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Human Medicines Division

Assessment report for paediatric studies submitted according to Article 46 of the Regulation (EC) No 1901/2006

Spinraza

Nusinersen

Procedure no: EMA/PAM/0000279100

Note

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

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Status of this report and steps taken for the assessment			
Current step	**Description**	**Planned date**	**Actual Date**
☐	Start of procedure	23 June 2025	23 June 2025
☐	CHMP Rapporteur AR	28 July 2025	29 July 2025
☐	CHMP comments	11 August 2025	n/a
☐	Updated CHMP Rapporteur AR	14 August 2025	n/a
☒	CHMP outcome	21 August 2025	21 August 2025

Administrative information

Procedure resources	
Rapporteur	**Fátima Ventura**

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1. Introduction

On 10 June 2025, the MAH submitted a completed paediatric study for Spinraza, in accordance with Article 46 of Regulation (EC) No1901/2006, as amended.

These data are also submitted as part of the post-authorisation measure PAM 46

2. Scientific discussion

2.1. Information on the development program

The MAH stated that An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy (NURTURE)Study Number 232SM201 is a stand alone study.

2.2. Information on the pharmaceutical formulation used in the study

2.3. Clinical aspects

2.3.1. Introduction

The MAH submitted the final report for:

- 232SM201: An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy.

2.3.2. Clinical study

Nusinersen (Spinraza; BIIB058, ISIS 396443) - An Open-Label Study to Assess the Efficacy, Safety, Tolerability, and Pharmacokinetics of Multiple Doses of ISIS 396443 Delivered Intrathecally to Subjects With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy.

Description

A Phase 2 open-label, multicenter, multinational, single-arm study to assess the long-term efficacy, safety, tolerability, PK, PD, and immunogenicity of multiple doses of nusinersen in participants with genetically diagnosed and presymptomatic SMA.

Methods

Study participants

This study planned to enroll up to 25 participants who were 6 weeks of age or younger at the time of the first dose administration and had genetic documentation of 5q *SMA* homozygous gene deletion or mutation or compound heterozygous mutation and 2 or 3 copies of the *SMN2* gene and were presymptomatic.

Participants permanently discontinued study treatment for any one of the following reasons:

- The participant's parent(s)/legal guardian(s) withdrew consent.
- The participant experienced a medical emergency that necessitated permanent

discontinuation of study treatment.

- At the discretion of the Investigator for medical reasons.
- At the discretion of the Investigator or Sponsor for noncompliance.

Participants were withdrawn from the study for any one of the following reasons:

- The participant's parent(s)/legal guardian(s) withdrew consent.
- The participant's parent(s)/legal guardian(s) were unwilling or unable to comply with the protocol.

The primary reason for discontinuation of study treatment or withdrawal from the study was recorded in the participant's CRF.

Treatments

Nusinersen (12-mg, IT bolus LP injection) was administered using a loading regimen (dosing on Days 1, 15, 29, and 64), followed by maintenance dosing once every 4 months beginning on Day 183. In previous versions of the protocol (Versions 1 through 5), the volume of the injection, and thus the dose, were adjusted based on the participant's age on the day of dosing, such that each participant received a 12-mg scaled equivalent dose based on CSF volume scaling. After implementation of Protocol Version 6, all participants were dosed with 12 mg (5 mL) of nusinersen, regardless of their age.

A summary of key information regarding the study treatment is provided in the Table below.

Summary of Treatment for Study 232SM201

Parameter	Description	Protocol and Section
Treatment Administered	In previous versions of the protocol (Versions 1 through 5), the volume of the injection and thus the dose were adjusted based on the participant's age on the day of dosing, such that each participant received a 12-mg scaled equivalent dose based on CSF volume scaling. After implementation of Protocol Version 6, all participants received 12 mg of nusinersen by IT LP administration. Participants received a loading regimen (dosing on Days 1, 15, 29, and 64), followed by maintenance dosing once every 4 months beginning on Day 183. 12 batches were used for this study (see Appendix 16.1.6, Table 1).	Protocol 232SM201 V9 Section 11.1
Comparator or Placebo Administered	Not applicable	Not applicable

Rationale for Study Dose	The nusinersen dose and dose interval were selected based on nonclinical data, consideration of the target tissue concentration anticipated for drug pharmacology, and safety data in the completed and ongoing clinical studies of nusinersen to date. The loading dose and maintenance dose intervals were based on nonclinical PK and pharmacology data. The loading dose interval was intended to achieve and maintain nusinersen spinal cord tissue levels that were predicted to be within the upper end of the pharmacologically active range (predicted to be approximately 30 µg/g lumbar and 10 µg/g cervical tissue concentrations) while at the same time considering participant safety and convenience for repeated LP IT injections. The maintenance dose interval was intended to maintain the spinal cord tissue levels of nusinersen at a steady-state level within the estimated pharmacologically active range. Because results from PK models showed similar concentrations and potential for higher efficacy with higher concentrations, the dosing regimen was adjusted to 12 mg (5 mL) regardless of age, which in result lowered the risk for dosing errors while still maintaining favorable safety margins. In previous versions of the protocol (Versions 1 through 5), the volume of the injection and thus the dose were adjusted based on the participant's age on the day of dosing, such that each participant received a 12-mg scaled equivalent dose based on CSF volume scaling.	Protocol 232SM201 V9 Section 4.5

Parameter	**Description**	**Protocol and Section**
Method of Treatment Assignment	This was a single-arm study. Therefore, randomization was not performed.	Not applicable
Dosage Modification	Dosage modifications were not permitted. In the event of a concurrent illness that would prevent the dosing procedure from being performed safely, an adjustment in the dose schedule could be permitted, with approval by the Medical Monitor.	Protocol 232SM201 V9 Section 11.2
Blinding	This was an open-label study. Therefore, no blinding was undertaken.	Protocol 232SM201 V9 Section 9.3
Prior and Concomitant Therapy	Participants who received prior treatment with an investigational drug given for the treatment of SMA, biological agent, or device were excluded from participation in the study. Participants with a history of gene therapy, prior ASO treatment, or cell transplantation were also excluded from the study. Approved concomitant therapies were used at the discretion of the Investigator. Any concomitant medications including SMA therapies were captured in the CRF.	Protocol 232SM201 V9 Section 8.2 and Section 11.5

Objective(s) & Outcomes/endpoints

Primary Objectives	**Primary Endpoints**
To examine the efficacy of multiple doses of nusinersen administered IT in preventing or delaying the need for respiratory intervention or death in infants with genetically diagnosed and presymptomatic SMA.	The time to death or respiratory intervention (invasive or noninvasive ventilation for ≥ 6 hours/day continuously for ≥ 7 days or tracheostomy).
Secondary Objectives	**Secondary Endpoints**
To examine the effects of nusinersen in infants with genetically diagnosed and presymptomatic SMA on the following: • Development of clinically manifested SMA as determined by a composite of clinical features seen in participants with SMA • Growth and function • Safety, tolerability, and PK	<u>Efficacy</u> • Proportion of participants developing clinically manifested SMA as defined by any of the following (all assessed at approximately 13 and 24 months of age, unless otherwise noted): ○ Age-adjusted weight < 5th percentile or decrease of ≥ 2 major weight growth curve percentiles (3rd, 5th, 10th, 25th, or 50th) or a percutaneous gastric tube placement for nutritional support ○ Failure to achieve the ability to sit without support ○ Failure to achieve standing with assistance ○ Failure to achieve hands-and-knees crawling ○ Failure to achieve walking with assistance by 24 months of age ○ Failure to achieve standing alone by 24 months of age ○ Failure to achieve walking alone by 24 months of age • Proportion of participants alive • Attainment of motor milestones assessed as part of the HINE (Section 2) at 13 and 24 months of age • Attainment of motor milestones as assessed by WHO criteria • Change from baseline in the CHOP INTEND motor function scale • Change from baseline in HFMSE • Change from baseline in growth parameters: weight for age/length, head circumference, chest circumference, head-to-chest circumference ratio, and arm circumference

	Safety • Incidence of AEs and/or SAEs • Change from baseline in clinical laboratory parameters, ECGs, and vital signs • Neurological examinations PK • CSF nusinersen concentrations • Plasma nusinersen concentrations

The exploratory endpoints of this study were as follows:

- Change from baseline in CMAP
- Change from baseline in 6MWT
- Change from baseline in the PDMS-2
- Measures of respiratory events: respiratory infections, hospitalizations for respiratory events, noninvasive and invasive ventilator use, and oxygen saturation
- Proportion of participants who develop thoracoabdominal asynchrony measured by RP
- Time to death or permanent ventilation (≥ 16-hour ventilation/day continuously for 21 days in the absence of an acute reversible event or tracheostomy)
- Time to death or ventilation (≥ 6-hour ventilation/day continuously for ≥ 1 day or tracheostomy)
- Change from baseline in cognitive assessments
- Change from baseline and maintenance in body composition and bone density (as assessed by DEXA)
- Proportion of participants who develop signs and symptoms of dysphagia (as measured using PASA questionnaire and HINE-1 suck/swallow)
- Change from the first assessment in SNAP
- Change from the first assessment in pVHI criteria and the Mobile Speech Battery
- Change from baseline in the quality-of-life questionnaires
- Plasma/CSF pNF-H concentrations and change in pNF-H concentrations over time

Statistical Methods

Analysis population:

Analysis populations were defined as follows:

- Intent-to-Treat (ITT) Set: all participants who received at least 1 dose of nusinersen. It should be noted that, in the protocol, the ITT Set was labeled as the Full Analysis Set but the definition has not changed.
- Per-Protocol Set (PPS): the subset of the ITT Set who completed the initial 4 doses of the drug, had a baseline and at least the Day 183 efficacy assessments, and had no notable protocol deviations that would be expected to affect the efficacy assessments.
- PK Set: participants who were dosed and for whom there was at least 1 evaluable post-dose PK sample.

Efficacy:

Efficacy analyses are presented separately for participants with 2 *SMN2* copies and those with 3 *SMN2* copies as detailed in the Statistical Analysis Plan.

Primary endpoint and analysis:

- The primary endpoint was time to death or respiratory intervention (invasive or noninvasive ventilation for ≥ 6 hours/day continuously for ≥ 7 days or tracheostomy).
- The time was the age of the participant at the first occurrence of either a respiratory intervention or death. A Kaplan-Meier survival curve of the time to respiratory intervention or death was presented and used to estimate the median time to event and corresponding 95% confidence interval (CI).

Secondary endpoints and analyses:

- The proportion of participants developing clinically manifested SMA as defined by age-adjusted weight, requirement for percutaneous gastric tube placement, and failure to achieve motor milestones as assessed by WHO criteria on Days 365 and 700 was presented with a corresponding 95% CI.
- A Kaplan-Meier survival curve was presented and used to estimate the proportion of participants alive at 13 months and 2, 3, 4, 5, 6, 7, and 8 years of age. Corresponding 95% CIs were presented.
- The total number of HINE-2 milestones achieved was summarized by visit.
- The proportion of participants who had the ability to perform WHO motor milestones was summarized by visit. The age at achievement of each milestone was reported by the caregiver and confirmed at the subsequent in-person assessment. The age of achievement will be summarized for each milestone.
- Change from baseline to each visit for the CHOP INTEND motor function was summarized using descriptive statistics. Once participants reached a score of 64, CHOP INTEND was no longer assessed.
- The HFMSE total score and change from baseline (first opportunity to perform) was summarized by visit and age using descriptive statistics.
- Change from baseline to each visit was summarized using descriptive statistics for the following

growth parameters: weight for age/length, head circumference, chest circumference, head-to-chest circumference ratio, and arm circumference.

Exploratory endpoints and analyses are described in the clinical study report.

PK:

- Results of CSF and plasma nusinersen concentrations were summarized by sampling time.

PD/Biomarker:

- Results from the PD assessment of plasma/CSF NF-L concentrations were summarized at each visit.

