length; mRNA = messenger ribonucleic acid; SMA = spinal muscular atrophy; SMN = survival motor neuron; SMN1 = survival motor neuron 1; SMN2 = survival motor neuron 2.~~

4.1. Overview of Spinal Muscular Atrophy

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. **Despite being a rare disease, SMA is one of the most common genetic causes of death in infants, with a reported birth prevalence ranging from 8.5 to 10.3 per 100,000 live births [Arkblad 2009; Jedrzejowska 2010; Prior 2010; Sugarman 2012; Tassie 2013].** ~~With an incidence of 1:6000 to 1:10,000 live births, SMA is the most common genetic cause of infant mortality, and a major cause of childhood morbidity due to weakness, in developed countries.~~ The natural history of SMA includes 4 major recognized phenotypes that are dependent on age of onset and achieved motor abilities. The most severe form, Type I SMA (equivalent to infantile-onset SMA), has a disease onset within the first few months of life; these children are never able to sit or walk and usually die from respiratory failure by 2 years of age. Patients with Type II SMA are able to sit but never walk unaided, with symptoms presenting between 6 and 18 months of age. Patients with Type III SMA are able to sit and walk but may become severely and increasingly disabled. Patients with

Type IV or adult-onset SMA have an age of onset over 18 years and have normal life expectancies.

In 95% of patients with SMA, a deletion in the survival motor neuron 1 (*SMN1*) gene on chromosome 5q11-q13 is found, with the remaining 5% attributable to small mutations in the same gene [Helmken 2003; Lefebvre 1995]. *SMN1* lies in the telomeric portion of an inverted duplication of a region of chromosome 5. The centromeric half of the duplication contains a homologous gene named survival motor neuron 2 (*SMN2*) that differs from *SMN1* by 5 to 11 nucleotides [Lorson 1999; Monani 1999]. The open reading frames for both genes encode proteins with identical amino acid sequences. Survival motor neuron *(SMN)* gene transcripts, similar to most mammalian transcripts, undergo alternative splicing in which certain exons are either included or excluded from the mature protein coding transcripts [Keren 2010]. In particular, exon 7 of the *SMN1* gene is alternatively spliced with 90% to 95% of the mature messenger ribonucleic acid (mRNA) transcripts derived from the *SMN1* gene containing exon 7, and 5% to 10% of transcripts missing exon 7. The transcripts missing exon 7 (often referred to as Δ7) produce a truncated protein that is defective and unstable [Cho and Dreyfuss 2010]. One of the 5 to 11 nucleotide differences between *SMN1* and *SMN2* is a C to T substitution, which occurs in exon 7 of the *SMN2* gene resulting in an alternative splicing pattern that favors skipping of exon 7. The result shows that as much as 90% of the transcripts produced from *SMN2* are missing exon 7. The remainder, *SMN2* transcripts containing exon 7, produces a full-length (FL) protein product identical to the SMN1 protein because the C to T substitution is silent. Humans have a variable copy number of the *SMN2* gene (0 to 8 copies) [Wirth 2006]. The number of *SMN2* copies and the resulting amount of FL-SMN protein expressed in patients with SMA (10% to 40% of normal SMN protein levels) correlate with SMA disease severity, thus, *SMN2* is a key modifier of disease phenotype [Coovert 1997; Feldkötter 2002; Lefebvre 1997; Prior 2004].

~~SMA is caused by loss of SMN protein due to a homozygous deletion or mutation or compound heterozygous mutation in the *SMN1* gene on chromosome 5q11-q13. Humans have a duplication of the chromosome region where *SMN1* is found, resulting in a second copy of the gene, *SMN2*. In *SMN2*, a C to T nucleotide substitution in exon 7 results in an alternative splicing event such that the majority of transcripts produced (~90%) lack exon 7 and a defective truncated protein is produced. The remainder, SMN2 transcripts containing exon 7, produces a full-length protein product identical to the SMN1 protein, since the C to T substitution is silent. Humans have a variable copy number of the *SMN2* gene (0 to 8 copies). The number of SMN2 copies and the resulting amount of full-length SMN protein expressed in patients with SMA (10% to 40% of normal SMN protein levels) correlate with SMA disease severity, and thus, *SMN2* is a key modifier of disease phenotype [Coovert 1997; Feldkötter 2002; Lefebvre 1997; Prior 2004].~~

4.2 Current Therapies for Spinal Muscular Atrophy

Nusinersen, also referred to as ISIS 396443 or Spinraza™, has been approved in the United States in December 2016 for the treatment of SMA in pediatric and adult patients. Current medical care is supportive and focused on respiratory support, nutritional support, and

management of resulting musculotendinous contractures and neuromuscular scoliosis through bracing, physical therapy, and surgery [Wang 2007].

~~There are currently no approved therapies for SMA. Current medical care is supportive and focused on respiratory support, nutritional support, and management of resulting musculotendinous contractures and neuromuscular scoliosis through bracing, physical therapy, and surgery [Wang 2007].~~

4.3 Nusinersen

4.3.1 Mechanism of Action

Nusinersen is a fully modified, 2′-*O*-(2-methoxyethyl) [2′-MOE] chimeric antisense oligonucleotide (ASO) drug designed to bind to a specific sequence in the intron downstream of exon 7 of the *SMN2* gene transcript. The region of the pre-mRNA targeted by nusinersen is normally occupied by heterogeneous nuclear ribonucleoproteins (hnRNP) A1/2 proteins, masking the U1 small nuclear ribonucleic acid (snRNA) binding site at the 5′-exon-intron junction of exon 7. U1 snRNA base pairs to the sequences that define the 5′-splice site, which is thought to be one of the first steps that initiate splicing of an intron. Nusinersen displaces the hnRNP A1/2 proteins from the pre-mRNA binding site, allowing U1 snRNA to bind to the exon-intron junction and promote assembly of the spliceosomal complex, thus promoting inclusion of exon 7 into the mRNA, which results in production of FL-SMN protein.

4.3.2 Chemistry

Chemically, nusinersen is a synthetic oligomer of 18 nucleotides (i.e., an 18-mer) that are connected sequentially by phosphorothioate linkages. Each of the 17 internucleotide linkages is a 3′-*O* to 5′-*O* phosphorothioate diester. The 18 sugar residues are uniformly modified with 2′-MOE). These MOE-modified nucleotides (1) increased affinity to the target mRNA [McKay 1999], (2) increased resistance to exonucleases and endonucleases (thereby increasing stability in tissue) [Geary 2003], and (3) ameliorated some of the high-dose toxicities associated with ASO containing only the phosphorothioate linkages [Henry 2000].

The sequence of nusinersen is written as follows:

5′-^{Me}U^{Me}CA^{Me}C^{Me}U^{Me}U^{Me}U^{Me}CA^{Me}UAA^{Me}UG^{Me}C^{Me}UGG-3′

where A and G are 2′-MOE nucleosides, ^{Me}C is 5-methyl-2′-MOE cytidine, and ^{Me}U designates 5-methyl-2′-MOE uridine.

4.3.4 Clinical Experience

Nusinersen~~ISIS 396443~~ has been evaluated in a completed open-label, single-ascending-dose, Phase 1 study designed to assess the safety, tolerability, and PK of **nusinersen**~~ISIS 396443~~ in

subjects with SMA (ISIS 396443-CS1). A single dose of **nusinersen**~~ISIS 396443~~ was administered by IT injection to **subjects** ~~2- to 14-year-old subjects~~ with SMA **2 to 14 years of age**. Four doses (1, 3, 6, and 9 mg) were evaluated sequentially. Each dose was studied in a cohort of 6 or 10 subjects, where all subjects received **nusinersen.**~~study treatment. In this study, all subjects completed dosing and follow-up visits per protocol. Overall, ISIS 396443 was well tolerated, and no safety concerns were identified up to the 9-mg dose given as a single IT injection. No serious adverse events (SAEs) or dose-limiting toxicities were reported in ISIS 396443-CS1. Adverse events (AEs) were mild or moderate in severity, and there was no relationship with ISIS 396443 dose. In addition, no ISIS 396443-related adverse changes in neurological examinations were reported despite intensive monitoring during the immediate postdosing period. CSF and plasma drug concentrations observed were generally consistent with predictions made from nonclinical monkey studies.~~

Nusinersen~~ISIS 396443~~ **was evaluated in 3 additional completed studies, ISIS 396443-CS2, ISIS 396443-CS3B, and ISIS 396443-CS10, and** ~~**ISIS 396443**~~ is ~~also~~ **currently** being evaluated in 5~~4~~ **additional** ongoing studies: ~~ISIS 396443-CS2, ISIS 396443-CS10,~~ ISIS 396443-CS12, ~~and~~ ISIS 396443-CS3A**, ISIS 396443-CS4, ISIS 396443-CS11, and 232SM202**.

Study ISIS 396443-CS2 **(completed) was** ~~is~~ an open-label, multiple-ascending-dose Phase 1/2a study designed to assess the safety, tolerability, and PK of **nusinersen**~~ISIS 396443~~ in subjects with SMA. Multiple doses of **nusinersen**~~ISIS 396443~~, ranging from 3 to 12 mg, **were**~~are~~ being administered by IT injection to ~~2- to 15-year-old~~ subjects with SMA **2 to 15 years of age**.

Study ISIS 396443-CS3B (completed) was a randomized, double-blind, sham-procedure controlled study designed to assess the clinical efficacy, safety, and PK of nusinersen in subjects with infantile-onset SMA. A 12-mg dose equivalent scaled by CSF volume was evaluated in infants with symptomatic SMA ≤7 months of age at Screening.

Study ISIS 396443-CS10 **(completed) was**~~is~~ an open-label, single-dose, redosing study of subjects with SMA who previously participated in Cohorts 2, 3, and 4 in **Study** ISIS 396443-CS1.

Study ISIS 396443-CS12 is an **ongoing** open-label study to assess the safety and tolerability of a single IT dose of **nusinersen**~~ISIS 396443~~ (12 mg) in subjects with SMA who previously participated in either **Study** ISIS 396443-CS2 or **Study** ISIS 396443-CS10.

Study ISIS 396443-CS3A is an **ongoing,** open-label, multiple-dose study designed to assess the safety, tolerability, and PK of **nusinersen**~~ISIS 396443~~ in subjects with infantile-onset SMA. Multiple doses of **nusinersen**~~ISIS 396443~~ are being administered by IT injection to symptomatic infants with SMA ≤7 months of age. Two doses (6- and 12-mg dose equivalent scaled by CSF volume) are being evaluated sequentially.

Study ISIS 396443-CS4 is an ongoing, randomized, double-blind, sham-procedure controlled study designed to assess the clinical efficacy, safety, and PK of nusinersen in subjects with later-onset SMA 2 to 12 years of age at Screening.

