

Open-label phase 4 trial evaluating nusinersen after onasemnogene abeparvovec in children with spinal muscular atrophy

Crystal M. Proud, ... , Bora Youn, Angela D. Paradis

J Clin Invest. 2025. <https://doi.org/10.1172/JCI193956>.

Clinical Research and Public Health

In-Press Preview

Clinical Research

Muscle biology

BACKGROUND. Spinal muscular atrophy (SMA) is a rare genetic neuromuscular disease caused by deletions or mutations of the *survival motor neuron 1* gene. Despite the availability of genetically-based treatments for SMA, functional impairments and weakness persist in treated symptomatic individuals. This study addresses whether additional treatment after gene transfer therapy could provide further clinical benefits.

METHODS. Interim Day 302 findings are described from the phase 4 open-label RESPOND trial evaluating nusinersen in participants aged ≤ 36 months who had suboptimal clinical status following onasemnogene abeparvovec (OA) treatment, as determined by the investigator.

RESULTS. Thirty-seven participants included in the interim analysis were symptomatic at the time of OA administration. Most (92%) had two survival motor neuron 2 gene copies. Age at first nusinersen dose (median [range]) was 9.1 (3–33) months for participants with two SMN2 copies and 34.2 (31–36) months for those with three SMN2 copies, while time from OA dose to first nusinersen dose (median [range]) was 6.3 (3–31) and 13.3 (10–22) months, respectively. Participants had elevated neurofilament light chain (NfL) levels and low compound muscle action potential (CMAP) amplitudes at baseline, suggesting active neurodegeneration and severe denervation at study entry. Improvements from baseline were observed across a range of outcomes at Day 302, including motor function outcomes (HINE-2 and CHOP-INTEND total score), achievement of independent sitting, NfL levels, CMAP, [...]

Find the latest version:

<https://jci.me/193956/pdf>



For submission to: *The Journal of Clinical Investigation*

Open-label phase 4 trial evaluating nusinersen after onasemnogene abeparvovec in children with spinal muscular atrophy

Crystal M. Proud,¹ Richard S. Finkel,² Julie A. Parsons,³ Riccardo Masson,⁴ John F. Brandsema,⁵
Nancy L. Kuntz,⁶ Richard Foster,⁷ Wenjing Li,⁸ Ross Littauer,⁸ Jihee Sohn,^{8*} Stephanie
Fradette,⁸ Bora Youn,⁸ Angela D. Paradis,⁸ on behalf of the RESPOND Study Group

¹*Neurology and Neuromuscular Medicine, Children's Hospital of The King's Daughters,
Norfolk, VA, USA*

²*Center for Experimental Neurotherapeutics, Department of Paediatric Medicine, St Jude
Children's Research Hospital, Memphis, TN, USA*

³*Department of Pediatrics Children's Hospital Colorado, University of Colorado School of
Medicine, Aurora, CO, USA*

⁴*Neuroimmunology and Neuromuscular Disease Unit, Fondazione IRCCS Istituto Neurologico
Carlo Besta, Milan, Italy*

⁵*Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA, USA*

⁶*Division of Neurology, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL,
USA*

⁷*Biogen, Maidenhead, Berkshire, UK*

⁸*Biogen, Cambridge, MA, USA*

22

23 *At the time of contributing to the work

24 Funding: This study was sponsored by Biogen (Cambridge, MA, USA).

25

26 **Short title:** Day 302 data for RESPOND study (30/40 characters including spaces)

27 **Corresponding authors:**

28 Crystal Proud

29 Address: Children's Hospital of The King's Daughters, Norfolk, VA, USA,

30 Phone number: +1 757 668 9920

31 Email address: Crystal.Proud@CHKD.ORG

32 Angela D Paradis

33 Address: Biogen, Cambridge, MA, USA

34 Phone number: +1 781 464 5020

35 Email address: angela.paradis@biogen.com

36 **Word count:** 10221 words (Recommended: 9000 words; max. 12 000 [incl. title page, full text,
37 references, figure legends, and tables])

38 **Number of figures and table:** 11 (9 figures, 2 tables)

39 **Number of supplementary figures and tables:** 4 (1 figure, 3 tables)

40 **Number of references:** 69

Abstract

Background: Spinal muscular atrophy (SMA) is a rare genetic neuromuscular disease caused by deletions or mutations of the *survival motor neuron 1* gene. Despite the availability of genetically-based treatments for SMA, functional impairments and weakness persist in treated symptomatic individuals. This study addresses whether additional treatment after gene transfer therapy could provide further clinical benefits.

Methods: Interim Day 302 findings are described from the phase 4 open-label RESPOND trial evaluating nusinersen in participants aged ≤ 36 months who had suboptimal clinical status following onasemnogene abeparvovec (OA) treatment, as determined by the investigator.

Results: Thirty-seven participants included in the interim analysis were symptomatic at the time of OA administration. Most (92%) had two *survival motor neuron 2* gene copies. Age at first nusinersen dose (median [range]) was 9.1 (3–33) months for participants with two *SMN2* copies and 34.2 (31–36) months for those with three *SMN2* copies, while time from OA dose to first nusinersen dose (median [range]) was 6.3 (3–31) and 13.3 (10–22) months, respectively. Participants had elevated neurofilament light chain (NfL) levels and low compound muscle action potential (CMAP) amplitudes at baseline, suggesting active neurodegeneration and severe denervation at study entry. Improvements from baseline were observed across a range of outcomes at Day 302, including motor function outcomes (HINE-2 and CHOP-INTEND total score), achievement of independent sitting, NfL levels, CMAP, and investigator- and caregiver-reported outcomes. Mean NfL levels decreased rapidly from baseline to Day 183 and remained low at Day 302. Mean ulnar and peroneal CMAP amplitudes increased. No safety concerns were identified.

Conclusion: Improvements in clinical and biomarker outcomes support the benefit of nusinersen treatment in infants and children with suboptimal clinical status following OA.

Trial registration: ClinicalTrials.gov ID, NCT04488133; EudraCT number, 2020-003492-18

Funding: This study was sponsored by Biogen (Cambridge, MA, USA).

Brief summary (25/25 words)

Improvements in clinical and biomarker outcomes were observed after nusinersen treatment in children with spinal muscular atrophy who had suboptimal clinical status following onasemnogene abeparvovec.

Keywords: compound muscle action potential; motor function, neurofilament; nusinersen; onasemnogene abeparvovec; spinal muscular atrophy

Introduction

Spinal muscular atrophy (SMA) is an autosomal-recessive, neuromuscular disease characterized by progressive neurodegeneration, muscle atrophy, and muscle weakness.(1-3) It is caused by deletions or mutations of the *survival motor neuron 1 (SMN1)* gene, reducing/stopping production of full-length SMN protein. The paralogous *survival motor neuron 2 (SMN2)* gene produces mostly truncated, dysfunctional SMN protein, with only approximately 10% of *SMN2* transcripts resulting in the production of full-length SMN protein.(1-3) SMN protein insufficiency leads to motor neuron degeneration and declining motor, bulbar, and respiratory function.(3) Greater *SMN2* copy numbers are generally associated with increased functional SMN protein and thus correlate with a milder SMA phenotype. Individuals with two *SMN2* copies typically develop a more severe form of the disease, with symptoms readily observable within a few weeks to months after birth.(4)

The approval of disease-modifying treatments (DMTs) over the past decade has notably improved the disease trajectories and outcomes for individuals with SMA. Nusinersen, the first DMT approved for SMA in the US, is an antisense oligonucleotide (ASO) administered intrathecally that modifies the splicing of *SMN2* precursor mRNA to increase levels of full-length SMN protein.(5-7) Nusinersen has demonstrated clinically meaningful and sustained efficacy in a range of presymptomatic and symptomatic individuals across the SMA spectrum, with a well-established safety profile from over 10 years in clinical trials and post-marketing settings.(8-11) The current approved dose for nusinersen in the US is four 12 mg loading doses (first three loading doses at 14-day intervals; fourth loading dose 30 days after the third dose) followed by 12 mg maintenance doses every 4 months.(7)

Onasemnogene abeparvovec (OA) is a one-time gene transfer therapy delivered intravenously. It uses an adeno-associated virus serotype 9 (AAV9) vector to deliver a copy of the human *SMN* gene.(12-14) Although OA therapy substantially improves survival and motor function, challenges such as motor delays or residual weakness may persist even with prompt OA treatment, particularly in individuals with two *SMN2* copies.(12, 15, 16)

Preclinical animal models and limited human postmortem studies suggest that the AAV9 vector transduces only a subset of motor neurons.(17-19) Motor neurons that are not transduced remain subject to the natural progression of this degenerative disease, which would be expected to impact clinical outcomes over time.(20-22) This raises the question of whether nusinersen-driven increases in SMN protein in untransduced motor neurons could contribute to broader target engagement and provide additional clinical benefits for individuals with SMA. However, due to the lack of clinical trials on the combined use of genetically-based therapies to date, there is limited data to guide treatment decisions for optimizing clinical outcomes in SMA.

RESPOND (NCT04488133) (23) is an ongoing study evaluating the clinical outcomes and safety of nusinersen administration in participants with SMA previously treated with OA. The objective of this interim analysis is to provide clinical, biomarker, and safety findings for RESPOND participants at Day 302 post nusinersen initiation.

Results

Participants

In total, 46 individuals were enrolled (**Figure 1**). The efficacy set included 37 participants who had received ≥ 1 dose of nusinersen and had the opportunity to complete the Day 302 assessment. The safety set included all 46 enrolled participants.

Baseline demographics and clinical characteristics

Of the 37 participants in the efficacy set, 34 (92%) had two *SMN2* copies, and 3 (8%) had three *SMN2* copies (**Table 1**). Among participants with two *SMN2* copies, 21 were aged ≤ 9 months at first nusinersen dose and 13 were aged > 9 months (**Supplementary Table 1**).

Age at first nusinersen dose and time from OA dose to first nusinersen dose varied greatly among participants (**Table 1 and Supplementary Table 1**). Median (range) age at first nusinersen dose was 9.1 (3–33) months for those with two *SMN2* copies and 34.2 (31–36) months for those with three *SMN2* copies. Median (range) time from OA dose to first nusinersen dose was 6.3 (3–31) months and 13.3 (10–22) months in each group, respectively.

Most of the 37 participants had multiple investigator-identified domains of suboptimal clinical status; 30 (88%) in the two *SMN2* copy group and 1 (33%) in the three *SMN2* copy group, respectively. Suboptimal clinical status in motor function was most commonly reported, with 94% in the two *SMN2* copy group and 100% in the three *SMN2* copy group. Suboptimal clinical status in swallowing or feeding ability for age and respiratory function was reported in 59% and 71% in the two *SMN2* copy group, and 33% and 0% in the three *SMN2* copy group. Detailed descriptions of suboptimal clinical status provided by investigators are summarized in

Supplementary Table 1.

The majority of participants with two *SMN2* copies (27 [79%]) were unable to sit without support at baseline (typical window for sitting achievement in healthy children: 3.8–9.2 months).(24) All three participants with three *SMN2* copies were able to sit without support, but none were able to walk without support (typical window for walking achievement in healthy children: 8.2–17.6 months).(24) Penetration or aspiration of thin liquids on swallow study was respectively observed in 26% and 33% of participants with two *SMN2* copies.

For ulnar compound muscle action potential (CMAP) amplitude, 27 (79%) participants in the two *SMN2* copy group and 1 (33%) in the three *SMN2* copy group had a baseline value of ≤ 1 mV. Additionally, 32 (94%) and 1 (33%) had a baseline value of ≤ 2 mV, respectively. For peroneal CMAP amplitude, 18 (58%) participants in the two *SMN2* copy group and 0 (0%) in the three *SMN2* copy group had a baseline value of ≤ 1 mV. Twenty-nine (94%) and two (67%) had a baseline value of ≤ 2 mV, respectively.