Safety:

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

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

The analysis of vital signs was focused on clinically relevant abnormalities. The number and percentage of participants with clinically relevant postbaseline abnormalities were presented. Summary statistics for actual values and change from baseline was also presented.

For ECGs, the number and percentage of participants with shifts from baseline normal to each of the categorical values denoting normal, abnormal, and abnormal (not AE) were summarized.

The number and percentage of participants with clinical changes in echocardiogram readings from the first assessment were summarized.

For immunogenicity, descriptive statistics of the incidence of anti-nusinersen plasma antibody concentrations were presented.

Results

Disposition of Participants

Thirty participants were screened at 17 study sites. Of the 30 participants screened, 5 did not meet the eligibility criteria for the study (Figure 1):

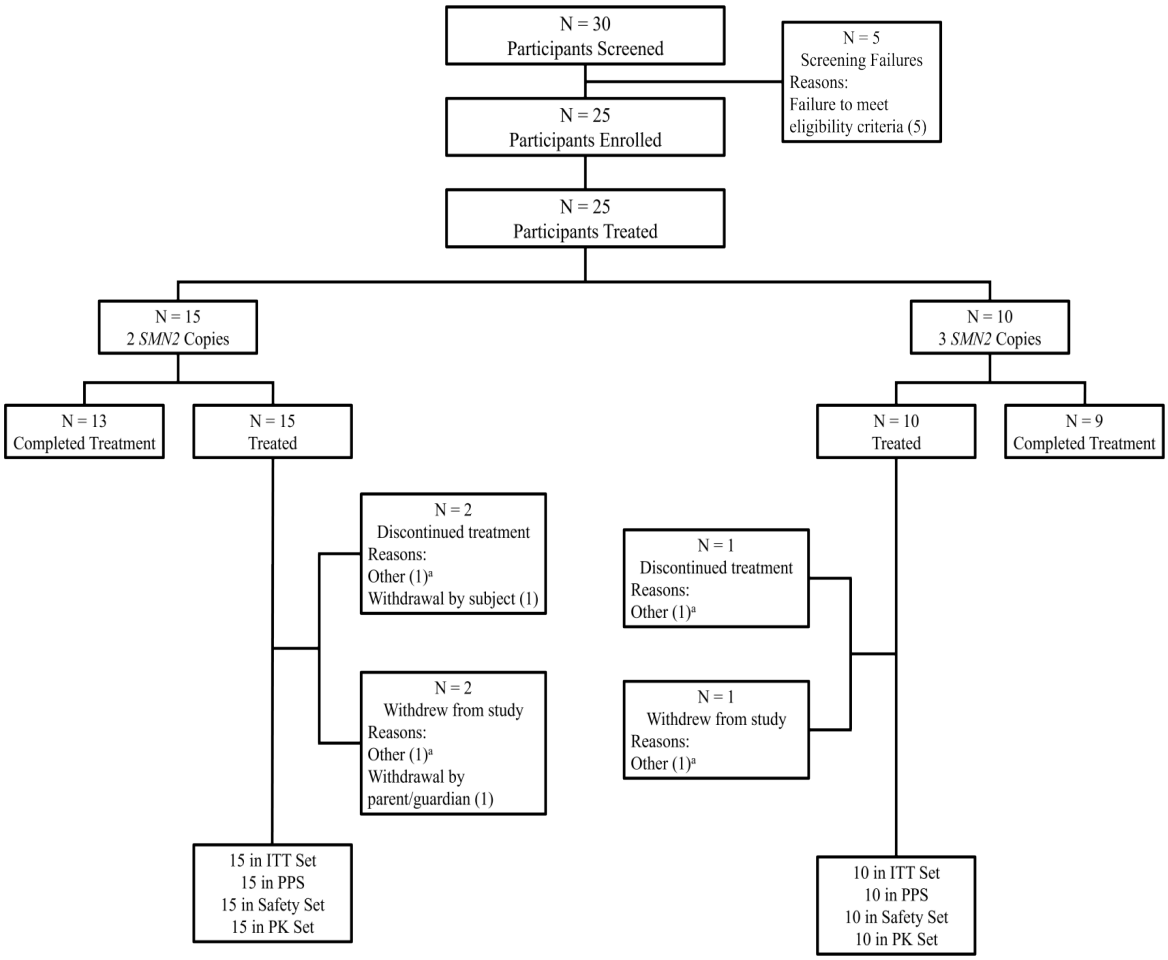
- Three participants did not meet the inclusion criterion of having an ulnar CMAP ≥ 1 mV at baseline and 2 of these participants also had clinical signs or symptoms at Screening or immediately prior to the first dose (Day 1) that were, in the opinion of the Investigator, strongly suggestive of SMA.
- One participant did not meet the inclusion criterion of having genetic documentation of 2 or 3 copies of *SMN2*.
- One participant did not meet the inclusion criterion of having genetic documentation of 5q *SMA* homozygous gene deletion or mutation or compound heterozygous mutation.

The remaining 25 participants (15 participants with 2 *SMN2* copies and 10 participants with 3 *SMN2* copies) were enrolled at 16 study sites. All 25 participants enrolled were dosed; these participants comprised the ITT Set. All completed the Day 183 Visit and met the criteria for inclusion in the PPS. Twenty-two participants (88%) completed the Day 2891/End of Study Visit. Three participants (12%) discontinued treatment and withdrew from the study. The first participant was treated on 20 May 2015, and the end of study date (last participant last visit) was 17 December 2024.

Figure 1: CONSORT Flow Diagram for Disposition of Participants

N = number of participants.

[a] The parent of the participant decided to withdraw due to study site closing.



Source: Section 14.1, Outputs 1 and 2; Appendix 16.2.1, Table 2; and Appendix 16.2.4, Table 1

Study Populations Analyzed

Safety and efficacy analyses were performed on the ITT Set (Table 1). The analysis of the primary endpoint was repeated using the PPS. The PPS and PK Set were the same as the ITT Set. A subgroup analysis was performed for participants with peroneal CMAP amplitude ≥ 2 mV and an absence of areflexia defined as HINE item for tendon reflexes > 0, as described in Appendix 16.1.9, Statistical Analysis Plan, Section 5.11.

No participants, visits, or observations were excluded from the efficacy analysis.

Table 1: Analysis Populations

Population	Definition
ITT Set	All participants who received at least 1 dose of nusinersen. It should be noted that, in the protocol, the ITT Set was labeled as the Full Analysis Set but the definition has not changed.
PPS	The subset of the ITT Set who completed the initial 4 doses of the drug, had a baseline and at least the Day 183 efficacy assessments, and had no notable protocol deviations that would be expected to affect the efficacy assessments.
PK Set	Participants who were dosed and for whom there was at least 1 evaluable postdose PK sample.

Demographics

Summaries of demographics at Screening are presented for participants in the ITT Set in Table 2. There were 12 males (48%; 8 participants with 2 SMN2 copies and 4 participants with 3 SMN2 copies) and 13 females (52%; 7 participants with 2 SMN2 copies and 6 participants with 3 SMN2 copies) in the ITT Set. Fourteen participants (56%) were White. Age at first dose ranged from 3 through 42 days, with a median of 22.0 days and a mean of 20.6 days. In participants with 2 *SMN2* copies, age at first dose ranged from 8 through 41 days, with a median of 19.0 days; in participants with 3 *SMN2* copies, age at first dose ranged from 3 through 42 days, with a median of 23.0 days. The majority of participants were from the US.

Demographic characteristics for the 16 participants (8 with 2 *SMN2* copies and 8 with 3 *SMN2* copies) with baseline peroneal CMAP amplitude ≥ 2 mV and an absence of areflexia were consistent with the overall ITT Set.

Table 2: Demography - ITT Set

Demography - ITT Set
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	ISIS 396443 2 *SMN2* copies	**ISIS 396443 3 *SMN2* copies**	**Total**
Number of subjects dosed	15	10	25
Age at first dose (days)			
<=14	6 (40)	3 (30)	9 (36)
>14 and <=28	7 (47)	5 (50)	12 (48)
>28	2 (13)	2 (20)	4 (16)
n	15	10	25
Mean	19.5	22.3	20.6
SD	9.29	12.45	10.51
Median	19.0	23.0	22.0
25%, 75% percentile	12.0, 27.0	12.0, 25.0	12.0, 25.0
Min, Max	8, 41	3, 42	3, 42
Se			
x	15	10	25
n			
Male	8 (53)	4 (40)	12 (48)
Female	7 (47)	6 (60)	13 (52)

NOTE: Numbers in parentheses are percentages based on the number of subjects dosed in each treatment arm with non-missing data.

Source: isis396443/232sm201/final-analysis/t-bl-demog.sas:t-bl-demog-itt.rtf **Run Date:** 29JAN2025

Demography - ITT Set

	ISIS 396443 2 *SMN2* copies	ISIS 396443 3 *SMN2* copies	Total
Ethnicit y n	15	10	25
Hispanic or Latino	2 (13)	0	2 (8)
Not Hispanic or Latino	10 (67)	9 (90)	19 (76)
Not reported	3 (20)	1 (10)	4 (16)
Rac e n	15	10	25
American Indian or Alaska native	1 (7)	0	1 (4)
Asian	1 (7)	2 (20)	3 (12)
Black	0	0	0
Native Hawaiian or other Pacific Islander	0	0	0
White	8 (53)	6 (60)	14 (56)
Other	2 (13)	1 (10)	3 (12)
Not reported due to confidentiality regulations	3 (20)	1 (10)	4 (16)
Count ry USA	9 (60)	7 (70)	16 (64)
Italy	2 (13)	1 (10)	3 (12)
Germany	1 (7)	0	1 (4)
Turkey	0	1 (10)	1 (4)
Australia	1 (7)	0	1 (4)
Taiwan	1 (7)	1 (10)	2 (8)
Qatar	1 (7)	0	1 (4)

NOTE: Numbers in parentheses are percentages based on the number of subjects dosed in each treatment arm with non-missing data.

Source: isis396443/232sm201/final-analysis/t-bl-demog.sas:t-bl-demog-itt.rtf **Run Date:** 29JAN2025

Baseline Disease Characteristics

All 25 enrolled participants had genetic documentation of 5q *SMA* homozygous gene deletion or mutation or compound heterozygous mutation but were presymptomatic. Of the 25 participants in the ITT Set, 15 had 2 *SMN2* copies and 10 had 3 *SMN2* copies by central laboratory testing. There was 1 set of twins with 2 *SMN2* copies. Additionally, 1 participant with 2 SMN2 copies and 1 participant with 3 *SMN2* copies had a twin; however, in both cases, the twin did not have *SMA* and was not enrolled in the study.

In the ITT Set, gestational age at birth ranged from 37 through 41 weeks, with a median of 39.0 weeks. Birth weight ranged from 2.14 through 4.36 kg, with a median of 3.290 kg. Weight at Screening ranged from 2.20 through 4.81 kg, with a median of 3.500 kg (the majority of participants (17 of 25 participants) were within the > 25th and ≤ 75th percentiles for weight for age at Screening [WHO 2006; WHO Multicentre Growth Reference Study Group 2006a, 2006b].

In participants with 2 *SMN2* copies, the majority of participants (9 of 15 participants) were within the > 25th and ≤ 75th percentile for weight for age at Screening. In participants with 3 *SMN2* copies, the majority of participants (8 of 10 participants) were within the > 25th and ≤ 50th percentile for weight for age at Screening. Two participants were below the 5th percentile for weight for age at Screening (1 participant with 2 *SMN2* copies and 1 participant with 3 *SMN2* copies). One participant (with 2 *SMN2* copies) weighed above the 95th percentile for weight for age at Screening. Length ranged from 42.5 through 59.1 cm, with a median of 51.70 cm. The majority of participants (14 of 25 participants) were within the > 10th and ≤ 50th percentiles for length for age at Screening. Two participants were below the 5th percentile for length for age at Screening (1 participant with 2 *SMN2* copies and 1 participant with 3 *SMN2* copies), and 2 participants (both with 2 *SMN2* copies) were above the 95th percentile for length for age at Screening. Head circumference ranged from 30.0 through 40.0 cm, with a median of 35.60 cm. Chest circumference ranged from 29.0 through 38.7 cm, with a median of 34.40 cm. Head-to-chest circumference ratio ranged from 0.9 through 1.2, with a median of 1.04. Overall, anthropometric parameters for all participants were consistent with normal development.

