



## Statistical Analysis Plan

Version No.: Final V1.0

**Study Title:** A phase 2, randomized, double-blind, sham-procedure controlled study to assess the safety and tolerability and explore the efficacy of ISIS 396443 (BIIB058) administered intrathecally in subjects with spinal muscular atrophy who are not eligible to participate in the clinical studies ISIS 396443-CS3B or ISIS 396443-CS4





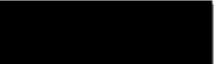
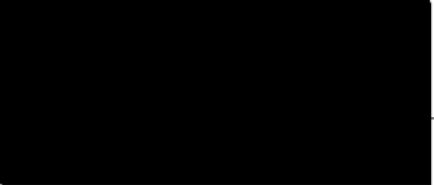
Part 2 and integrated Part 1 and 2 analyses

Name of Study Treatment: ISIS 396443

Protocol No.: 232SM202 / NCT02462759.

Study Phase: Phase 2

## APPROVAL

This document has been reviewed and approved by:		
 SMT Statistician		<u>16 July 2018</u> Date
 CDT Statistician (Printed Name)	 Signature	<u>16 JUL 2018</u> Date
 Study Medical Lead (Printed Name)		<u>16 JUL 2018</u> Date

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## LIST OF ABBREVIATIONS

ACEND	assessment of Caregiver Experience with Neuromuscular Disease
AE	adverse event
ALT/SGPT	alanine aminotransferase/serum glutamic pyruvic transaminase
aPTT	activated partial thromboplastin time
AST/SGOT	aspartate aminotransferase/serum glutamic-oxaloacetic transaminase
ATC	anatomical therapeutic class
BLQ	below the lower limit of quantification
BUN	blood urea nitrogen
CPK	creatine phosphokinase
CRF	case report form
CSF	cerebrospinal fluid
CV	coefficient of variation
DSMB	data safety monitoring board
HINE	hammersmith infant neurological examination
IM	immunogenicity
INR	international normalized ratio
IT	intrathecal
LLN	lower limit of normal
LLOQ	lower limit of quantification
LP	lumbar-puncture
MedDRA	medical dictionary for regulatory activities
pNf-H	phosphorylated neurofilament heavy chain subunit

PK	pharmacokinetics
PT	preferred term
PTT	partial thromboplastin time
RBC	red blood cell
SAE	serious adverse event
SD	standard deviation
SMA	spinal muscular atrophy
SOC	system organ class
ULN	upper limit of normal
WBC	white blood cell
WHO	world health organization
WHODrug	world health organization drug dictionary

## 1 Study Design

This is a Phase 2 multicenter study conducted in 2 parts.

Part 1 was originally designed as a randomized, double-blind, sham-procedure controlled study to assess the safety and tolerability and explore the efficacy of intrathecally administered Nusinersen (also known as ISIS 396443 and BIIB058) over a period of approximately 14 months (from the first dose until the End of Part 1 Evaluation). Up to 21 subjects were randomized in a ratio of 2:1 to receive Nusinersen by intrathecal lumbar puncture (LP) injection or a sham-procedure control. Randomization was stratified based on age at onset of clinical signs and symptoms consistent with SMA: >6 months versus ≤6 months. Part 1 of the study was stopped early due to the efficacy results seen in the ISIS 396443-CS3B study and the planned analysis of Part 1 is described in a separate Statistical Analysis Plan and corresponding CSR.

Part 2 study procedures will be determined based on the treatment assignment in Part 1.

For subjects who were randomized to receive Nusinersen in Part 1, Part 2 Day 1 will occur following the End of Part 1 Evaluation or approximately 4 months following the last injection of Nusinersen in Part 1. On Part 2 Day 1, subjects will undergo predose evaluations according to the schedule of events and then receive their first dose of Nusinersen for Part 2 (next maintenance dose). Subjects will return to the study site on Days 120, 239, 358, 477, 596, and 715 for follow-up evaluations and subsequent maintenance dose injections. For subjects who were receiving sham procedure in Part 1, Part 2 Day 1 will occur following the End of Part 1 Evaluation or as soon as possible following the End of Part 1 Evaluation. At Day 1, subjects will undergo predose evaluations according to the schedule of events and then receive their first dose of Nusinersen (first loading dose). Subjects will return to the study site on Days 15, 29, 64, 183, 302, 421, 540, 659, and 778 for follow-up evaluations and subsequent injections. All subjects will terminate early Part 2 for enrollment in open-label extension study, ISIS 396443-CS11.

This statistical analysis plan (SAP) describes the analyses planned for the entire study (integration of Part 1 and Part 2) and additionally some summaries of Part 2 data only.

### 1.1 Screening

After informed consent is obtained, subjects undergo a Screening evaluation no greater than 28 days prior to administration of the first dose or first sham procedure at which time their eligibility for the study will be examined.



## 1.2 Study treatment

In Part 1 of the study, each subject was administered a single intrathecal bolus (1 to 3 minutes) LP injection of Nusinersen or sham procedure on Days 1, 15, 29, 64, 183, and 302 by dedicated study personnel who were unblinded to treatment. The study treatment administration was performed in a dedicated room, and key study personnel and the parents were not present during the procedure to ensure blinding.

In Part 2 of the study, all subjects received Nusinersen. Subjects randomized to receive sham in Part 1 of the study received loading doses in Part 2 and after that a maintenance dose (every 120 days) and subjects randomized to receive Nusinersen in Part 1 of the study continued on maintenance doses. Treatment administration was not blinded.

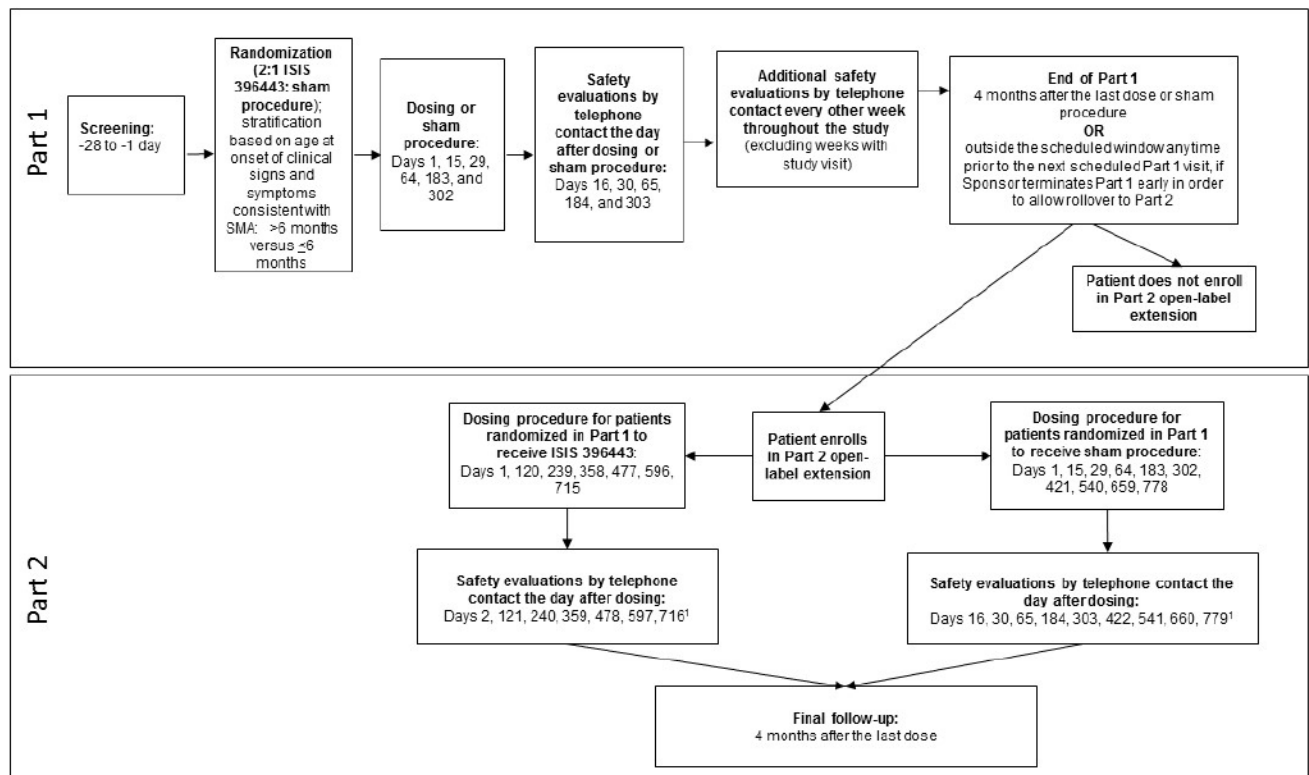
## 1.3 Follow-Up

Part 1 was terminated early after an interim analysis of a phase 3 study in infants with SMA (ISIS 396443-CS3B) demonstrated a positive benefit-risk profile for Nusinersen. Subjects returned to the study site for a follow-up evaluation (Final Study Visit) approximately 4 months after the last dose of study treatment in Part 1 and all eligible were enrolled in Part 2. Part 2 will terminate early as well to allow subjects to enroll in another long term Phase 3 open-label extension study, ISIS 396443-CS11. Subjects who terminate early from Part 2 of the study will be encouraged to complete the Part 2 Early Termination Evaluation assessments at the time of withdrawal.