Changes in motor function and motor milestones

Improvements in motor function and milestones were observed from baseline to Day 302. In the two *SMN2* copy group, the mean Hammersmith Infant Neurological Examination section 2 (HINE-2) total score increased by 8.1 points, from 4.9 points at baseline to 13.0 points at Day 302 for those with two *SMN2* copies (**Figure 2**). Similarly, the mean Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) total score increased by 7.9 points, from 42.2 points at baseline to 49.7 points at Day 302 in this group (**Figure 3**); 24 (75%) participants achieved a ≥ 4 -point increase. Of 27 participants unable to sit at baseline, 14 (52%) achieved sitting by Day 302, as per the World Health Organization (WHO) motor milestones assessment (**Figure 4**).

Improvements were observed across motor function and milestones outcomes for both the younger (aged ≤ 9 months) and older (aged > 9 months) groups with two *SMN2* copies (**Supplementary Table 2**). The mean change in HINE-2 total scores from baseline to Day 302 was +8.7 points in the younger group and +6.9 points in the older group with two *SMN2* copies. The mean change in CHOP-INTEND total scores was +9.3 points and +5.4 points in each group, respectively, with 17 (81%) and 7 (64%) achieving a ≥ 4 -point increase.

Among the three participants with three *SMN2* copies, HINE-2 total scores generally remained stable. Since all three participants were ≥ 2 years old and had attained sitting at baseline, CHOP-INTEND was not assessed.

Changes in biomarkers

Baseline plasma neurofilament light chain (NfL) levels were elevated compared to serum NfL levels previously reported in neurologically healthy children of similar ages, indicating active neurodegeneration at study entry (**Figure 5**). (25, 26) Mean plasma NfL levels rapidly decreased from baseline to Day 183 and remained low at Day 302, showing a 79% reduction from baseline at Day 302 in the two *SMN2* copy group (**Figures 6, A and B**). Reductions in plasma NfL levels were observed regardless of age at nusinersen initiation or time since OA administration (**Figures 6, C and D, and Supplementary Table 2**). Similar patterns of reduction were observed in cerebrospinal fluid (CSF) NfL levels, with an 82% reduction during the same period (**Supplementary Figure 1**). Among participants in the three *SMN2* copy group, one participant with an elevated baseline plasma NfL level experienced a reduction similar to that observed in the two *SMN2* copy group. The levels remained low over time for the other two participants in this group.

Improvements in mean ulnar and peroneal CMAP amplitudes were observed from baseline at Day 302, with mean increases of 0.4 mV and 0.6 mV for ulnar and peroneal amplitudes, respectively (**Figures 7, A and B**) in the two *SMN2* copy group. The pattern of improvement in CMAP was similar regardless of age at nusinersen initiation and time from OA dose (**Figures 7, C and D, and Supplementary Table 2**). Ulnar and peroneal CMAP amplitudes increased in two of the three participants in the three *SMN2* copy group, while one participant with the highest baseline value experienced a slight decrease.

Changes in investigator- and caregiver-reported outcomes

In the two *SMN2* copy group, improvements were reported by Day 183 for 30 (94%) participants with investigator-reported suboptimal motor function, eight (40%) with suboptimal swallowing or feeding ability, and eight (33%) with suboptimal respiratory function at baseline. No changes were reported by one (3%), 11 (55%), and 15 (63%) participants, respectively. In the three *SMN2* copy group, all three (100%) participants with suboptimal motor function and one (100%) with suboptimal swallowing or feeding ability at baseline reported improvement at Day 183 (**Figure 8A**). The majority of caregivers observed improvements in suboptimal motor function and swallowing or feeding ability, while respiratory function remained unchanged in many participants (**Figure 8B**).

Most investigators and caregivers observed global improvements as assessed by Clinical Global Impression of Change (CGI-C) at Day 302, reporting that participants had ‘very much improved’, ‘much improved’, or ‘minimally improved’ (**Figure 9**). Seventy percent of participants with two *SMN2* copies and all participants with three *SMN2* copies were assessed by the investigators as either ‘very much improved’ or ‘much improved’. ‘No change from baseline’

was reported by the investigator for one participant with two *SMN2* copies (3%) and no cases of worsening were reported.

Safety and tolerability

The majority of adverse events (AEs) reported were mild to moderate in severity (**Table 2**). Overall, serious AEs were reported in 17 (37%) participants, none of which were considered related to nusinersen by the investigator. Mild AEs of proteinuria that occurred in three (7%) participants were considered related to nusinersen by the investigator; all three participants continued to receive nusinersen treatment. One death from respiratory arrest was reported, which was not considered related to nusinersen by the investigator. The event happened after completing the last dose of nusinersen in the study, but prior to completing the end of study visit (a non-dosing visit).

The most common AEs reported by $\geq 15\%$ of participants were upper respiratory tract infections, pyrexia, pneumonia, viral upper respiratory infections, and vomiting. No clinically relevant trends related to nusinersen in hematology, blood chemistry, urinalysis, coagulation, vital signs, ECGs, or liver function tests were observed.

Discussion

Although treatment with OA has improved survival and motor function in children with SMA, preclinical animal models and human post-mortem studies suggest that motor neuron transduction remains incomplete, with reported rates varying between 40% and 90%.(17-19) The consequences of untransduced motor neurons may not become apparent for years, as motor neuron degeneration precedes clinical symptomatology in SMA.(27) The RESPOND study is based on the hypothesis that administering nusinersen could increase SMN protein in the untransduced motor neurons, leading to additional clinical benefits for individuals with SMA.

Results from the RESPOND study demonstrate that (1) consistent with the investigator's assessment of suboptimal clinical status, participants showed active neurodegeneration and severe denervation at study entry despite prior treatment with OA, and (2) participants experienced improvements in clinical and biomarker outcomes following initiation of nusinersen with no new safety concerns identified. Our study addresses a critical and timely clinical question regarding the need for additional therapies in individuals with SMA who show suboptimal clinical status after OA. The use of NfL and CMAP as objective biomarkers to support clinical observations enhances biological understanding and suggests a potential approach to identifying patients suitable for early add-on treatment, with the goal of optimizing outcomes.

Growing evidence from real-world cohort studies indicates that many individuals treated with OA have suboptimal clinical status.(12, 15, 16, 28-30) Data from larger international registries indicate that motor delays are common in children treated with OA at an older age after symptom onset, but delays are also observed in younger children identified through newborn screening. (12, 28) Two recent multicenter U.S. studies similarly reported ongoing disability, motor delays,

or suboptimal outcomes in many OA-treated children. Suboptimal outcomes were particularly common (~50%) among children with two *SMN2* copies, though it was also seen in some with three copies, many of whom required additional *SMN2*-modifying therapy.(29, 30)

Most participants in RESPOND exhibited multiple domains of suboptimal clinical status at baseline as assessed by the investigator. Nearly all participants, including those with three *SMN2* copies, reported suboptimal motor function. These subjective assessments by investigators were further supported by objective measurements of elevated NfL levels and low CMAPs at baseline. Despite the expected limited potential for improvement given their baseline characteristics, participants demonstrated measurable improvements across various outcomes during the study. At the time of study design, greater improvements were expected in younger participants who received nusinersen as early as possible in their disease course. Prior studies suggest that treatment may be most effective before significant motor neuron loss occurs, while enough neurons remain to respond to SMN protein restoration.(30) Nevertheless, the study enrolled participants with a wide range of age at first nusinersen dose (2 to 36 months) given the lack of consensus on the optimal therapeutic window. Age subgroup analyses used a predefined cutoff of 9 months at first nusinersen dose, based on the typical developmental window for detecting delays in sitting without support. Although mean motor function score changes were slightly greater in the younger group than in the older group, improvements in clinical and biomarker outcomes were observed in both age groups, suggesting that the older group with a longer time since OA also experienced improvements. Therefore, the main analyses combined all participants with two *SMN2* copies.

At Day 302, nearly all participants (35 [95%]) demonstrated improvements in motor function as measured by HINE-2 total motor milestone scores or CHOP-INTEND total scores. Two

participants with three *SMN2* copies, who had the highest HINE-2 total scores at baseline, did not report numerical improvements at Day 302. However, their investigators reported improvements in their suboptimal motor function at Day 183, and both investigators and caregivers reported improvements in CGI-C at Day 302. To our knowledge, no threshold has been established to define a meaningful response in HINE-2 total scores. However, since HINE-2 total scores reflect the achievement of key developmental milestones, any improvement may be considered meaningful for these children and their caregivers.(31)

Due to differences in study design, participant characteristics, and prior treatment status, the HINE-2 and CHOP-INTEND total score results from this study cannot be directly compared to other trials of nusinersen or OA. For example, symptomatic, treatment-naïve participants in the ENDEAR trial had a severe disease burden with low mean baseline HINE-2 and CHOP-INTEND scores (1.3 and 27.9 points, respectively).(32) Nevertheless, among treated participants with 2 *SMN2* copies, mean improvements in the HINE-2 total score were relatively greater in RESPOND than in ENDEAR (+8.1 vs. +4.6 points at Day 302), suggesting greater motor milestone gains in RESPOND. In contrast, mean improvements in CHOP-INTEND, a motor skill assessment more suitable for weaker infants and younger children,(33) were greater in ENDEAR (+11.3 vs. +7.9 points).(32) In the STRIVE and STRIVE-EU studies of symptomatic participants with 2 *SMN2* copies treated with OA, the greatest CHOP-INTEND improvements were seen within the first 3 to 6 months of OA treatment, after which gains slowed.(13, 14). As only a few participants in STRIVE and STRIVE-EU had CHOP-INTEND data beyond 12 months, and HINE-2 data were not collected in these studies, further contextualization with our study is limited. Many RESPOND participants enrolled at least several months after receiving OA (median time from OA: 6.3 months in participants with 2 *SMN2* copies).

Although age at baseline largely overlapped with or exceeded the typical window for the sitting achievement in healthy children (3.8–9.2 months),(24) most participants (27/37 [73%]) were unable to sit independently at baseline. Of the 10 participants who could sit, only two achieved this milestone within the normal developmental window. These findings highlight the ongoing unmet need in this population, despite having received treatment with OA. Among the participants who could not sit independently at baseline, 14/27 (52%) gained the ability to do so by Day 302, representing a clinically relevant benefit for these individuals.

None of the three participants with three *SMN2* copies (age range: 30.8–35.7 months) were able to walk without support at baseline, despite their age exceeding the typical window for walking achievement in healthy children (8.2–17.6 months).(24) Achievements in these key motor milestones will continue to be evaluated during the study.

In addition to measurable motor function improvements, biomarker data supported the additional benefit of nusinersen for patients previously treated with OA. Our findings highlight the potential of neurofilament levels to serve as a key biomarker for assessing disease activity and monitoring treatment response in SMA. Neurofilaments are structural proteins that are released into the interstitial fluid following axonal damage or neuronal degeneration,(34, 35) providing quantitative and real-time information on the extent of ongoing neuro-axonal injury.(36)

Neurofilaments have been studied extensively as a biomarker across a wide range of neurodegenerative diseases.(36) In SMA, neurofilament levels are elevated – with the highest levels observed in the youngest patients with the more severe forms of the disease.(37-40) Similarly in amyotrophic lateral sclerosis (ALS), levels of neurofilament are prognostic for disease progression and survival.(41) In the case of *SOD1*-ALS, treatment-driven reductions preceded and predicted clinical benefit over time.(42)

321 In the RESPOND study, most participants exhibited elevated NfL levels at baseline compared to
322 the levels reported in neurologically healthy children,(25, 26) suggesting active
323 neurodegeneration at study entry, consistent with the study hypothesis. In a study estimating age-
324 specific reference levels for serum NfL in neurologically healthy children, the following 5% and
325 95% percentile levels were reported: 5.0 and 18.2 pg/mL for ages 0–1 year, 4.5 and 16.6 pg/mL
326 for ages 1–2 years, and 4.1 and 15.0 pg/mL for ages 2–4 years.(25) Similar serum NfL levels
327 were reported in another study that reported 99th percentiles of 22, 20.4, and 18.9 pg/mL for
328 those aged 1, 2, and 3 years, respectively.(26) Relatively higher levels in younger infants and
329 children are likely due to high cell turnover during neuronal migration and differentiation in the
330 developing brain.(25, 37, 39)

331 Although caution is needed when comparing values across studies due to known variations in
332 analytical methods, as well as differences between serum and plasma levels, baseline plasma
333 NfL levels observed in the RESPOND study (median [range]: 93.6 [13–483] pg/mL) are
334 substantially higher than serum NfL levels previously reported for neurologically healthy
335 children in these studies,(25, 26) which also used single molecular array (Simoa) immunoassay.
336 As NfL levels are expected to be approximately 5–20% higher in serum as compared to
337 plasma,(43-45) our findings of relative elevation of NfL in plasma are likely conservative.