Compared with participants with 2 *SMN2* copies, participants with 3 *SMN2* copies had higher median baseline values for the CHOP INTEND, HINE motor milestones, and CMAP amplitude assessments.

In the ITT Set, baseline CHOP INTEND total score ranged from 25 through 60 with a median of 50.0 (median of 45.0 for participants with 2 *SMN2* copies and 53.5 for participants with 3 *SMN2* copies). Twenty-three participants (14 participants with 2 *SMN2* copies and 9 participants with 3 *SMN2* copies) had a CHOP INTEND total score between 25 and 60, and 2 participants (1 participant with 2 *SMN2* copies and 1 participant with 3 *SMN2* copies) had scores between 60 and 64.

Baseline motor milestones were assessed using Section 2 of the HINE, which evaluates neuromuscular development in 8 motor milestone components (voluntary grasp, ability to kick [in supine], head control, rolling, sitting, crawling, standing, and walking). At baseline, participants with 3 *SMN2* copies exhibited more advanced HINE motor milestones than participants with 2 *SMN2* copies. For example, 2 participants (20%) with 3 *SMN2* copies and 1 participant (7%) with 2 *SMN2* copies maintained their head upright all the time; 7 participants (70%) with 3 *SMN2* copies and 9 participants (60%) with 2 *SMN2* copies had the ability to kick upward (vertically); 1 participant (10%) with 3 *SMN2* copies and no participants with 2 *SMN2* copies could crawl on elbow; and 3 participants (30%) with 3 *SMN2* copies and 2 participants (13%) with 2 *SMN2* copies could support weight, as would be expected based on their age at the time of the assessment. No participants in either *SMN2* copy cohort achieved more advanced milestones, such as rolling to side, at baseline, and this would not be expected in healthy infants until 4 months or older.

In the ITT Set, baseline ulnar nerve CMAP amplitude ranged from 1.0 through 6.7 mV with a median of 2.65 mV (median of 2.30 mV for participants with 2 *SMN2* copies and 2.90 mV for participants with 3 *SMN2* copies). Baseline peroneal nerve CMAP amplitude ranged from 0.2 through 9.7 mV with a median of 3.30 mV (median of 3.20 mV for participants with 2 *SMN2* copies and 4.00 mV for participants with 3 *SMN2* copies).

The median (range) total plasma (n = 14) and CSF (n = 21) concentrations of NF-L at baseline were 543.0 (7 through 1160) and 9720.0 (146 through 23,600) pg/mL, respectively. Median baseline concentrations of both plasma and CSF NF-L were higher in participants with 2 *SMN2* copies (678.5 and 12,250.0 pg/mL, respectively) than in participants with 3 *SMN2* copies (12.9 and 383.0 pg/mL, respectively).

For the 16 participants (8 participants with 2 *SMN2* copies and 8 participants with 3 *SMN2* copies) with baseline peroneal CMAP amplitude ≥ 2 mV and an absence of areflexia, baseline CHOP INTEND total score ranged from 35 through 60 with a median of 54.5 (median of 54.5 for participants with 2 *SMN2* copies and 52.0 for participants with 3 *SMN2* copies). Baseline ulnar nerve CMAP amplitude ranged from 1.5 through 6.7 mV with a median of 3.20 mV (median of 3.20 mV for participants with 2 *SMN2* copies and 3.40 mV for participants with 3 *SMN2* copies). Baseline peroneal nerve CMAP amplitude ranged from 2.5 through 9.7 mV with a median of 4.0 mV (median of 3.55 mV for participants with 2 *SMN2* copies and 4.20 mV for participants with 3 *SMN2* copies).

SMA Data Siblings

The collection of SMA data of affected siblings was added in Protocol Version 4. Data were available for a total of 25 evaluable siblings with SMA of 19 participants in the ITT Set. Sibling data related to *SMN2* copy number, age at SMA symptom onset, age when enrolled in a clinical trial or initiated on commercial nusinersen, survival, ventilator use, and age when sitting and walking were achieved are presented in Appendix 16.2.6, Table 8.

SMN2 copy number was available for 17 untreated siblings of 14 participants who received nusinersen in this study. Eight of the 14 treated participants had 2 *SMN2* copies, 5 participants had 3 *SMN2* copies, and 1 participant had 3 *SMN2* copies at exon 7 and 2 SMN2 copies at exon 8. Consistent with the concordance often observed in siblings with SMA [Jones 2020; Medrano 2016], the reported *SMN2* copy number for the untreated siblings was the same as that of the treated participants.

Baseline Medical History

In the ITT Set, 11 participants (44%) had a reported medical history finding. Baseline medical history by SOC and PT included the following:

- Pregnancy, puerperium, and perinatal conditions: jaundice neonatal (5 participants [20%])
- Congenital, familial, and genetic disorders: macrocephaly, polydactyly, and tethered oral tissue (1 participant [4%] each)
- Ear and labyrinth disorders: middle ear effusion (1 participant [4%])
- Hepatobiliary disorders: hyperbilirubinemia (1 participant [4%])
- Metabolism and nutrition disorders: hypoglycaemia (1 participant [4%])
- Musculoskeletal and connective tissue disorders: short stature (1 participant [4%])
- Surgical and medical procedures: circumcision (1 participant [4%])

Prior and Concomitant Treatments

Dose modifications were not permitted.

Concomitant Medications

All 25 participants received at least 1 concomitant medication during the study. The most commonly used concomitant medications were paracetamol and ibuprofen (24 participants [96%]); propofol (23 participants [92%]); lidocaine/prilocaine and sodium chloride (19 participants [76%]); midazolam (18 participants [72%]); amoxicillin, ondansetron, and sevoflurane (17 participants [68%]); and lidocaine and nitrous oxide (16 participants [64%]). Lidocaine was primarily used as a prophylactic for LP pain whereas paracetamol was used for a variety of reasons, including fever and pain. None of the concomitant medications were used under an experimental protocol.

Ancillary Procedures

All 25 participants underwent at least 1 ancillary procedure. By PT, the most common ancillary procedures were chest X-ray and SARS-COV-2 test (15 participants [60%] each). Five participants (20%) had echocardiograms, and 5 participants (20%), all with 2 copies of SMN2, underwent an ancillary procedure of gastrostomy.

Protocol Deviations

Deviations were departures from the currently approved version of the protocol. A list of potential protocol deviations was generated by the SMT after the protocol was finalized. Major deviations are considered changes from the protocol that can affect data integrity or participant safety.

All deviations (major and minor) were captured by the Clinical Monitors on a Protocol Deviation log during routine monitoring visits. Periodically during the course of the study, key study team members (e.g., Biostatistician, Statistical Programmer, Data Manager, and Medical Director) review protocol deviations identified from the clinical database via programmed data listings and edit checks, in addition to deviations captured in the Protocol Deviation log, to identify trends that required retraining at study sites or a protocol amendment and to identify major protocol deviations and determine whether they affect the planned statistical analysis.

A summary of the major protocol deviations that occurred on study for participants in the ITT Set is presented in Section 14.1, Output 13. Protocol deviations were mostly minor and consisted of deviations in laboratory assessment criteria, study procedures criteria, visit schedule criteria, informed consent, and other criteria. All 25 participants in the ITT Set had at least 1 major protocol deviation. The most common major deviations were laboratory assessment criteria, study procedures criteria, and visit schedule criteria. No participant discontinued study treatment due to the protocol deviations. The major protocol deviations did not affect data integrity.

Serious Breaches of Good Clinical Practice

No serious breaches of GCP occurred during the study.

Exposure and Study Treatment Compliance

Exposure

Participants received loading doses of nusinersen (12 mg scaled equivalent) by IT injection on Days 1, 15, 29, and 64, followed by maintenance doses of nusinersen (12 mg scaled equivalent; upon implementation of Protocol Version 6, all participants received fixed 12-mg doses) by IT injection approximately every 4 months until Day 2801, for a total of 27 doses.

Twenty-two participants (88%) received all 27 doses, 2 participants (8%) received 20 doses, and 1 participant (4%) received 21 doses. The mean and median numbers of doses were 26.2 and 27.0, respectively. All participants were on the study for at least 5 years, 24 participants (96%) were on study for at least 6 years, 22 participants (88%) were on study for at least 7 years, and 2 participants (8%) were on study for at least 8 years. The mean (median) time on study was 2820.4 (2892.0) days (range: 2155 through 3024 days), corresponding to 7.72 (7.92) years (range: 5.9 through 8.3 years), with a total of 193.05 participant-years on study.

Dose Modification

Dose modifications were not permitted.

COVID-19 Impact

Due to the COVID-19 pandemic, 9 of the 25 participants (36%) were unable to be dosed as planned at their original study site. Four participants received at least 1 dose at an alternative study site, and 5 participants received commercial dose for at least 1 visit.

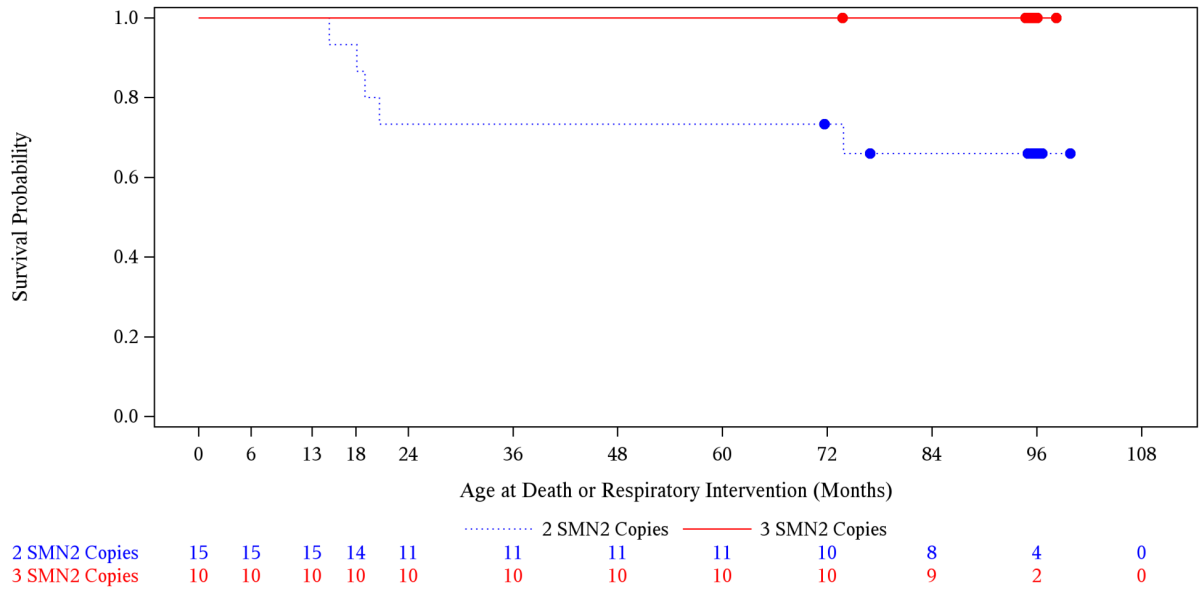
Efficacy results

Efficacy analyses are presented separately for participants with 2 SMN2 copies and those with 3 SMN2 copies as detailed in the Statistical Analysis Plan.