### 1.4 Schematic of events

The schedule of events is as follows

Figure 1: Study Schematic 1



**Schedule of events Part1**

Table 1: Part 1 Schedule of Activities																	
Activities Study Period	Screen <sup>1</sup>	Part 1 Treatment/Follow-Up															
		Days -28 to -1	Day 1			Day 2 <sup>2</sup>	Day 15 (±1 day)			Day 16 <sup>2</sup>	Day 29 (±1 day)			Day 30 <sup>2</sup>	Days 64, 183, 302 (±7 days)		
Predose	LP		Postdose	Predose	LP		Postdose	Predose	LP		Postdose	Predose	LP		Postdose		
Informed Consent	X																
Inclusion/Exclusion Criteria	X	X															
Medical History	X																
Vital Signs <sup>6</sup>	X	X		4X <sup>7</sup>	X <sup>8</sup>	X		X		X		4X <sup>7</sup>		X		4X <sup>7</sup>	X
Weight	X	X			X	X				X				X			X
Growth Parameters <sup>9</sup>	X	X				X				X				X			X
Physical Examination	X	X				X				X				X			X
Ventilator Use	X	X			X	X			X	X			X	X			X
Neurological Examination <sup>10</sup>	X	X		X <sup>11</sup>	X	X		X <sup>11</sup>		X		X <sup>11</sup>		X		X <sup>11</sup>	X
ECG	X				X							X					X
Safety Laboratory Tests <sup>12</sup>	X												X <sup>13</sup>				X <sup>14</sup>
Coagulation Laboratory Tests	X																

**Schedule of events Part1**

Table 1: Part 1 Schedule of Activities																		
Activities Study Period	Screen <sup>1</sup>	Part 1 Treatment/Follow-Up																
Study Day	Days -28 to -1	Day 1			Day 2 <sup>2</sup>	Day 15 (±1 day)			Day 16 <sup>2</sup>	Day 29 (±1 day)			Day 30 <sup>2</sup>	Days 64, 183, 302 (±7 days)			Days 65, 184, 303 <sup>2</sup>	End of Part 1 <sup>3,4,5</sup>
		Predose	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		
Immunogenicity Sample		X											X				X	
CSF PK <sup>15</sup>		X				X				X				X				
Plasma PK				X <sup>16</sup>										X <sup>17</sup>			X	
Study Treatment Injection or Sham Procedure			X				X				X				X			
Inpatient Stay <sup>18</sup>				X														
Telephone Contact for Safety Monitoring								X					X				X	
HINE Motor Milestone		X												X			X	
Clinical Global Impression of Change														X			X	
Con Med Recording <sup>19</sup>		X-----X																
Adverse Event Collection <sup>20</sup>		X-----X																

AE = adverse event; Bi-PAP = bilevel positive airway pressure; Con Med = concomitant medication; CSF = cerebrospinal fluid; ECG = electrocardiogram; HINE = Hammersmith Infant Neurological Examination; LP = lumbar puncture; PK = pharmacokinetic(s); SAE = serious adverse event; SMA = spinal muscular atrophy; *SMN2* = survival motor neuron 2

- <sup>1</sup> A blood sample will be collected at Screening for *SMN2* copy number 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.
- <sup>2</sup> After the injection of study treatment or sham procedure on Day 1, subjects will remain at the study site for at least 24 hours (Day 2) for safety monitoring. On the day following the injection of study treatment or sham procedure on Days 15, 29, 64, 183, and 302, there will be safety monitoring through telephone contact. During that telephone contact (i.e., Days 16, 30, 65, 184 and 303), changes in concomitant medications and AEs, information on the subject's daily ventilator/Bi-PAP use, and health status will be recorded.
- <sup>3</sup> End of Part 1 is one of the following: Part 1 Final Follow-up Evaluation (Day 422 [ $\pm 7$  days]) according to the study schedule, early Part 1 Final Follow-up Evaluation to allow for rollover into Part 2, or Early Termination Evaluation for subjects who withdraw from the study during Part 1.
- <sup>4</sup> For subjects not transitioning into Part 2, the date of the End of Part 1 Evaluation assessments will be the end of study. For subjects who transition to Part 2, the date of the End of Part 1 will be the date of first dose in Part 2.
- <sup>5</sup> At the End of Part 1 evaluation, in order to allow transition to Part 2, subjects will be unblinded.
- <sup>6</sup> Vital signs include resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, pulse oximetry, and transcutaneous carbon dioxide. Pulse oximetry and transcutaneous carbon dioxide will be measured predose at each evaluation. For subjects who are not receiving noninvasive ventilation, pulse oximetry will be measured overnight once a week at home.
- <sup>7</sup> Vital signs (resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, and pulse oximetry) will be collected at 4 timepoints: 1, 2, 4, and 6 hours (all  $\pm 15$  minutes) after the injection of study treatment.
- <sup>8</sup> Vital signs (resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, and pulse oximetry) will be collected within 20 to 24 hours after the injection of study treatment or sham procedure.
- <sup>9</sup> At the evaluations scheduled for injection of study treatment or sham procedure, subjects will be assessed for the following growth parameters: body length, head circumference, chest circumference, and arm circumference.
- <sup>10</sup> Neurological examinations include assessment of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes.
- <sup>11</sup> Neurological examinations will occur between 4 to 6 hours after the injection of study treatment or sham procedure.
- <sup>12</sup> Blood chemistry, hematology, and urinalysis panels.
- <sup>13</sup> Samples for safety laboratory tests will be collected before the injection of study treatment or sham procedure on Days 64 and 183 only.
- <sup>14</sup> End of Part 1 safety laboratory tests may be performed locally in addition to centrally to accommodate the Part 2 Day 1 dosing.

<sup>15</sup>Refer to **Error! Reference source not found.** for CSF PK sample schedule. The CSF samples will be analyzed for Nusinersen concentrations. The remaining CSF sample will be stored for optional future biomarker analyses.

<sup>16</sup>Blood samples for PK assessment will be collected 4 hours ( $\pm 1$  hour) after the injection of study treatment or sham procedure only on Day 1. Refer to **Error! Reference source not found.** for the plasma PK sample schedule.

<sup>17</sup>Blood samples for PK assessment will be collected before the injection of study treatment or sham procedure on Days 64 and 183 only. Refer to **Error! Reference source not found.** for the plasma PK sample schedule.

<sup>18</sup>Overnight stay (at least 24 hours) is required after the first injection of study treatment or sham procedure. Following all subsequent injections or sham procedures, a stay of at least 6 hours at the study site is required; overnight stays are optional on these days.

<sup>19</sup>In addition to concomitant medications, ancillary procedures will be recorded.

<sup>20</sup>AEs and SAEs will be collected in the case report form from the time of signing of the informed consent form as described in Section and Section 15.3.2 of the protocol.

**Schedule of events Part 2**

Table 1: Part 2 Schedule of Activities for Subjects Randomized to Receive Nusinersen in Part 1									
Study Period	Part 2 Treatment/Follow-Up								
Study Day	Day 1			Day 2 <sup>1</sup>	Days 120, 239, 358, 477, 596, 715 ( $\pm 7$ days) <sup>2</sup>			Days 121, 240, 359, 478, 597, 716 <sup>1</sup>	Part 2 Final Follow-Up (Day 835 [ $\pm 7$ days]) <sup>3</sup> or Part 2 Early Termination <sup>4</sup>
	Predose <sup>5</sup>	LP	Postdose		Predose	LP	Postdose		
Informed Consent	X <sup>5</sup>								
Inclusion/ Exclusion Criteria	X <sup>5</sup>								
Vital Signs <sup>6</sup>	X <sup>7</sup>		X <sup>8</sup>		X		X <sup>8</sup>		X
Weight	X <sup>7</sup>				X				X
Growth Parameters <sup>9</sup>	X <sup>7</sup>				X				X
Physical Examination	X <sup>7</sup>				X				X
Ventilator Use	X <sup>7</sup>			X	X			X	X
Neurological Examination <sup>10</sup>	X <sup>7,11</sup>		X <sup>11</sup>		X <sup>11</sup>		X <sup>11</sup>		X
ECG	X <sup>7</sup>				X <sup>12</sup>				X <sup>12</sup>
Safety Laboratory Tests <sup>13</sup>	X <sup>7</sup>				X <sup>14</sup>				X
Immunogenicity Sample	X <sup>7</sup>				X <sup>15</sup>				X
CSF PK <sup>16</sup>	X <sup>5,7</sup>				X				
Plasma PK <sup>17</sup>			X <sup>17</sup>		X <sup>17</sup>				X



**Schedule of events Part 2**

Table 1: Part 2 Schedule of Activities for Subjects Randomized to Receive Nusinersen in Part 1									
Study Period	Part 2 Treatment/Follow-Up								
Study Day	Day 1			Day 2 <sup>1</sup>	Days 120, 239, 358, 477, 596, 715 ( $\pm 7$ days) <sup>2</sup>			Days 121, 240, 359, 478, 597, 716 <sup>1</sup>	Part 2 Final Follow-Up (Day 835 [ $\pm 7$ days]) <sup>3</sup> or Part 2 Early Termination <sup>4</sup>
	Predose <sup>5</sup>	LP	Postdose		Predose	LP	Postdose		
Study Treatment Injection		X				X			
Telephone Contact for Safety Monitoring (+3 Days)				X				X	
HINE and WHO Motor Milestone					X				X
Clinical Global Impression of Change and ACEND					X				X
Optional Sibling SMA Data Collection <sup>18</sup>	X								X
Con Med Recording <sup>19</sup>	X-----X								
Adverse Event Collection <sup>20</sup>	X-----X								

ACEND = Assessment of Caregiver Experience with Neuromuscular Disease; AE = adverse event; Bi-PAP = bilevel positive airway pressure; Con Med = concomitant medication; CSF = cerebrospinal fluid; ECG = electrocardiogram; HINE = Hammersmith Infant Neurological Examination; LP = lumbar puncture; PK = pharmacokinetic(s); SAE = serious adverse event; WHO = World Health Organization.

<sup>1</sup> There will be safety monitoring through telephone contact on the day following the injection of study treatment (i.e., Days 2, 121, 240, 359, 478, 597, and 716). During that telephone contact, changes in concomitant medications and AEs, information on the subject's daily ventilator/Bi-PAP use, and health status will be recorded.