338 Nearly all RESPOND participants experienced a rapid decline in NfL levels during the loading
339 phase of nusinersen, followed by relative stabilization thereafter – a pattern consistently
340 observed in other nusinersen studies.(37, 38, 46) This pattern was observed regardless of age at
341 baseline or time from OA, suggesting that these reductions are not fully driven by age-related
342 declines in NfL levels or transient increases in NfL levels after OA.

Limited data are available on changes in NfL among individuals receiving OA. Evidence suggests that NfL levels increase for up to approximately six months after OA administration.(47-50) The transient increase in NfL may reflect an inflammatory response in the central nervous system caused by the AAV9 vector or AAV-induced toxicity.(47-50)

Approximately half of the participants in the study had more than 6 months between OA and first nusinersen dose (median time from OA: 6.3 months in the two *SMN2* copy group and 13.3 months in the three *SMN2* copy group). Given that these participants had passed the period of transient neurofilament increases after OA, the reductions observed following nusinersen initiation can be largely attributed to nusinersen, rather than resolution of the OA-related elevations.

Consistent with elevations in plasma NfL, RESPOND participants had low CMAP values at baseline, indicating severe denervation at study entry. Median values of ulnar and peroneal amplitudes were 0.6 mV and 0.9 mV at baseline for those with two *SMN2* copies, with 79% and 58% of participants showing amplitudes below 1 mV, respectively. In children without neurological disease, mean ulnar and peroneal CMAP values increase from 3.11 mV and 2.68 mV at 1 to 6 months of age, respectively, to 4.55 mV and 3.69 mV at 12 to 24 months of age.(51) In the natural history of untreated infantile-onset SMA, CMAP amplitudes decline over time with low values (< 1.0 mV) reported for nearly all individuals.(52) The threshold of CMAP amplitude used in prior clinical trials enrolling presymptomatic infants with SMA ranged between 1 and 2 mV.(10)

In prior studies of nusinersen or OA, increases in CMAP have been generally reported following treatments.(10, 53-55) In the START and STRIVE-US studies, symptomatic participants with two *SMN2* copies treated with intravenous OA showed mean peroneal CMAP amplitude

increases from baseline (mean age: 3.6 months) up to 18 months of age. However, wide variability in CMAP values was observed during follow-up.(54) In another study that examined the potential utility of CMAP in predicting motor recovery after OA, individuals with baseline CMAP values of < 0.5 mV were less likely to achieve independent sitting after OA.(53)

Although there is no consensus on defining the threshold for low CMAP values or a clinically meaningful increased response, the low baseline values observed in the RESPOND study likely indicate suboptimal response to OA and a risk of limited potential improvements without additional intervention. Despite the extent of denervation at baseline, improvements in CMAP were observed regardless of age and time from OA, indicating electrophysiologic response to treatment with additional nusinersen, coinciding with motor function gains in other measures.

Improvements were also consistently observed for investigator- and caregiver-reported outcomes. Most participants with suboptimal motor function at baseline improved following initiation of nusinersen, as assessed by both investigators and caregivers. Caregivers reported improvements in suboptimal swallowing/feeding ability more frequently than investigators. As caregivers are primarily responsible for providing meals to their child, they may be more perceptive of benefits in this domain. Suboptimal respiratory function remained stable for most participants. Changes will continue to be evaluated at the end of the study for these domains.

Given the limited data on the safety of additional therapies and the potential for new safety concerns in individuals receiving nusinersen after OA treatment, evaluating safety was also an important objective of this study. Nusinersen has generally been well tolerated in clinical trials and post-marketing safety studies, with most reported AEs and SAEs consistent with those typically observed in individuals with SMA or associated with lumbar puncture procedures.(56) Key safety concerns of OA treatment include the risk of liver toxicity and thrombocytopenia.

(13, 14) Careful monitoring of liver function and coagulation parameters including platelets was performed throughout the study. AEs and SAEs in this study have generally been consistent with prior experience with nusinersen and with reported data from OA trials, with no new safety concerns identified. Given the increasing use of combination or additional therapies and the underlying pathology in this patient population, where impaired function of peripheral organs may occur,(57) future research on multiple SMA treatments should continue to carefully evaluate safety.

There are limited studies examining additional treatment after OA in clinical trials and post-marketing settings.(15, 58-62) Most studies were single-center and observational with small sample sizes. In a recent observational study of 23 presymptomatic children, the authors concluded that preemptive dual therapy with either risdiplam or nusinersen after OA was well-tolerated but provided limited or no benefit compared with OA monotherapy.(15) This study included presymptomatic children with mostly normal muscle ultrasound at baseline. Most children in the dual therapy group received risdiplam shortly after OA (n = 6); only one received nusinersen for a limited time.(15) RESPOND participants may be more comparable to the subgroup of three children who initially received OA monotherapy but later received nusinersen due to motor delays or residual weakness. However, limited outcomes were reported for this small group. A recent multicenter, retrospective case series reported on 20 children who received risdiplam following OA due to suboptimal clinical status.(62) Compared to our study, the children in this case series had a longer average time from OA administration (15.2 months) and were older at the time of additional therapy initiation (mean age: 24.9 months). While the authors reported improvements in some children after risdiplam treatment, the findings based on electronic health records were limited by variability in data collection and outcome reporting. No

biomarker data were available to help understand the underlying pathology.(62) Differences in study populations and treatments preclude direct comparisons of outcomes between these studies. However, both the observational studies and RESPOND show the considerable unmet clinical need after OA, especially for those with two *SMN2* copies. Most participants with two *SMN2* copies in the observational study experienced widespread degenerative changes on muscle ultrasound during follow-up.(15)

As none of the currently available DMTs result in a cure for SMA, combination and sequential therapies are increasingly being used in practice to optimize clinical outcomes. (63, 64) However, assessing the impact of such approaches is challenging due to several factors, including the lack of randomized controlled trials and clinical variability. Our study focused specifically on the outcomes of individuals treated with nusinersen following OA. Other therapeutic combinations or sequences were considered outside the scope of this study. While risdiplam is another *SMN2*-targeting therapy that may also be used after OA, its mechanism of action and safety profile differ from those of nusinersen. Therefore, these therapies should not be considered interchangeable in terms of efficacy or safety.

Our study has several limitations. First, since RESPOND is an open-label trial without a comparator group, not all observed improvements can be directly attributed to nusinersen treatment as all participants had received OA prior to enrollment, which likely provides ongoing production of SMN protein in those motor neurons that are transduced. Placebo effects or expectation bias from evaluators and caregivers may also have influenced the reported improvements in investigator- and caregiver-assessed outcomes. The study was not designed as a comparative study because recruiting participants to undergo a sham procedure would not have been feasible. The study was not statistically powered to detect significant differences. Second,

at the time of study design, there was no clear consensus on what constitutes suboptimal clinical status after OA, particularly given the variability in participant age and clinical characteristics. Accordingly, the definition was based on investigators' assessments, which could be subjective. Third, details of symptoms at the time of OA could not be examined as participants received OA months to years prior to study enrollment. Fourth, there is no consensus on clinically meaningful thresholds for many clinical and biomarker outcomes in this study, especially among individuals previously treated with other DMTs. To help contextualize the findings, we provided reference values based on those typically observed in neurologically healthy children and/or untreated individuals with SMA. Fifth, given the wide variability in participant characteristics, the limited sample size to account for this variability, and the near-universal improvements in both biomarker and clinical outcomes, correlation or predictive analyses between these outcomes could not be conducted.

Despite the study's limitations, objectively and quantitatively assessed biomarkers can enhance the understanding of real-time disease activity, contextualize the clinical outcomes and complement the findings from a single-arm trial when a randomized trial is not feasible. As clinicians navigate decisions on sequencing or combining treatments for SMA in the absence of clinical trial data, our findings can support informed decision-making to optimize outcomes.

Conclusion

In summary, improvements in clinical and biomarker outcomes support the benefit of nusinersen treatment in infants and children with suboptimal clinical status following OA.

Materials and methods

Study design

RESPOND is a phase 4, multicenter, single-arm, open-label study evaluating nusinersen in infants and children with SMA who have previously received OA and have suboptimal clinical status as determined by the investigator (**Figure 1**).

The study adheres to the ethical principles outlined in the Declaration of Helsinki and signed informed consent was collected from the parent or guardian of all participants.

Participants

Participants were included if they (1) had genetic documentation of 5q SMA, (2) were aged ≤ 36 months at first nusinersen dose, (3) had an *SMN2* copy number of ≥ 1 , (4) received OA ≥ 2 months prior to the first nusinersen dose, (5) had received intravenous OA per the approved label or local/regional regulations including the steroid regimen and monitoring, (6) had suboptimal clinical status as determined by the investigator in ≥ 1 of the following domains: motor function, swallowing or feeding ability for age, respiratory function, or other (**Supplementary Table 1**), and (7) had alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels ≤ 2 times the upper limit of normal at screening and within 7 days prior to dosing. Those with severe or serious adverse events (AEs) related to OA treatment that were ongoing during screening were excluded. Investigators were asked to provide a detailed description of each participant's suboptimal clinical status at baseline. Sex was not considered as a biological variable.

Participants received intrathecal nusinersen per the current label (7): 12 mg administered as four loading doses on Days 1, 15, 29, and 64, followed by a maintenance dose every 4 months until Day 659. Participants had a post-treatment follow-up visit on Day 778.

For clinical and biomarker outcomes, participants who received ≥ 1 dose of nusinersen and had the opportunity to complete the Day 302 assessment at the time of data-cut were included (efficacy set). For safety outcomes, all participants who received ≥ 1 dose of nusinersen were included (safety set). Data-cut dates were June 26, 2023 for neurofilament outcomes and October 18, 2023 for all other outcomes.

The full list of investigators, study site personnel, and study sites is included in **Supplementary Methods**.

Outcomes

Motor function and milestones

HINE-2 total motor milestone score was assessed as the primary endpoint. HINE-2 total score comprises the following eight motor milestone categories: voluntary grasp, ability to kick in supine position, head control, rolling, sitting, crawling, standing, and walking. Total score ranges from 0 to 26, with a higher score indicating better motor function.(65)

CHOP-INTEND motor function scale was assessed in participants who were < 2 years of age or those who were 2 to ≤ 3 years of age if they had not achieved independent sitting prior to screening. CHOP-INTEND total scores range from 0 to 64, with higher scores indicating better motor function.(33) The achievement of independent sitting during the study was assessed using the WHO motor milestone criteria.(24)

Biomarkers

Levels of NfL, a marker of axonal injury and neurodegeneration, were examined as a measure of disease activity and treatment response.(37-40) Plasma and CSF NfL levels were measured with

a single molecular array (Simoa) immunoassay via the HD-X Analyzer (Quanterix, Lexington, MA, USA). Measurements were performed using the NfL v2 Advantage kit from Quanterix (Product# 104073; Lot# 503808).

CMAP, an electrophysiological biomarker, was assessed to obtain physiologic information about motor units.(52, 66) CMAP measures the motor response to supramaximal electrical stimulation of a peripheral nerve, with lower CMAP amplitudes reflecting a reduced number of motor axons comprising the motor unit.(67) CMAP amplitude was assessed for the ulnar nerve recorded at the abductor digiti minimi muscle, and the peroneal/fibular nerve recorded at the anterior tibialis muscles.

Investigator- and caregiver-reported outcomes

Investigators and caregivers assessed suboptimal clinical status at baseline based on the following domains: motor function (e.g., motor delays, limited spontaneous or antigravity movement, hypotonia), swallowing or feeding ability for participant age (e.g., fatigue during feeding, weakness in sucking/swallowing, the need for a nasogastric tube), respiratory function (e.g., paradoxical breathing, chest deformity, need for non-invasive ventilation, increased respiratory rate, inadequate cough), or any other area of suboptimal clinical status. Changes in the identified domains relative to participants' status on Day 1 were evaluated at later time points.