Primary endpoint

- Five participants (all with 2 SMN2 copies) met the primary endpoint of the study and had respiratory intervention (defined as either invasive or non-invasive ventilation for ≥ 6 hours/day continuously for ≥ 7 days or tracheostomy). No participants died or required tracheostomy.
- Four of the 5 participants who met the primary endpoint required respiratory intervention in the context of acute, reversible infections. All 5 of these participants continued to gain motor milestones
- None of the participants required permanent ventilation (≥ 16-hour ventilation/day continuously for > 21 days in the absence of an acute reversible event or tracheostomy).
- Only the 5 participants who reached the primary endpoint of the study received ventilation for ≥ 6 hours/day continuously for ≥ 1 day or tracheostomy.

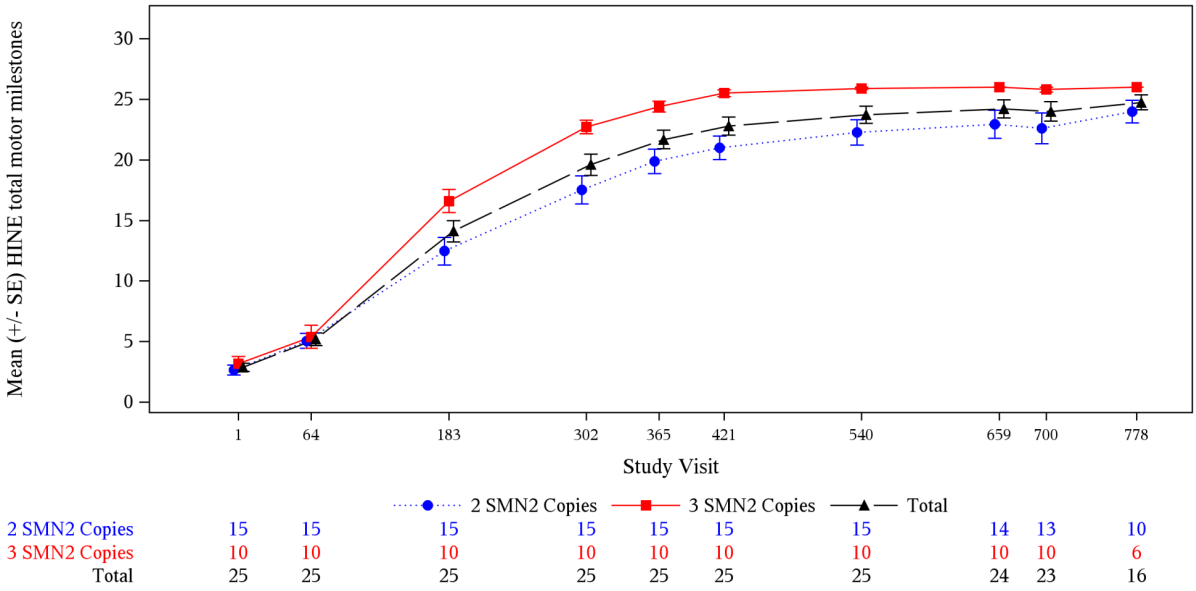
Figure 2: Kaplan-Meier Plot For Age at Death or Respiratory Intervention by SMN2 Copy Number - ITT Set~



Secondary endpoints

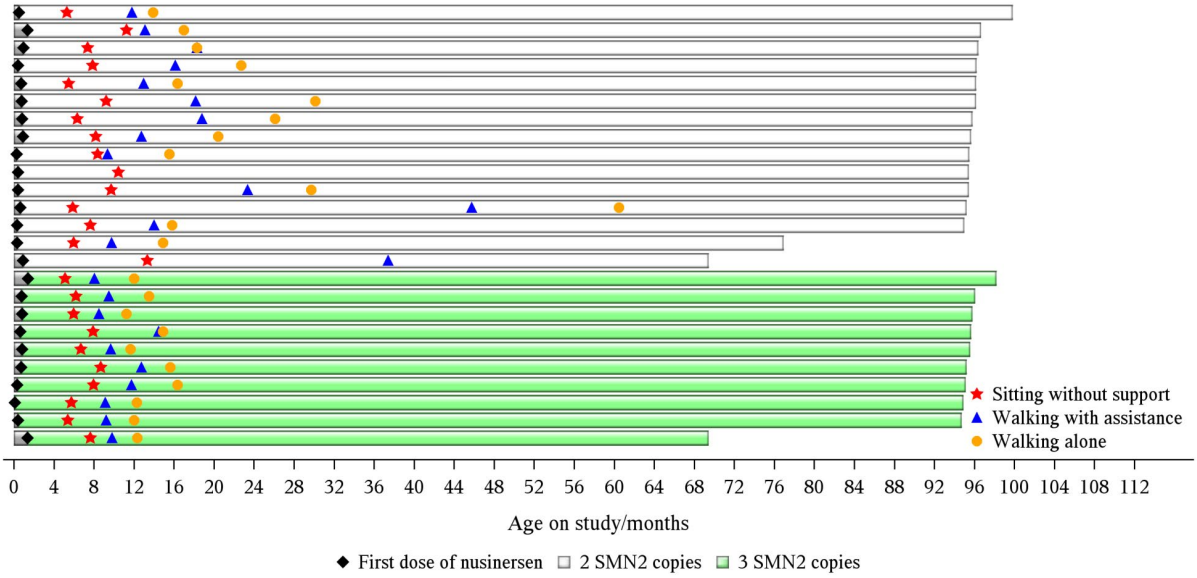
- Protocol-predefined **symptoms of SMA were observed in 12 participants** (10 participants with 2 *SMN2* copies and 2 participants with 3 *SMN2* copies**) on Day 365** (13 months of age) **and in 7 participants** (all with 2 *SMN2* copies) **on Day 700** (24 months of age). The most common reason was weight < 5th percentile, decrease of ≥ 2 major weight growth curve percentiles, or failure to achieve age-appropriate milestones. All of these participants continued to grow, gain weight, and achieve milestones over time.
- Five of the 6 participants who did not achieve WHO motor milestones on time on Day 700 also failed on another criteria of clinical manifestation of SMA.
- On Day 365, for participants with 2 *SMN2* copies, 5 (33%) had weight below the 5th percentile and 5 (33%) had weight dropping 2 or more major percentiles compared to baseline. No participants with 3 *SMN2* copies met either of these criteria. On Day 700, for participants with 2 *SMN2* copies, 4 (27%) had weight below the 5th percentile and 5 (33%) had weight dropping 2 or more major percentiles compared to baseline. No participants with 3 *SMN2* copies met either of these criteria.
- HINE motor milestones were only assessed and are reported up to Day 778. Achievement of HINE motor milestones increased from baseline to Day 659 and remained stable to Day 778. The mean total score in participants with 2 *SMN2* copies was 23.9, ranging from 16 through 26. All participants with 3 *SMN2* copies reached the maximum total score of 26 as of the last assessment. All 25 participants achieved the maximum level for head control (i.e., all the time maintained upright), sitting (i.e., stable sit or pivot), voluntary grasp (i.e., pincer grasp), rolling (i.e., supine to prone or prone to supine), and kicking (i.e., touches toes). Eleven of 15 participants (73%) with 2 *SMN2* copies and all 10 participants (100%) with 3 *SMN2* copies achieved the maximum level for crawling and standing, and **10 of 15 participants (67%) with 2 *SMN2* copies and all 10 participants (100%) with 3 *SMN2* copies achieved the maximum level for walking**.

Figure 3: Mean HINE Total Motor Milestones Over Time - ITT Set



- Achievement of WHO motor milestones increased steadily from baseline, and all participants who achieved a milestone on study were able to demonstrate the milestone at the last study visit. At the last observed visit, all 15 participants with 2 *SMN2* copies achieved the WHO motor milestones of sitting without support and standing with assistance. The majority of participants with 2 *SMN2* copies achieved hands-and-knees crawling (93%), walking with assistance (93%), standing alone (93%), and all walking alone (87%). All 10 participants with 3 *SMN2* copies achieved all 6 WHO motor milestones.

Figure 4: Achievement of WHO Motor Milestones Based on Caregiver Observation With Confirmation by Study Site at Subsequent Visit - ITT Set



- From baseline to the last study visit, all 15 participants with 2 *SMN2* copies and all 10 participants

with 3 *SMN2* copies in the ITT Set achieved improvements in the CHOP INTEND total score. Twelve of 15 participants (80%) with 2 *SMN2* copies and 10 of 10 participants (100%) with 3 *SMN2* copies) achieved the highest attainable CHOP INTEND score of 64 at any point during the study. Participants with 3 *SMN2* copies had higher scores than participants with 2 *SMN2* copies at most visits, but the final change from baseline was generally similar in both cohorts.

Figure 5: Kaplan-Meier Plot for Days at First Achieved Total CHOP INTEND Score of 64 - ITT Set

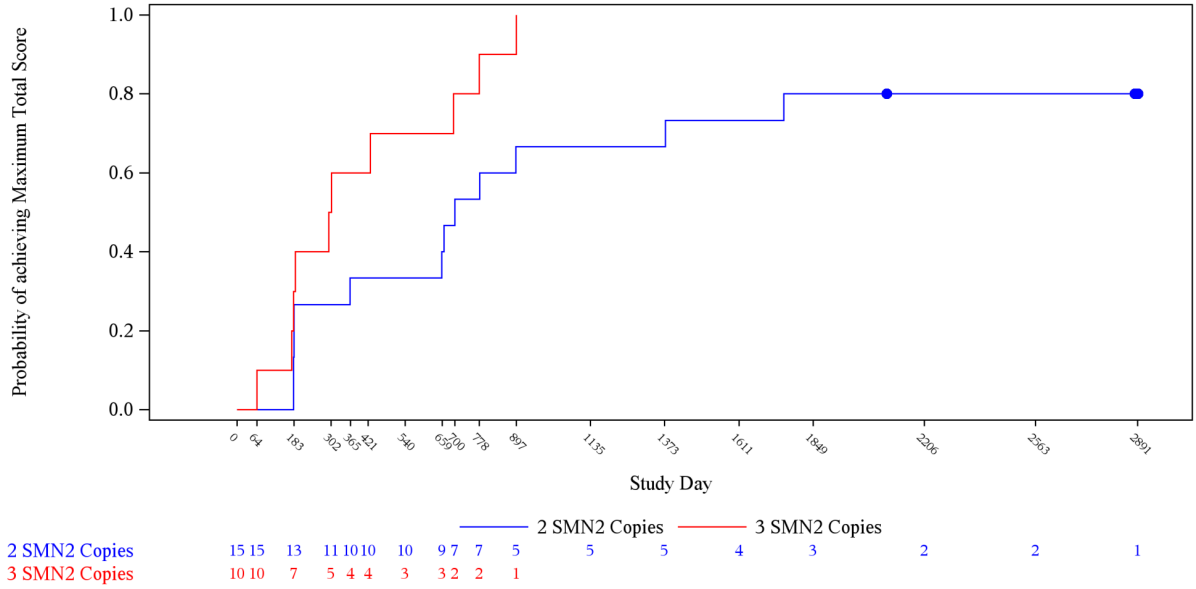
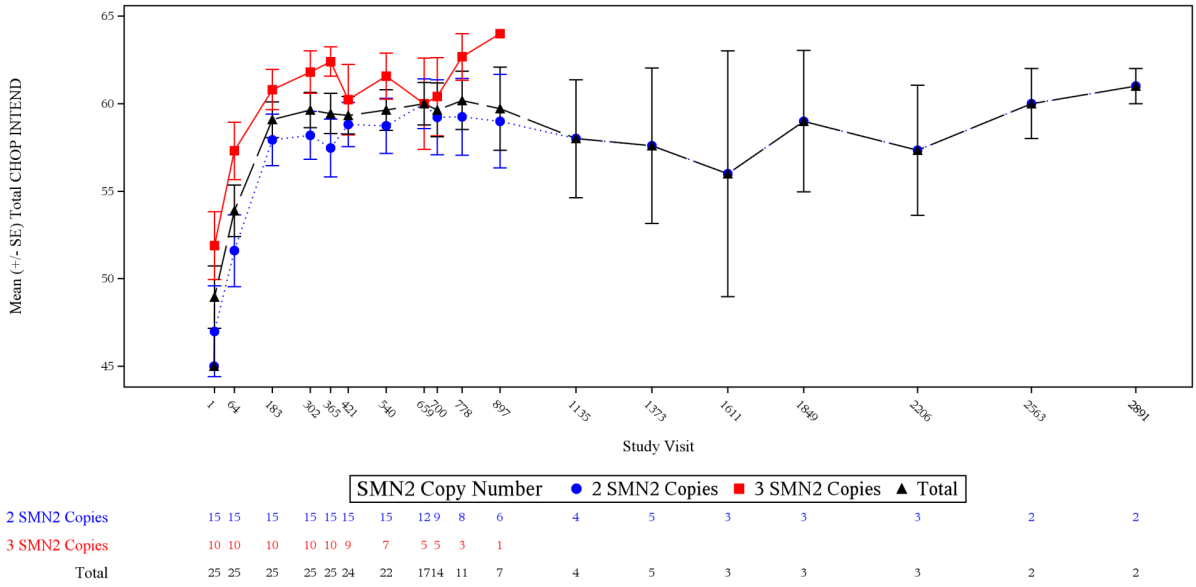