Study ISIS 396443-CS11 is an ongoing, open-label, extension study being conducted to evaluate the long-term safety, tolerability, and efficacy of nusinersen administered IT to subjects with SMA who previously participated in investigational studies of nusinersen.

Study 232SM202 is an ongoing, randomized, double-blind, sham-procedure controlled study to assess the safety and tolerability and to explore the efficacy of nusinersen administered by IT injection in subjects with SMA who are not eligible to participate in the Studies ISIS 396443-CS3B or ISIS 396443-CS4.

The primary support for the safety and efficacy of nusinersen in the treatment of SMA is derived from the final analysis of Study ISIS 396443-CS3B, the sham-controlled study in subjects with infantile-onset SMA, and the pre-planned interim analysis of Study ISIS 396443-CS4, the sham-controlled study in subjects with later-onset SMA. Subjects receiving nusinersen achieved statistically significant improvements in motor function compared with subjects in the control arms.

~~Nusinersen has a favorable safety profile.~~ **In Study ISIS 396443-CS4, there was a greater change from Baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) score at 15 months for subjects with later-onset SMA who received nusinersen relative to subjects in the control group. In Study ISIS 396443-CS3B, there was greater improvement in motor milestones, as assessed by Section 2 of the Hammersmith Infant Neurological Examination (HINE), for subjects with symptomatic infantile-onset SMA who received nusinersen relative to subjects in the control group.**

Uncontrolled studies involving subjects with infantile-onset SMA (Study ISIS 396443-CS3A) and later-onset SMA (longitudinal analyses of Studies ISIS 396443-CS2 and ISIS 396443-CS12) are highly supportive of the results of the pivotal efficacy studies (Studies ISIS 396443-CS3B and ISIS 396443-CS4) and provide evidence of the long-term benefit of treatment with nusinersen.

See the Investigator's Brochure for detailed information on clinical studies.

Rationale: This section was updated to reflect the most recent information regarding completed and ongoing studies of nusinersen in subjects with SMA.

This change also affects Section 4.5, Rationale for Dose and Schedule Selection.

Section 4.5, Rationale for Dose and Schedule Selection

Change: The dosing language was updated to reflect a fixed dose (12 mg [5 mL]) of nusinersen; additional support for maintenance dose was also included.

Now reads:

The **nusinersen**~~ISIS 396443~~ dose and dose interval for this study were selected based on nonclinical toxicology and PK observations from ~~monkey~~ studies **in monkeys** using single-dose and repeat-dosing IT administration, consideration of the target tissue concentration anticipated for drug pharmacology, and safety data in the completed and ongoing clinical studies of **nusinersen**~~ISIS 396443~~ to date. Based on **the** pharmacology and PK results in SMA transgenic mice, it **was**~~is~~ estimated that a target spinal cord tissue concentration between **2**~~1~~ and 10 µg/g will produce 50% to 90% *SMN2* exon 7 inclusion. Nonclinical studies in juvenile monkeys receiving IT doses of **nusinersen**~~ISIS 396443~~ showed a resulting gradient of distribution of **nusinersen**~~ISIS 396443~~ along the spinal cord, with mean lumbar spinal cord concentrations ranging from 1.6- to 2.3-fold and 2.0- to 3.5-fold higher than thoracic and cervical spinal cord levels, respectively. The dose selected for this multiple-dose clinical study (equivalent to 12 mg of **nusinersen**~~ISIS 396443~~) is predicted to achieve levels at the high end of this range (approximately 10 µg/g lumbar and 3 µg/g cervical spinal cord tissue concentrations) after the first dose. ~~The loading dose interval (i.e., doses on Days 1, 15, 29, and 64) was selected based on the nonclinical PK and pharmacology data in order to achieve and maintain ISIS 396443 spinal cord tissue levels that are predicted to be above or within the upper end of the pharmacologically active range by Day 64 (approximately 30 µg/g lumbar and 10 µg/g cervical tissue concentration), while at the same time considering subject safety and convenience for repeated IT injections by lumbar puncture (LP). The maintenance dose interval (once every 4 months or 119 days) was selected based on the estimated spinal tissue and CSF drug half-life (4 to 6 months) and was selected to maintain spinal cord tissue levels of ISIS 396443 at a steady-state level within the estimated pharmacologically active range. Maintenance doses will be given on Days 183, 302, 421, 540, 659, and 778, for a total of 10 doses over the study period.~~

The loading dose interval (i.e., doses on Days 1, 15, 29, and 64) was selected as the dose interval based on the nonclinical PK and pharmacology data to achieve and maintain nusinersen spinal cord tissue levels that are predicted to be within the upper end of the pharmacologically active range by Day 64 (predicted to be approximately 30 µg/g lumbar and 10 µg/g cervical tissue concentrations), while at the same time considering subject safety and convenience for repeated lumbar puncture (LP) IT injections.

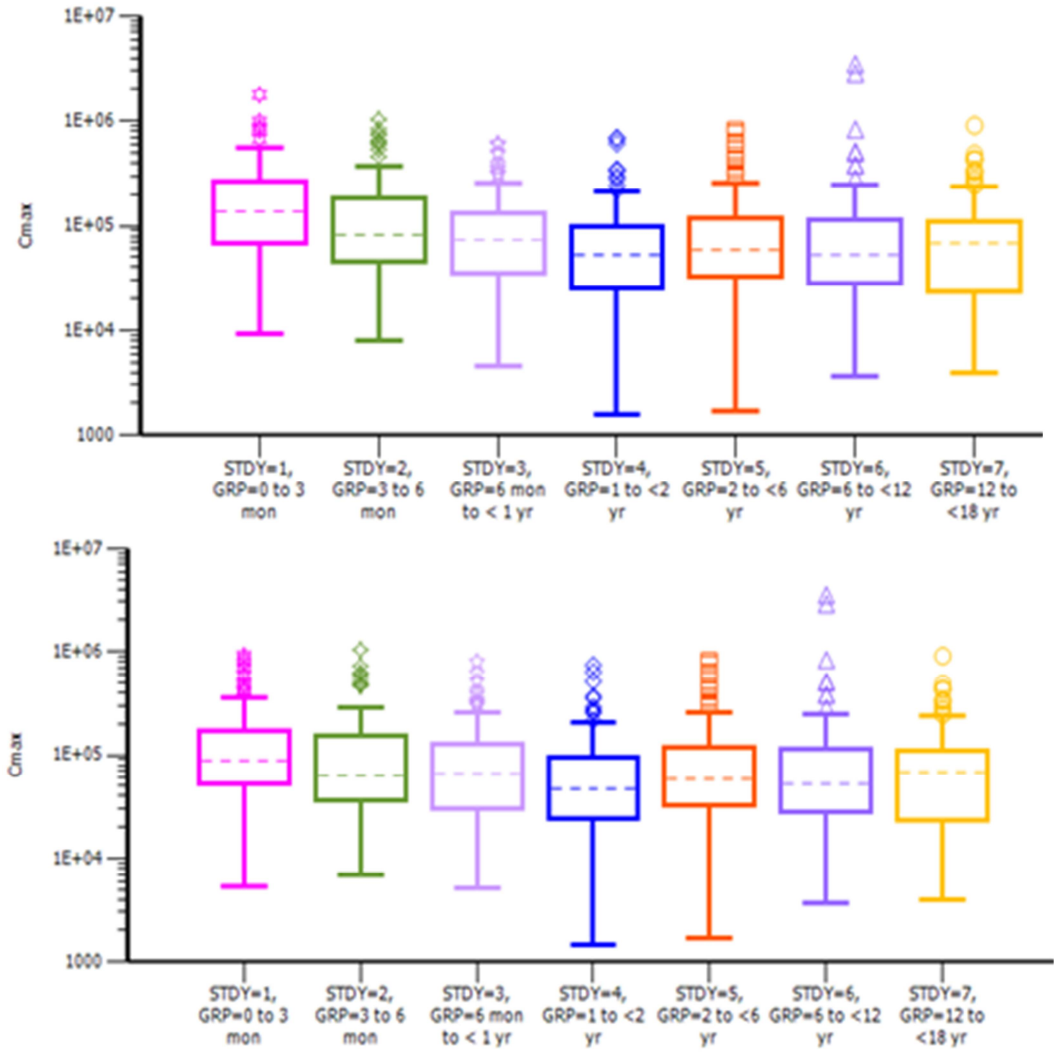
The maintenance dose interval (once every 4 months or 119 days) was selected based on nonclinical PK and pharmacology data and clinical PK data from subjects in ongoing and completed clinical studies, with the goal of maintaining the spinal cord tissue levels of nusinersen at a steady-state level within the estimated pharmacologically active range. The elimination half-life of nusinersen from human CSF is approximately 135 to 177 days and was estimated based on a limited number of postdose levels. Although CNS tissue half-life cannot be measured in humans, the median terminal elimination half-life was measured in the CNS tissue of adult monkeys and was found to be 116 days (approximately 4 months). Because the site of action of nusinersen is within the CNS tissues, these findings support maintenance doses administered every 4 months.

Nusinersen will be administered as an IT injection. **All subjects will be dosed with 12 mg (5 mL) regardless of age. Because results from PK models showed similar concentrations and potential for higher efficacy with higher concentrations, the dosing regimen was adjusted to 12 mg (5 mL) regardless of age, which in result will lower the risk for dosing errors while still maintaining favorable safety margins. Dosing instructions and details regarding administration will be provided in the Directions for Handling and Administration (DHA). In previous versions of the protocol (Versions 1 through 5), the volume of the injection, and thus the dose, was adjusted based on the subject's age on the day of dosing, such that each subject received a 12-mg scaled equivalent dose based on CSF volume scaling. Thus, younger subjects were given a lower dose of nusinersen, achieved by injecting a smaller volume that was proportional to the estimated CSF volume for age, such that the dose volume was equivalent to 5 mL for a 2-year-old child to adult.** ~~ISIS 396443 will be administered as an IT injection. The volume of the injection, and thus the dose, will be adjusted based on the subject's age on the day of dosing, such that each subject will receive a 12-mg scaled equivalent dose based on CSF volume scaling. Thus, younger subjects will be given a lower dose of drug, achieved by injecting a smaller volume that is proportional to the estimated CSF volume for age, such that the dose volume will be equivalent to 5 mL for a 2-year-old child to adult.~~

Rationale: The update to a fixed dose (12 mg [5 mL]) of nusinersen is strongly supported by results from PK models, which showed similar concentrations and potential for higher efficacy with higher concentrations.