CGI-C was assessed by both the investigator and caregiver using a 7-point scale, ranging from 'very much improved' to 'very much worsened', to rate overall improvement since study enrollment.

Bulbar function assessments

The ability to tolerate swallowing thin liquids was assessed using a video fluoroscopic swallow study (VFSS). The VFSS is a radiographic procedure that provides a direct, dynamic view of oral, pharyngeal, and upper esophageal function during swallowing.(68) The most severe form of bolus airway entry observed during the VFSS exam was documented.

Safety and tolerability

Safety and tolerability were assessed by examining the incidence of AEs overall, by severity, and by relationship to nusinersen. Other safety assessments included clinical laboratory parameters, electrocardiograms (ECGs), and vital signs.

Statistical methods

Analyses were stratified by *SMN2* copy number, a strong prognostic modifier of the disease,(67) to assess clinical and biomarker outcomes. Additional subgroup analyses by age at first nusinersen dose were conducted using a prespecified cut-off (≤ 9 months, > 9 months at first nusinersen dose) in the two *SMN2* copy group to examine potential differences in outcomes by age. Outcomes were summarized using descriptive statistics. Mean changes from baseline at Day 302 were obtained for each outcome, except in individuals with three *SMN2* copies due to the small sample size ($n = 3$). Individual trajectories for all participants are shown in the figures where possible.

Per the protocol, the investigator and caregiver assessments of suboptimal clinical status were performed at Days 1, 183, and 540, and at the end of the study, but not at Day 302. Accordingly, changes at Day 183 are summarized for this outcome in this interim analysis. The VFSS was performed on Day 1 and at the end of the study, so only baseline data are presented.

Study approval

RESPOND was conducted in accordance with the protocol, the principles of applicable International Council for Harmonisation and Good Clinical Practice guidelines, and the requirements of Clinical Trials Regulation (EU) No. 536/2014 or, if applicable, Clinical Trials Directive 2001/20/EC. The study adheres to the ethical principles outlined in the Declaration of Helsinki and signed informed consent was collected from the parent or guardian of all participants. Investigators obtained ethics committee approval of the protocol, informed consent form, and other required study documents prior to starting the study.

551

552 **Data availability**

553 Requests for the materials and data supporting this manuscript should be submitted to
554 <https://vivli.org/>.

555

556 **Author contributions**

557 **CP:** contributed to the concept and study design, analyzed and interpreted the data, and reviewed
558 and revised the manuscript for intellectual content.

559 **RSF:** contributed to the concept and study design, analyzed and interpreted the data, and
560 reviewed and revised the manuscript for intellectual content.

561 **JAP:** contributed to the concept and study design, analyzed and interpreted the data, and
562 reviewed and revised the manuscript for intellectual content.

563 **RM:** contributed to the concept and study design, analyzed and interpreted the data, and
564 reviewed and revised the manuscript for intellectual content.

JFB: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

NLK: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

JS: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

RF: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

WL: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

RL: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

SF: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

BY: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

ADP: contributed to the concept and study design, analyzed and interpreted the data, and reviewed and revised the manuscript for intellectual content.

Acknowledgments: The authors thank the participants and their family members, investigators, clinical monitors, study coordinators, physical therapists, and laboratory technicians. The authors

also thank Sowmya Chary, Corinne Makepeace, Nicole Harris, and Carrie Rubel for their contribution to the biomarker and safety findings. We also thank the peer reviewers for their comments which substantially improved the manuscript. See Supplemental Methods for RESPOND Study Group details.

Medical writing support: Writing and editorial support for the preparation of this manuscript was provided by Kirsty Walters, PhD, of Oxford PharmaGenesis, Oxford, UK; funding was provided by Biogen.

Funding: This study was sponsored by Biogen (Cambridge, MA, USA).

Competing interests

CP: Principal investigator of studies sponsored by Biogen, Biohaven, Genentech, Novartis Gene Therapies, and Scholar Rock. Participates on advisory boards and as a consultant for Biogen, Genentech/Roche, Novartis Gene Therapies, and Scholar Rock. Serves as a speaker for Biogen and Novartis Gene Therapies.

RSF: grants from Biogen, Dyne, Genentech, Genethon, Italfarmaco, Roche, Sarepta, Scholar Rock and Novartis; royalty payments from Children's Hospital of Philadelphia for licensing fees obtained for use of the CHOP-INTEND motor function scale; advisory board consultant for Astellas, Biogen, Dyne, Genentech, Italfarmaco, Novartis, ReveraGen, Roche, Sarepta, and Scholar Rock; honoraria and travel support from Novartis; patent addendum signed over to Roche; participant of University of Texas Southwestern Lafora Body study and Ionis Angelman

607 Study; editorial board member for Neuromuscular Disorders and the Journal of Neuromuscular
608 Diseases; steering committee member of the Pediatric Neuromuscular Clinical Research
609 Network for SMA.

610 **JAP:** principal investigator for clinical studies for Biogen, Biohaven, Genentech, Novartis, PTC
611 Therapeutics, and Scholar Rock; adviser and consultant for Biogen, Genentech, Novartis, and
612 Scholar Rock; travel support from MDA and Cure SMA; served as a speaker for Astellas and
613 Ultragenyx.

614 **RM:** principal investigator sponsored by Roche; received consultancy fees and travel support
615 from Biogen, Novartis, PTC, and Roche; fees for educational events from Biogen, Novartis, and
616 Roche; participation in Advisory boards for Roche and Novartis.

617 **JFB:** site investigator for clinical trials with Alexion, Astellas, AveXis/Novartis, Biogen,
618 Biohaven, CSL Behring, Fibrogen, Genentech/Roche, Italfarmaco, Janssen, Pfizer, PTC
619 Therapeutics, Sarepta, and Scholar Rock; consultant for Alexion, Argenx, AveXis/Novartis,
620 Biogen, CSL Behring, Dyne, Edgewise, Fibrogen, Genentech/Roche, ITF, Janssen, NS Pharma,
621 PTC Therapeutics, Sarepta, Scholar Rock, and Takeda; support for travel from and serves as a
622 medical advisory council member for Cure SMA; participation in advisory boards/data safety
623 monitoring for Edgewise and SickKids Neuromuscular.

624 **NLK:** site principal investigator for clinical research studies for Argenx, Astellas, Biogen,
625 Catalyst, Genentech, Sarepta, and Scholar Rock; advisory boards for Argenx, Astellas, Catalyst,
626 Genentech, Sarepta, and Scholar Rock; speaker for Sarepta.

627 **JS:** employee of Biogen at the time of contributing to the work.

628 **RF, WL, RL, SF, BY, and ADP:** employees of Biogen and may own stock in the company.

629 **References**

- 630 1. Lunn MR, and Wang CH. Spinal muscular atrophy. *Lancet*. 2008;371(9630):2120-33.
- 631 2. Monani UR, et al. A single nucleotide difference that alters splicing patterns distinguishes
- 632 the SMA gene SMN1 from the copy gene SMN2. *Hum Mol Genet*. 1999;8(7):1177-83.
- 633 3. Mercuri E, et al. Spinal muscular atrophy. *Nat Rev Dis Primers*. 2022;8(1):52.
- 634 4. Calucho M, et al. Correlation between SMA type and SMN2 copy number revisited: An
- 635 analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases.
- 636 *Neuromuscul Disord*. 2018;28(3):208-15.
- 637 5. Chiriboga CA, et al. Results from a phase 1 study of nusinersen (ISIS-SMN(Rx)) in
- 638 children with spinal muscular atrophy. *Neurology*. 2016;86(10):890-7.
- 639 6. Finkel RS, et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a
- 640 phase 2, open-label, dose-escalation study. *Lancet*. 2016;388(10063):3017-26.
- 641 7. Biogen Inc. SPINRAZA (nusinersen) injection (Labeling-Package Insert). U.S. Food and
- 642 Drug Administration website. Available from:
- 643 https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/209531s013s014lbl.pdf
- 644 (Accessed January 2025).
- 645 8. Mercuri E, et al. Nusinersen versus Sham Control in Later-Onset Spinal Muscular
- 646 Atrophy. *N Engl J Med*. 2018;378(7):625-35.
- 647 9. Gunther R, et al. Long-term efficacy and safety of nusinersen in adults with 5q spinal
- 648 muscular atrophy: a prospective European multinational observational study. *Lancet Reg*
- 649 *Health Eur*. 2024;39:100862.
- 650 10. Crawford TO, et al. Continued benefit of nusinersen initiated in the presymptomatic stage
- 651 of spinal muscular atrophy: 5-year update of the NURTURE study. *Muscle Nerve*.
- 652 2023;68(2):157-70.
- 653 11. Finkel RS, et al. DEVOTE Study Exploring Higher Dose of Nusinersen in Spinal
- 654 Muscular Atrophy: Study Design and Part A Results. *J Neuromuscul Dis*.
- 655 2023;10(5):813-23.
- 656 12. Weiß C, et al. Efficacy and safety of gene therapy with onasemnogene abeparvovec in
- 657 children with spinal muscular atrophy in the D-A-CH-region: a population-based
- 658 observational study. *Lancet Reg Health Eur*. 2024;47:101092.
- 659 13. Day JW, et al. Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset
- 660 spinal muscular atrophy in patients with two copies of SMN2 (STRIVE): an open-label,
- 661 single-arm, multicentre, phase 3 trial. *Lancet Neurol*. 2021;20(4):284-93.
- 662 14. Mercuri E, et al. Onasemnogene abeparvovec gene therapy for symptomatic infantile-
- 663 onset spinal muscular atrophy type 1 (STRIVE-EU): an open-label, single-arm,
- 664 multicentre, phase 3 trial. *The Lancet Neurology*. 2021;20(10):832-41.
- 665 15. Matesanz SE, et al. Preemptive dual therapy for children at risk for infantile-onset spinal
- 666 muscular atrophy. *Ann Clin Transl Neurol*. 2024;11(7):1868-78.
- 667 16. Kichula EA, et al. Expert recommendations and clinical considerations in the use of
- 668 onasemnogene abeparvovec gene therapy for spinal muscular atrophy. *Muscle Nerve*.
- 669 2021;64(4):413-27.
- 670 17. Foust KD, et al. Intravascular AAV9 preferentially targets neonatal neurons and adult
- 671 astrocytes. *Nat Biotechnol*. 2009;27(1):59-65.
- 672 18. Thomsen G, et al. Biodistribution of onasemnogene abeparvovec DNA, mRNA and SMN
- 673 protein in human tissue. *Nat Med*. 2021;27(10):1701-11.