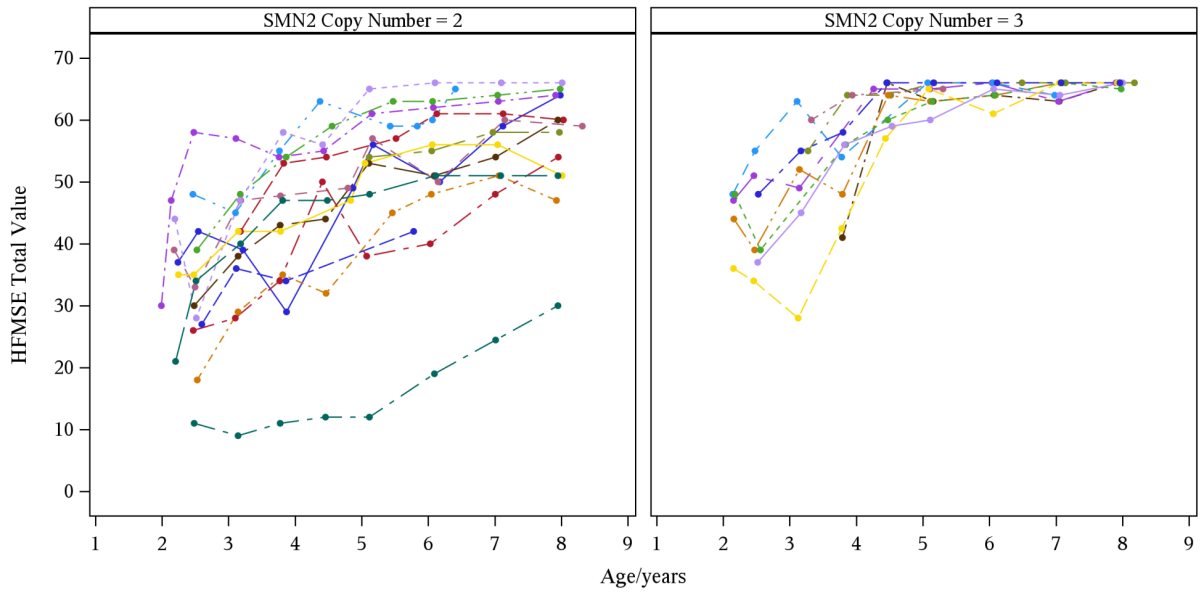
Figure 6: Mean Total CHOP INTEND Over Time - ITT Set



- HFMSE was collected from 2 years of age but could have been initiated at later visits depending on the ability of the participants. Baseline was defined as the first evaluable assessment. At first evaluable assessment after 2 years of age, the mean total HFMSE scores were 34.5 and 46.4 for participants with 2 *SMN2* copies and 3 *SMN2* copies, respectively. Forty-eight months after the first assessment, the mean score for participants with 2 *SMN2* copies increased to 52.3 of a

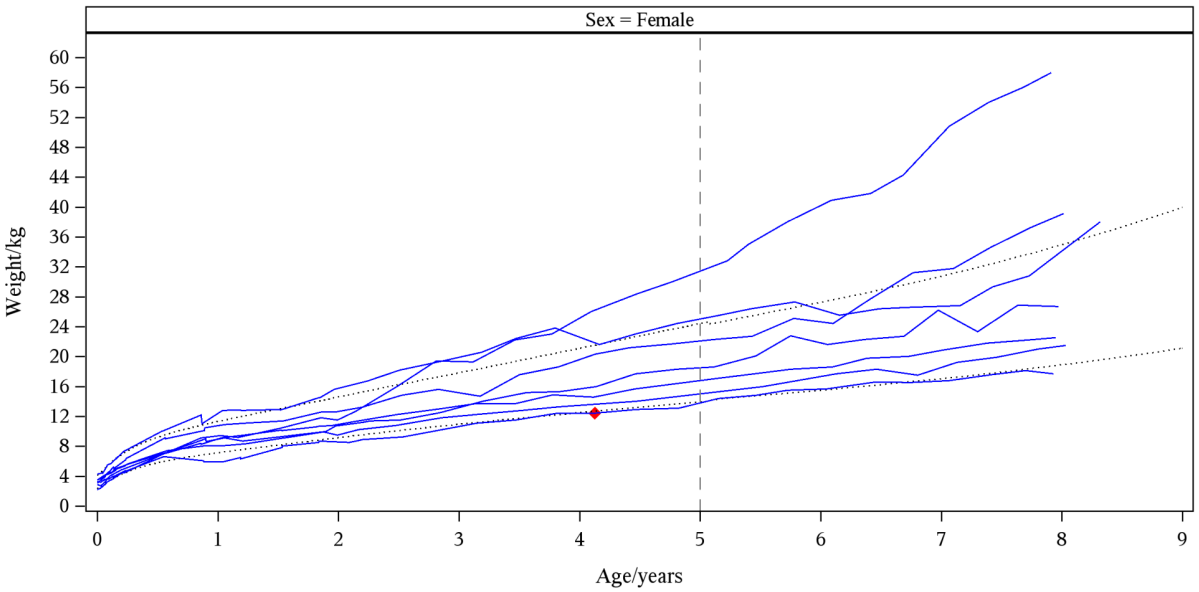
maximum possible score of 66 (mean change from baseline of 18.7) and the mean score for participants with 3 *SMN2* copies increased to 64.9 of a maximum possible score of 66 (mean change from baseline of 20.0). Considering the last study visit, on Day 2891 (rather than using the baseline of the first evaluable assessment), the mean (minimum, maximum) score for participants with 2 *SMN2* copies was 56.1 (30, 66) of a maximum possible score of 66 and the mean score for participants with 3 *SMN2* copies was 65.9 (65, 66) of a maximum possible score of 66.

Figure 7: Spaghetti Plots of HFMSE Total Score Over Time and by SMN2 Copy Number – ITT Set



- Mean increases in weight, body length, head circumference, and chest circumference as assessed by the mean changes from baseline were observed for the 25 nusinersen-treated participants at every study visit.

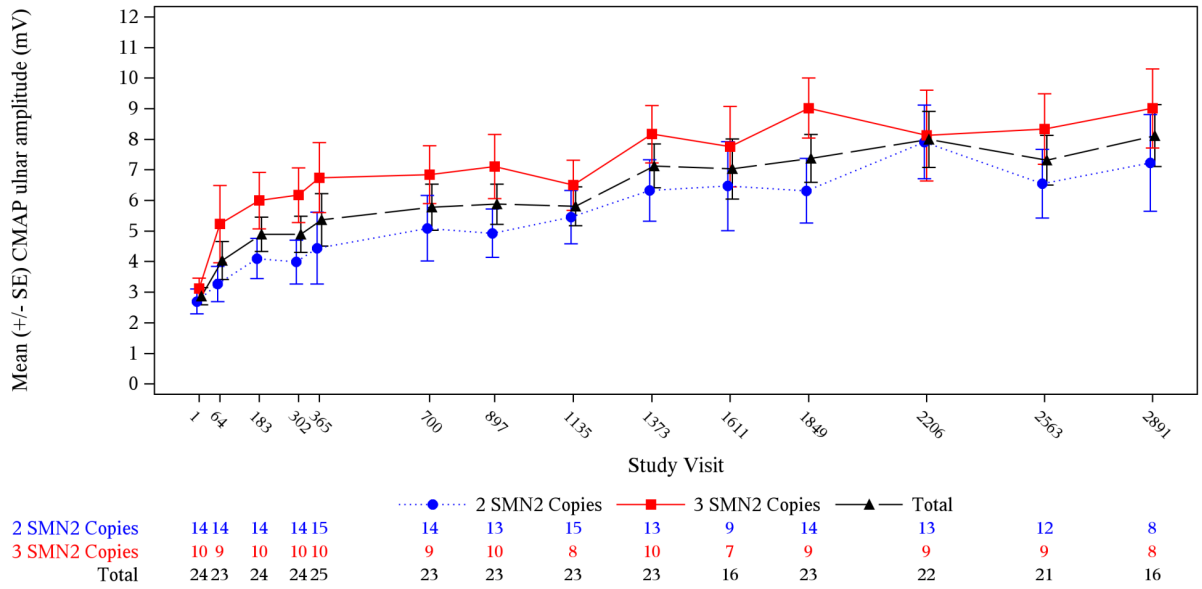
Figure 8: Growth Parameters: Individual Plots of Weight Over Age by SMN2 Copy Number and Gender - ITT Set



- Five participants, all with 2 *SMN2* copies, had a percutaneous gastric tube inserted during the study.
- Eight of 15 participants with 2 *SMN2* copies had peroneal (anterior tibialis) measured at baseline ≥ 2 mV and reflex present on HINE-1 assessment. Eight of 10 participants with 3 *SMN2* copies had peroneal (anterior tibialis) measured at baseline ≥ 2 mV and reflex present on HINE-1 assessment.
    - One of the 8 participants with 2 *SMN2* copies and none of the 8 participants with 3 *SMN2* copies required respiratory intervention.
    - All of the 8 participants with 2 *SMN2* copies and all of the 8 participants with 3 *SMN2* copies achieved all WHO motor milestones.
    - None of the 8 participants with 2 *SMN2* copies and none of the 8 participants with 3 *SMN2* copies required a percutaneous endoscopic gastrostomy (PEG) tube prior to 24 months of age.
    - One of the 8 participants with 2 *SMN2* copies and none of the 8 participants with 3 *SMN2* copies displayed clinically manifested SMA symptoms (as per secondary endpoint) by 24 months of age.
- Ulnar CMAP amplitudes at baseline had a wide range across participants with 2 and 3 *SMN2* copies (1.0 through 6.7 mV and 1.8 through 4.9 mV, respectively). Mean CMAP amplitudes were 2.69 and 3.11 mV, respectively.
- Ulnar and peroneal mean CMAP amplitudes increased over the course of the study in participants with 2 and 3 *SMN2* copies. Improvement in ulnar CMAP amplitudes in participants with 3 *SMN2* copies was consistently higher throughout the study compared to those with 2 *SMN2* copies. For peroneal CMAP amplitudes, mean values were similar in participants with 2 and 3 *SMN2* copies up to Day 1611. After Day 1611, mean peroneal CMAP values stabilized in participants with 3 *SMN2* copies while they continued to increase in participants with 2 *SMN2* copies. Mean CMAP amplitudes did not decrease compared with baseline, which is normally associated with the