- 
- <sup>2</sup> If the study continues beyond Year 2 (i.e., beyond Day 715), then from Day 835 onwards, subjects will maintain evaluations for study treatment injection every 4 months with the assessments for those additional timepoints to alternate between the assessments done on Day 120 and those done on Day 239. Day 120 assessments would be performed at Day 835 and Day 239 assessments would be performed at Day 954. The alternating pattern would continue until the end of the study. Part 2 Final Follow-up Evaluation would then occur approximately 4 months after the last injection of study treatment.
- <sup>3</sup> The Part 2 Final Follow-up Evaluation will occur 4 months after the last injection of study treatment, which will either be on Day 716 or later, if Part 2 of the study continues beyond Year 2.
- <sup>4</sup> Subjects who terminate early will be encouraged to complete the safety evaluations scheduled for the Part 2 Early Termination Evaluation.
- <sup>5</sup> Inclusion/Exclusion Criteria, Informed Consent, and CSF PK must be performed on Part 2 Day 1.
- <sup>6</sup> Vital signs include resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, pulse oximetry, and transcutaneous carbon dioxide. Pulse oximetry and transcutaneous carbon dioxide will be measured predose at each evaluation.
- <sup>7</sup> If Part 2 Day 1 study treatment injection is delayed more than 24 hours after the End of Part 1 visit, the following procedures must be done on Part 2 Day 1 predose: vital signs, weight, growth parameters, physical examination, ventilator use, neurological examination, and CSF PK. If Part 2 Day 1 study treatment injection is delayed more than 24 hours after End of Part 1 due to an AE, SAE, or other safety concern, or if Part 2 Day 1 study treatment injection is delayed more than 30 days after End of Part 1 for any reason, the following procedures must be done on Part 2 Day 1 predose: vital signs, weight, growth parameters, physical examination, ventilator use, neurological examination, ECG, CSF PK, and safety laboratory tests. Immunogenicity testing must also be repeated predose if Part 2 Day 1 study treatment injection is delayed more than 30 days after End of Part 1 for any reason.
- <sup>8</sup> Vital signs (resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, and pulse oximetry) will be collected 1 hour ( $\pm 15$  minutes) after the injection of study treatment.
- <sup>9</sup> At the evaluations scheduled for injection of study treatment, subjects will be assessed for the following growth parameters: body length, head circumference, chest circumference, and arm circumference. Growth parameters and weight are to be collected at the same time.
- <sup>10</sup> Neurological examinations include assessment of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes. If sedation was used, please allow sufficient recovery time for the subject to be fully engaged in the examination because it is important that the data collected truly reflect the subject's neurological performance.
- <sup>11</sup> Predose and postdose.
- <sup>12</sup> ECG will be performed on Days 239, 477, and 715, and at the Part 2 Final Follow-up Evaluation. ECG must be performed before obtaining blood samples for safety laboratory tests.
- <sup>13</sup> Blood chemistry, hematology, urinalysis, urine total protein panels, and coagulation parameters. Urine total protein and coagulation parameters will be assessed by local laboratories.
- <sup>14</sup> Samples for safety laboratory tests will be collected before the injection of study treatment at each dosing visit.
- <sup>15</sup> Blood samples for immunogenicity testing will be collected before the injection of study treatment on Days 239, 477, and 715.
- <sup>16</sup> Refer to **Error! Reference source not found.** for CSF PK sample schedule. The CSF samples will be analyzed for Nusinersen concentrations. The remaining CSF sample will be stored for optional future biomarker analyses.
- <sup>17</sup> Blood samples for PK assessment will be collected 4 hours ( $\pm 1$  hour) after the injection of study treatment only on Day 1. Refer to **Error! Reference source not found.** for the plasma PK sample schedule. Plasma PK samples will be collected predose on Days 239, 477, and 715.
- <sup>18</sup> The optional assessment of sibling SMA data collection will be performed only after obtaining consent.

<sup>19</sup>In addition to concomitant medications, ancillary procedures will be recorded.

<sup>20</sup>AEs and SAEs will be collected in the case report form from the time of signing of the informed consent form as described in Section and Section 15.3.2 of the protocol.

**Schedule of events Part 2**

Table 2: Part 2 Schedule of Activities for Subjects Randomized to Receive Sham Procedure in Part 1																	
Study Period	Part 2 Treatment/Follow-Up																
Study Day	Day 1			Day 2 <sup>1</sup>	Day 15 (±1 day)			Day 16 <sup>1</sup>	Day 29 (±1 day)			Day 30 <sup>1</sup>	Days 64, 183, 302, 421, 540, 659, 778 (±7 days) <sup>2</sup>			Days 65, 184, 303, 422, 541, 660, 779 <sup>1</sup>	Part 2 Final Follow-Up (Day 897 [±7 days]) <sup>3</sup> or Part 2 Early Termination <sup>4</sup>
	Predose <sup>5</sup>	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		
Informed Consent	X <sup>5</sup>																
Inclusion/Exclusion Criteria	X <sup>5</sup>																
Vital Signs <sup>6</sup>	X <sup>7</sup>		X <sup>8</sup>	X <sup>9</sup>	X		X <sup>8</sup>		X		X <sup>8</sup>		X		X <sup>8</sup>		X
Weight	X <sup>7</sup>			X	X				X				X				X
Growth Parameters <sup>10</sup>	X <sup>7</sup>				X				X				X				X
Physical Examination	X <sup>7</sup>				X				X				X				X
Ventilator Use	X <sup>7</sup>			X	X			X	X			X	X			X	X
Neurological Examination <sup>11</sup>	X <sup>7</sup>		X <sup>12</sup>	X	X		X <sup>12</sup>		X		X <sup>12</sup>		X		X <sup>12</sup>		X
ECG	X <sup>7</sup>			X							X		X <sup>13</sup>				X <sup>13</sup>
Safety Laboratory Tests <sup>14</sup>	X <sup>7</sup>				X				X				X <sup>15</sup>				X

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Study Period	Part 2 Treatment/Follow-Up																
Study Day	Day 1			Day 2 <sup>1</sup>	Day 15 (±1 day)			Day 16 <sup>1</sup>	Day 29 (±1 day)			Day 30 <sup>1</sup>	Days 64, 183, 302, 421, 540, 659, 778 (±7 days) <sup>2</sup>			Days 65, 184, 303, 422, 541, 660, 779 <sup>1</sup>	Part 2 Final Follow-Up (Day 897 [±7 days]) <sup>3</sup> or Part 2 Early Termination <sup>4</sup>
	Predose <sup>5</sup>	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		
Immunogenicity Sample	X <sup>7</sup>												X <sup>16</sup>				X
CSF PK <sup>17</sup>	X <sup>5,7</sup>				X				X				X				
Plasma PK <sup>18</sup>			X <sup>18</sup>										X <sup>18</sup>				X
Study Treatment Injection		X				X				X				X			
Inpatient Stay <sup>19</sup>			X														
Telephone Contact for Safety Monitoring (+3 Days)								X				X				X	
HINE and WHO Motor Milestone													X				X
Clinical Global Impression of Change and ACEND													X				X

**Schedule of events Part 2**

Table 2: Part 2 Schedule of Activities for Subjects Randomized to Receive Sham Procedure in Part 1																	
Study Period	Part 2 Treatment/Follow-Up																
Study Day	Day 1			Day 2 <sup>1</sup>	Day 15 (±1 day)			Day 16 <sup>1</sup>	Day 29 (±1 day)			Day 30 <sup>1</sup>	Days 64, 183, 302, 421, 540, 659, 778 (±7 days) <sup>2</sup>			Days 65, 184, 303, 422, 541, 660, 779 <sup>1</sup>	Part 2 Final Follow-Up (Day 897 [±7 days]) <sup>3</sup> or Part 2 Early Termination <sup>4</sup>
	Predose <sup>5</sup>	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		Predose	LP	Postdose		
Optional Sibling SMA Data Collection <sup>20</sup>	X																X
Con Med Recording <sup>21</sup>	X-----X																
Adverse Event Collection <sup>22</sup>	X-----X																

ACEND = Assessment of Caregiver Experience with Neuromuscular Disease; AE = adverse event; Bi-PAP = bilevel positive airway pressure; Con Med = concomitant medication; CSF = cerebrospinal fluid; ECG = electrocardiogram; HINE = Hammersmith Infant Neurological Examination; LP = lumbar puncture; PK = pharmacokinetic(s); SAE = serious adverse event; WHO = World Health Organization.

<sup>1</sup> After the injection of study treatment on Day 1, subjects will remain at the study site for at least 24 hours (Day 2) for safety monitoring. There will be safety monitoring through telephone contact on the day following the injection of study treatment (i.e., Days 16, 30, 65, 184, 303, 422, 541, 660, and 779). During that telephone contact, changes in concomitant medications and AEs, information on the subject's daily ventilator/Bi-PAP use, and health status will be recorded.

<sup>2</sup> If the study continues beyond Year 2 (i.e., beyond Day 778), then from Day 897 onwards, subjects will maintain evaluations for study treatment injection every 4 months with the schedule of events for those additional timepoints to alternate between the assessments done on Day 183 and those done on Day 302. Day 302 assessments would be performed at Day 897 and Day 183 assessments would be performed at Day 1016. That pattern would continue until the end of the study. Part 2 Final Follow-up Evaluation would then occur approximately 4 months after the last injection of study treatment.

- <sup>3</sup> The Part 2 Final Follow-up Evaluation will occur 4 months after the last injection of study treatment, which will either be on Day 778 or later, if Part 2 of the study continues beyond Year 2.
- <sup>4</sup> Subjects who terminate early will be encouraged to complete the safety evaluations scheduled for the Part 2 Early Termination Evaluation.
- <sup>5</sup> Inclusion/Exclusion Criteria, Informed Consent, and CSF PK must be performed on Part 2 Day 1 predose.
- <sup>6</sup> Vital signs include resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, pulse oximetry, and transcutaneous carbon dioxide. Pulse oximetry and transcutaneous carbon dioxide will be measured predose at each evaluation.
- <sup>7</sup> If Part 2 Day 1 study treatment injection is delayed more than 24 hours after the End of Part 1 visit, the following procedures must be done on Part 2 Day 1 predose: vital signs, weight, growth parameters, physical examination, ventilator use, neurological examination, and CSF PK. If Part 2 Day 1 study treatment injection is delayed more than 24 hours after End of Part 1 due to an AE, SAE, or other safety concern, or if Part 2 Day 1 study treatment injection is delayed more than 30 days after End of Part 1 for any reason, the following procedures must be done on Part 2 Day 1 predose: vital signs, weight, growth parameters, physical examination, ventilator use, neurological examination, ECG, CSF PK, and safety laboratory tests. Immunogenicity testing must also be repeated if Part 2 Day 1 study treatment injection is delayed more than 30 days after End of Part 1 for any reason.
- <sup>8</sup> Vital signs (resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, and pulse oximetry) will be collected at 4 timepoints: 1, 2, 4, and 6 hours (all  $\pm 15$  minutes) after the injection of study treatment only on Part 2 Day 1. On subsequent study days, vital signs will be collected 1 hour after the injection of study treatment.
- <sup>9</sup> Vital signs (resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, and pulse oximetry) will be collected on Day 2 within 20 to 24 hours after the injection of study treatment only.
- <sup>10</sup> At the evaluations scheduled for injection of study treatment, subjects will be assessed for the following growth parameters: body length, head circumference, chest circumference, and arm circumference. Growth parameters and weight are to be collected at the same time.
- <sup>11</sup> Neurological examinations include assessment of mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes. If sedation was used, please allow sufficient recovery time for the subject to be fully engaged in the examination because it is important that the data collected truly reflect the subject's neurological performance.
- <sup>12</sup> Predose and postdose.
- <sup>13</sup> ECG will be performed at Days 64, 183, 540, and 778, and at the Part 2 Final Follow-up Evaluation. ECG must be performed before obtaining blood samples for safety laboratory tests.
- <sup>14</sup> Blood chemistry, hematology, urinalysis, urine total protein panels, and coagulation parameters. Urine total protein and coagulation parameters will be assessed by local laboratories.
- <sup>15</sup> Samples for safety laboratory tests will be collected before the injection of study treatment at each dosing visit.
- <sup>16</sup> Blood samples for immunogenicity testing will be collected before the injection of study treatment on Days 64, 183, 302, 421, 540, 659, and 778.
- <sup>17</sup> Refer to [Error! Reference source not found.](#) for CSF PK sample schedule. The CSF samples will be analyzed for Nusinersen concentrations. The remaining CSF sample will be stored for optional future biomarker analyses.
- <sup>18</sup> Blood samples for PK assessment will be collected 4 hours ( $\pm 1$  hour) after the injection of study treatment only on Day 1. Refer to [Error! Reference source not found.](#) for the plasma PK sample schedule. Plasma PK samples will be collected predose on Days 64, 183, 540, and 778.
- <sup>19</sup> Overnight stay (at least 24 hours) is required after the first injection of study treatment. Following all subsequent injections, a stay of at least 1 hour at the study site is required; overnight stays are optional on these days.