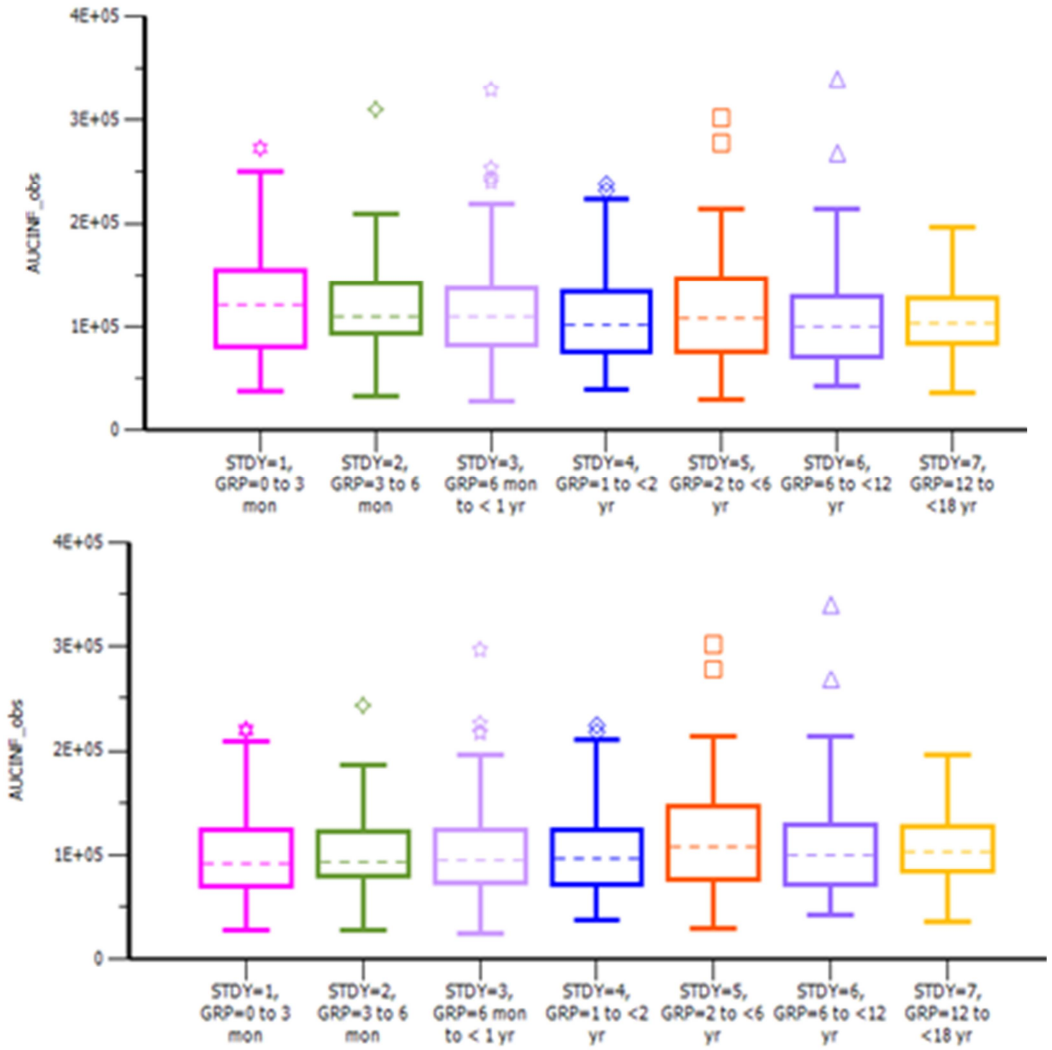
The population PK model (using n = 100 per age group) suggests that there is a similar area under the concentration-time curve (AUC) from zero to infinity across all age groups, but a higher maximum observed concentration (C_{max}) in the youngest age group (i.e., <3 months), with the fixed dose (relative to the age-adjusted dose; Figure 1 and Figure 2). There was high variation in the prediction of C_{max} as a result of only trough cerebrospinal fluid (CSF) data being available for population pharmacokinetic (PK) model analysis (percent coefficient variation >100%) and distribution of parameters across age groups that largely overlap, regardless of whether fixed or age-adjusted dosing was used. Given the fact that the AUC is similar regardless of fixed or age-adjusted dose and that there is substantial overlap in the range of estimates for each age group, neither method is likely to produce clinically relevant differences in exposure across different age groups.

Figure 1: CSF C_{max} (ng/mL) Plots in Different Age Groups After Single Fixed (Top) or Age-Adjusted Dose (Bottom) [Semi-Logarithmic Scale]



C_{max} = maximum plasma concentration; CSF = cerebrospinal fluid; GRP = group; mon = month; STDY = study; yr = year.

Figure 2: CSF AUC_{inf} (ng × hr/mL) Plots in Different Age Groups After Single Fixed (Top) or Age-Adjusted Dose (Bottom) [Semi-Logarithmic Scale]



AUC_{inf} = area under the plasma concentration-time curve from zero to infinity; CSF = cerebrospinal fluid; GRP = group; mon = month; STDY = study; yr = year.

This change also affects Section 5.2, Schedule of Activities: Day 897 Through Day 1820 (End of Study), Section 7.1, Study Overview, and Section 11.1, Regimen.

Section 6.2.2, Secondary Endpoints

Change: Secondary endpoints of change from Baseline in Hammersmith Functional Motor Scale – Expanded (HFMSE) and neurological examinations were added.

Now reads:

Efficacy

The secondary efficacy endpoints of this study are as follows (all assessed at approximately 13 and 24 months of age, unless otherwise noted):

- Proportion of subjects developing clinically manifested SMA as defined by any of the following:
    - Age-adjusted weight <5th percentile or decrease of ≥2 major weight growth curve percentiles (3rd, 5th, 10th, 25th, or 50th) or a percutaneous gastric tube placement for nutritional support
    - Failure to achieve the ability to sit without support
    - Failure to achieve standing with assistance
    - Failure to achieve hands-and-knees crawling
    - Failure to achieve walking with assistance by 24 months of age
    - Failure to achieve standing alone by 24 months of age
    - Failure to achieve walking alone by 24 months of age
- Proportion of subjects alive
- Attainment of motor milestones assessed as part of the HINE (Section 2)
- Attainment of motor milestones as assessed by World Health Organization (WHO) criteria
- Change from Baseline in the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) motor function scale
- **Change from Baseline in HFMSE**
- Change from Baseline in growth parameters: weight for age/length, head circumference, chest circumference, head to chest circumference ratio, and arm circumference

Safety

The secondary safety endpoints of this study are as follows:

- Incidence of AEs and/or SAEs

- Change from Baseline in clinical laboratory parameters, electrocardiograms (ECGs), and vital signs

- **Neurological examinations**

Rationale: The secondary efficacy and safety endpoints of change from Baseline in HFMSE and neurological examinations were added to more thoroughly assess motor development and neurological changes in subjects with presymptomatic SMA who are treated with nusinersen.

This change also affects Section 5, Schedule of Activities for Study 232SM201.

[redacted]

[redacted]

This change also affects Section 5, Schedule of Activities for Study 232SM201 and Section 13.1, Efficacy Assessments.

Section 11.1, Regimen

Change: Language describing the age-based dosing was removed and updated with language detailing the fixed dose. Language describing delay or missing of a loading dose was added.

Now reads:

~~The volume of the injection, and thus, the dose, will be adjusted for the subject's age on the day of dosing, such that each subject will receive a 12-mg scaled equivalent dose based on CSF volume scaling (Table 3). Thus, younger subjects will be given a lower dose of drug, achieved by injecting a smaller volume that is proportional to estimated CSF volume for age, such that dose volume will be equivalent to 5 mL for a 2-year-old child to adult. All subjects' age must be equivalent to ≥37 weeks gestational age at the time of the first dose.~~

~~Table 1: ISIS 396443 Dose Volume to be Injected~~

~~Age~~	~~Estimated CSF Volume (mL)~~	~~Injection Volume (mL)~~	~~Dose (mg)~~
~~0-3 months (0-90 days)~~	~~120~~	~~4~~	~~9.6~~
~~3-6 months (91-182 days)~~	~~130~~	~~4.3~~	~~10.3~~
~~6-12 months (183-365 days)~~	~~135~~	~~4.5~~	~~10.8~~
~~12-24 months (366-730 days)~~	~~140~~	~~4.7~~	~~11.3~~
~~>24 months (>730 days)~~	~~150~~	~~5.0~~	~~12.0~~

~~Source: [Matsuzawa 2001]~~
~~CSF = cerebrospinal fluid.~~

All subjects will be dosed with 12 mg (5 mL) regardless of age.

Note that injections should not occur within 72 hours after an immunization.

If a loading dose is delayed or missed, nusinersen should be administered as soon as possible, with at least 14 days between doses, and dosing continued at the prescribed dosing frequency. In the maintenance phase, if a planned dose is delayed or missed, nusinersen should be administered as soon as possible and dosing continued at the prescribed dosing frequency.

Rationale: This section was updated to reflect current recommendations for missed loading doses or missed/delayed maintenance doses; specifically, nusinersen should be administered with at least 14 days between doses as nonclinical and clinical testing has not occurred with dosing more frequently than every 14 days.

Section 13.1, Efficacy Assessments

Change: Efficacy assessments were revised and added to this section.

Now reads:

The following clinical assessments will be performed to evaluate the efficacy of **nusinersen**~~ISIS 396443~~:

- Survival
- [redacted]
- [redacted]
- Growth parameters
- WHO motor milestones
- HINE motor milestones **(Screening through Day 779 only)**
- CHOP INTEND motor function scale
- **HFMSE**
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

Videotaping of all motor milestone and motor function assessments will be optional.

If a subject has or had a sibling with SMA and if consent is given, data for the sibling will be collected at **5 timepoints during the course of the study**: **at** Screening (or the **first**~~next~~ visit after the implementation of ~~this amendment~~ **Protocol Version 4**), **Day 897, Day 1254, Day 1611,** and **at the end of the study** ~~Day 868~~ (or Early Termination **Visit**). Data to be collected from siblings with SMA will be nonbiologic and **noninvasive** ~~non-invasive~~ and will include historical data for *SMN2* gene copy number, sibling treatment history, etc. (see Table 1 **and Table 3** for timing of sibling data collection).

SMA is caused by loss of SMN protein due to a homozygous deletion or mutation or compound heterozygous mutation in the *SMN1* gene on chromosome 5q11-q13. Thus, patients with SMA are completely dependent on the amount of SMN protein produced by the *SMN2* gene. Genetic modifiers, such as the number of copies of the *SMN2* gene, are known to impact the eventual phenotype of SMA. The incidence and prevalence of SMA and its subtypes have been reported to vary based on country. One hypothesis for these variances is a difference in the frequency of certain genetic mutations that may be associated with different racial and ethnic groups within each country. Therefore, the race/ethnicity of subjects is collected as part of the medical history, where local regulations allow.

[redacted]

13.1.2 Growth Parameters

Growth parameters of body length and/or height (for all subjects), head circumference (for subjects up to 36 months of age), chest circumference (for subjects up to 36 months of age), and arm circumference (for subjects up to 36 months of age) will be measured at every onsite visit throughout the study.