19. Meyer K, et al. Improving single injection CSF delivery of AAV9-mediated gene therapy for SMA: a dose-response study in mice and nonhuman primates. *Mol Ther*. 2015;23(3):477-87.
20. Yeo CJJ, et al. Challenges and opportunities in spinal muscular atrophy therapeutics. *Lancet Neurol*. 2024;23(2):205-18.
21. Day JW, et al. Advances and limitations for the treatment of spinal muscular atrophy. *BMC Pediatrics*. 2022;22(1):632.
22. Arbab M, et al. Base editing rescue of spinal muscular atrophy in cells and in mice. *Science*. 2023;380(6642):eadg6518.
23. ClinicalTrials.gov ID: NCT04488133. A Study to Learn About the Effect of Nusinersen (BIIB058) Given as Injections to Children With Spinal Muscular Atrophy (SMA) Who Were Previously Treated With Onasemnogene Apeparvovec (RESPOND) Available from: <https://clinicaltrials.gov/study/NCT04488133>.
24. WHO Motor Development Study: windows of achievement for six gross motor development milestones. *Acta Paediatr Suppl*. 2006;450:86-95.
25. Bayoumy S, et al. Neurofilament light protein as a biomarker for spinal muscular atrophy: a review and reference ranges. *Clin Chem Lab Med*. 2024;62(7):1252-65.
26. Abdelhak A, et al. Serum neurofilament light chain reference database for individual application in paediatric care: a retrospective modelling and validation study. *Lancet Neurol*. 2023;22(9):826-33.
27. Sumner CJ, and Crawford TO. Early treatment is a lifeline for infants with SMA. *Nat Med*. 2022;28(7):1348-9.
28. Servais L, et al. Real-World Outcomes in Patients with Spinal Muscular Atrophy Treated with Onasemnogene Apeparvovec Monotherapy: Findings from the RESTORE Registry. *J Neuromuscul Dis*. 2024;11(2):425-42.
29. Goedeker NL, et al. Outcomes of early-treated infants with spinal muscular atrophy: A multicenter, retrospective cohort study. *Muscle Nerve*. 2024;70(6):1247-56.
30. Waldrop MA, et al. Continued safety and long-term effectiveness of onasemnogene apeparvovec in Ohio. *Neuromuscul Disord*. 2024;34:41-8.
31. Clinical Review Report: Nusinersen (Spinraza): (Biogen Canada Inc.): Indication: Treatment of patients with 5q SMA [Internet]. Ottawa (ON): Canadian Agency for Drugs and Technologies in Health; 2018 Jan. Table 17, Validity and Minimal Clinically Important Differences of Outcome Measures. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK533982/table/cl.app5.tab1/>. 2018.
32. Finkel RS, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. *N Engl J Med*. 2017;377(18):1723-32.
33. Glanzman AM, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): test development and reliability. *Neuromuscul Disord*. 2010;20(3):155-61.
34. Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. *J Neurol Sci*. 2005;233(1-2):183-98.
35. Xu Z, et al. Neurofilaments as Biomarkers for Amyotrophic Lateral Sclerosis: A Systematic Review and Meta-Analysis. *PLoS One*. 2016;11(10):e0164625.
36. Khalil M, et al. Neurofilaments as biomarkers in neurological disorders - towards clinical application. *Nat Rev Neurol*. 2024;20(5):269-87.

37. Darras BT, et al. Neurofilament as a potential biomarker for spinal muscular atrophy. *Ann Clin Transl Neurol.* 2019;6(5):932-44.
38. De Vivo DC, et al. Nusinersen initiated in infants during the presymptomatic stage of spinal muscular atrophy: Interim efficacy and safety results from the Phase 2 NURTURE study. *Neuromuscul Disord.* 2019;29(11):842-56.
39. Pino MG, et al. Update on Biomarkers in Spinal Muscular Atrophy. *Biomark Insights.* 2021;16:11772719211035643.
40. Nitz E, et al. Serum neurofilament light chain in pediatric spinal muscular atrophy patients and healthy children. *Ann Clin Transl Neurol.* 2021;8(10):2013-24.
41. Thompson AG, et al. Multicentre appraisal of amyotrophic lateral sclerosis biofluid biomarkers shows primacy of blood neurofilament light chain. *Brain Commun.* 2022;4(1):fcac029.
42. Miller T, et al. Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. *N Engl J Med.* 2020;383(2):109-19.
43. Kapoor R, et al. Serum neurofilament light as a biomarker in progressive multiple sclerosis. *Neurology.* 2020;95(10):436-44.
44. Barro C, et al. Blood neurofilament light: a critical review of its application to neurologic disease. *Ann Clin Transl Neurol.* 2020;7(12):2508-23.
45. Kwon HS, et al. Comparing Neurofilament Light Chain Levels in Serum and Plasma. *Dement Neurocogn Disord.* 2023;22(3):109-11.
46. Olsson B, et al. NFL is a marker of treatment response in children with SMA treated with nusinersen. *J Neurol.* 2019;266(9):2129-36.
47. Flotats-Bastardas M, et al. Paradoxical increase of neurofilaments in SMA patients treated with onasemnogene abeparvovec-xioi. *Front Neurol.* 2023;14:1269406.
48. Alves CRR, et al. Implications of circulating neurofilaments for spinal muscular atrophy treatment early in life: A case series. *Mol Ther Methods Clin Dev.* 2021;23:524-38.
49. Johnson EW, et al. Neurofilament light chain and dorsal root ganglia injury after adeno-associated virus 9 gene therapy in nonhuman primates. *Mol Ther Methods Clin Dev.* 2023;28:208-19.
50. Fader KA, et al. Circulating neurofilament light chain as a promising biomarker of AAV-induced dorsal root ganglia toxicity in nonclinical toxicology species. *Mol Ther Methods Clin Dev.* 2022;25:264-77.
51. Garcia A, et al. Peripheral motor and sensory nerve conduction studies in normal infants and children. *Clin Neurophysiol.* 2000;111(3):513-20.
52. Swoboda KJ, et al. Natural history of denervation in SMA: relation to age, SMN2 copy number, and function. *Ann Neurol.* 2005;57(5):704-12.
53. Barrois R, et al. A new score combining compound muscle action potential (CMAP) amplitudes and motor score is predictive of motor outcome after AVXS-101 (Onasemnogene Abeparvovec) SMA therapy. *Neuromuscul Disord.* 2023;33(4):309-14.
54. McGill B, et al. VP226 Post-hoc analysis of compound motor action potential from clinical trials of intravenous onasemnogene abeparvovec for spinal muscular atrophy. *Neuromuscular Disorders.* 2023;33:S91-S2.
55. Sumner C, et al. P225 Longitudinal changes in compound muscle action potential and their association with motor function in infantile-onset SMA children in ENDEAR/SHINE. *Neuromuscular Disorders.* 2023;33:S91.

56. Finkel RS, et al. Final Safety and Efficacy Data From the SHINE Study in Participants With Infantile-Onset and Later-Onset SMA. Presented at the Annual Cure SMA Research and Clinical Care Meeting, June 5–7, 2024. 2024.
57. Leow DM, et al. Hepatocyte-intrinsic SMN deficiency drives metabolic dysfunction and liver steatosis in spinal muscular atrophy. *J Clin Invest*. 2024;134(12).
58. Harada Y, et al. Combination molecular therapies for type 1 spinal muscular atrophy. *Muscle Nerve*. 2020;62(4):550-4.
59. Lee BH, et al. Combination therapy with nusinersen and AVXS-101 in SMA type 1. *Neurology*. 2019;93(14):640-1.
60. Mendell JR, et al. Five-Year Extension Results of the Phase 1 START Trial of Onasemnogene Apeparvovec in Spinal Muscular Atrophy. *JAMA Neurology*. 2021;78(7):834-41.
61. Oechsl K, and Cartwright MS. Combination therapy with onasemnogene and risdiplam in spinal muscular atrophy type 1. *Muscle Nerve*. 2021;64(4):487-90.
62. Svoboda MD, et al. Risdiplam treatment following onasemnogene abeparvovec in individuals with spinal muscular atrophy: a multicenter case series. *BMC Neurol*. 2025;25(1):283.
63. Schroth MK, et al. Spinal Muscular Atrophy Update in Best Practices: Recommendations for Treatment Considerations. *Neurol Clin Pract*. 2025;15(1):e200374.
64. Proud CM, et al. Combination disease-modifying treatment in spinal muscular atrophy: A proposed classification. *Ann Clin Transl Neurol*. 2023;10(11):2155-60.
65. Bishop KM, et al. Motor milestone assessment of infants with spinal muscular atrophy using the hammsmith infant neurological Exam-Part 2: Experience from a nusinersen clinical study. *Muscle Nerve*. 2018;57(1):142-6.
66. Lewelt A, et al. Compound muscle action potential and motor function in children with spinal muscular atrophy. *Muscle Nerve*. 2010;42(5):703-8.
67. Finkel RS, and Benatar M. Pre-symptomatic spinal muscular atrophy: a proposed nosology. *Brain*. 2022;145(7):2247-9.
68. Martin-Harris B, and Jones B. The videofluorographic swallowing study. *Phys Med Rehabil Clin N Am*. 2008;19(4):769-85, viii.
69. Mercuri E, et al. Longitudinal natural history of type I spinal muscular atrophy: a critical review. *Orphanet J Rare Dis*. 2020;15(1):84.

Figures and Tables

Table 1. Baseline demographics and clinical characteristics (efficacy set)

	Two <i>SMN2</i> copies (n = 34)	Three <i>SMN2</i> copies (n = 3)
Male/female, n (%)	23 (67.6) / 11 (32.3)	2 (66.7) / 1 (33.3)
Age at SMA symptom onset, months, median (range)	0.9 (0.0–5.0)	6.0 (5.0–18.0)
Age at SMA diagnosis, months, median (range)	1.3 (0.0–7.0)	15.0 (6.0–23.0)
Age at OA dosing, months, median (range)	2.1 (0.5–9.2)	17.5 (13.6–24.0)
Age at first nusinersen dose, months, median (range)	9.1 (3.2–33.3)	34.2 (30.8–35.7)
Time from OA dose to first nusinersen dose, months, median (range)	6.3 (2.6–31.3)	13.3 (10.2–22.2)
Suboptimal clinical status at baseline per investigator, n (%)		

Motor function	32 (94.1)	3 (100)
Swallowing or feeding ability for age	20 (58.8)	1 (33.3)
Respiratory function	24 (70.6)	0
Other	4 (11.8)	0
Sitting without support at screening, n (%) ^a	7 (20.6)	3 (100)
Walking without support at screening, n (%) ^a	0	0
HINE-2 total score, median (range)	4.0 (0–18) ^b	19.0 (12–24)
CHOP-INTEND total score, median (range)	42.0 (22–64) ^b	NA
Plasma NfL, pg/mL		
Mean (SD)	127.4 (97.1) ^c	49.5 (62.4)
Median (range)	95.5 (27–483) ^c	14.3 (13–122)
CMAP ulnar amplitude, mV, ^d median (range)	0.6 (0.0–2.7)	5.6 (0.9–7.0)
≤ 1 mV, n (%)	27 (79.4)	1 (33.3)
CMAP peroneal amplitude, mV, ^e median (range)	0.9 (0.1–2.9) ^c	1.6 (1.5–4.0)

≤ 1 mV, n (%)	18 (58.1)	0
Most severe form of bolus airway entry for thin liquids assessed by VFSS, n (%) ^f		
None	11 (40.7)	2 (100)
Penetration	7 (25.9)	0
Aspiration	9 (33.3)	0

^aWHO 1st and 99th percentiles for achievement in healthy children of sitting without support and walking without support are 3.8–9.2 and 8.2–17.6 months, respectively.(24) ^bn = 33. ^cn = 31. ^dUlnar nerve recorded at the abductor digiti minimi muscle. ^ePeroneal/fibular nerve recorded at the anterior tibialis muscle. ^fAssessed in participants treated at sites that perform VFSS, n = 27 in two *SMN2* copy group and n = 2 in three *SMN2* copy group.

CHOP-INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CMAP = compound muscle action potential; HINE-2 = Hammersmith Infant Neurological Examination section 2; NA = not applicable; NfL = neurofilament light chain; OA = onasemnogene abeparvovec; SD = standard deviation; SMA = spinal muscular atrophy; *SMN2* = *survival motor neuron 2*; VFSS = video fluoroscopic swallow study.