<sup>20</sup>The optional assessment of sibling SMA data collection will be performed only after obtaining consent.

<sup>21</sup>In addition to concomitant medications, ancillary procedures will be recorded.

<sup>22</sup>AEs and SAEs will be collected in the case report form from the time of signing of the informed consent form described in Section and Section 15.3.2 of the protocol.



### PK Part1

<b>Table 3 Part 1 Pharmacokinetic Sampling Schedule<sup>1</sup></b>				
<b>Treatment Period</b>	<b>Part 1 Study Day</b>	<b>Timepoints</b>	<b>Blood Collection (mL)</b>	<b>CSF Collection (mL)<sup>2</sup></b>
Multiple Dose: LP Injection	Day 1	Predose	NA	0.5
		4 h ( $\pm$ 1 h) postdose	0.5	NA
	Day 15	Predose	NA	0.5
	Day 29	Predose	NA	0.5
	Day 64	Predose	0.5	0.5
	Day 183	Predose	0.5	0.5
	Day 302	Predose	NA	0.5
	End of Part 1	NA	0.5	NA

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

<sup>1</sup> Details on sampling, preparation, and shipment are included in the Study Laboratory Manual.

<sup>2</sup> Upon consent, the collected CSF samples from the study subjects will be stored for future investigation of possible biomarkers of SMA disease (e.g., CSF mRNA, CSF protein, CSF microRNA panel), for profiling of study drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, and immunogenicity assessments (including assay development and validation purposes), or for assessing other actions of Nusinersen with plasma and CSF constituents.

### PK Part2

<b>Table 4 Part 2 Pharmacokinetic Sampling Schedule for Subjects Randomized to Receive Nusinersen in Part 1<sup>1</sup></b>				
<b>Treatment Period</b>	<b>Part 2 Study Day</b>	<b>Timepoints</b>	<b>Blood Collection (mL)</b>	<b>CSF Collection (mL)<sup>2</sup></b>
Multiple Dose: LP Injection	Day 1	Predose	NA	0.5
		4 h ( $\pm$ 1 h) postdose	0.5	NA

**PK Part2**

	Day 120	Predose	NA	0.5
	Day 239	Predose	0.5	0.5
	Day 358	Predose	NA	0.5
	Day 477	Predose	0.5	0.5
	Day 596	Predose	NA	0.5
	Day 715	Predose	0.5	0.5
	Part 2 Final Follow-Up	NA	0.5	NA

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

<sup>1</sup> Details on sampling, preparation, and shipment are included in the Study Laboratory Manual.

<sup>2</sup> Upon consent, the collected CSF samples from the study subjects will be stored for future investigation of possible biomarkers of SMA disease (e.g., CSF mRNA, CSF protein, CSF microRNA panel), for profiling of study drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, and immunogenicity assessments (including assay development and validation purposes), or for assessing other actions of Nusinersen with plasma and CSF constituents.

**PK Part 2**

<b>Table 5 Part 2 Pharmacokinetic Sampling Schedule for Subjects Randomized to Receive Sham Procedure in Part 1<sup>1</sup></b>				
<b>Treatment Period</b>	<b>Part 2 Study Day</b>	<b>Timepoints</b>	<b>Blood Collection (mL)</b>	<b>CSF Collection (mL)<sup>2</sup></b>
Multiple Dose: LP Injection	Day 1	Predose	NA	0.5
		4 h ( $\pm$ 1 h) postdose	0.5	NA
	Day 15	Predose	NA	0.5
	Day 29	Predose	NA	0.5
	Day 64	Predose	0.5	0.5
	Day 183	Predose	0.5	0.5
	Day 302	Predose	NA	0.5
	Day 421	Predose	NA	0.5

## PK Part 2

	Day 540	Predose	0.5	0.5
	Day 659	Predose	NA	0.5
	Day 778	Predose	0.5	0.5
	Part 2 Final Follow-Up	NA	0.5	NA

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

<sup>1</sup> Details on sampling, preparation, and shipment are included in the Study Laboratory Manual.

<sup>2</sup> Upon consent, the collected CSF samples from the study subjects will be stored for future investigation of possible biomarkers of SMA disease (e.g., CSF mRNA, CSF protein, CSF microRNA panel), for profiling of study drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, and immunogenicity assessments (including assay development and validation purposes), or for assessing other actions of Nusinersen with plasma and CSF constituents.

## Description of Objectives and Endpoints

### 1.1 Primary Objectives and Endpoints

#### Part 1

The primary objective of Part 1 of this study is:

- To assess the safety and tolerability of Nusinersen in subjects with SMA who are not eligible to participate in the clinical studies ISIS 396443-CS3B or ISIS 396443-CS4.

The endpoints that relate to this objective are:

- Incidence of adverse events (AEs) and serious adverse events (SAEs)
- Change from baseline (see [Section 5](#)) in clinical laboratory parameters, electrocardiograms (ECGs), vital signs, and growth parameters
- Change from baseline (see [Section 5](#)) in neurological examination outcomes

#### Part 2

The primary objective of Part 2 of this study is:

- To assess the long-term safety and tolerability of Nusinersen in subjects with SMA who participated in Part 1 and completed their End of Part 1 Evaluation assessments.

The endpoints that relate to this objective are:

- Incidence of AEs and SAEs
- Change from baseline (see [Section 5](#)) in clinical laboratory parameters, ECGs, and vital signs
- Change from baseline (see [Section 5](#)) in neurological examination outcomes
- Coagulation parameters (activated partial thromboplastin time [aPTT], partial thromboplastin time [PTT], and international normalized ratio [INR]) and urine total protein

## **1.2 Secondary Objectives and Endpoint**

The secondary objective and endpoint of Part 1 of this study are as follows:

- To examine the pharmacokinetics (PK) of Nusinersen in subjects with SMA who are not eligible to participate in the clinical studies ISIS 396443-CS3B or ISIS 396443-CS4
- Nusinersen concentrations in plasma and cerebrospinal fluid (CSF)

The secondary objective and endpoint of Part 2 of this study are as follows:

- To examine the PK of Nusinersen in subjects with SMA who participated in Part 1 and completed their End of Part 1 Evaluation assessments
- Nusinersen concentrations in plasma and CSF
- Plasma antibodies to Nusinersen

## **1.3 Exploratory endpoints**

### **Part 1**

The exploratory objective of Part 1 of this study is:

- To explore the efficacy of Nusinersen in subjects with SMA who are not eligible to participate in the clinical studies ISIS 396443-CS3B or ISIS 396443-CS4.

The endpoints that relate to this objective are:

- Change from baseline in ventilator use
- Attainment of motor milestones assessed by Section 2 of the Hammersmith Infant Neurological Examination (HINE)

- Change from baseline in growth parameters (body length, head circumference, chest circumference, arm circumference, weight for age, weight for length, and head to chest circumference ratio) through final safety follow-up evaluation
- Clinical Global Impression of Change (physician and caregiver assessment) through final safety follow-up evaluation
- Plasma antibodies to Nusinersen

## **Part 2**

The exploratory objective of Part 2 of this study is:

- To explore the efficacy of Nusinersen in subjects with SMA who participated in Part 1 and completed their End of Part 1 Evaluation assessments.

The endpoints that relate to this objective are:

- Change from baseline in ventilator use
- Attainment of motor milestones assessed by World Health Organization (WHO) motor milestones and Section 2 of the HINE
- Change from baseline in growth parameters (body length, head circumference, chest circumference, arm circumference, weight for age, weight for length, and head to chest circumference ratio) through Part 2 Final Follow-up Evaluation
- Clinical Global Impression of Change (physician and caregiver assessment) through Part 2 Final Follow-up Evaluation and Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)

## **2 Statistical Methods: General Considerations**

In this SAP, the terms “control” and “previous control” refer to the set of subjects who were randomized to undergo the sham procedure during the Part 1.

In order to distinguish nominal visit names from duration defined in days, visit names will be referred to as “Day 15”, “Day 29”, etc., and “15 days” or “29 days” etc. will be used to define time intervals.

Due to emergent data from the Nusinersen clinical development program, the subjects in Part 1 ended the study early as follows: subjects randomized to sham terminated Part 1 first irrespective of Part 1 planned dosing days and subjects randomized to Nusinersen transitioned to Part 2 accordingly to their specific dose schedule. Part 2 was also terminated early to allow subjects to

enter the ISIS 396443-CS11 study. For these reasons, the actual study day associated with visits Part 1 and Part 2 will vary widely amongst subjects. Therefore, a windowing schema will be implemented for by visit analyses (see [section 4.1](#))

The safety population will include all subjects who are randomized and receive at least 1 dose of study treatment or sham procedure. Subjects randomized to receive sham procedure but incorrectly treated with Nusinersen will be counted in the ISIS 396443 group from the first dose of Nusinersen received.

The Efficacy set at each visit will be defined as the subset of subjects in the Safety set who has had the opportunity to be assessed at that visit (actual visit for Part 2 or windowed visit for integrated analysis see [section 4.1](#))

The PK population will include all subjects who are randomized and have at least 1 evaluable post dose or post sham-procedure PK sample.

The immunogenicity population will include all subjects who are randomized and have at least 1 evaluable post dose or post sham-procedure immunogenicity data.

For all time-to-event endpoints, (e.g., time to death or permanent ventilation), the date of first dose/sham administration will be used as the reference time point. If the date of first dose/sham procedure is incomplete, the date of randomization will be used.

Summary statistics will be presented throughout. For continuous endpoints, summary statistics will generally include number of subjects with data, mean, standard deviation, median, minimum and maximum. The mean changes from baseline will be estimated with 95% confidence intervals. For categorical endpoints, summary statistics will generally include: number of subjects dosed, number of subjects with data, and the percentage of those with data in each category. Frequency distributions will be presented as appropriate.