Additional parameters of weight-for-age, weight-for-length, and head-to-chest circumference ratio will be calculated.

13.1.3 Motor Milestones

The assessments that are performed at a given visit will depend on the subject's age at that visit and current motor abilities. For the purposes of this protocol, ambulatory will be defined as any subject who has achieved independent walking as defined by the WHO Motor Milestones criteria (Test Item #6 – Walking Alone). Videotaping of the WHO and/or HINE motor milestone assessments will be optional.

For all subjects, motor milestones will be assessed using the WHO Motor Milestones criteria [WHO Multicentre Growth Reference Study Group 2006; Wijnhoven 2004].

For subjects <2 years of age who have not yet achieved independent walking, motor milestones will be assessed using Section 2 of the HINE, which is composed of 8 motor milestone categories as follows: voluntary grasp, ability to kick in supine position, head control, rolling, sitting, crawling, standing, and walking. Within each motor milestone category, there are 3 to 5 levels that can be achieved. All 8 motor milestones will be tested during each assessment. A subject whose results after testing all appear in the first column (no grasp, no kicking, unable to maintain head upright, and so on) has not achieved any motor milestone. Motor milestone achievement is depicted by movement from the left side of Table 4 to the right side of the table, as denoted by the Milestone Level Progression arrow in the table [Haataja 1999].

Table 4: Hammersmith Infant Neurological Examination Section 2 - Motor Milestones

Motor Milestone Category	Milestone Level Progression (Age Expected in Heathy Infants[1]) →				
Voluntary grasp	No grasp	Uses whole hand	Finger and thumb; immature grasp	Pincer grasp	
Ability to kick (in supine)	No kicking	Kicks horizontal; legs do not lift	Upward (vertically) [3 months]	Touches leg (4 to 5 months)	Touches toes (5 to 6 months)
Head control	Unable to maintain upright (<3 months)	Wobbles (4 months)	All the time upright (5 months)		
Rolling	No rolling	Rolling to side (4 months)	Prone to supine (6 months)	Supine to prone (7 months)	
Sitting	Cannot sit	Sit with support at hips (4 months)	Props (6 months)	Stable sit (7 months)	Pivots (rotates) [10 months]
Crawling	Does not lift head	On elbow (3 months)	On outstretched hand (4 to 5 months)	Crawling flat on abdomen (8 months)	On hands and knees (10 months)
Standing	Does not support weight	Supports weight (4 to 5 months)	Stands with support (8 months)	Stands unaided (12 months)	
Walking	No walking	Bouncing (6 months)	Cruising (holding on) [11 months]	Walking independently (15 months)	

[1] Values for healthy infants in [Haataja 1999].

The proportion of motor milestone responders is defined based on the 8 motor milestones categories, with the exclusion of voluntary grasp using the assessment at the later study visits, as follows:

i. Subject demonstrates at least a 2-point increase in the motor milestone category of ability to kick or achievement of the maximal score on that category (touching toes) or a 1-point increase in the motor milestones of head control, rolling, sitting, crawling, standing, or walking; AND

ii. Among the 8 motor milestone categories with the exclusion of voluntary grasp, subject demonstrates improvement (defined in [i]) in more categories than worsening. Note: For the category of ability to kick, similar to the definition of improvement in (i) mentioned previously, worsening is defined as at least a 2-point decrease or decrease to the lowest possible score of no kicking. For the other 6 categories, worsening is defined as at least a 1-point decrease.

13.1.4 Motor Function Assessments

Motor function assessments include all assessments listed in Section 13.1.4.1, Section 13.1.4.2, and Section 13.1.4.3. The assessments that are performed at a given visit will depend on the subject's age at that visit and current motor abilities.

Videotaping of all motor function assessments will be optional.

13.1.4.1 Children's Hospital of Philadelphia Infant Test for Neuromuscular Disease

CHOP INTEND will be assessed in subjects with infantile-onset SMA until they have a maximum score of 64. Once a score of 64 is achieved, CHOP INTEND will no longer be assessed.

The CHOP INTEND test was specifically designed to evaluate the motor skills of infants with significant motor weakness, including infants with SMA [Glanzman 2010]. The CHOP INTEND test captures neck, trunk, and proximal and distal limb strength in 14 elicited and 2 observational items. The CHOP INTEND has been established as a safe and reliable infant motor measure in infantile-onset SMA and has been validated [Glanzman 2011].

13.1.4.2 Hammersmith Functional Motor Scale - Expanded

All subjects ≥2 years of age will be evaluated using the HFMSE for the duration of the study. Subjects who are ≥2 years of age but have not yet achieved the maximum score of 64 with CHOP INTEND will be assessed with both until a CHOP INTEND maximum score of 64 is achieved. The HFMSE should be performed after the CHOP INTEND with an approximately 15-minute rest period in between to allow the subject to be fully engaged with both assessments.

The HFMSE is a reliable and validated tool used to assess motor function in children with SMA. The scale was originally developed with 20 scored activities and was devised for use in children with Type II and Type III SMA with limited ambulation to give objective information on motor ability and clinical progression [Main 2003]. The expanded scale includes an additional module of 13 items developed to allow for evaluation of ambulatory patients with SMA [O'Hagen 2007]. The HFMSE has been shown to be highly correlated with other clinical assessments and has shown good test-retest reliability.

[redacted]

[redacted]

[redacted]

DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

[redacted]

Section 14.1, Clinical Safety Assessments

Change: Subsections to describe neurological examination, electrocardiogram (ECG) assessment, physical examination procedures, and vital signs were added.

Now reads:

The following clinical assessments will be performed to evaluate the safety profile of ~~ISIS 396443~~**nusinersen**:

- Neurological examinations**: Sections 1 and 3 of the HINE (will be collected predose, 3 and 6 hours after dosing from Screening through Day 779, and 1 hour after dosing from Day 897 through Day 1820) or standard neurological examinations**
- Physical examinations and weight
- Vital sign measurements: temperature, pulse rate, resting systolic and diastolic blood pressure, and respiratory rate
- Pulse oximetry
- 12-Lead ECGs
- Concomitant therapy recording
- AE recording

14.1.1 Neurological Examinations

Neurological examinations will be performed predose and approximately 3 and 6 hours after dosing from Day 1 through Day 779 and predose and approximately 1 hour after dosing from Day 897 through Day 1820. Please note: If sedation was used, please allow sufficient recovery time for the subject to be fully engaged in the examination. It is important that the data collected truly reflect the subject's neurological performance.

The HINE (Sections 1 and 3) will be conducted in all subjects ≤24 months of age. This standard examination (developed by [Dubowitz and Dubowitz 1981]) is a quantitative scorable method for assessing the neurological development of infants between 2 and 24 months of age. The examination includes assessment of cranial nerve functions, posture, movements, tone, and reflexes.

For all subjects >24 months of age, standard neurological examinations, which include assessments of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be conducted.

14.1.2 Electrocardiograms

ECGs will be performed for all subjects at Screening, Day 2, Day 29 postdose, Day 365, Day 700, Day 897, Day 1254, Day 1611, Day 1820, and at the Early Termination Visit.

After the ECG is completed, an initial local read of the ECG should occur before the ECG is sent for central read (all ECGs will be centrally read). If the subject's initial ECG results show a QTc interval of ≥500 ms, then the ECG should be repeated (prior to the subject leaving the visit). If the second ECG QTc again reads ≥500 ms, the physician should use his or her best clinical judgement to address the condition.

Additional ECGs may be performed per the judgement of the Investigator, as deemed clinically necessary.

14.1.3. Physical Examinations

Physical examinations will be performed for all subjects at every onsite visit throughout the study. Any abnormal findings observed during physical examinations will be captured as AEs and reported according to Section 15.

Videotaping of the physical examinations will be optional.

14.1.4. Vital Signs

Vital signs include systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature. Additionally, pulse oximetry will be collected. From Screening through Day 779, vital signs and pulse oximetry will be collected predose and at 4 timepoints postdose: 1, 2, 4, and 6 hours. From Day 897 through Day 1820, vital signs and pulse oximetry will

be taken predose and approximately 1 hour postdose at every onsite visit throughout the study.

Rationale: This language was added to thoroughly monitor the neurologic condition, ECG readings, physical condition, and vital signs of subjects with presymptomatic SMA who received nusinersen.

This change also affects Section 5, Schedule of Activities for Study 232SM201.

Section 14.2, Laboratory Safety Assessments

Change: The section was revised to detail when laboratory safety assessments will be performed, and the specific analytes were detailed in Appendix A. Language regarding coagulation testing was added. Additional hematology, serum chemistry, and coagulation testing visits were added to the schedule.

Now reads:

~~The following laboratory tests will be performed to evaluate the safety profile of ISIS 396443:~~

- ~~Hematology: red blood cells, hemoglobin, hematocrit, platelets, white blood cells, white blood cell differential~~
- ~~Blood chemistry: total protein, albumin, creatinine, creatine phosphokinase, blood urea nitrogen, total bilirubin (direct and indirect), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glucose, calcium, phosphorus, chloride, sodium, potassium~~
- ~~Urinalysis: specific gravity, pH, protein, glucose, ketones, bilirubin, red blood cells, white blood cells, epithelial cells, bacteria, casts, crystals~~
- ~~Cystatin C (for subjects with Screening weight of ≥3 kg)~~

~~Coagulation (performed at local labs): aPTT, PT, INR~~

Laboratory measurements of serum chemistry, hematology, urinalysis, and urine total protein will be collected at Screening, Day 15, Day 29, and every 4 months thereafter (i.e., Days 64, 183, 302, 421, 540, 659, 778, 897, 1016, 1135, 1254, 1373, 1492, 1611, and 1730), as well as on Day 365, Day 700, and Day 1820 (End of Study)/Early Termination Visit. The laboratory analytes to be measured are shown in Appendix A. For urinary protein concentration >0.2 g/L, repeat testing and further evaluation should be considered.

Due to blood collection volume limitations for newborns, the laboratory safety assessments described above will be performed according to the schedules listed in Table **7**~~5~~ and Table **8**~~6~~.

Coagulation testing will be conducted at each visit at the time of the implementation of this amendment (Protocol Version 6), and results must be reviewed prior to dosing. Coagulation testing was not performed at every visit in previous versions of the protocol (Versions 1 through 5); therefore, not all subjects will have coagulation results prior to all doses.

Table **7~~5~~**: **Laboratory Safety** Blood Collection Schedule for Subjects with a Screening Weight of ≥3 kg

Panel/Test	Screening[1]	Day 1	**Day 15**	Day 29	Day 64	**Days**~~Day~~183, **302, 421, 540, 659, 778** ~~D183, D302, D421, D540, D659, D778~~ (±7D)	**Days 365, 700, 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1820** ~~D365, D700, D868~~ (±7D), and Early Term
Hematology	X		**X**	X	X	X	X
Serum Chemistry	X		**X**	X	X	X	X
Cystatin C	X				X[2]	X[2]	X
Coagulation	X~~[3]~~	**X**	**X**	**X**	**X**	**X**	**X**

D = day(s); Term = termination.