Table 2. Adverse events (safety set)

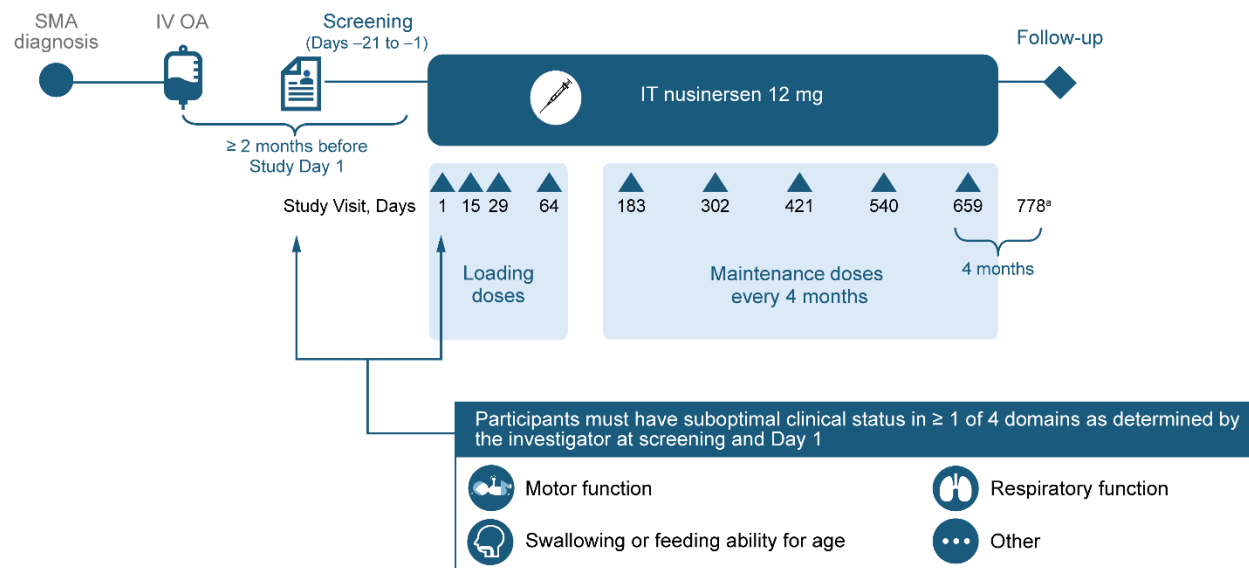
	Overall population (N = 46)
Time on study, days, median (range)	561.5 (29–818)
Any AE, n (%)	37 (80.4)
Mild	17 (37.0)
Moderate	11 (23.9)
Severe ^a	9 (19.6)
AE considered related to study drug by investigator, n (%)	
Mild	3 (6.5) ^b
Moderate	0
Severe	0
Serious AE, n (%)	17 (37.0) ^c
AE leading to study or drug withdrawal, n (%)	1 (2.2) ^d
Deaths, n (%)	1 (2.2) ^d
Most common AEs (reported in $\geq 15\%$ of participants) by preferred term, n (%)	
Upper respiratory tract infection	12 (26.1)
Pyrexia	8 (17.4)
Pneumonia	7 (15.2)
Viral upper respiratory tract infection	7 (15.2)
Vomiting	7 (15.2)

^aSevere AEs by System Organ Class: infections and infestations; respiratory, thoracic and mediastinal disorders; metabolism and nutrition disorders; general disorders and administration site conditions; injury, poisoning and procedural complications; musculoskeletal and connective tissue disorders. ^bMild AEs of proteinuria in three participants were considered related to the study drug by the investigator. All participants continued to receive nusinersen treatment. ^cNone of the serious AEs were considered related to study drug, and all events were resolved. All participants continued treatment with nusinersen. ^dOne death from respiratory arrest at Day 728; the event was not considered related to nusinersen by the investigator.

AE = adverse event.

Figures and figure legends

Study design



Participant disposition

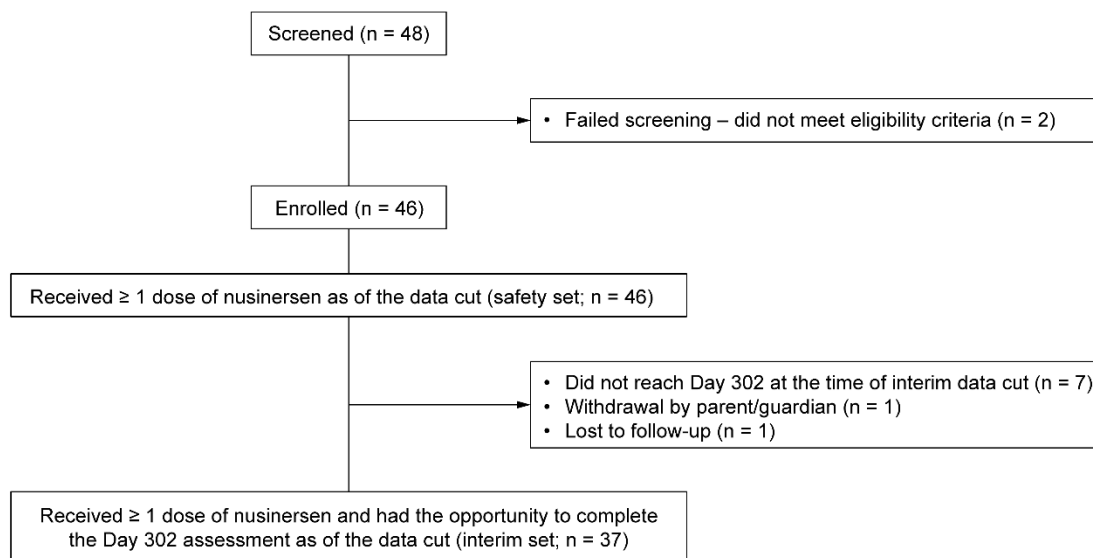
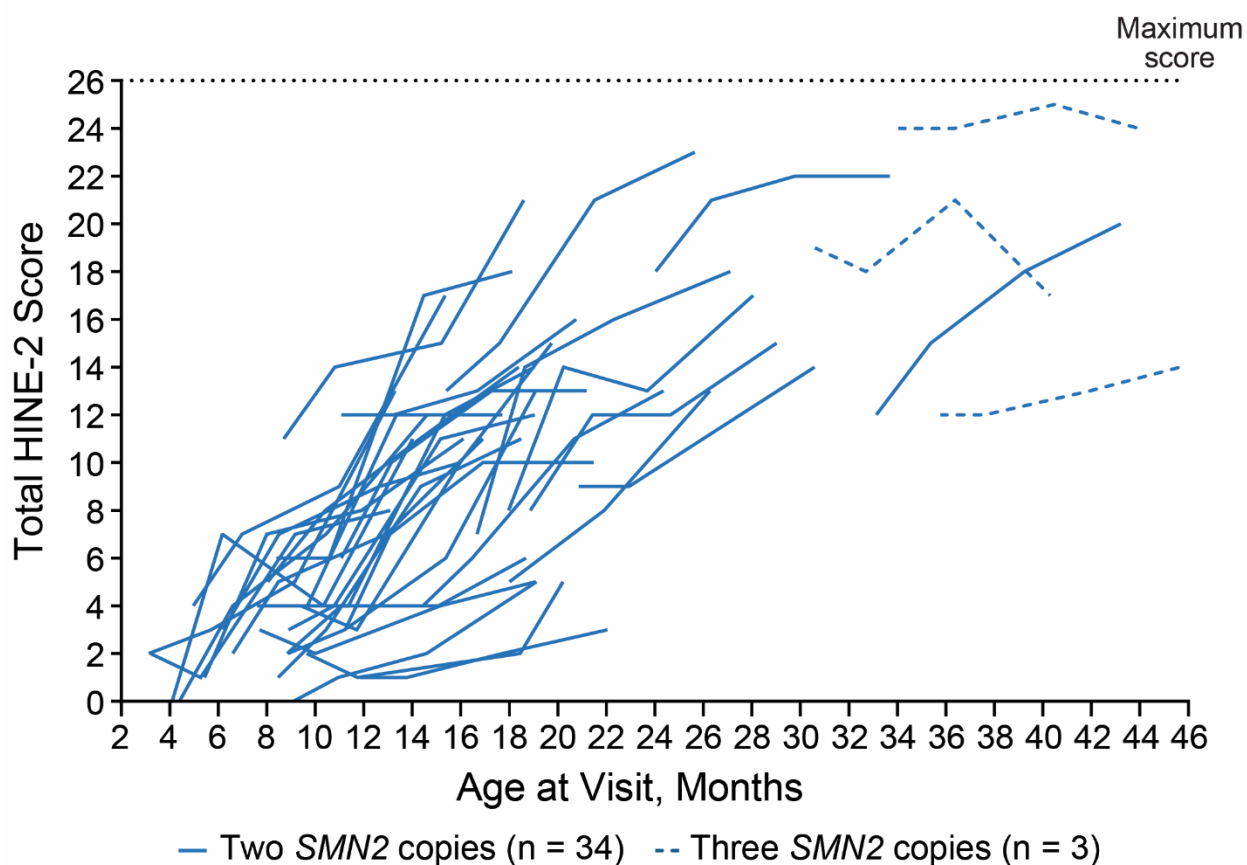


Figure 1. Study design and participant disposition

^aOr 4 months from last dose.

IT = intrathecal; IV = intravenous; OA = onasemnogene abeparvovec; SMA = spinal muscular atrophy.

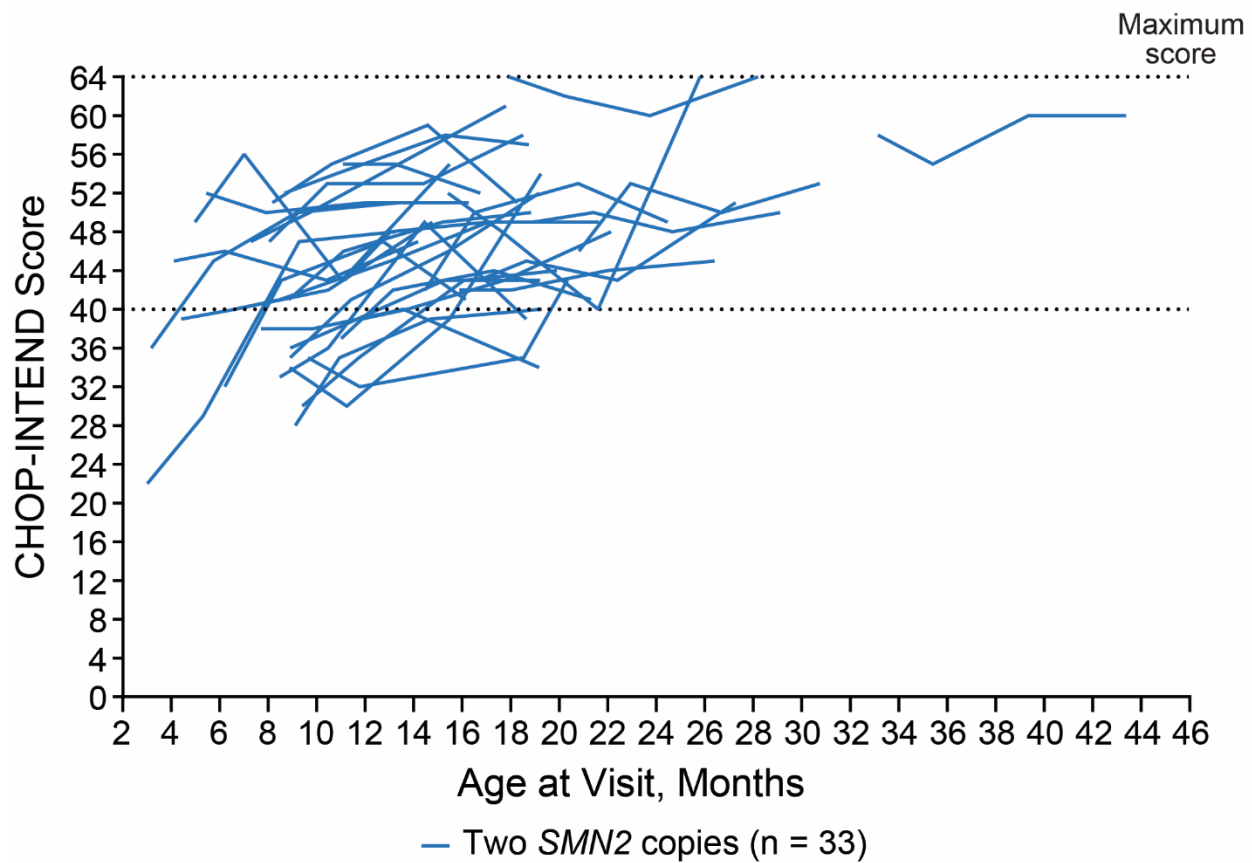


	Actual Mean (SD) HINE-2 Scores		Mean (SD) Change From Baseline to Day 302 (n = 33)
	Baseline (n = 33)	Day 302 (n = 34)	
Two <i>SMN2</i> copies	4.9 (4.4)	13.0 (4.9)	8.1 (3.1)

Figure 2. Changes in HINE-2 total score by age at visit

All data from baseline to Day 302 are shown for participants with post-baseline scores. Mean scores and change from baseline not shown for participants with three *SMN2* copies due to small sample size.