The statistical software, SAS® version 9.4 or above, will be used for all summaries and statistical analyses.

For Part 2, a limited number of summaries will be produced. The approach will be to preserve the Part 1 study groupings as follows for Safety and Efficacy population:

- Previous control.
- Previous ISIS 396443.
- Total (previous control and previous ISIS 396443).

For the integrated analyses, the Safety displays will be grouped as follows:

- Previous control, Part 1, from their first sham procedure in Part 1.
- Previous control, Part 2, from their first ISIS 396443 dose in Part 2.
- Previous ISIS 396443, Part 1 and 2, from their first ISIS 396443 dose in Part 1.
- Total (Previous control Part 2 and previous ISIS 396443 Part 1 and 2)

For the integrated analyses, the Efficacy displays will be grouped as follows:

- Previous control, Part 1, from their first sham procedure in Part 1.
- Previous control, Part 2, from their first ISIS 396443 dose in Part 2.
- Previous control, Part 1 and 2, from their first sham procedure in Part 1.
- Previous ISIS 396443, Part 1 and 2, from their first ISIS 396443 dose in Part 1.

### **3 Study Subjects**

#### **3.1 Subject Accountability**

The number of subjects who were randomized, split by stratification factor (age of symptom onset: <6 and  $\geq$ 6 months), who were dosed, who completed Part 1 but not in Part 2, who completed Part 2, who discontinued treatment along with the reasons withdrawing from the study, will be presented.

Listings of those subjects who withdrew from the study and the reasons for discontinuation/withdrawal will be presented. Subjects who died during the study will be listed separately.

#### **3.2 Demography and Baseline Disease Characteristics**

Baseline data (demography, birth characteristics and SMN history, medical history and baseline characteristics) will be summarized. Medical history was collected only at screening of Part 1.

Demography includes age at Part 1 first dose ( $\leq$ 7,  $>7\leq$ 18,  $>18$  months to 2 years,  $\geq$ 2 to  $<$  3 years,  $\geq$ 3 to  $<$ 4 years,  $\geq$ 4 to 5 years, and  $>5$  years) and age at Part 2 first dose (similar categories), sex, ethnicity, and race. SMA history will include age at SMA onset (months), age at SMA diagnosis (months), and disease duration.

Baseline disease characteristics will include HINE motor milestones and growth parameters – these assessments are further described in [Section 4](#).

Baseline will generally be defined as the closest measurement before the first dose/sham procedure. For Part 2, the baseline could be the predose assessment of Day 1, or the last assessment in Part 1, depending on the actual schedule of events in Part 2 see section 1.4.

Demographic and baseline disease characteristics will be presented for the Safety set as well as both repeated by age of SMA onset (< 6 months, >= 6 months).

### **3.3 Extent of Exposure**

The number of doses and or/ sham procedures will be summarized using frequency distributions. The amount of Nusinersen received will be summarized using summary statistics.

Overall time on study will be defined as the total number of days a subject is known to be followed on study and will be calculated as follows:

Overall time on study = (Last date on study) – (Date of first dose or first sham procedure) + 1.

The last date on study is defined as the date of the latest visit or evaluation or telephone contact, or time of death from all available data for a given subject.

Given the long half-life of Nusinersen, subjects are considered to be exposed to study drug from the time the first dose was administered to the last day of follow-up. Essentially, exposure is equivalent to time on study.

The dosing and time on study will be summarized only for Part 2 and integrated data.

### **3.4 Concomitant therapy**

A concomitant therapy is any drug or substance administered between Screening and or End of study Evaluation.

Throughout the study, Investigators or designated licensed physicians involved in the study may prescribe concomitant medications or treatments deemed necessary for treatment of AEs or to provide adequate supportive care. 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 (e.g., albuterol/ salbutamol, riluzole, carnitine, sodium phenylbutyrate, valproate, hydroxyurea). All concomitant medications will be coded using the World Health Organization drug dictionary (WHO Drug).

A concomitant 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 End of Part 2. These will be collected on the ancillary procedure page of the eCRF and will be coded using MedDRA.

For the purposes of analysis, a concomitant therapy (including medication or procedure) is defined as any therapy that was taken or administered on or after the first injection of Nusinersen or sham procedure. For subjects receiving the sham procedure in Part 1 who subsequently roll over onto Nusinersen in Part 2, an additional definition will be applied and therapies will be considered to be concomitant medication if they were taken on or after the first dose of Nusinersen in Part 2. This definition includes therapies that were started prior to the initiation of injection of Nusinersen or sham procedure if their use continued on or after the first injection of Nusinersen. In order to define concomitant therapies with missing start or stop dates, the following additional criteria will be used:

- if both the start and stop dates of a particular therapy were missing, that therapy is considered concomitant;
- if the start date of a therapy was missing and the stop date of that therapy fell on or after the date of dosing, that therapy is considered concomitant;
- if the start date of a therapy was prior to the date of dosing and the stop date of that therapy was missing and the therapy was listed as continuing, that therapy is considered concomitant;
- if the start date of a therapy was prior to the date of dosing and the stop date of that therapy was missing and the therapy was listed as *not* continuing, that therapy is considered concomitant, or
- if the start or stop date of a therapy is partial, it will be considered concomitant where it is not possible to rule out that it was not taken concomitantly.

The number and percentage of subjects who were taking each type of concomitant medication at baseline and during the study will be presented. The number and percentage of subjects taking each type of ancillary procedure will be presented by preferred term.

### **3.5 Protocol deviations**

Protocol deviations identified during site monitoring will be captured in a protocol deviation log and categorized as major or minor deviations. These deviations will be listed. Major protocol deviations will be summarized for only Part 2 as part of this SAP.

## 4 Efficacy data (exploratory)

### 4.1 Visit Windowing

#### *Part 2 analysis*

Part 2 will terminate early and the end of study visit will occur at different time points, for this reason the end of study visit will be re-labeled and windowed as follow:

- If the actual study day falls within +/- 7 days of any of Part 2 Day xx visit, the end of study visit will be re-labelled as the corresponding scheduled visit.
- Otherwise, the end of study visit will be re-labelled as an unscheduled visit.

#### *Integrated analysis*

The HINE motor milestones, growth parameters and CGI, laboratory data, CSF and Plasma concentrations integrated analyses will be windowed as follows:

Study days  $\leq 1$  will be labelled Baseline

- Study days  $> 1$  to  $\leq 22$  will be labelled Day 15
- Study days  $> 22$  to  $\leq 47$  will be labelled Day 29
- Study days  $> 47$  to  $\leq 123$  will be labelled Day 64
- Study days  $> 123$  to  $\leq 242$  will be labelled Day 183
- Study days  $> 242$  to  $\leq 362$  will be labelled Day 302
- Study days  $> 362$  to  $\leq 482$  will be labelled Day 422
- Study days  $> 482$  to  $\leq 600$  will be labelled Day 540
- Study days  $> 600$  to  $\leq 719$  will be labelled Day 659
- Study days  $> 719$  to  $\leq 838$  will be labelled Day 778
- Study days  $> 838$  to  $\leq 958$  will be labelled Day 898
- Study days  $> 958$  to  $\leq 1078$  will be labelled Day 1018
- Study days  $> 1078$  to  $\leq 1198$  will be labelled Day 1138

If multiple results are allocated into the same window then the closest non-missing result to the target date will be chosen.

In order to account for the denominator across the analysis visits, an Efficacy set for each windowed analysis visit (Day X) will be defined as: a subset of subjects in the Safety set who have the opportunity to be assessed at the Day X visit, where the lower bound for the windowed analysis visit are denoted: L= lower. Specifically, it will include all subjects with a time difference of at

least L days between the date of first dose and the end of Part 2. A subject who has died or withdrawn will be included provided that there is a difference of at least L days between the date of first dose and the end of Part 2. In a situation where the subject was known to have withdrawn from the study at a date within the window due to rolling over to commercial drug then they will be excluded from the corresponding Efficacy set and subsequent analysis visit Efficacy sets.

An evaluable set will be defined for each analysis visit as the subset of subjects in the Efficacy set at the analysis visit who meet the following criteria:

- Value assigned from the windowing
- Subject who died or discontinued from the study – no imputation of efficacy data will be performed for such subjects and they will be considered non-responders.
- No value assigned from windowing, but ongoing in study with a subsequent assessment assigned to a later analysis visit. In this situation, a value will be imputed as the value from the analysis visit prior to the missing visit.

A couple of considerations as part of this final criterion are as follows:

- In a situation where a subject does not have a windowed value at analysis visit X and is known to discontinue at the next visit, X+1, without an efficacy assessment, the subject would not be included within the denominator for the responder analysis at visit X, but they would be included as a non-responder at visit X+1.
- In a situation where a subject does not have a windowed value at analysis visit X and is known to discontinue at the next visit, X+1, with an efficacy assessment prior to the discontinuation, the subject would be included within the denominator for the responder analysis at visit X and the last observed value will be utilized, and then they would be included as a non-responder at visit X+1.

## 4.2 Ventilation data

Ventilator use during the study will be tracked at study visits, with details collected using a ventilation diary or other available sources. Caregivers will initiate collecting ventilation diary data for all subjects. Note, once the first entry of ventilation use has been recorded on the diary, then the diary will then need to be completed until the end of the study. If the daily number of hours of ventilation and the type is the same for consecutive days, then the first and last day date will be captured, together with the daily hours and type of ventilation – invasive/non-invasive (for prophylaxis or treatment). For days on which ventilator use was not recorded, the number of hours of use will be imputed using the greater of the daily values among the days that flank the missing day(s).

#### 4.2.1 Change in Ventilator use

In the statistical analysis of Part 1, for each subject the mean number of hours of ventilator was calculated amongst the periods. These periods were created from the first dose of Nusinersen or sham procedure to the nominal visit. (example Day 1: > from Day 1 to Day 15 visit; Day 15: from Day 15 inclusive to Day 29 visit). In Part 2 for each subject the mean number of hours will be calculated for a monthly interval (30 days) starting from the first dose of Nusinersen or sham procedure. The intervals will be: month 1 from Day 1 inclusive to Day 30, Month 2 from Day 30 inclusive to Day 60, and so on.

The change from baseline over time will be presented and a plot of both total and change from baseline will be presented.

For each subject, the total number of hours of ventilation support required during the study will be calculated and prorated according to number of days on study. The percentage of time on ventilator support in total and by type either invasive prophylactic, invasive treatment, or non-invasive prophylactic and not invasive treatment will be presented.