[1] Screening laboratory tests will be collected over a two or more day period.

[2] At Day 183 or when the subject's weight is ≥5.4 kg, whichever comes first, the blood collection schedule will revert to that outlined in Section 5.

~~[3] Coagulation testing must be reviewed prior to dosing on Day 1.~~

Table **8~~6~~**: Safety Labs Blood Collection Schedule for Subjects with a Screening Weight of <3 kg

Panel/Test	Screening[1]	Day 1	**Day 15**	Day 29	Day 64	**Days**~~Day~~ 183, **302, 421, 540, 659, 778** ~~D183, D302, D421, D540, D659, D778~~ (±7D)	**Days 365, 700, 897, 1016, 1135, 1254, 1373, 1492, 1611, 1730, 1820** ~~D365, D700, D868~~ (±7D), and Early Term
Hematology	X		**X**	**X**	**X**	X	**X**
Serum Chemistry	X		**X**	X	X	X	**X**
Coagulation	**X**	**X**~~[2]~~	**X**	**X**	**X**	**X**	**X**

D = day(s); Term = termination.

[1] Screening laboratory tests will be collected over a two or more day period.

~~[2] Coagulation testing must be performed and reviewed prior to dosing on Day 1.~~

Rationale: These assessments were added to thoroughly evaluate coagulation parameters in subjects with presymptomatic SMA who received nusinersen.

This change also affects Section 5, Schedule of Activities for Study 232SM201 and led to the creation of Appendix A, Laboratory Analytes.

Section 15.1.1, Adverse Event

Change: The definition of adverse events was revised.

Now Reads:

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including **an**~~a clinically significant~~ abnormal laboratory finding~~, for example~~), symptom, or disease temporally associated with the use of a~~ medicinal (investigational) product, whether or not related to the~~ medicinal (investigational) product.

Determination of whether an abnormal laboratory value**, vital sign result, and/or ECG result** meets the definition of an AE will be made by the Investigator. Abnormal **results**~~laboratory findings that~~ are ~~considered by the Investigator as~~ not **considered**~~clinically significant should not be reported as~~ AEs **unless 1 or more of**~~. Although abnormal laboratory values are typically not considered AEs,~~ the following **criteria are met**~~considerations may result in an abnormal laboratory value being considered an AE~~:

- **The**~~A laboratory test~~ result ~~that~~ meets the criteria for an SAE
- **The**~~A laboratory test~~ result ~~that~~ requires the subject to receive specific corrective therapy
- **The result is considered by**~~A laboratory abnormality that~~ the Investigator ~~considers~~ to be clinically significant

Rationale: The language was updated to be consistent with the current protocol template for Biogen-sponsored studies.

Section 15.4.1, Overdose

Change: The section was expanded to define the conditions when a dosing error will be considered an overdose.

Now reads:

~~An overdose is any dose of study treatment given to a subject or taken by a subject that exceeds the dose described in the protocol, and must be documented as a protocol deviation.~~ **All dosing errors (including but not limited to route of administration, wrong dose, etc.) must be reported as protocol deviations.** A brief description should be provided in the deviation, including whether the subject was symptomatic **or not** ~~(with a list of symptoms) or asymptomatic~~. **Dosing details should be captured on the Dosing CRF.**

A dosing error will be considered an overdose when any of the following conditions are met:

- **A dose given exceeds the dose level described in the protocol and Drug Handling Guidelines**
- **Dosing frequency exceeds 4 doses in a 2-month period (and, thus, results in a higher than acceptable cumulative dose)**
- **Any time study treatment is administered less than 2 weeks from the previous dose**

Overdoses are not considered AEs and should not be recorded as an AE on the CRF **unless an AE or an SAE occurs.** ~~; however, all~~**All** overdoses **(regardless of whether or not they result in an AE) must be recorded on an overdose form and faxed** ~~must be reported to the Medical Monitor~~ **to the Sponsor (or designee)** within 24 hours of the site becoming aware of the overdose. ~~An overdose must be reported to the Medical Monitor even if the overdose does not result in an AE. If an overdose results in an AE, then the AE must be recorded.~~ If an overdose results in an SAE, ~~then~~ **both** the SAE ~~form~~ **and overdose forms** must be completed and faxed **to the Sponsor (or designee). Should an overdose occurs, the Investigator or designee must contact the Medical Monitor within 24 hours; refer to the Study Reference Guide for the complete contact** ~~SynteractHCR Safety. All study treatment-related dosing~~ information ~~must be recorded on the dosing CRF~~.

Rationale: This text was added to address this potential event and to collect information on overdoses.

Section 16, Statistical Methods and Determination of Sample Size

Change: The definition of study baselines were revised.

Now Reads:

In general, continuous variables will be summarized by descriptive statistics, including number, mean, median, standard deviation, minimum, and maximum. Categorical variables will be presented with the number and percentage in each category.

The Full Analysis Set (FAS) is defined as all subjects who receive at least 1 dose of **nusinersen**~~ISIS 396443~~.

The Per-Protocol Set (PPS) will include the subset of the FAS who complete at least the initial 4 doses of study **treatment**~~drug~~, have a baseline and at least the Day 183 efficacy assessments, and who have no significant protocol deviations that would be expected to affect efficacy assessments.

~~Baseline will be defined as the closest available assessment on or prior to Day 1 Predose.~~

A number of the endpoints are age specific and, therefore, over the course of the study as subjects get older, different scales will be utilized. Three baselines will be defined. The first baseline will be defined as the closest available assessment on or prior to Day 1 predose when the child is <6 weeks of age. The second baseline will be defined as on or prior to the Day 700 visit when the child is approximately 2 years of age. The third baseline will be defined as on or prior to Day 897 predose visit when the child is approximately 2.5 years of age.

The objectives of the study and the endpoints to be analyzed are listed in Section 6.

Rationale: The language was added to clarify that different baselines will be utilized, which will be further defined in the Statistical Analysis Plan.

Section 16.1, Efficacy

Change: The section was updated to include analysis plan for additional assessments.

Now Reads:

16.1.1. Analysis Population

The primary analysis of efficacy will be performed on subjects in the FAS with 2 SMN2 copies. Analyses will be repeated in the FAS and PPS, as well as in the PPS with 2 SMN2 copies. **For the longitudinal analyses of certain endpoints, it may be necessary to define additional efficacy sets.**

16.1.2.2. Analysis of the Secondary Endpoints

~~For~~**Age: <6 weeks to 2 years**

During this period, ~~the purposes of analysis, the secondary endpoints are classified into the following:~~

- ~~Achievement of milestones at approximately~~ 13- and 24 ~~months of age~~
- ~~Other~~

~~Achievement~~ of Milestones

-Month Visit is of most interest. The visit at Day 365 will be used for the assessment of the **endpoint at** 13 ~~month milestone~~ **months** and the visit at Day 700 will be used for the assessment of the ~~24 month milestone~~ **endpoint at 24 months**. The proportion of subjects meeting the criteria for the following at the 13- and 24-Month Visit will be presented with a corresponding 95% CI:

- Clinically manifested SMA defined by any one of the following:
    - If at the 13- or 24-Month Visit, the subject's weight has dropped below the 5th percentile according to WHO criteria [WHO 2014][WHO 2014]; or if compared to Baseline, a subject has decreased ≥2 major weight growth curve percentiles (3rd, 5th, 10th, 25th, or 50th) according to WHO criteria; or if a percutaneous gastric tube has been required at any point up to and including the 13- or 24-Month Visit, as documented in the concomitant procedures in the CRF
    - Failure to demonstrate the following as assessed by the WHO motor milestones:
        - At 13 and 24 months of age: ability to sit without support, standing with assistance, hands and knees crawling
        - Additionally at 24 months of age: walking with assistance, standing alone, and/or walking alone
    - Subjects who discontinue or die on or before the 13- or 24-Month Visit
- Achievement of each individual motor milestone as assessed by the HINE (Section 2) and the WHO motor milestones. Subjects who discontinue prior to the 13- or 24-Month Visit will be counted in the denominator.

~~Other~~

Age: 2 to 5 years

During this period, the 2.5, 3, 4 and 5 years of age visits are of the most interest. The Day 897 visit will be used for 2.5 years, Day 1135 will be used for 3 years, Day 1492 will be used for 4 years, and Day 1820 will be used for 5 years.

The proportion of subjects who have the ability to perform WHO motor milestones at Baseline and maintain this ability up to 5 years of age will be summarized by visit.

The HFMSE total score and change from Baseline will be summarized by visit using descriptive statistics.

Age: <6 weeks to 5 years

A Kaplan-Meier survival curve will be presented and used to estimate the proportion of subjects alive at 13 ~~and 24~~ months **and 2, 3, 4, and 5 years** of age. Corresponding 95% CIs will also be presented.

~~For the following endpoints, the 13- and 24-Month Visit is of most interest, but, where appropriate, changes from Baseline to each visit will be presented. If necessary, windowing will be used so subjects are similarly aged at each visit.~~

- ~~Change from Baseline to each visit for the CHOP INTEND motor function will be summarized using descriptive statistics.~~

- Change from Baseline to each visit will be summarized using descriptive statistics for the following growth parameters: weight for age/length, head circumference, chest circumference, head to chest circumference ratio, and arm circumference.

- **Change from Baseline to each visit for the CHOP INTEND motor function will be summarized using descriptive statistics. The maintenance of the ability to achieve a threshold of total score of ≥50 over time will also be presented.**

- **The actual value and change from Baseline in CSF survival protein concentration will be summarized.**

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Section 19.2, Study Committees

Change: The section was revised to remove reference to an independent Data and Safety Monitoring Board (DSMB).

Now Reads:

Safety data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor. ~~Safety data will also be reviewed on an ongoing basis by an independent Data and Safety Monitoring Board (DSMB).~~ The **Sponsor and Medical Monitor**~~DSMB~~ will ~~be assembled to~~ review safety, tolerability, and efficacy (as needed) data collected on **nusinersen**~~ISIS 396443~~ during this study~~. Based on its ongoing assessment of the safety and tolerability of ISIS 396443, the DSMB will provide recommendations to the Sponsor for modifying, stopping, or continuing the study as planned~~.