HINE-2 = Hammersmith Infant Neurological Examination section 2; SD = standard deviation; *SMN2* = *survival motor neuron 2*.



	Actual Mean (SD) CHOP-INTEND Scores		Mean (SD) Change From Baseline to Day 302 (n = 32)	n (%) with ≥ 40 Points	
	Baseline (n = 33)	Day 302 (n = 32)		Baseline (n = 33)	Day 302 (n = 32)
Two <i>SMN2</i> copies	42.2 (9.2)	49.7 (7.2)	7.9 (6.4)	19 (57.6)	30 (93.8)

Figure 3. Changes in CHOP-INTEND total score by age at visit.

All data from baseline to Day 302 are shown for participants with post-baseline scores. CHOP-INTEND was not administered to four participants per protocol because they were ≥ 2 years of age at the time of informed consent and had attained sitting without support (n = 1 in the two *SMN2* copy group, and n = 3 in the three *SMN2* copy group). An improvement up to or above 40 points in CHOP-INTEND is very uncommon in the natural history of infantile-onset SMA and would indicate a favorable treatment effect.⁽⁶⁹⁾

CHOP-INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; SD = standard deviation; *SMN2* = *survival motor neuron 2*.

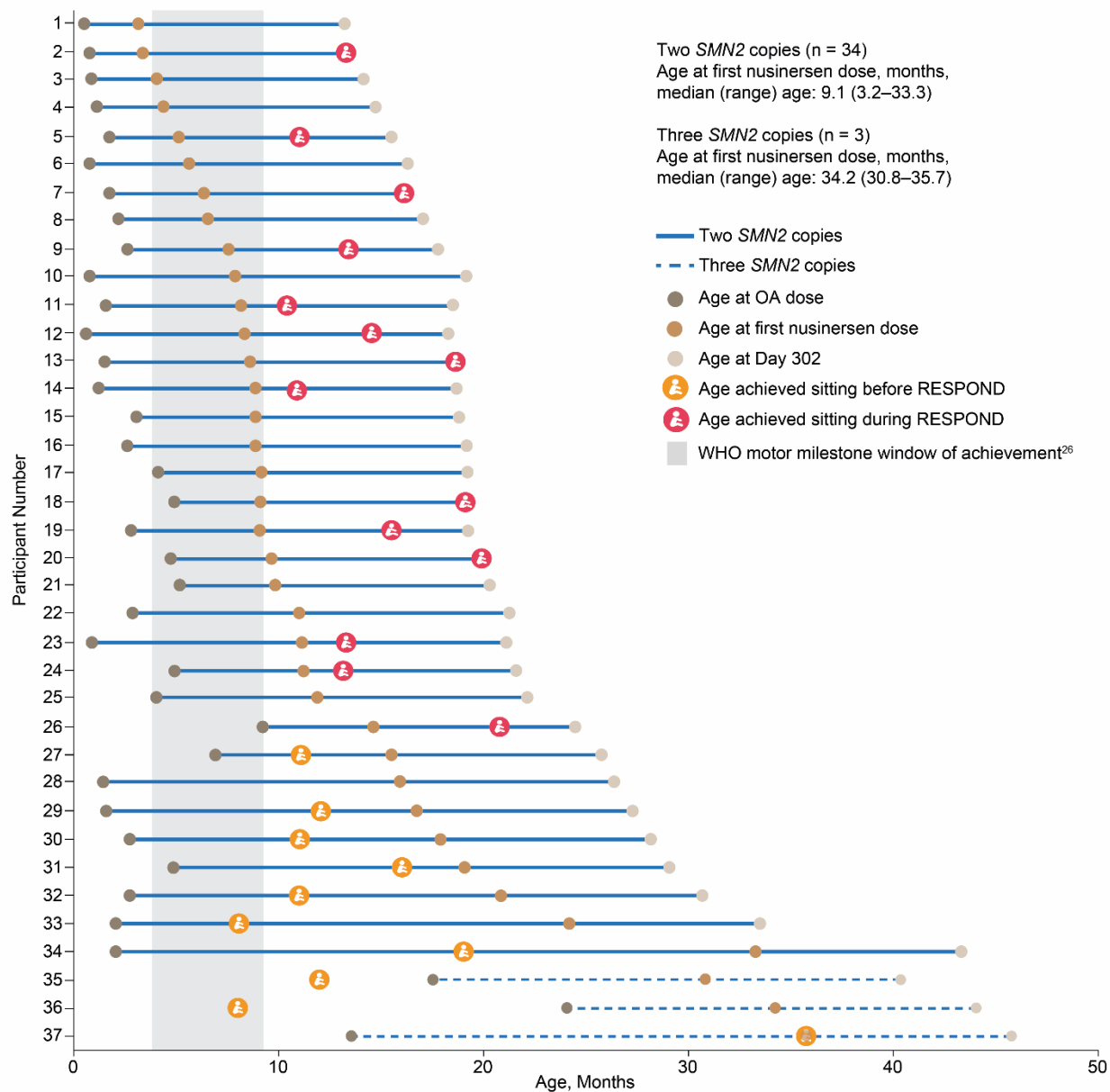
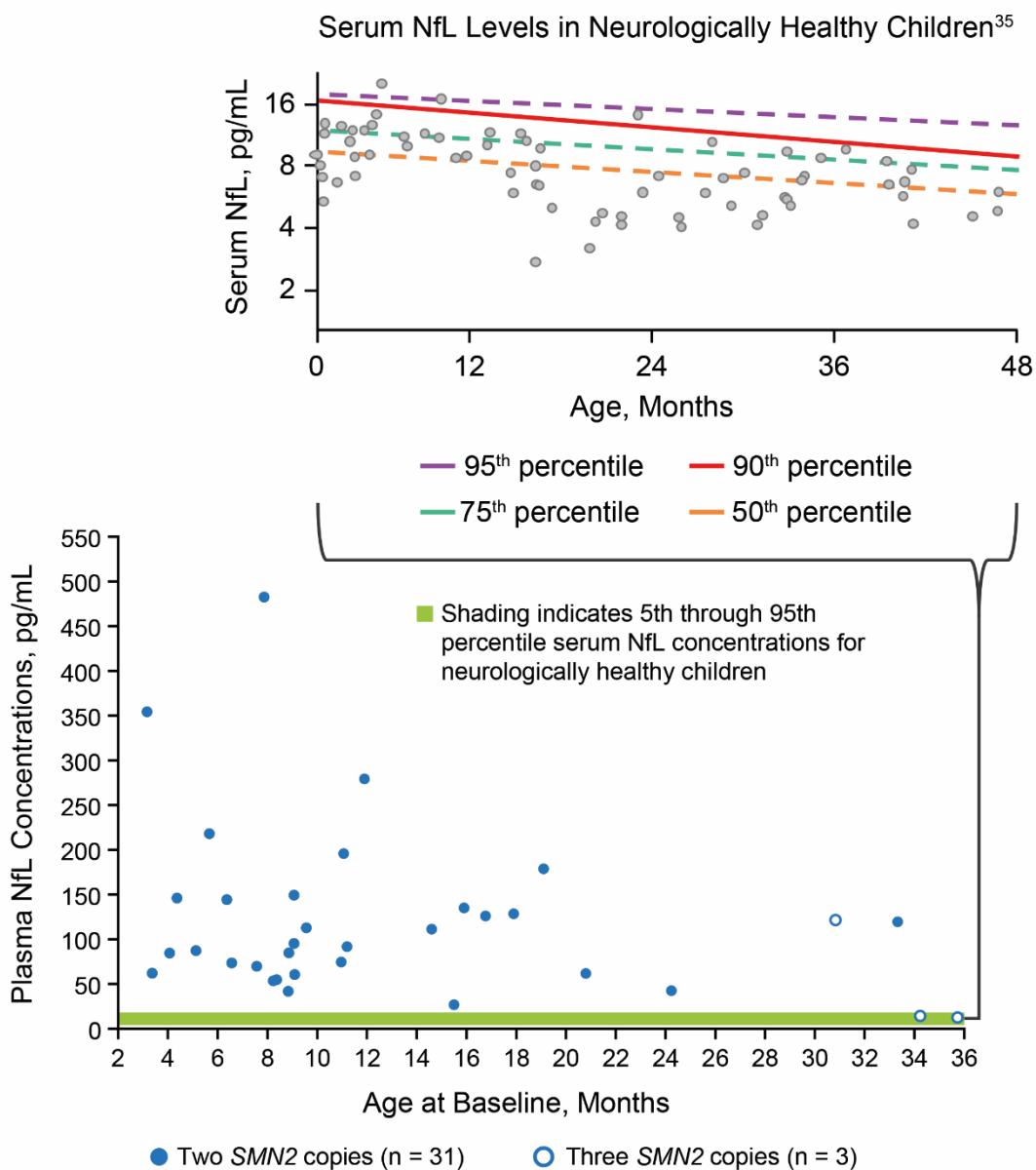


Figure 4. Achievement of independent sitting (WHO motor milestone) at Day 302.

Shading indicates the WHO motor milestone 1st and 99th percentiles of age at achievement for typically healthy developing children (3.8 and 9.2 months for sitting without support).⁽²⁴⁾ Only the achievement of sitting without support was examined in the interim analysis; other milestones will be evaluated at the end of the study.

OA = onasemnogene abeparvovec; SMN2 = *survival motor neuron 2*; WHO = World Health Organization.



Baseline Plasma NfL, pg/mL	Two <i>SMN2</i> copies (n = 31)	Three <i>SMN2</i> copies (n = 3)
Mean (SD)	127.4 (97.1)	49.5 (62.4)
Median (range)	95.5 (27–483)	14.3 (13–122)

Figure 5. Baseline plasma NfL concentrations by age.

‘Serum NfL levels in neurologically healthy children’ figure adapted from Bayoumy S et al.(25)

Data represent participants with non-missing baseline values. Although caution is needed when comparing values across studies due to known variations in analytical methods, as well as differences between serum and plasma levels, the baseline plasma NfL levels observed in the RESPOND study (range: 13–483 pg/mL) are substantially higher than the serum NfL levels previously reported for neurologically healthy children,(25, 26) which also used single molecular array (Simoa) immunoassay. NfL levels are expected to be approximately 5–20% higher in serum as compared to plasma.(43-45)

NfL = neurofilament light chain; SD = standard deviation; *SMN2* = *survival motor neuron 2*.