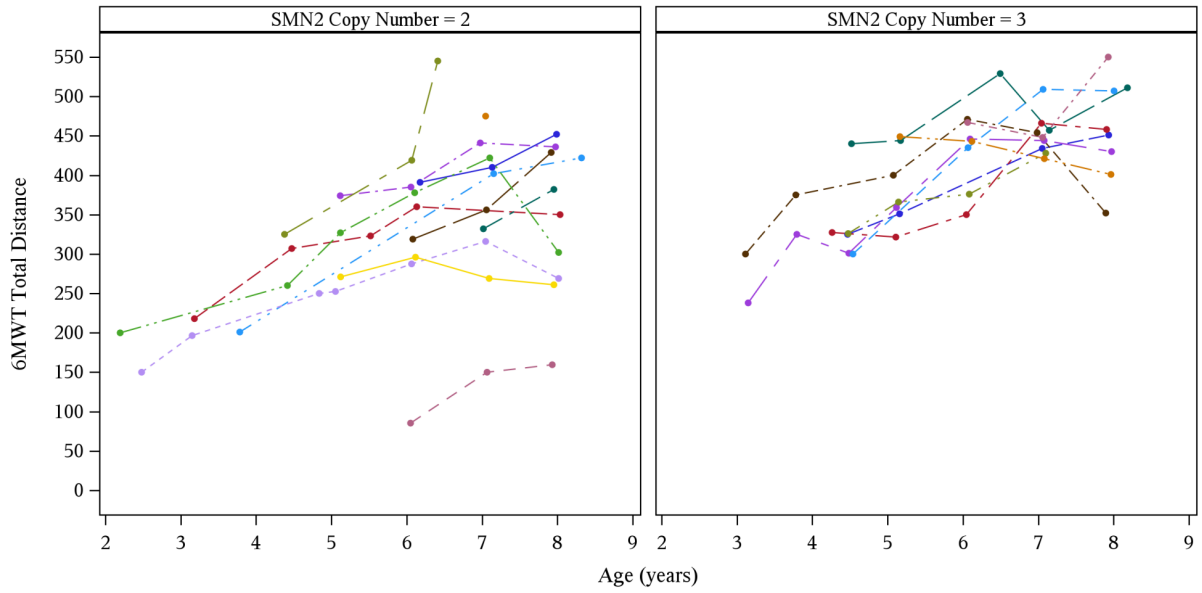
development of SMA.

Figure 9: Mean CMAP - Ulnar Amplitude (mV) Over Time - ITT Set



- Participants were able to comply with and undertake 6MWT assessments at different times. Mean 6MWT distances, on average, increased with time and were higher in participants with 3 *SMN2* copies compared to those with 2 *SMN2* copies.

Figure 10: Spaghetti Plot of 6MWT Distance Over Time and by SMN2 Copy Number For Valid 6MWT Tests - ITT Set



- Annualized rates of respiratory events over time were higher in participants with 2 *SMN2* copies than in those with 3 *SMN2* copies. Rates decreased over the study in participants with 2 *SMN2* copies and remained low but constant in participants with 3 *SMN2* copies.

- Serious respiratory events occurred in participants with 2 *SMN2* copies but not in those with 3 *SMN2* copies. Annualized rates decreased over the study in participants with 2 *SMN2* copies.

- Choking, respiratory distress, and respiratory failure were the preferred terms (PTs) driving the AEs in participants with 2 *SMN2* copies, and these terms are in keeping with symptoms of the SMA disease.

- Based on the HINE-1 item suck/swallow assessment up to Day 788, 12 of 15 participants with 2 *SMN2* copies maintained a normal suck/swallow function from baseline to the last assessment. None of the participants with 3 *SMN2* copies showed a decline in suck/swallow function.

- Five participants with 2 *SMN2* copies demonstrated consistent bulbar disability on the PASA questionnaire.

Other participants with 2 *SMN2* copies demonstrated more consistent retention of normal function and did not require a gastric tube. Participants with 3 *SMN2* copies maintained normal or near-normal scores.

Safety results

The table below summarizes the safety results.

Summary of Safety Results

Safety Assessment	**Key Results**
Deaths	No deaths were reported during the study.
SAEs	• Fourteen participants (56%) experienced at least 1 SAE, none of which were assessed as possibly related or related to study treatment by the Investigator. • No participants experienced SAEs that led to discontinuation of study treatment or withdrawal from the study. • The SAEs that occurred in > 5% of participants were pneumonia (20%); respiratory syncytial virus infection and choking (12% each); and respiratory syncytial virus bronchiolitis, upper respiratory tract infection, respiratory distress, respiratory failure, dehydration, pyrexia, and tonsillectomy (8% each).
AEs That Led to Discontinuation of Study Treatment	No participants experienced AEs that led to discontinuation of study treatment or withdrawal from the study.
AEs	• All 25 participants experienced at least 1 AE; the most common AEs (reported in > 70% of participants) were pyrexia, upper respiratory tract infection, cough, and nasopharyngitis. • Eight of the 25 participants (32%) experienced a severe AE on the study. Thirteen participants (52%) experienced an AE with the maximum severity of moderate, and the remaining 4 participants (16%) experienced an AE with the maximum severity of mild. No participants experienced AEs considered by the Investigator to be related to study treatment. Eleven participants (44%) experienced AEs considered to be possibly related to study treatment, and 12 participants (48%) experienced AEs considered to be related to the LP procedure.

Safety Assessment	Key Results
Laboratory Parameters, ECGs, and Vital Signs	• Overall, results for haematology, blood chemistry, urinalysis, coagulation, vital signs, and ECGs were not suggestive of any new safety concerns. • No participants had an AE of thrombocytopenia. No participants had an AE associated with coagulation disorders. • Two participants (8%) had an AE of protein urine present. Both events were mild in severity and assessed as possibly related to study treatment by the Investigator. Two participants (8%) had an AE of proteinuria. One event was mild in severity and assessed as possibly related to study treatment. The other event was moderate in severity assessed as unlikely related to study treatment. There were no AEs of acute kidney injury.
Neurological Examinations	Results for neurological assessments were not suggestive of any new safety concerns. The majority of AEs related to neurologic examinations were events expected in the natural history of SMA such as muscle weakness, gait disturbance, or hypotonia.
Immunological Assessments	Five of 25 participants (20%) had treatment- emergent anti-nusinersen antibodies, of which 1 participant (4%) had transient anti-nusinersen antibodies and 4 participants (16%) had persistent anti-nusinersen antibodies. No discernible effects of ADAs on efficacy or safety were observed.

Adverse Events

Overall Summary of Adverse Events

All AEs were classified and coded using MedDRA version 27.1. If a participant had the same event more than once, it was counted only once in the incidence for that event. All AEs were analysed based on the principle of treatment emergence. An AE was regarded as treatment emergent if it was present prior to the first dose of study treatment and subsequently worsened or was not present prior to the first dose of study treatment but subsequently appeared.

All 25 participants experienced at least 1 AE.

Overall summary of adverse events - ITT Set

Page: 1 of 1

	ISIS 396443 2 SMN2 copies	ISIS 396443 3 SMN2 copies	Total
Number of subjects dosed	15 (100)	10 (100)	25 (100)
Number of subjects with an event	15 (100)	10 (100)	25 (100)
Number of subjects with a moderate or severe event	15 (100)	6 (60)	21 (84)
Number of subjects with a severe event	7 (47)	1 (10)	8 (32)
Number of subjects with an event, possibly related or related to:			
Study treatment	5 (33)	6 (60)	11 (44)
LP procedure	9 (60)	6 (60)	15 (60)
Number of subjects with an event related to:			
Study treatment	0	0	0
LP procedure	6 (40)	6 (60)	12 (48)
Number of subjects with a serious event	10 (67)	4 (40)	14 (56)
Number of subjects with a serious event related to study treatment	0	0	0
Number of subjects discontinuing treatment due to an event	0	0	0
Number of subjects withdrawing from study due to an event	0	0	0

NOTE: Numbers in parentheses are percentages.
LP = Lumbar puncture
Source: isis396443/232sm201/final-analysis/t-ae-overall.sas **Run Date:** 06MAR2025

The most common AEs by SOC (≥ 50% of participants) were Infections and infestations (24 participants [96%] with 433 events); Respiratory, thoracic, and mediastinal disorders (24 participants [96%] with 218 events); General disorders and administration site conditions (23 participants [92%] with 140 events); Gastrointestinal disorders (22 participants [88%] with 121 events); Nervous system disorders (22 participants [88%] with 76 events); Investigations (20 participants [80%] with 62 events); Injury, poisoning and procedural complications (18 participants [72%] with 95 events); Skin and subcutaneous tissue disorders (18 participants [72%] with 58 events); and Metabolism and nutrition disorders (15 participants [60%] with 33 events).

By PT, the most common AEs (reported in > 70% of participants) were pyrexia (22 participants [88%] with 120 events), upper respiratory tract infection (18 participants [72%] with 71 events), nasopharyngitis (18 participants [72%] with 68 events), and cough (18 participants [72%] with 61 events). In total, 1394 events were reported across all participants. The most common AE by number of events was pyrexia (120 events [9%] in 22 participants).

Analysis by Severity

The severity of each AE was assessed by the Investigator as mild, moderate, or severe. If a participant experienced the same AE multiple times, the event with the maximum severity was counted.

Eight of the 25 participants (32%) experienced a severe AE. Thirteen participants (52%) experienced an AE with the maximum severity of moderate, and the remaining 4 participants (16%) experienced an AE with the maximum severity of mild.

The most common SOCs for severe AEs were Infections and infestations (5 participants [20%]) and Respiratory, thoracic and mediastinal disorders (3 participants [12%]). Severe events in all other SOCs were reported for individual participants only. By PT, the most common severe AE was pneumonia (2 participants [8%]), with all other severe AEs reported for individual participants only.

Incidence of severe adverse events - ITT set

Page: 1 of 2

	ISIS 396443 2 SMN2 copies	ISIS 396443 3 SMN2 copies	Total
Number of subjects dosed	15 (100)	10 (100)	25 (100)
Number of subjects with an severe event	7 (47)	1 (10)	8 (32)
Infections and infestations	5 (33)	0	5 (20)
Pneumonia	2 (13)	0	2 (8)
Coronavirus infection	1 (7)	0	1 (4)
Enterovirus infection	1 (7)	0	1 (4)
Gastrointestinal viral infection	1 (7)	0	1 (4)
Pneumonia aspiration	1 (7)	0	1 (4)
Pneumonia pseudomonal	1 (7)	0	1 (4)
Pneumonia respiratory syncytial viral	1 (7)	0	1 (4)
Respiratory syncytial virus bronchiolitis	1 (7)	0	1 (4)
Upper respiratory tract infection	1 (7)	0	1 (4)
Respiratory, thoracic and mediastinal disorders	3 (20)	0	3 (12)
Cyanosis central	1 (7)	0	1 (4)
Respiratory distress	1 (7)	0	1 (4)
Respiratory failure	1 (7)	0	1 (4)
Gastrointestinal disorders	1 (7)	0	1 (4)
Faecaloma	1 (7)	0	1 (4)
General disorders and administration site conditions	1 (7)	0	1 (4)
Pyrexia	1 (7)	0	1 (4)

NOTE 1: Numbers in parentheses are percentages.
2: A subject was counted only once within each system organ class and preferred term (MedDRA version 27.1).
3:System organ class and preferred term are presented in decreasing frequency of the table's rightmost column.
Source: isis396443/232sm201/final-analysis/t-ae-sev.sas **Run Date:** 29JAN2025

Incidence of severe adverse events - ITT set

Page: 2 of 2

	ISIS 396443 2 SMN2 copies	ISIS 396443 3 SMN2 copies	Total
Immune system disorders	1 (7)	0	1 (4)
Anaphylactic reaction	1 (7)	0	1 (4)
Injury, poisoning and procedural complications	0	1 (10)	1 (4)
Post lumbar puncture syndrome	0	1 (10)	1 (4)
Investigations	1 (7)	0	1 (4)
Respirovirus test positive	1 (7)	0	1 (4)
Nervous system disorders	1 (7)	0	1 (4)
Loss of consciousness	1 (7)	0	1 (4)
Skin and subcutaneous tissue disorders	1 (7)	0	1 (4)
Superficial inflammatory dermatosis	1 (7)	0	1 (4)

NOTE 1: Numbers in parentheses are percentages.
2: A subject was counted only once within each system organ class and preferred term (MedDRA version 27.1).
3:System organ class and preferred term are presented in decreasing frequency of the table's rightmost column.
Source: isis396443/232sm201/final-analysis/t-ae-sev.sas **Run Date:** 29JAN2025

Analysis by Relationship to Study Treatment

The Investigator assessed the relationship of each AE to study treatment as not related, unlikely related, possibly related, or related. If a participant experienced the same AE multiple times, the event with the most conservative assessment was counted.