The different respiratory aids/therapies used will be listed

#### 4.2.2 Time to Death or Permanent Ventilation

Permanent ventilation is defined as tracheostomy or  $\geq 16$  hours of ventilatory support per day continuously for  $> 21$  days in the absence of an acute reversible event. This endpoint was not pre-specified in the protocol.

Time to permanent ventilation and acute reversible events will be determined by medical review. For all time-to-event endpoints (e.g., time to death or permanent ventilation), the date of first dose/sham administration will be used as the reference time point. If the date of first dose/sham procedure is incomplete, the date of randomization will be used. Only events that were adjudicated will be included in the analysis. Subjects who do not meet the endpoint definition will be censored at the last occasion the subject was seen (either in-person visit or by telephone contact), irrespective of whether the subject has completed a full course of treatment and whether the subject has completed the study or withdrawn prematurely. However, in the event that a subject has begun a ventilation diary, the latest entry in the diary will be used as the date of censoring. Of note, once a ventilation diary has been started, the diary will then need to be completed every day until the end of the study.

The median times to death or permanent ventilation and associated 95% confidence limits will be estimated using the Kaplan-Meier method. The proportion of subjects who meet such an event will be estimated from the Kaplan-Meier curve.

### 4.2.3 Survival

Survival rates over time will be estimated from the Kaplan-Meier curve for time to death based on the Safety set.

### 4.3 Growth parameters

Growth parameters comprise length for age, weight for age, weight for length, head circumference for age, chest circumference, head to chest circumference ratio, and arm circumference are to be assessed through the study.

The WHO child growth standards (WHO Child Growth Standards, 2006) will be used to determine the percentiles for each parameter. The WHO provides a SAS macro (SAS igrowup package) which can be downloaded from a website [WHO Anthro] and this will be utilized to calculate the percentiles for each child.

The change from baseline to each windowed analysis visit will be summarized using descriptive statistics for the following growth parameters: weight for age, weight for length/height, head circumference, chest circumference, head to chest circumference ration and arm circumference. Presentations will be made for all groups.

Two additional analyses will be performed examining the change between WHO percentiles (Sproule 2012). In the first analysis, subjects with weight below the 5<sup>th</sup> percentile based on WHO growth charts, in the second, subjects with decreased growth velocity resulting in falling more than major percentiles (3<sup>rd</sup>, 5<sup>th</sup>, 10<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup>, 90<sup>th</sup>, 95<sup>th</sup>, 97<sup>th</sup>) over a 6-month period will be used. Both analyses will be presented by windowed analysis visit by index treatment group and overall.

Growth parameters will also be summarized by age of SMA onset ( $\leq 6$  months,  $>6$  months).

#### *Missing values*

No imputation will be performed for missing data.

### 4.4 Number of Hospitalizations

The number and reason for hospitalizations per subject will be summarized.

### 4.5 Motor milestones

Motor milestones assessments are made using HINE, Section 2 and WHO motor milestones (after V4 of the protocol). Each assessment is described in further detail below.

#### 4.5.1 HINE motor milestones

HINE Section 2 comprises eight tests for motor milestones: head control, sitting, voluntary grasp, ability to kick in supine position, rolling, crawling, standing, and walking.

There are 26 possible motor milestones that can be achieved using this schema (presented below). A subject whose results all appear in the first column (unable to maintain head upright, cannot sit, no grasp, etc.) has not achieved any motor milestone. A subject whose results all appear in the second column (head wobbles, sits with support at hips, uses the whole hand for a voluntary grasp, etc.) has achieved 8 motor milestones. A subject whose results all appear in the third column (head maintained upright all the time, props when sitting, index finger and thumb but immature grasp, etc.) has achieved 16 motor milestones.

*Attainment of motor milestones assessed by Section 2 of the Hammersmith Infant Neurological Examination (HINE)*

For the integrated analysis, the HINE motor milestones analyses will be presented by windowed analysis visits (see section 4.1).

The definition of a motor milestone responder is based upon the motor milestones categories in Section 2 of the HINE, with the exclusion of voluntary grasp as follows:

- Subject demonstrates at least a 2-point increase in the category of ability to kick or increase to the maximal score on that category (touching toes), or a 1-point increase in the motor milestones category of head control, rolling, sitting, crawling, standing, or walking AND
- Among the motor milestone categories, with the exclusion of voluntary grasp, there are more categories where there is improvement, as defined in (i) below, than there are categories where there is worsening (as defined below).
- Subjects who die or withdraw from the study on or prior to the visit will be counted as non-responders and will be included in the denominator

Improvement in motor milestones is defined as a 2-point increase in the total motor milestones score, where voluntary grasp is excluded. For example, (i) a 1-point increase in head control and a 1-point increase in rolling is considered a response, (ii) a 2-point increase in rolling is considered a response, (iii) a 2-point increase in rolling with a 1-point decrease in head control is considered a non-response. Worsening is defined as at least a 2-point decrease or a decrease to the lowest possible score of no kicking. For the other 6 categories, worsening is defined as at least 1-point decrease.

To illustrate this responder definition, examples are provided below. In these examples, it is assumed that there are no changes in motor milestone categories other than those stated.

- A subject with a 2-point increase of ability to kick, a 1-point increase in rolling, and a 1-point decrease in head control is a responder.
- A subject with a 1-point increase of ability to kick from “touches legs” to “touches toes” is a responder.
- A subject with a 1-point increase of head control and a 1-point decrease rolling is a non-responder.
- A subject with a 1-point increase of rolling and a 2-point decrease in ability to kick is a non-responder.
- A subject with a 1-point increase of rolling and a 1-point decrease of ability to kick from “upward vertically” to “kicks horizontal, legs do not lift” is a responder.
- A subject with a 1-point increase of rolling and a 1-point decrease of ability to kick from “kicks horizontal, legs do not lift” to “no kicking” is a non-responder.
- A subject with a 2-point increase of voluntary grasp is a non-responder.
- A subject with a 1-point increase of rolling and a 2-point decrease of voluntary grasp is a responder.

The response will be conducted on data from each visit and windowed visit separately for Part 2 and integrated analysis respectively (see section 4.1 for details about the windowing).

*Additional presentations of HINE motor milestones*

- The above responder analysis will be repeated for the subjects that are responder at last available assessment. For those subjects that are responders, the analysis visits when first respond occurred will be also displayed.
- The proportion of subjects who have achieved the follow milestones:
  - Full head control,
  - Stable sit or pivots,
  - Stand unaided,
  - Walk independently
 will be presented by visit and at the last available assessment (for the integrated analysis)
- The proportion of subjects who have achieved individual milestones will be presented by visit.

The shift from baseline to last available assessment (for the integrated analysis).

- The total motor milestones score achieved over time will be presented (for Part 2 only and the integrated analysis) .

- The change from baseline over time will be presented and a plot of both total and change from baseline will be presented (for Part 2 only and the integrated analysis) .

The analysis of motor milestones responders and summaries of total motor milestones score will also be conducted by age of SMA onset ( $\leq 6$  months,  $>6$  months).

#### Missing data

##### *Part 2 analysis*

For Part 2 HINE motor milestones, Baseline will be retrieved from Part 1 and it will be the last assessment in Part 1.

Some general rules for the approaches to imputation of missing data are described in the following sections.

- 1) If a subject has any milestones missing at baseline then the missing milestone will be imputed using the median baseline value within the age of SMA onset ( $\leq 6$  months,  $>6$  months). for the correspondent milestone level
- 2) If a subject has any missing milestones at any visit that is not last visit, then the missing milestone will be imputed using a linear interpolation at the milestone level for any actual scheduled study visits.
- 3) If a subject has any missing milestones at last visit, then the missing milestone will be imputed using the lowest observed value for milestone assigned within the age of SMA onset ( $\leq 6$  months,  $>6$  months) and group to which the subject belongs at that visit (e.g., if a previous control subject's last visit is Day 120, then the at lowest observed value for the previous control group at Day 120 is used for the non-missing values for the specific motor milestone).

##### *Integrated analysis*

For the integrated analysis, these rules will be applied:

For subjects randomized to Nusinersen in Part 1 then Part 1 and Part 2 will be considered as a one period and any data available will be used for any imputation. However, for subjects who were randomized to receive Sham in Part 1 then the two periods (Part 1 and 2) will be considered separately and no imputation will be allowed i.e., no interpolation between the 2 periods.



The imputation of missing data will follow these rules:

- 1) If a subject has any milestones missing at baseline then the missing milestone will be imputed using the median baseline value within the age of SMA onset ( $\leq 6$  months,  $>6$  months). for the correspondent milestone level
- 2) If a subject has any missing milestones at any other visit that is not last visit then the missing milestone will be imputed using a linear interpolation at the milestone level for any actual scheduled study visits (for last visit, please see bullet point 4).
- 3) Perform windowing selecting the closest non-missing milestone to the target date
- 4) If a subject has a least one milestone observed but some milestones are missing, then any missing milestones at the last visit will be imputed using the lowest observed value for that milestone, assigned within the age of SMA onset ( $\leq 6$  months,  $>6$  months) to which the subject belongs, at that windowed analysis visit. If only a date is available and all of the milestones are missing, then no imputation will be performed.

#### 4.5.2 WHO motor milestones

The WHO motor milestones are a set of six milestones in motor development, all of which would be expected to be attained by age 18 months in healthy children. The individual milestones are:

- Sitting without support
- Standing with assistance
- Hands and knees crawling
- Walking with assistance
- Standing alone
- Walking alone

The motor milestones are assessed at visits, after the V4 protocol approval at sites, using the WHO motor milestone criteria [WHO Multicentre Growth Reference Study Group 2006]. As part of the assessment, the examiner records an overall rating of the subject's emotional state and then for each milestone one of the following four classifications:

- No (inability) – Child tried but failed to perform the milestone
- No (refusal) – Child refused to perform despite being calm and alert
- Yes – Child was able to perform the milestone

- Unable to test – Could not be tested because of irritability, drowsiness or sickness.

In an effort try and attach greater precision to the dates of first achievement of milestones, the caregivers are recording the first date they observe the child achieving each milestone and this is additionally captured in the CRF

#### *Missing values*

No imputation will be performed for missing data.

### **4.6 Clinical Global Impression of Change (Investigator and Caregiver assessments)**

The Clinical Global Impression of Change (CGIC) is assessed at specific visit. At each visit the investigator and the subject's caregiver each score how the subject has changed compared to 'admission to the project'. The assessment is scored on a 7- point ordinal scale (1= Very much improved, 2 = "Much improved", 3 = "Minimally improved", 4= "No change from baseline", 5= "Minimally worse", 6= "Much worse", 7 = "Very much worse").