Rationale: Given the established safety profile, the DSMB will no longer be assembled. Going forward, ongoing safety review will be conducted by the Sponsor and Medical Monitor.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- The Ionis logo was replaced with the Biogen logo on the title page.
- The Sponsor Signatory information was updated, and a signature page was added.
- The List of Abbreviations was updated.
- When referring to "study treatment," "nusinersen" is used in place of "ISIS 396443". When referring to the protocol title or previous ("index") studies, ISIS 396443 is used. The term "study treatment" refers to administration of nusinersen or the sham procedure during the blinded portion of the study.
- The phrase "Study Reference Manual" was replaced with "Study Reference Guide".
- Section 7.2.2, Treatment, was updated to specify that an overnight stay is required for the first injection but is otherwise optional.
- Section 10.1, Discontinuation of Study Treatment, was updated to detail that the primary reason for discontinuation of study treatment must be recorded.
- Section 10.2, Withdrawal of Subjects From Study, was updated to detail that subjects who withdraw from the study will not be replaced.
- Section 11.5.2, Concomitant/Ancillary Procedures, was updated to include the word "ancillary."
- Section 12, Study Treatment Management, was updated to include a sentence describing the manufacture, handling, and storage of study treatment in accordance with applicable Good Manufacturing Practice.
- Section 12.1, Nusinersen, was revised to remove a sentence stating that ISIS396443 is manufactured by Isis Pharmaceuticals Inc.
- Section 12.1.2, Nusinersen Storage, was revised to include information regarding the Directions for Handling and Administration.
- Section 12.1.3, Nusinersen Handling and Disposal, was updated to include guidance for onsite destruction.
- Section 12.1.4, Nusinersen Accountability, was updated to include language regarding the loss of nusinersen.

- Section 15.1.2, Serious Adverse Events, was updated to be consistent with the current protocol template for Biogen-sponsored studies.

- Section 15.3.1, Adverse Events, and Section 15.5.1, Investigator, were updated to state that the Investigator will assess the subject at every visit and record new adverse events (AEs) or updates to previously reported AEs on the case report form (CRF).

- Section 15.3.2, Serious Adverse Events; Section 15.3.3, Immediate Reporting of Serious Adverse Events; Section 15.3.3.1, Deaths; Section 15.4.1, Overdose; and Section 15.5.1, The Investigator; were revised to clarify that serious AEs, deaths, and overdose should be reported to the safety vendor listed in the Study Reference Guide and not to SynteractHCR Safety.

- Section 15.5, Safety Responsibilities, was revised to add that AE follow-up information and resolution should be recorded on the CRF by the Investigator.

- Section 17, Ethical Requirements; Section 17.1, Declaration of Helsinki; Section 17.3, Subject Information and Consent; and Section 17.4, Subject Data Protection; were updated to be consistent with the current protocol template for Biogen-sponsored studies.

- Section 18, Administrative Procedures, was expanded to add information regarding quality assurance and monitoring of the study.

- Section 20, References, was revised to reflect the changes in references cites throughout the protocol.

- Typographical and formatting errors were corrected.

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AMENDMENT SUMMARY

Biogen Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 5

Date: 15 December 2015

EUDRA CT Number: 2014-002098-12

Version 5 of the protocol has been prepared for this amendment, which supersedes Version 4.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 232SM201 is to allow formal data reviews and interim analysis(es) to be performed during the course of the study.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 16.7, Interim Analysis

Now reads:

Interim data analyses may be performed after approximately 10 subjects have been enrolled. Further details about these analyses will be described in the Statistical Analysis Plan. ~~No formal interim analyses will be conducted.~~

Rationale: The purpose of the interim analysis(es) is to provide study sponsors and regulators with information on the status of this ongoing study.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- The Biogen Therapeutic Area was updated.
- The primary and secondary contacts for urgent medical issues were updated.

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AMENDMENT SUMMARY

Biogen Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 4

Date: 30 September 2015

EUDRA CT Number: 2014-002098-12

Version 4 of the protocol has been prepared for this amendment, which supersedes Version 3 dated 18 December 2014.

PRIMARY REASON FOR AMENDMENT

[redacted]

Now reads:

Table 1: Schedule of Activities

~~Note: Monitoring telephone calls will occur on a monthly (± 7 days) basis starting on Day 94 and continuing to the end of the study.[1]~~

Study Period	**Screen[21]**	**Treatment/Follow-Up**																
Study Day	**D -21 to D -1**	**D1**			**D2**	**D15 (±1D)**			**D16**	**D29 (±1D)**			**D30**	**D64, D183, D302, D421, D540, D659, D778 (±7D)**			**D65, D184 D303, D422, D541, D660, D779[32]**	**D365, D700, D868 (±7D) and Early Term**
		Pre-Dose	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		
Informed Consent	X																	
Inclusion/Exclusion Criteria	X	X[43]																
Medical History	X																	
Sibling SMA Data[4]	**X**																	**X**
Vital Signs & Pulse Oximetry[5]	X	X		4X[6]	X[7]	X		4X[6]	X	X		4X[6]	X	X		4X[6]	X	X
Weight	X	X			X	X			X	X			X	X			X	X
Growth Parameters[8]	X													X[9]				X
Physical Examination	X	X				X				X				X				X
Ventilator Use	X	X			X	X			X	X			X	X			X	X
Neurological Examination[10]	X	X		2X[11]	X[7]	X		2X[11]	X	X		2X[11]	X	X		2X[11]	X	X
ECG	X				X							X						X
Safety Laboratory Tests[12]	X									X				X[9]				X
Coagulation Labs	X[13]																	
Immunogenicity		X												X[9]				X

Study Period	Screen [21]	Treatment/Follow-Up																
Study Day	**D -21 to D -1**	**D1**			**D2**	**D15 (±1D)**			**D16**	**D29 (±1D)**			**D30**	**D64, D183, D302, D421, D540, D659, D778 (±7D)**			**D65, D184 D303, D422, D541, D660, D779[32]**	**D365, D700, D868 (±7D) and Early Term**
		Pre-Dose	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		
CSF PK [redacted]		X				X				X				X				
[redacted]																		
Plasma PK[14]				X										X[9]				
[redacted]																		
Study Treatment Injection[1618]			X				X[1719]				X[1719]				X[1719]			
In-Patient Stay (24 hours)[1719]				X														
CHOP INTEND[20]	X[1821]													X[9]				X
HINE Motor Milestones	X[1821]													X[9]				X
WHO Motor Milestones[22]	X[21]													X[9]				X
[redacted]																		
Con Med Recording	X[2025]----------X																	
Adverse Event Collection	X[2126]---------- X																	

AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted] Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; DNA = deoxyribonucleic acid; ECG = electrocardiogram; HINE = Hammersmith Infant Neurological Exam; ICF = informed consent form; LP = lumbar puncture; PK = pharmacokinetic(s); RNA = ribonucleic acid; SAE = serious adverse event; SMA = spinal muscular atrophy; [redacted] Term = termination; WHO = World Health Organization.

[1] Note: Monitoring telephone calls will occur on a monthly (± 7 days) basis starting on Day 94 and continuing to the end of the study. At telephone contact, changes in concomitant medications and AEs will be recorded, as well as information on the subject's ventilator use and SMA disease status.

[21] A blood sample will be collected at Screening for *SMN2* copy number analysis only from those subjects without genetic documentation of *SMN2* copy number. For all other subjects, a blood sample will be collected at any time during the study for analysis of *SMN2* copy number by the central laboratory.

~~3~~[2]These safety monitoring visits will occur on the day after the subject receives an injection of study treatment.
~~4~~[3]Investigator to check for signs and symptoms consistent with SMA
[4] **If a subject has or had a sibling with SMA and if consent is given, data for the sibling will be collected at Screening (or the next visit after the implementation of this amendment) and at the Day 868 (or Early Termination) visit. Data to be collected will be nonbiologic and non-invasive and will include historical data for *SMN2* gene copy number, sibling treatment history, etc.**
[5] Resting blood pressure, pulse, respiratory rate, temperature, and pulse oximetry awake. Pulse oximetry asleep will also be assessed at Screening only.
[6] Vital signs collected at 4 timepoints: 1, 2, 4, and 6 hours after dosing. Pulse oximetry will not be collected postdose.
[7] Conducted within 20 to 24 hours after dosing.
[8] Length, weight for age/length, head circumference, chest circumference, head to chest circumference ratio, and arm circumference.
[9] These assessments may be performed up to 7 days prior to dosing, if necessary.
[10]Neurological examinations consist of Sections 1 and 3 of the HINE.
[11]Neurological examinations conducted at 2 timepoints: 3 and 6 hours after dosing.
[12]Serum chemistry, hematology, and urinalysis panels; refer to ~~Table 4~~ **Table 5** and ~~Table 5~~ **Table 6** for a list of analytes and collection timepoints based on the subject's weight at Screening. For subjects ≥3 kg at Screening, once they reach a weight of 5.4 kg or the Day 183 visit, whichever happens first, blood samples for all analytes should be collected according to the above schedule. The blood sample for Cystatin C analysis will not be collected at any timepoint for subjects with a Screening weight of <3 kg. Urinalysis will be conducted according to the above schedule, independent of a subject's weight.
[13]Coagulation testing will be conducted at D1 pre-dose for subjects with a Screening weight of <3 kg. For all subjects, results must be reviewed prior to dosing on Day 1.
[14]Refer to Table 2 for the detailed PK sampling schedule.
[redacted]
[16]**A blood sample for RNA and DNA assessments will be drawn on Day 184. For subjects who have already completed Day 184 at the time of the implementation of this amendment, blood samples should be obtained at the next visit.**
[17]**A blood sample for RNA and DNA assessments will be drawn on Day 868 or Early Termination visit.**
[18]Injections should not occur within 72 hours after an immunization.
~~17~~[19]Overnight stay is required for the first injection, but is optional for all subsequent injections, at the discretion of the Investigator.
~~18~~[20]**Videotaping during the CHOP INTEND test will be optional.**
[21]The CHOP INTEND, HINE, and WHO motor milestone assessments will be conducted as part of the initial screening assessments. If they are conducted within 7 days prior to dosing, they will only need to be performed once; otherwise, they will need to be repeated within 7 days prior to dosing.
~~19~~[22]**WHO Major Milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments made by the subject's caregiver.**
[redacted]
~~20~~[25]In addition to concomitant medications, ancillary procedures will be recorded.
~~21~~[26]AEs and SAEs will be collected in the case report form from the time of signing the ICF as described in Section 15.3.1 and in Section 15.3.2.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 3, Synopsis

The Synopsis was revised to reflect changes made throughout the protocol.

Section 4, Schedule of Events for Study 232SM201

Change: The Schedule of Activities (SoA) was updated to include sibling SMA data collection at Screening and Day 868/Early Termination (see Table 1 for addition to SoA and the following footnote).

Now reads: If a subject has or had a sibling with SMA and if consent is given, data for the sibling will be collected at Screening (or the next visit after the implementation of this amendment) and at the Day 868 (or Early Termination) visit. Data to be collected will be nonbiologic and non-invasive and will include historical data for *SMN2* gene copy number, sibling treatment history, etc.