No participants experienced AEs considered related to study treatment, as assessed by the Investigator. Eleven participants (44%) experienced AEs considered to be possibly related to study treatment, 5 participants (20%) experienced AEs with the strongest relationship considered to be unlikely related to study treatment, and 9 participants (36%) experienced only AEs considered to be not related to study treatment.

The following AEs were considered to be possibly related to study treatment: hyperreflexia and protein urine present (2 participants [8%] each) and alanine aminotransferase increased, aspartate aminotransferase increased, blood alkaline phosphatase increased, blood calcium increased, clonus, dermatitis allergic, eosinophil count increased, extensor plantar response, lymphocyte count increased, muscular weakness, musculoskeletal procedural complication, platelet count increased, post lumbar puncture syndrome, proteinuria, pyrexia, rash, tachycardia, and white blood cell count increased (1 participant [4%] each).

Incidence of related or possibly related adverse events by preferred term - ITT Set

Page: 1 of 1

	ISIS 396443 2 SMN2 copies	ISIS 396443 3 SMN2 copies	Total
Number of subjects dosed	15 (100)	10 (100)	25 (100)
Number of subjects with an related event	5 (33)	6 (60)	11 (44)
Hyperreflexia	1 (7)	1 (10)	2 (8)
Protein urine present	1 (7)	1 (10)	2 (8)
Alanine aminotransferase increased	1 (7)	0	1 (4)
Aspartate aminotransferase increased	1 (7)	0	1 (4)
Blood alkaline phosphatase increased	1 (7)	0	1 (4)
Blood calcium increased	1 (7)	0	1 (4)
Clonus	1 (7)	0	1 (4)
Dermatitis allergic	0	1 (10)	1 (4)
Eosinophil count increased	1 (7)	0	1 (4)
Extensor plantar response	1 (7)	0	1 (4)
Lymphocyte count increased	1 (7)	0	1 (4)
Muscular weakness	1 (7)	0	1 (4)
Musculoskeletal procedural complication	1 (7)	0	1 (4)
Platelet count increased	0	1 (10)	1 (4)
Post lumbar puncture syndrome	0	1 (10)	1 (4)
Proteinuria	1 (7)	0	1 (4)
Pyrexia	1 (7)	0	1 (4)
Rash	0	1 (10)	1 (4)
Tachycardia	0	1 (10)	1 (4)
White blood cell count increased	1 (7)	0	1 (4)

NOTE 1: Numbers in parentheses are percentages.
2: A subject was counted only once within each preferred term (MedDRA version 27.1).
3: Preferred terms are presented in decreasing frequency of the table's rightmost column.

Analysis by Relationship to Lumbar Puncture Procedure

The Investigator assessed the relationship of each AE to the LP procedure as not related, unlikely related, possibly related, or related. In cases where 1 participant had multiple AEs related to LP, the AE with the strongest relationship to the LP procedure was used in the calculation of incidence by relationship to the LP procedure.

Twelve participants (48%) experienced an AE considered related to the LP procedure, as assessed by the Investigator. Three participants (12%) experienced an AE with the strongest relationship of possibly related to LP, 1 participant (4%) experienced an AE with the strongest relationship of unlikely related to LP, and 9 participants (36%) experienced only AEs considered not related to LP. AEs considered by the Investigator to be related to the LP procedure included post lumbar puncture syndrome (6 participants [24%]); procedural pain (5 participants [20%]); and post procedural complication, musculoskeletal procedural complication, post procedural swelling, procedural vomiting, and subdural hematoma (1 participant [4%] each). All AEs considered by the Investigator to be related to the LP procedure resolved.

Deaths, Serious Adverse Events, and Other Significant Events

Deaths

No deaths were reported during the study.

Serious Adverse Events

Fourteen participants (56%) experienced at least 1 SAE, with a total of 71 SAEs reported. The most frequently reported SAEs were pneumonia (5 participants [20%]); respiratory syncytial virus infection and choking (3 participants [12%] each); and respiratory syncytial virus bronchiolitis, upper respiratory tract infection, respiratory distress, respiratory failure, dehydration, pyrexia, and tonsillectomy (2 participants [8%] each). All other SAEs were reported for 1 participant each (4%).

All SAEs resolved or downgraded to nonserious AEs. No treatment-emergent SAEs were assessed as possibly related or related to study treatment by the Investigator. No participants experienced SAEs that led to discontinuation from study treatment or withdrawal from the study. No participants experienced SAEs with a fatal outcome.

Adverse Events of Special Interest

There were no AEs of special interest specified in the protocol for this study.

Discontinuation of Study Treatment Due to Adverse Events

No participants experienced AEs that led to discontinuation of study treatment.

Withdrawal From Study Due to Adverse Events

No participants experienced AEs that led to withdrawal from the study.

Dose Modifications Due to Adverse Events

Dose modifications were not permitted.

Clinical Laboratory Results

Laboratory assessments, including haematology, blood chemistry, urinalysis, and coagulation were conducted according to the Schedule of Activities. Results of laboratory assessments were summarized using shift tables, presenting changes relative to each parameter's normal range. Each participant's laboratory values were classified according to whether the test result was "low" (i.e., below the lower limit of normal), "normal" (within the normal range), or "high" (i.e., above the ULN). If a participant was missing a baseline value but had a postbaseline value, then the baseline assessment was labelled as "unknown." Likewise, if a participant had a baseline value but had no postbaseline values, then the minimum and maximum were labelled as "unknown." Postbaseline laboratory results are defined as any assessment taken after the first dose, including data collected from local laboratories.

Laboratory actual values and changes from baseline were also presented. Laboratory values outside the normal range were identified in the listings.

Haematology Results

Overall, there were no clinically meaningful changes over time for the haematology analytes. In general, parameter shifts from baseline to abnormal values for individual participants were mild and transient.

AEs associated with shifts in haematology values included anaemia in 7 participants (28%); neutropenia, iron deficiency anaemia, lymphocyte count increased, neutrophil count increased, platelet count increased, and white blood cell count increased in 2 participants (8%) each; and eosinophilia, hypochromic anaemia, lymphadenopathy, lymphocytosis, neutrophilia, eosinophil count increased, and haemoglobin decreased in 1 participant (4%) each.

Of the 7 participants with the event of anaemia, 5 participants experienced events with the maximum severity of mild and 2 participants experienced moderate events of anaemia, with some participants experiencing > 1 event. Six participants experienced events assessed as not related to study treatment, and 1 participant experienced an event assessed as unlikely related to study treatment). At the end of the study, events in 5 participants resolved and events in 3 participants were ongoing.

One event of neutropenia was assessed as mild in severity, and 1 event was assessed as moderate in severity. All other AEs associated with haematology parameters were mild in severity and were considered to be unlikely or not related to study treatment.

Although participants experienced shifts in platelet counts and platelet count increased was reported as an AE in 2 participants, counts generally remained stable over time and there were no AEs of thrombocytopenia reported.

Blood Chemistry Results

Liver Function Tests

Examination of postbaseline values relative to ULNs for liver enzymes showed the following:

- Eleven of 25 participants (44%) had ALT values > ULN. Two participants (8%) had ALT values > 10 × ULN, and 1 participant (4%) had ALT values > 20× ULN.
- Ten of 25 participants (40%) had AST values > ULN. Two participants (8%) had AST values > 5× ULN, and 1 participant (4%) had AST values > 10× ULN.
- Seven of 25 participants (28%) had total bilirubin values > ULN. Three participants (12%) had total bilirubin values > 2 × ULN.
- No participants had AST or ALT ≥ 3 × ULN with concurrent total bilirubin > 1.5 × ULN.
- Eight of 25 participants (32%) had alkaline phosphate values > ULN. Three participants (12%) had alkaline phosphate values > 1.5 × ULN.

AEs associated with changes in liver function test parameters were AST increased (2 participants [8%]), blood alkaline phosphatase increased (2 participants [8%]), and ALT increased (1 participant [4%]). All of these events resolved without the need for treatment discontinuation.

Kidney Function Tests

Five of 25 participants (20%) had a shift in creatinine from baseline to high; however, there were no AEs of acute kidney injury reported. There was 1 AE of cystatin C increased; this event was mild in severity and assessed as unlikely to be related to study treatment by the Investigator. The dose was not changed, and the participant recovered.

Other Blood Chemistry Tests

AEs associated with changes in clinical laboratory parameters were hypocalcaemia, blood calcium increased, blood glucose decreased, hypoglycaemia, and vitamin D deficiency. All events resolved without the need for study treatment modification, with the exception of 2 events of vitamin D deficiency in 2 participants, which were ongoing as of the end of the study. Both ongoing events of vitamin D deficiency were mild and considered to be not related to study treatment.

Urinalysis Results

Seventeen of 25 participants (68%) had at least 1 positive postbaseline urinary protein result, and 13 of 25 participants (52%) had at least 2 positive postbaseline urinary protein results. Eight of 25 participants (32%) had 2 or more consecutive positive urinary protein results.

Two participants (8%) experienced AEs of protein urine present; both events were mild in severity and assessed as possibly related to study treatment by the Investigator. Two participants (8%) experienced AEs of proteinuria; the event in 1 participant (4%) was mild in severity and assessed as possibly related to study treatment. The other event in 1 participant (4%) was moderate in severity and assessed as unlikely related to study treatment.

Coagulation Results

There were no clinically meaningful changes over time for the coagulation studies. No participants experienced an AE associated with coagulation disorders.

Vital Sign Measurements, Physical Examination Findings, and Other Observations Related to Safety

Vital Sign Measurements

With the exception of pyrexia, AEs associated with vital sign abnormalities were infrequently reported. Twenty-two participants (88%) had at least 1 AE of pyrexia, 6 participants (24%) had AEs of tachycardia, 4 participants (16%) had AEs of respiratory disorder, 3 participants (12%) had AEs of dyspnoea, and 2 participants each (8%) had AEs of respiratory distress, respiratory failure, and breath sounds abnormal. All other events were reported by 1 participant (4%) and included hypertension, heart rate increased, body temperature increased, oxygen saturation decreased, and acute respiratory failure.

ECG Findings

No clinically significant abnormal ECG results, including AEs of prolonged QT interval, were reported.

Echocardiogram Findings

One participant (4%) with data available had a clinically significant abnormal echocardiogram finding. AEs of aortic dilatation and pulmonary artery dilatation were reported for the same participant. Both events were mild, not considered by the Investigator to be related to study treatment, and ongoing as of the end of the study.

Neurological Examinations

The HINE (Sections 1 and 3) was conducted in all participants ≤ 24 months of age. This standard examination is a quantitative scorable method for assessing the neurological development of infants between 2 and 24 months of age. The examination includes assessment of cranial nerve functions, posture, movements, tone, and reflexes. For all participants > 24 months of age, standard neurological examinations were conducted, including mental status, level of consciousness, sensory function, motor function, cranial nerve function, and reflexes.