In order to investigate response on these scales, three definitions of responder will be defined: "Much improved": Responder ( $\leq 2$ ) versus Non-responder ( $\geq 3$ ), "Any improvement": Responder ( $\leq 3$ ) versus Non-responder ( $\geq 4$ ), "No worsening": Responder ( $\leq 4$ ) versus Non-responder ( $\geq 5$ ). At the start of Part 2 for subjects that were previously on sham, the question was changed to be how the subject has changed compared to 'admission to Part 2, while for subjects remaining on Nusinersen, the question remained how the subject has changed compared to 'admission to the Part 1.'

The proportion of subjects who were responders based on the "Much improved", 'Any improvement' and 'No worsening' definition of responder will be presented by windowed analysis visit.

These analyses will be performed separately for investigators and caregivers' responses. The analyses will also be stratified based on age of SMA onset ( $\leq 6$  months,  $>6$  months). In Part 2 the data will be collected referring to the admission to Part 1 or 2 respectively depending on the original treatment in Part 1: previous Sham subjects will be scored in comparison to 'admission to Part 2', previous Nusinersen subjects will be scored in comparison to 'admission to Part 1

#### *Missing values*

No imputation will be performed for missing data.

## 4.7 ACEND

The ACEND includes a total of seven domains assessing physical impact (including feeding/grooming/dressing, sitting/play, transfers, and mobility) and general caregiver impact (including time, emotion, and finance) and each domain comprises several items. All the ACEND scores will be listed and not summarized for this analysis being the data collected only after the latest version of the protocol.

The ACEND includes a total of seven domains assessing physical impact (including feeding/grooming/dressing, sitting/play, transfers, and mobility) and general caregiver impact (including time, emotion, and finance) and each domain comprises several items. The total score for a domain with  $n$  items, each item assessed on ordinal scale from 1 to  $z$ , is derived as follows: 100 multiplied by (Mean of the  $n$  items in the domain - 1) divided by ( $z-1$ ). This total score will be on a scale of 0 to 100 with a higher score indicating a greater impact on the caregiver. At least two items for the time domain and one item for the remaining domains need to be non-missing for a total to be calculated; else the total score will be set to be missing.

## 4.8 Biomarkers

CSF and plasma samples collected as outlined in the schedule of events will be tested for pNf-H concentration.

Baseline pNf-H is defined as the most recent non-missing measurement collected prior to the first dose or Day 1. If any pNf-H measurement is non-missing prior to the first dose, the value closest to and prior to the first dose is used as baseline value. If no pNf-H measurement is available prior to the first dose but any non-missing pNf-H measurement on Day 1 is collected, the value closest to the first dose on Day 1 is used as baseline value.

### 4.8.1 pNf-H concentration and change in pNf-H

Baseline pNf-H concentrations will be summarized overall, by age of onset using descriptive statistics. Spearman correlation coefficients between baseline pNf-H and one of the baseline measurements (Baseline weight, Age at first dose, gestational age, HINE motor milestones score) and the corresponding  $p$ -values testing whether such correlation is equal to zero will be presented

Summary tables will be presented for pNf-H concentration, change and percentage change from baseline pNf-H by visit. A plot of mean over time will be presented for pNf-H concentration on the semi-logarithmic scale. The mean change from baseline and percentage change from baseline, respectively will be presented on the original scale.

#### **4.8.2 Change in CSF SMN protein concentration**

An appropriate assay for SMN protein in the CSF has not been identified. Once available, analyses will be performed to explore and characterize this across the study endpoints.

#### **4.9 Sibling SMA Data Collection**

If any subject has or had a sibling(s) with SMA and if consent is given, data for the sibling(s) will be collected at Day 1 and at the End of Study Evaluation/Early Termination Visit. Data to be collected from siblings with SMA will be non-biologic and noninvasive and will include historical data for SMN2 gene copy number and sibling treatment history.

Part of protocol amendment 4 incorporated retrospective and potential prospective capture of data on siblings of subjects enrolled into protocol 232SM202 who were affected by SMA. Depending on the amount and completeness of data collected it may be possible to perform some analyses comparing subjects in protocol 232SM202 who were dosed versus siblings. Differences in age at symptom onset, age at death and highest motor function attained between such siblings will be explored.

#### **4.10 Interim Analyses**

No formal interim analyses will be conducted.

### **5 Safety Data**

Analyses of safety data will include adverse events (Section 5.1), laboratory data (Section 5.2), ECGs (Section 5.3), vital signs (Section 5.4), and neurological examinations (Section 5.5).

Analyses of safety data will be based on the Safety Set. Baseline is defined as the last non-missing results prior to the first dose of Nusinersen or sham procedure.

#### **5.1 Clinical adverse events**

All adverse events will be analyzed based on the principle of treatment emergence. An adverse event will be regarded as treatment emergent if it was present prior to receiving the first dose of Nusinersen or sham procedure and subsequently worsened in severity, or was not present prior to receiving the first dose of Nusinersen or sham procedure but subsequently appeared. For subjects receiving the sham procedure in Part 1, an additional treatment emergent definition will be used to define if the adverse event is treatment emergent to the first dose of Nusinersen in Part 2.

In the situation where change in severity (but no change in seriousness) occurs for an adverse event, study sites are instructed to enter an end date and start a new record for the adverse event. In the new record, the changed severity is to be recorded and the start date will be set to be the end date of the previous record. These records will be programmatically linked by preferred term and start/end date. Consider three scenarios:

- The first AE record occurs prior to the first dose, the second AE record occurs on or after the first dose, and the severity increases: only the second record will be counted as treatment-emergent.
- The first AE record occurs prior to the first dose, the second AE record occurs on or after the first dose, and the severity decreases: neither record will be counted as treatment-emergent.
- Both records occur on or after the first dose: if the AE severity on the second record is worse than the severity on the first record, then both records will be counted as treatment-emergent. However, if the severity decreases, then only the first record will be counted as treatment-emergent.

Of note, when counting the total number of treatment-emergent events, events linked through change in severity will still be counted as separate events.

For events with missing start or stop dates, the following criteria will be used for the purpose of identifying treatment-emergent adverse events:

- if both the start and stop dates for a particular event are missing, then the event is considered to have occurred on or after the first dose of study treatment;
- if the start date for a particular event is missing and the stop date/time falls after the first dose date/time, then the event is considered to have occurred on or after the first dose of study treatment; and
- if the start time is missing and the start date is same as the first dosing date, then the event is considered to have occurred on or after the first dose of study treatment.

If it cannot be determined whether an event has occurred on or after dosing due to a missing or partial date, then the event will be assumed to have occurred on or after the first dose for the purpose of identifying treatment-emergent AEs.

Due to the long half-life of Nusinersen, analyses of treatment-emergent AEs will include all events reported during the study.

AEs will be coded using the MedDRA dictionary. This coding system provides more than five levels to classify adverse events. In general, adverse events will be presented by system organ class and preferred terms but other classifications may be used if warranted.

The incidence of treatment-emergent AEs will be summarized. A subject having the same AE more than once will be counted only once in the incidence for that AE. The summary tables will include incidence estimates for overall system organ class as well as for preferred terms within each system organ class. Incidence will be presented by decreasing order by system organ class and by decreasing order by preferred term within each system organ class. The most common AEs, i.e., those that occurred in at least 3 subjects, will be presented. Upon examination of the actual data, different cut-offs may be used if it is deemed more appropriate.

#### **5.1.1 Adverse events by severity**

The investigator is to record the severity of each adverse event as mild, moderate, or severe. If a subject experiences the same AE multiple times, the event with the worst severity will be counted. The incidence within each category will be presented. The incidence of severe events will be summarized.

#### **5.1.2 Adverse events by relationship to study treatment**

The investigator is to record the degree to which each AE is related to study drug (not related, unlikely or remotely related, possibly related, and related). If a subject experiences the same AE multiple times, the event with the strongest relationship to study drug will be counted. The incidence within each category will be presented. The incidence of drug-related events (those categorized as possibly related or related) will be summarized.

#### **5.1.3 Serious adverse events**

The incidence of treatment-emergent SAEs will be summarized by time of onset by 3-month time intervals. All SAEs will be listed including any that occurred prior to commencement of study treatment.

#### **5.1.4 Adverse events that led to discontinuation from treatment and/or withdrawal from study**

The incidence of AEs that led to discontinuation of study treatment and those that led to withdrawal from the study will be presented.

### **5.1.5 Deaths**

The incidence of death will be summarized. All deaths will be listed including cause of death. The incidence of events that led to death will be presented.

### **5.1.6 Presentations**

The following presentations will be shown for the integrated analysis:

- an overall summary showing, the number and percentage of subjects with an AE, a moderate or severe event, a severe event, a possibly or related event, a related event, a serious event, an event that led to discontinuation of study drug, and an event that led to withdrawal from the study, repeated also by age at symptom onset
- incidence by primary system organ class and preferred term, repeated also by age at symptom onset
- Frequency of adverse events by system organ class and preferred term, repeated also by age at symptom onset
- incidence of mild, moderate and severe events by primary system organ class and preferred term
- incidence of severe events by primary system organ class and preferred term
- incidence of not related, unlikely to be related, possibly related, and related events by primary system organ class and preferred term
- incidence of drug-related events by primary system organ class and preferred term
- incidence of SAEs by primary system organ class and preferred term and a listing of SAEs
- incidence of death and a listing of each death
- incidence of events that led to death
- incidence of events leading to discontinuation of study drug by primary system organ class and preferred term and a listing of such events
- incidence of events leading to withdrawal from the study by primary system organ class and preferred term and a listing of such events
- incidence of events of special interest – AEs following dosing procedure/LP, AEs by antibody status etc., by primary system organ class and preferred term and a listing of such events.

To avoid the potential for misleading interpretation of analysis of AEs, no statistical testing will be performed.

Only the following presentations will be shown for Part 2 analysis:

- an overall summary showing, the number and percentage of subjects with an AE, a moderate or severe event, a severe event, a possibly or related event, a related event, a serious event, an event that led to discontinuation of study drug, and an event that led to withdrawal from the study
- incidence by primary system organ class and preferred term
- Incidence of serious adverse events by primary system organ class and preferred term and a listing of SAEs.
- Frequency of adverse events by system organ class and preferred term

#### **5.1.7 Subgroup analysis**

For the integrated analysis, the overall summary of Adverse events, the incidence of all AEs and SAEs by primary system organ class and preferred term tables will be repeated by age of onset ( $\leq 6$  months,  $>6$  months).