Rationale: The purpose of capturing sibling SMA data (onset, progression, and treatment, if applicable), is to better understand SMA and its similarities or differences within and across families. Sibling data will be used as a natural comparison for the progression of the disease.

Section 4, Schedule of Events for Study 232SM201

Change: A footnote was added to the SoA to indicate that optional videotaping during the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) test will be conducted (see Table 1 for the footnote).

Now reads: **Videotaping during the CHOP INTEND test will be optional**.

Rationale: The purpose of videotaping the subject during the CHOP INTEND test is to further examine the effect of the study drug on the subject and to assess the consistency of the examination between physical therapists, visits, sites, and for ongoing physical therapist training.

Section 8.1, Inclusion Criteria

Change: Inclusion criterion 5 was updated to provide additional clarification regarding the location of the compound muscle action potential (CMAP) assessment.**Now reads**: Inclusion Criteria

5. **Ulnar** CMAP ≥1 mV at Baseline.

Rationale: Ulnar CMAP was specified in the amended criterion to provide guidance for study sites when assessing study eligibility, as both ulnar and anterior tibialis CMAP have been performed previously. This text is intended to clarify that data from ulnar CMAP readings should be used to confirm study eligibility.

This change also affects Section 4, Schedule of Events for Study 232SM201.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- The Clinical Trial Review Board signature page was removed.
- The Therapeutic Area for Neurology (signatory) was updated.
- The signatory for Isis Pharmaceuticals was updated.
- Title page and Section 1, Sponsor Information: The Biogen addresses were updated.
- Section 1, Sponsor Information: The name of the Sponsor was updated.
- Section 4, Schedule of Events for Study 232SM201: A note describing monitoring telephone calls and an associated footnote were moved to the footnotes section of the SoA.
- Section 4, Schedule of Events for Study 232SM201: A footnote was added to the SoA indicating that WHO Major Milestone assessments will be performed by the Investigator or physical therapist at study visits independently of the assessments performed by the subject's caregiver.
- [redacted]
- Section 4, Schedule of Events for Study 232SM201: A footnote was added to Table 2 to clarify that 4 to 5 mL of CSF will be collected, but only 0.5 mL of CSF will be used for PK assessments.
- Section 8, Selection of Subjects: Text was added to exclusion criterion 2 to clarify the timing of observation of clinical signs and symptoms.
- Section 11.1, Study Treatment Use: Fluoroscopy was added as an option for the lumbar puncture procedure.
- Section 13.1, Efficacy Assessments: Language was added to describe capturing sibling SMA data at Screening and the Day 868/Early Termination visit, and the title of the section was updated.
- [redacted]

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AMENDMENT SUMMARY

Biogen Idec Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 3

Date: 18 December 2014

EUDRA CT Number: 2014-002098-12

Version 3 of the protocol has been prepared for this amendment, which supersedes Version 2

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 232SM201 is to accommodate the inclusion of twins with spinal muscular atrophy (SMA). The main change in the protocol that has been made to accommodate these subjects is the addition of a specific gestational age requirement for twins (34 to 42 weeks) due to the lower mean gestational age for twins.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 8.1, Inclusion Criteria

Now reads:

8. Gestational age of 37 to 42 weeks **for singleton births; gestational age of 34 to 42 weeks for twins.**

Rationale: Uncomplicated pregnancies of singletons deliver babies at 37 weeks or greater of gestational age. However, in pregnancies with normal twins, it is recommended to deliver babies in some cases as early as 34 weeks. In fact, the mean gestational age for singleton births is 38.7 weeks, whereas for twins, the mean gestational age is 35.3 weeks. Therefore, this change allows for babies with SMA born from normal pregnancies with twins to participate in this study.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 4, Schedule of Events for Study 232SM201

Change: Table 1, Schedule of Activities, was modified.

Now reads:

Table 1: Schedule of Activities

Note: Monitoring telephone calls will occur on a monthly (±7 days) basis starting on Day 94 and continuing to the end of the study.[1]

Study Period	**Screen[2]**	**Treatment/Follow-Up**																
Study Day	**D -21 to D -1**	**D1**			**D2**	**D15 (±1D)**			**D16**	**D29 (±1D)**			**D30**	**D64, D183, D302, D421, D540, D659, D778 (±7D)**			**D65, D184 D303, D422, D541, D660, D779[3]**	**D365, D700, D868 (±7D) and Early Term**
		Pre-Dose	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		**Pre-Dose**	**LP**	**Post-Dose**		
Informed Consent	X																	
Inclusion/Exclusion Criteria	X	X[4]																
Medical History	X																	
Vital Signs & Pulse Oximetry[5]	X	X		4X[6]	X[7]	X		4X[6]	X	X		4X[6]	X	X		4X[6]	X	X
Weight	X	X			X	X			X	X			X	X			X	X
Growth Parameters[8]	X													X[9]				X
Physical Examination	X	X				X				X				X				X
Ventilator Use	X	X			X	X			X	X			X	X			X	X
Neurological Examination[10]	X	X		2X[11]	X[7]	X		2X[11]	X	X		2X[11]	X	X		2X[11]	X	X
ECG	X				X							X						X
Safety Laboratory Tests[12]	X									X				X[9]				X
Coagulation Labs	X[13]																	
Immunogenicity		X												X[9]				X
CSF PK [redacted]		X				X				X				X				
[redacted]																		
Plasma PK~~[13]~~[14]				X	~~X~~									X[9,~~15~~]		~~X~~[~~15~~]		
Study Treatment Injection[16]			X				X[17]				X[17]				X[17]			
In-Patient Stay (24 hours)[17]				X														

Study Period	Screen[2]	Treatment/Follow-Up															
Study Day	D -21 to D -1	D1			D2	D15 (±1D)			D16	D29 (±1D)			D30	D64, D183, D302, D421, D540, D659, D778 (±7D)			D65, D184 D303, D422, D541, D660, D779[3]	D365, D700, D868 (±7D) and Early Term
		Pre-Dose	LP	Post-Dose		Pre-Dose	LP	Post-Dose		Pre-Dose	LP	Post-Dose		Pre-Dose	LP	Post-Dose		
CHOP INTEND	X[18]													X[9]				X
HINE Motor Milestones	X[18]													X[9]				X
WHO Motor Milestones	X[18]													X[9]				X
[redacted]																		
Con Med Recording	X[20] ---------- X																	
Adverse Event Collection	X[21]---------- X																	

AE = adverse event; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; [redacted]; Con Med = concomitant medication; CSF = cerebrospinal fluid; D = day; ECG = electrocardiogram; HINE = Hammersmith Infant Neurological Exam; ICF = informed consent form; LP = lumbar puncture; PK = pharmacokinetic(s); SAE = serious adverse event; SMA = spinal muscular atrophy; [redacted] Term = termination; WHO = World Health Organization

[1] At telephone contact, changes in concomitant medications and AEs will be recorded, as well as information on the subject's ventilator use and SMA disease status.

[2] A blood sample will be collected at Screening for *SMN2* copy number analysis only from those subjects without genetic documentation of *SMN2* copy number. For all other subjects, a blood sample will be collected at any time during the study for analysis of *SMN2* copy number by the central laboratory.

[3] These safety monitoring visits will occur on the day after the subject receives an injection of study treatment.

[4] Investigator to check for signs and symptoms consistent with SMA.

[5] Resting blood pressure, pulse, respiratory rate, temperature, and pulse oximetry awake. Pulse oximetry asleep will also be assessed at Screening only.

[6] Vital signs collected at 4 timepoints: 1, 2, 4, and 6 hours after dosing. Pulse oximetry will not be collected postdose.

[7] Conducted within 20 to 24 hours after dosing.

[8] Length, weight for age/length, head circumference, chest circumference, head to chest circumference ratio, and arm circumference.

[9] These assessments may be performed up to 7 days prior to dosing, if necessary.

[10] Neurological examinations consist of Sections 1 and 3 of the HINE.

[11] Neurological examinations conducted at 2 timepoints: 3 and 6 hours after dosing.

[12] Serum chemistry, hematology, and urinalysis panels**; refer to** Table 3 **and** 4 **for a list of analytes and collection timepoints based on the subject's weight at Screening. For subjects ≥3 kg at Screening, once they reach a weight of 5.4 kg or the Day 183 visit, whichever happens first, blood samples for all analytes should be collected according to the above schedule. The blood sample for Cystatin C analysis will not be collected at any timepoint for subjects with a Screening weight of <3 kg. Urinalysis will be conducted according to the above schedule, independent of the subject's weight.**

[13] **Coagulation testing will be conducted at D1 pre-dose for subjects with a Screening weight of <3 kg. For all subjects, results must be reviewed prior to dosing on Day 1.**

[~~13~~14] Refer to Table 2 for **the detailed** PK sampling schedule.

[redacted]

[15] ~~Plasma PK collected before the injection of study treatment only on Study Days 64, 183, 302, 540, 659, and 778. Plasma PK collected 4 h (±1 h) after the injection of study treatment only on Study Day 64~~.

[16] Injections should not occur within 72 hours after an immunization.

[17] Overnight stay is required for the first injection, but is optional for all subsequent injections, at the discretion of the Investigator.

[18] The CHOP INTEND, HINE, and WHO motor milestone assessments will be conducted as part of the initial screening assessments. If they are conducted within 7 days prior to dosing, they will only need to be performed once; otherwise, they will need to be repeated within 7 days prior to dosing.

[redacted].

[20] In addition to concomitant medications, ancillary procedures will be recorded.

[21] AEs and SAEs will be collected in the case report form from the time of signing the ICF as described in Section 15.3.1 and in Section 15.3.2.

Rationale: The change in timing of coagulation testing permits collection and review of coagulation test results prior to dosing given the low blood volume restriction in this infantile population.

Change: Table 2, Pharmacokinetic Sampling Schedule, was modified.

Now reads:

Table 2: Pharmacokinetic Sampling Schedule

Treatment Period	Study Day	Timepoints	Blood Collection (mL)	CSF Collection (mL)
Multiple Dose: LP Injection	D1	Predose	NA	0.5
		~~1 h (±1 h)~~	~~0.35~~	~~NA~~
		4 h (±1 hr)	0.5~~35~~	NA
	~~D2~~	~~20 to 24 h (±4 h)~~	~~0.35~~	~~NA~~
	D15	Predose	NA	0.5
	D29	Predose	NA	0.5
	D64	Predose	0.5~~35~~	0.5
		~~4 h (±1 h)~~	~~0.35~~	~~NA~~
	D183	Predose	0.5~~35~~	0.5
	D302	Predose	0.5~~35~~	0.5
	D421	Predose	**0.5**~~NA~~	0.5
	D540	Predose	0.5~~35~~	0.5
	D659	Predose	0.5~~35~~	0.5
	D778	Predose	0.5~~35~~	0.5

Note: Details on sampling, preparation, and shipment are included in the Study Laboratory Manual.