Results for neurological assessments were not suggestive of any new safety concerns. The majority of AEs related to neurologic examinations were events expected in the natural history of SMA such as muscle weakness, gait disturbance, or hypotonia.

Immunological Assessments

Five of 25 participants (20%) had treatment-emergent anti-nusinersen antibodies, of which 1 participant (4%) had transient anti-nusinersen antibodies and 4 participants (16%) had persistent anti-nusinersen antibodies. No discernible effects of ADAs on efficacy or safety were observed.

Summary of antidrug antibody status - ITT Set
Page: 1 of 2

Antibody status	ISIS 396443 2 SMN2 copies	ISIS 396443 3 SMN2 copies	Total
Number of subjects dosed	15 (100)	10 (100)	25 (100)
Baseline status (a)			
Positive	0	0	0
Negative	13 (87)	8 (80)	21 (84)
Unknown	2 (13)	2 (20)	4 (16)
Baseline positive			
Treatment-emergent positive (b)	0	0	0
Negative	0	0	0
No evaluable sample post baseline	0	0	0
Baseline negative			
Treatment-emergent positive (c)	4 (27)	1 (10)	5 (20)
Negative	9 (60)	7 (70)	16 (64)
No evaluable sample post baseline	0	0	0
Baseline unknown			
Positive post baseline (c)	0	0	0
Negative	2 (13)	2 (20)	4 (16)
No evaluable sample post baseline	0	0	0
Subjects with persistent drug antibodies (d)	4 (27)	0	4 (16)
Subjects with transient drug antibodies (e)	0	1 (10)	1 (4)

Note:(a) Baseline value is defined as the immunogenicity data collected prior to first dose.
(b) A subject is counted as treatment-emergent positive if this subject has at least one post-baseline sample with a>2-fold increase in titer.
(c) A subject is counted as treatment-emergent positive if this subject has at least one confirmed positive post-treatment.
(d) A subject is counted having persistent drug antibodies if this subject has one positive test followed by another one more than 100 days after the first positive test or with one or more positive samples no more than 100 days after the first positive.
(e) A subject is counted having transient drug antibodies if this subject is not in the scenarios of persistent drug antibodies.
(f) The number is used as denominator of the rows below.
Source: isis396443/232sm201/final-analysis/t-ant-all.sas **Run Date:** 02APR2025

Summary of antidrug antibody status - ITT Set
Page: 2 of 2

Antibody status	ISIS 396443 2 SMN2 copies	ISIS 396443 3 SMN2 copies	Total
Number of patients with negative or positive or unknown status at baseline and at least one evaluable sample post baseline (f)	15 (100)	10 (100)	25 (100)
Subjects defined as treatment emergent positive	4/15 (27)	1/10 (10)	5/25 (20)
Subjects with transient drug antibodies (a)	0/15	1/10 (10)	1/25 (4)
Subjects with persistent drug antibodies (a)	4/15 (27)	0/10	4/25 (16)

Note:(a) Baseline value is defined as the immunogenicity data collected prior to first dose.
(b) A subject is counted as treatment-emergent positive if this subject has at least one post-baseline sample with a>2-fold increase in titer.
(c) A subject is counted as treatment-emergent positive if this subject has at least one confirmed positive post-treatment.
(d) A subject is counted having persistent drug antibodies if this subject has one positive test followed by another one more than 100 days after the first positive test or with one or more positive samples no more than 100 days after the first positive.
(e) A subject is counted having transient drug antibodies if this subject is not in the scenarios of persistent drug antibodies.
(f) The number is used as denominator of the rows below.
Source: isis396443/232sm201/final-analysis/t-ant-all.sas **Run Date:** 02APR2025

The incidence of AEs selected by anaphylactic reaction SMQ, angioedema SMQ, and hypersensitivity SMQ was evaluated for participants by antibody status postbaseline. No new safety concerns were identified after comparing AE frequency in these SMQ results between participants with positive anti-nusinersen antibody status and participants with negative anti-nusinersen antibody status.

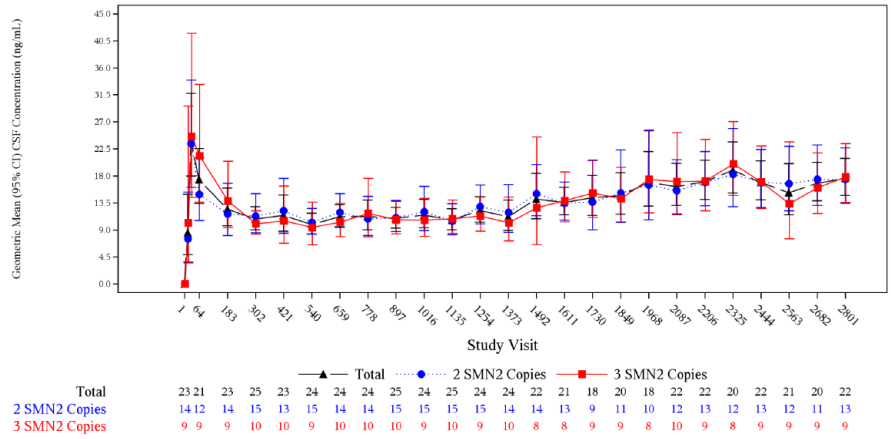
Pharmacokinetic results

CSF and Plasma samples were collected at protocol designated times for ISIS 396443 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 Set was the same as the ITT Set, thus considering all 25 enrolled participants.

CSF Nusinersen Concentrations

The geometric mean nusinersen CSF concentrations are plotted over time in Figure 11. After 2 loading doses, nusinersen CSF concentrations accumulated approximately 2.0-fold with a predose mean (SD) of 29.40 (20.104) ng/mL on Day 29. With additional dosing, predose mean (SD) CSF concentrations declined and were maintained at 14.62 (8.860) ng/mL on Day 183 and 14.49 (5.164) ng/mL on Day 1611. The mean values from Days 183 through 1611 ranged from 10.70 through 16.68 ng/mL. The mean (SD) predose CSF concentration on the last sampling day (Day 2801) was 19.07 (8.666) ng/mL.

Figure 11: Plot of CSF Nusinersen Geometric Mean (95% CI) Over Time - PK Set

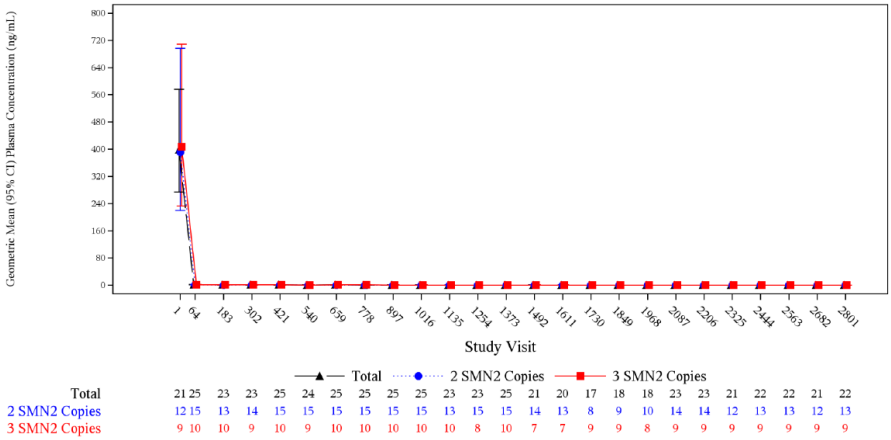


Interparticipant variabilities (%CV) in nusinersen CSF concentrations (Days 15 through 2801) were low to high and ranged from 35% to 78%. Nusinersen CSF concentrations were similar between participants with 2 and 3 *SMN2* copies.

Plasma Nusinersen Concentrations

The geometric mean plasma nusinersen concentrations are plotted over time in Figure 12. As expected, there was no accumulation in plasma concentration of nusinersen after multiple doses. The highest mean (SD) plasma concentration (524.58 [387.425] ng/mL) was observed after the first PK sample at 4 hours postdose on Day 1 and approximated the maximum concentration observed at 2 hours postdose in previous studies. Mean (SD) plasma concentrations declined rapidly by Day 64 to 1.70 (0.736) ng/mL. The mean (SD) plasma concentration on the last sampling day (Day 2801) was 0.41 (0.155) ng/mL.

Figure 12: Plot of Plasma Nusinersen Geometric Mean (95% CI) Over Time - PK Set



Interparticipant variabilities (%CV) plasma nusinersen concentrations (Days 64 through 2801) were low to high and ranged from 25% to 61%. Nusinersen plasma concentrations were similar between participants with 2 and 3 *SMN2* copies.

2.3.3. Discussion on clinical aspects

The MAH has presented the final report for study 232SM201.

Pharmacokinetics

Regarding PK, although no definitive PK analysis was made and only a descriptive one was presented, the observed values are in accordance to what was previously known and described in the SmPC of Spinraza: A CSF steady state occurring at around 7-8 years of treatment and no accumulation of plasma concentrations over time. No major additional findings were observed.

Efficacy

The use of Nusinersen in pre-symptomatic patients (or paucisymptomatic patients with *SMN2* two copies, which as a group were lower performers at the time of enrolment as compared to patients with 3 *SMN2* copies) has shown that it has a significant impact on the disease expression and prognosis. Only 5 out of 25 patients (and all in *SMN2* two copies population) have achieved the primary endpoint, and four in relation to an intercurrent event, recovering afterwards.

All patients have acquired and retained milestones. For those with *SMN2* two copies, ambulation without help does not occur according to natural history of the disease, and was achieved in 87% of these patients. In the *SMN2* three copies population, all patients achieved the 6 (WHO) motor milestones and there were no signs of a halt or reversion of acquired milestones.

Dysphagia was observed in three of the *SMN2* two copies population only, which would be expected in 100% of this population.

Overall, it is reasonable to consider that start of treatment in pre-symptomatic patients results in greater milestone acquisition than in natural history, with signs of sustained improvement or stabilisation and virtually no worsening not related to intercurrent illnesses.

There was no comparison to a delayed treatment start. However, if the results are compared to the patients who participated in the pivotal trials that led to MAH, it is evident that the achievement of milestones occurs earlier on average and the improvement is more sustained.

Safety

The safety profile of nusinersen in this study was consistent with the known safety profile of nusinersen 12 mg and previous clinical findings. Overall, no new safety concerns were identified during the study, being most AEs and SAEs consistent with those commonly observed in paediatric or SMA populations.

In this small study (25 patients), all participants experienced at least 1 AE; the most common AEs (reported in > 70% of participants) were pyrexia, upper respiratory tract infection, cough, and nasopharyngitis. No participants experienced AEs considered to be related to study treatment, as assessed by the Investigator. Eleven participants (44%) experienced AEs considered by the Investigator to be possibly related to study treatment, and 12 participants (48%) experienced AEs considered by the Investigator to be related to the LP procedure.

No deaths were reported, and no participants experienced AEs that led to discontinuation of study treatment or withdrawal from the study. A total of 14 (56%) participants experienced at least 1 SAE, none of which were assessed as possibly related or related to study treatment by the Investigator. No participants experienced SAEs that led to discontinuation of study treatment or withdrawal from the study. The SAEs that occurred in > 5% of participants were pneumonia (20%); respiratory syncytial virus infection and choking (12% each); and respiratory syncytial virus bronchiolitis, upper respiratory tract infection, respiratory distress, respiratory failure, dehydration, pyrexia, and tonsillectomy (8% each).

Overall, results for haematology, blood chemistry, urinalysis, coagulation, vital signs, ECG, and neurological assessments were not suggestive of any new safety concerns. No discernible effects of ADAs on safety were observed.

3. CHMP overall conclusion and recommendation

The results of study 232SM201 show that nusinersen is efficacious in the pre-symptomatic population with 2 or 3 *SMN2* copies.

☒ **Fulfilled:**

No regulatory action required.

4. Request for supplementary information

Not applicable