## **5.2 Clinical laboratory data**

The following clinical laboratory parameters are to be assessed:

- Blood chemistry: total protein, albumin, creatinine, cystatin C, creatine phosphokinase, blood urea nitrogen, total serum bilirubin (direct and indirect), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glucose, calcium, phosphorus, chloride, sodium, potassium, bicarbonate, creatine kinase (MB [isoenzyme expressed in the myocardium], BB [isoenzyme predominantly expressed in the brain], and MM [isoenzyme expressed in skeletal muscle]), gamma-glutamyl transferase
- Hematology: red blood cells, hemoglobin, hematocrit, platelets, white blood cells, white blood cell differential
- Urinalysis: specific gravity, pH, protein, glucose, ketones, bilirubin, red blood cells, white blood cells, epithelial cells, bacteria, casts, crystals
- Total urine protein
- Coagulation parameters (aPTT, PTT, and INR) will be collected.

As described below, laboratory data will be examined using an analysis of “shifts” and changed from baseline by windowed visits. However, further analyses may be undertaken to more fully characterize potential laboratory safety signals, such as assessment of timing, recovery, reversibility, association with AEs, etc.



Each subject's laboratory values will be classified according to whether the test result is "low" (i.e., below the lower limit of normal [LLN]), "normal" (within the normal range), or "high" (i.e., above the upper limit of normal [ULN]). If a subject is missing a baseline value but had a post-baseline value, then the baseline assessment is labeled as "unknown". Likewise, if a subject had a baseline value but had no post-baseline values, then the minimum and maximum are labeled as "unknown". Post-baseline laboratory results are defined as any assessment taken after the first dose, including data collected from local laboratories. The shifts (relative to the normal range) from baseline to the minimum and maximum post-baseline values will be presented. Should a treatment affect a laboratory parameter, that parameter could be affected at different times for different subjects. Therefore, these analyses present the most extreme values for each subject. For many laboratory parameters, the effect could be in either direction, (i.e., an increase or a decrease), so both the maximum and minimum values have been analyzed. Based on values from the laboratory parameters, the shifts (relative to the normal range) from baseline to low and high will be calculated. If a subject's value shifts, it can change from normal to either low or high, from low to normal or high, from high to normal or low, or from unknown to low, normal, or high. For each parameter, the incidence of shift to low will be summarized using the minimum post-baseline values. Shift to low includes subjects with a normal, high, or unknown baseline value and at least one post-baseline value of the given test. Similarly, the incidence of shift to high will be summarized using the maximum post-baseline values. Shift to high includes subjects with a low, normal, or unknown baseline value and at least one post-baseline value.

For many parameters processed centrally, the laboratory has additional ranges, e.g., a notification to the site by telefacsimile that the subject's result is far enough outside the normal range to warrant notification, and/or a panic alert involving a telephone call made to the investigational site to notify them that the subject's result is outside of the normal range with potentially critical implications for the subject (see the table below). With the exception of liver function tests, should such ranges be available for a parameter, these additional categories will be presented in listings. For shift tables of laboratory parameters, those additional categories will be combined with the conventional categories. Specifically:

- LT (Low – telefacsimile) and LP (Low panic) will be classified as L (Lower limit of normal)
- HT (High – telefacsimile) and HP (High panic) will be classified as H (Upper limit of normal)

For liver function tests, the additional categories will be defined to present the baseline and post-baseline values as within the upper limit of normal, >1 to < 3 x ULN, >3 to 5 x ULN, > 5 to 10 x ULN, >10 to 20x ULN. For shift tables of laboratory parameters, additional categories will be combined with the conventional categories. Specifically, >20x ULN for ALT/AST; >1 to 1.5 x ULN, >1.5 to 2 x ULN, and >2 x ULN for total bilirubin; and >1 to 1.5 x ULN and >1.5 x ULN for alkaline phosphatase. In addition, shift from baseline in ALT and AST will be summarized by elevation in bilirubin (maximum post-baseline bilirubin >1.5 x ULN and >2 x ULN).

The minimum, median and maximum versus time profiles (windowed visits) for some laboratory parameters of interest will be presented graphically.

### **5.3 ECG**

ECGs are assessed at a central reading laboratory, and the results provided back to sites to be entered into the eCRF. ECG qualitative results include an overall interpretation of 'normal', 'abnormal but not clinically significant' or 'abnormal and clinically significant'. Quantitative results will not be captured in the clinical database.

The number and percentage of subjects with shifts from normal to each of the categorical values denoting an abnormal scan (abnormal not AE, abnormal AE) will be summarized by treatment group. A listing of subjects with abnormal status in ECG will be presented.

### **5.4 Vital signs**

Vital signs are to be measured at Screening, pre-dosing and at various timepoints post-dosing on dosing days. At each of these times, resting systolic and diastolic blood pressure, pulse rate, respiratory rate, temperature, pulse oximetry, and pre-dosing transcutaneous carbon dioxide will be measured.

The number and percentage of subjects meeting selected criteria post-baseline and outliers will be summarized only for the integrated analysis.

### **5.5 Neurological examinations**

Neurological items, comprising mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes, will be assessed. These assessments will be done at Screening, pre-dose, and post-dose.

The result collected for the majority of the tests is 'normal' or 'abnormal', however the assessment of sensations is reported as 'present' or 'absent' and the assessment of reflexes is captured on an ordinal scale. For each test, changes secondary to SMA will be recorded. For each test and post-dosing time point, the number and proportion of subjects who moved from 'Normal' to 'Abnormal'

will be presented, and the number and proportion who move from ‘Present’ to ‘Absent’ will be presented for sensations. For the assessment of reflexes, decreases (from pre-dosing 1 to post-dosing 0, 2 to 1. etc.) will be presented. Only changes not deemed secondary to SMA will be presented in the integrated analysis. The percentage of the different results for each test will be presented graphically by visit.

### **5.6 Interim safety analyses**

No formal interim analyses are planned for safety but data will be reviewed on an ongoing basis by the Sponsor and the Medical Monitor.

## **6 Pharmacokinetic data**

CSF and plasma samples will be collected at protocol designated times for Nusinersen or sham procedure pharmacokinetic assessments in the Pharmacokinetic Population. The Pharmacokinetic Population includes all subjects who are dosed and for which there is at least one evaluable post-dose procedure pharmacokinetic sample.

The PK analysis will be on integrated data and the same windowing approach used for the efficacy analysis will be used.

### **6.1 CSF concentration data**

CSF concentrations of Nusinersen or sham procedure, along with the scheduled (nominal) and actual sampling times (i.e., time from IT dosing) will be listed (when applicable) for each subject and day. Differences between scheduled and actual sampling times will also be listed for all subjects. Percentage differences between actual administered dose and nominal dose will also be listed.

CSF concentrations below the lower limit of quantification (LLOQ) will be indicated by “BLQ”. For the purpose of calculating typical descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) for CSF concentrations, all BLQ values will be set to zero. Mean CSF concentrations that are BLQ will be presented as BLQ, and the SD and %CV will be reported as not applicable. Summary statistics of CSF concentrations of Nusinersen or sham procedure CSF concentrations will be tabulated by windowed visit for the integrated analysis only. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from descriptive statistics if there are large deviations between scheduled and actual sampling days or times, or large deviations between actual dose and nominal dose.

CSF concentrations of Nusinersen and sham procedure CSF concentration versus time (actual) profiles for each subject, as well as the mean ( $\pm$ SE) CSF concentration versus time (scheduled) profiles will be presented graphically on linear and semilogarithmic scales. Samples may be excluded from the mean plots if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

Due to the limited CSF samples collected no CSF pharmacokinetic parameters will be calculated.

## 6.2 Plasma concentration data

Plasma concentrations of Nusinersen, along with the scheduled (nominal) and actual sampling times (i.e., time from IT dosing) will be listed (when applicable) for each subject and day. Percentage differences between scheduled and actual sampling times will also be listed for all subjects. Percentage differences between actual administered dose and nominal dose will also be listed.

Plasma concentrations below the lower limit of quantification (LLOQ) will be indicated by “BLQ”. For the purpose of calculating typical descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) for plasma concentrations, all BLQ values will be set to zero. Mean plasma concentrations that are BLQ will be presented as BLQ, and the SD and %CV will be reported as not applicable. Summary statistics of plasma concentration of Nusinersen and sham procedure plasma concentrations will be tabulated by windowed visit for the integrated analysis only. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from descriptive statistics if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

Plasma concentration of Nusinersen and sham procedure plasma concentration versus time (actual) profiles for each subject, as well as the mean ( $\pm$ SE) plasma concentration versus time (scheduled) profiles will be presented graphically on linear and semilogarithmic scales. Samples may be excluded from the mean plots if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

## 7 Immunogenicity data

Immunogenicity (IM) testing (anti-Nusinersen antibody positivity), using designated plasma samples collected from each study subject, is planned to be conducted and reported. Immunogenicity plasma samples collected at other time points for Nusinersen concentration determinations may also be evaluated for IM testing if of further interest and deemed warranted by the pharmacokineticist. An individual sample result will be designated ‘antibody positive’

based on both positive screening (from Part 1) and confirmation assay results (i.e., confirmed positive result), and otherwise will be deemed ‘antibody negative’. A study subject will be given ‘antibody positive’ status if they have at least one confirmed positive sample result at any time during the treatment or post-treatment evaluation periods. A study subject will be given ‘antibody negative’ status if all evaluated IM sample results are antibody negative and they have at least one evaluable IM result later during the treatment and post-treatment evaluation periods. Otherwise, a study subject will be given ‘antibody inconclusive’ status.

The IM incidence and IM incidence rate for the overall treatment and post-treatment evaluation period, will be determined and appropriately summarized, by group, as the number of and percentage of evaluated subjects with antibody negative antibody positive, and antibody inconclusive status. In addition, in antibody positive study subjects, antibody titers of any antibody positive samples will be reported (listed) and appropriately summarized across subjects and by treatment (e.g., at each evaluated time point, or by observed peak titer values, etc.) at the discretion of the designated study pharmacokineticist and/or statistician.

Antibody positivity in anti-Nusinersen antibody-positive subjects will also be designated (when possible) as being either ‘persistent’ or ‘transient’, but only for those subjects in whom at least one confirmed antibody positive sample result is followed (> 100 days later) by at least one

additional IM sample assessment (which can be either a confirmed positive or negative result(s)). Those study patients meeting the above criteria and with at least 2 confirmed positive IM results occurring greater than 100 days apart (~5 immunoglobulin half-lives) or one or more positive samples and no other samples more than 100 days after the first positive sample will be considered to have a ‘persistent’ antibody response. All other subjects meeting the above criteria will be considered to have a ‘transient’ antibody response.

## **8 Sample size justification**

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

## **9 References**

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