[redacted]

CSF = cerebrospinal fluid; D = day; h = hour; mRNA = messenger ribonucleic acid; LP = lumbar puncture; NA = not applicable (no collection scheduled); SMA = spinal muscular atrophy.

Rationale: The pharmacokinetic (PK) sampling schedule was updated to provide lower total blood collection volumes for infants with lower weight and to reflect that only plasma peak concentrations on D1 and trough levels on and after D64 are sufficient for population PK analysis. Additionally, volume for collection of each PK sample was adjusted to 0.5 mL because tubes are not available to accurately collect a volume of 0.35 mL. The 0.5-mL collections still provide a total blood draw volume that adheres to the limits relevant to this infantile population.

Section 8.1, Inclusion Criteria

Change: Criterion #7 was modified.

Now reads:

7. Body weight ≥~~>~~3rd percentile for age using appropriate country-specific guidelines. ~~at Screening.~~

Rationale: The phrase "at Screening" was removed as all criteria must be met at Screening to be included in the study. The ">" operator symbol was previously assigned in error and was changed to the "≥" operator symbol.

Section 8.2, Exclusion Criteria

Change: Criterion #1 was modified.

Now reads:

1. Hypoxemia (oxygen saturation <96% awake or asleep **without any supplemental oxygen or respiratory support, or for altitudes >1000 m, oxygen saturation of <92% awake or asleep without any supplemental oxygen or respiratory support**).

Rationale: The threshold for this test was adjusted to account for sites at higher elevations.

Change: Criterion #9 was added.

Now reads:

9. Diagnosis of neonatal Respiratory Distress Syndrome requiring surfactant replacement therapy or invasive ventilatory support.

Rationale: This exclusion criterion was added as the risk for Respiratory Distress Syndrome (RDS) increases with lower gestational age. RDS can decrease respiratory function and therefore could adversely impact ascertainment of the primary endpoint.

Section 11.1, Regimen

Change: A clarifying sentence was added.

Now reads:

The volume of the injection, and thus, the dose, will be adjusted for the subject's age on the day of dosing, such that each subject will receive a 12-mg scaled equivalent dose based on CSF volume scaling (Table 3). Thus, younger subjects will be given a lower dose of drug, achieved by injecting a smaller volume that is proportional to estimated CSF volume for age, such that dose volume will be equivalent to 5 mL for a 2-year-old child to adult. **All subjects' age must be equivalent to ≥37 weeks gestational age at the time of the first dose.**

Rationale: This sentence was added to clarify that no subject can be dosed prior to reaching age equivalent to ≥37 weeks of gestational age. This requirement will allow twins born between 34 and 37 weeks of gestation the opportunity to grow in size and to attain cerebrospinal fluid volume approximately equivalent to infants born ≥37 weeks of gestation at the time of the first dose.

Section 14.2, Laboratory Safety Assessments

Change: Clarity regarding blood collection and testing for infants with differing body weight categories at Screening was added.

Now reads:

The following laboratory tests will be performed to evaluate the safety profile of ISIS 396443:

- Hematology: red blood cells, hemoglobin, hematocrit, platelets, white blood cells, white blood cell differential
- Blood chemistry: total protein, albumin, creatinine, ~~cystatin C,~~ creatine phosphokinase, blood urea nitrogen, total bilirubin (direct and indirect), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glucose, calcium, phosphorus, chloride, sodium, potassium
- Urinalysis: specific gravity, pH, protein, glucose, ketones, bilirubin, red blood cells, white blood cells, epithelial cells, bacteria, casts, crystals

- **Cystatin C (for subjects with Screening weight of ≥3 kg)**
- **Coagulation (performed at local labs): aPTT, PT, INR**

Due to blood collection volume limitations for newborns, the laboratory safety assessments described above will be performed according to the schedules listed in Table 3 and 4.

Table 3: Safety Labs Blood Collection Schedule for Subjects with a Screening Weight of ≥3 kg

Panel/Test	Screening[1]	Day 1	Day 29	Day 64	Day 183, D183, D302, D421, D540, D659, D778 (±7D)	D365, D700, D868 (±7D), and Early Term
Hematology	X		X	X	X	X
Serum Chemistry	X		X	X	X	X
Cystatin C	X			X[2]	X[2]	X
Coagulation	X[3]					

[1] **Screening labs will be collected over a 2 or more day period.**
[2] **At Day 183 or when the subject's weight is ≥5.4 kg, whichever comes first, the blood collection schedule will revert to that outlined in Section 4.**
[3] **Coagulation testing must be reviewed prior to dosing on Day 1.**

Table 4: Safety Labs Blood Collection Schedule for Subjects with a Screening Weight of < 3 kg

Panel/Test	Screening[1]	Day 1	Day 29	Day 64	Day 183, D183, D302, D421, D540, D659, D778 (±7D)	D365, D700, D868 (±7D), and Early Term
Hematology	X				X	X
Serum Chemistry	X		X	X	X	X
Coagulation		X[2]				

[1] **Screening labs will be collected over a 2 or more day period.**
[2] **Coagulation testing must be performed and reviewed prior to dosing on Day 1.**

Rationale: These tabular summaries of the sampling schedule for subjects with different weight categories clarify the requirements for each category due to blood volume restrictions at lower Screening weights. Cystatin C testing was removed for infants <3 kg at Screening due to blood collection volume restrictions.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.

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AMENDMENT SUMMARY

Biogen Idec Protocol 232SM201

An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy

Version 2

Date: 23 October 2014

EUDRA CT Number: 2014-002098-12

Version 2 of the protocol has been prepared for this amendment, which supersedes Version 1.

PRIMARY REASON FOR AMENDMENT

The primary reason for this amendment to Protocol 232SM201 is to update the sponsor information.

New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 1, Sponsor Information

Now reads:

Isis Pharmaceuticals, Inc. (Isis) is the Sponsor of the study in the United States. Biogen Idec Inc. (Biogen Idec) is the Sponsor of the study in the Rest of World. Biogen Idec (or designee) will be responsible for managing the study globally.

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Primary contact for urgent medical issues:
Quintiles Medical Monitor: [redacted] ~~, MD~~ [redacted] **, MD**
Cell phone: [redacted]
~~Cell phone:~~ [redacted]
Quintiles medical emergency: +1 973-659-6677 or +1 570-819-8565

Secondary contact for urgent medical issues:
Biogen Idec Medical Director: [redacted], MD, MPH
Cell phone: [redacted]

Refer to the Study Reference Manual for complete contact information.

Rationale: Biogen Idec will manage the study in Australia; therefore, the Biogen Australia affiliate location was added to the sponsor information page.

The medical director representing the clinical research organization (Quintiles) managing the conduct of this study has changed; therefore, the name and the phone number of the Quintiles medical director have been updated.

This change also affects Section 15.4.2, Medical Emergency.

SUMMARY OF MAJOR CHANGES TO THE PROTOCOL

Changes to the protocol are presented chronologically. New text is shown in **bold** type; deleted text is shown with a ~~strikethrough~~.

Section 15.3.1, Adverse Events

Change: It was added that adverse events (AEs) will be collected on the case report form (CRF) from the time of signing the informed consent form (ICF).

Now reads:

For subjects who receive study treatment, any AE experienced ~~by the subject~~ between the time of ~~first dose of study treatment~~**signing the ICF** and Final Study Visit/Telephone Call is to be recorded on the CRF, regardless of the severity of the event or its relationship to study treatment. **For subjects who never receive study treatment, no AEs need to be recorded on the applicable CRF.**

An AE that is ongoing when the subject completes or discontinues the study should be followed by the Investigator, if possible, until the event has resolved or stabilized.

Rationale: To be consistent across all the studies in the spinal muscular atrophy program, the AEs will be collected in the CRF from the time of signing the ICF instead of from the first dose.

This change also affects Section 4, Schedule of Events for Study 232SM201, Table 1 (Schedule of Activities), footnote 21.

Section 15.3.2, Serious Adverse Events

Change: It was added that the serious adverse events (SAEs) will be also be collected in the CRF from the time of signing the ICF.

Now reads:

For subjects who receive study treatment, any SAE experienced ~~by the subject~~ between the time of ~~the~~ signing ~~of~~ the ICF and Final Study Visit/Telephone Call is to be recorded on an SAE form **and on the applicable CRF,** regardless of the severity of the event or its relationship to study treatment. **For subjects who never receive study treatment, any SAE occurring between the time of signing the ICF and Final Study Visit/Telephone Call must be recorded on an SAE form, regardless of the severity of the event or its relationship to study treatment; however, the SAE does not need to be recorded on the applicable CRF**.

SAEs must be reported to SynteractHCR Safety (Fax: +1-760-268-6500; Email: safetyfax@synteracthcr.com) within 24 hours as described in Section 15.3.3. Follow-up information regarding an SAE also must be reported with 24 hours.

Subjects will be followed for all SAEs until their Final Study Visit/Telephone Call. Thereafter, the event should be reported to SynteractHCR Safety only if the Investigator considers the SAE to be related to study treatment.

Any SAE that is ongoing when the subject completes or discontinues the study will be followed by the Investigator until the event has resolved, stabilized, or returned to baseline status.

Rationale: Additional language was added to ensure that besides recording the SAEs in the safety database from the signing of the ICF, the SAEs will also be recorded in the clinical database via the CRFs.

This change also affects Section 4, Schedule of Events for Study 232SM201, Table 1 (Schedule of Activities), footnote 21.

SUMMARY OF MINOR CHANGES TO THE PROTOCOL

The following minor changes were made to the protocol, as appropriate:

- The version number and date were updated throughout the protocol.
- Based on the pharmacokinetic (PK) sampling schedule in Section 4, Schedule of Events for Study 232SM201, Table 1 (Schedule of Activities), the PK sample collection at 4 h (±1 h) after the injection of study treatment will occur only on Study Day 64. A statement was added to Section 4, Schedule of Events for Study 232SM201, Table 1 (Schedule of Activities), footnote 15 to clarify this schedule. Footnote 15 in Table 1 now reads: Plasma PK ~~only~~ collected **before the injection of study treatment only** on Study Days 64, 183, 302, 540, 659, and 778. **Plasma PK collected 4 h (±1 h) after the injection of study treatment only on Study Day 64.**
- To be consistent with Section 11.5.2, Concomitant Procedures, footnote 20 was added to Section 4, Schedule of Events for Study 232SM201, Table 1 (Schedule of Activities) to clarify that "**In addition to concomitant medications, ancillary procedures will be recorded."**
- In Section 8.2, Exclusion Criteria, Exclusion Criterion 8, and Section 11.5.1, Concomitant Therapy, Disallowed Concomitant Therapy, olesoxime was removed from the examples provided for the experimental agents that the subjects were prohibited from receiving during the study.