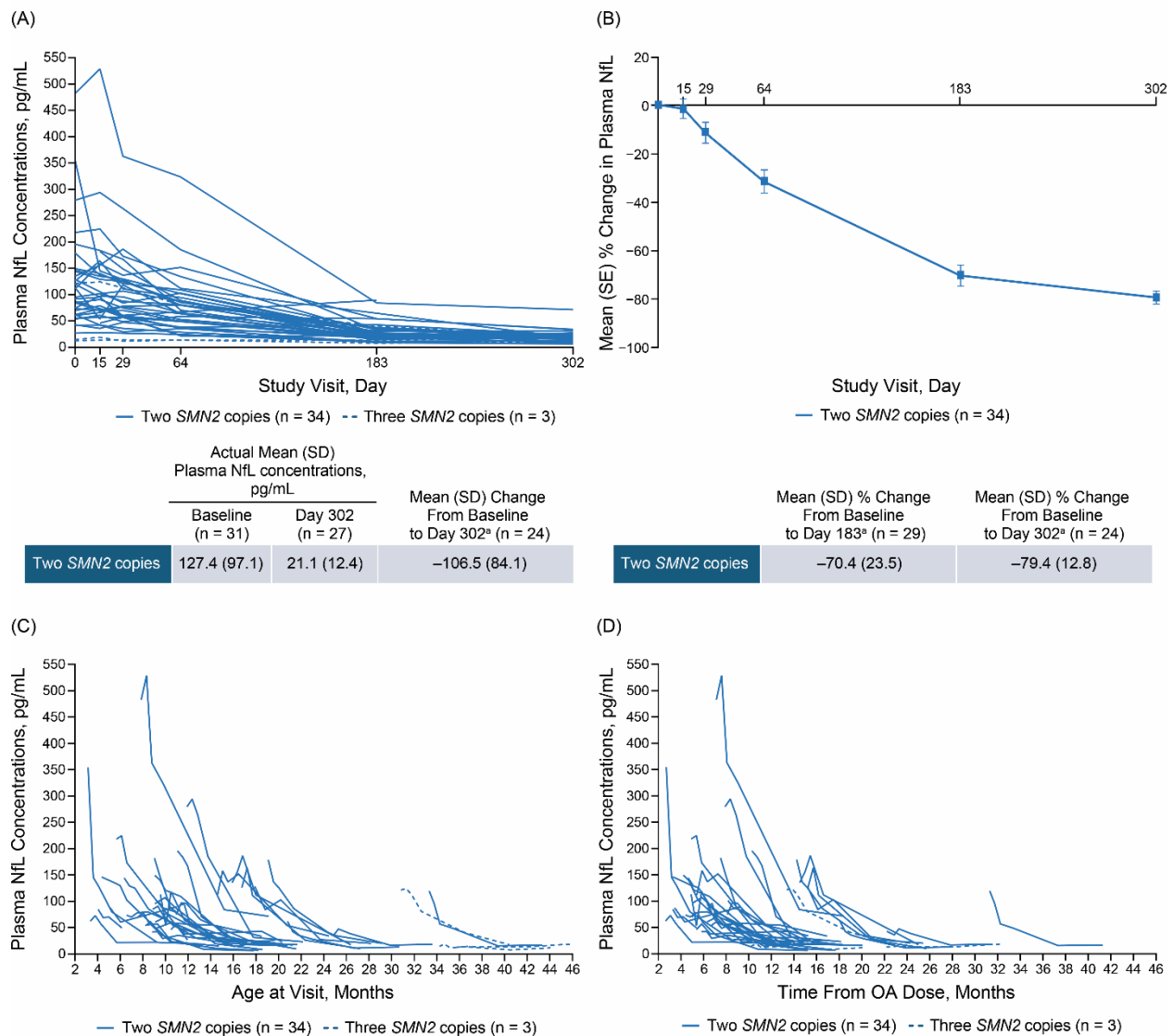


Figure 6. Changes in plasma NfL concentrations: (A) absolute changes by study visit, (B) percent changes by study visit, (C) absolute changes by age at visit, (D) absolute changes by time from OA.

All data from baseline to Day 302 are shown for participants with post-baseline scores. Individual participant trajectories may overlap. Descriptive statistics are not shown for participants with three *SMN2* copies due to small sample size.

^aMean (SD) changes were calculated for participants who had assessments at each time point at the time of the NfL data-cut.

NfL = neurofilament light chain; OA, onasemnogene abeparvovec; SE = standard error; SD = standard deviation;

SMN2 = survival motor neuron 2.

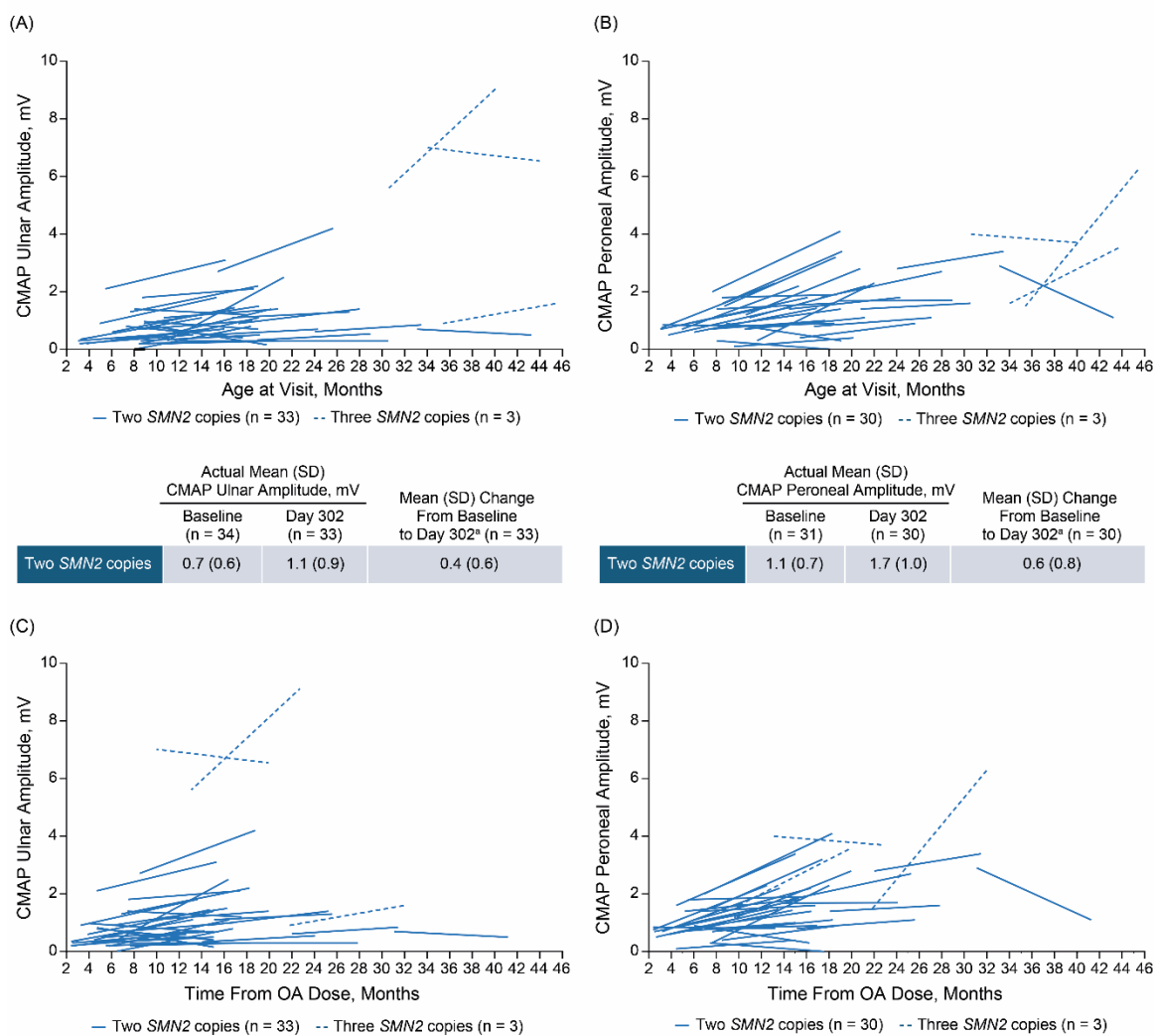


Figure 7. Changes in ulnar and peroneal CMAP amplitude by age at visit (**A** and **B**) and time from OA (**C** and **D**).

All data from baseline to Day 302 are shown for participants with post-baseline scores. Descriptive statistics are not shown for participants with three *SMN2* copies due to small sample size.

^aMean (SD) changes were calculated for participants who had assessments at each time point at the time of the data-cut.

CMAP = compound muscle action potential; SD = standard deviation; *SMN2* = *survival motor neuron 2*.

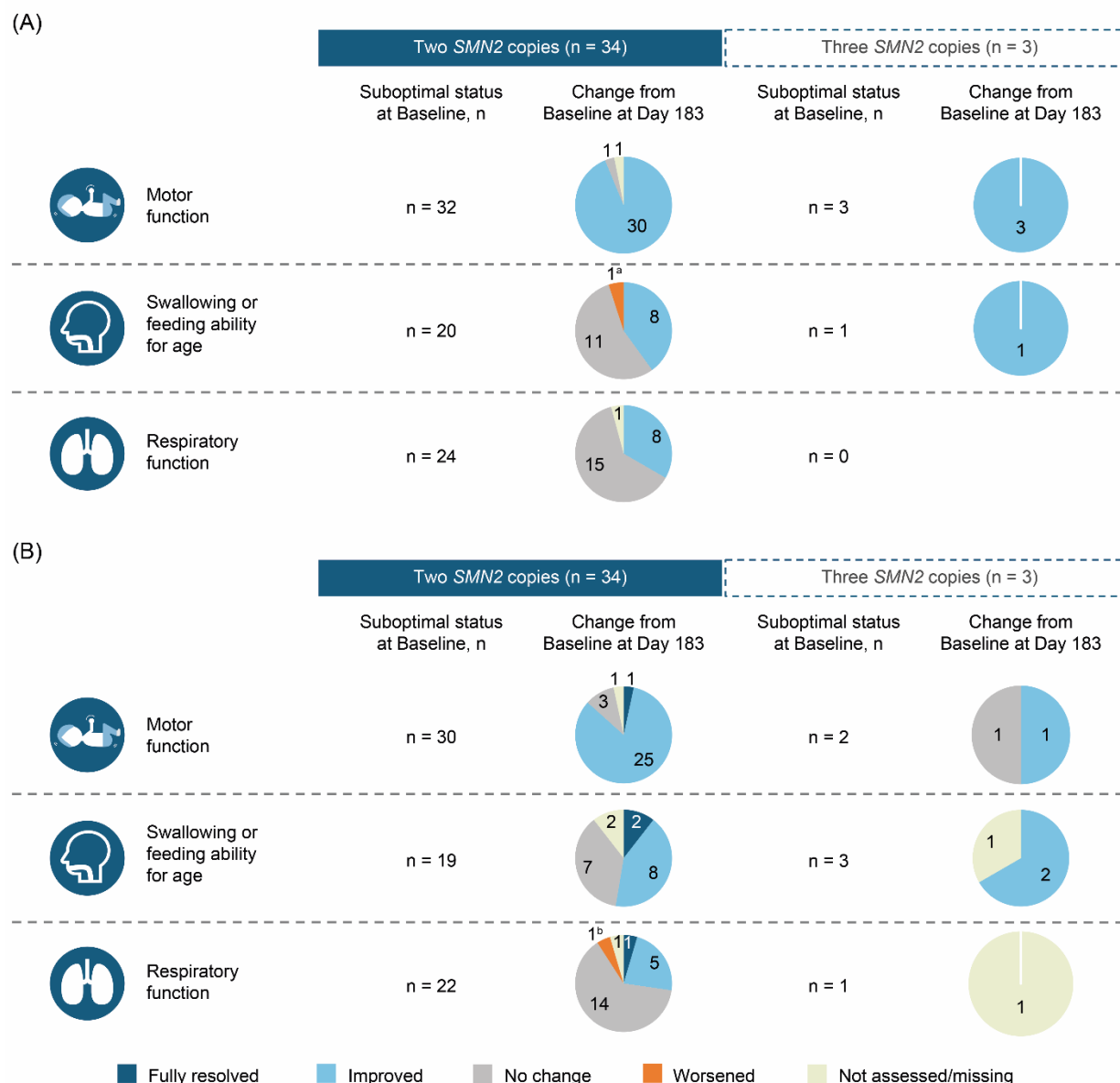


Figure 8. Changes in (A) investigator and (B) caregiver assessment of suboptimal clinical status at Day 183.

Changes at Day 183 are reported for this outcome as the assessment was not performed at Day 302 per the protocol. The language used on the form for caregiver was "strength and ability to move (example: unable to sit at the appropriate age)", "ability to breathe (example: requires ventilator support)", "ability to swallow (example: chokes when drinking liquids)", and "other".

In Panel A, four participants with two *SMN2* copies were reported to have suboptimal status in ‘other’ category at Baseline. At Day 183, no change was reported in one participant and the three other participants were not assessed with this domain at Day 183. In Panel B, six participants with two *SMN2* copies, and one participant with three *SMN2* copies were reported to have suboptimal status in ‘other’ category at baseline. At Day 183, two participants with two *SMN2* copies were reported to have ‘improved’, the other four were ‘not assessed’ with this domain, and the participant with three *SMN2* copies was reported to have ‘improved’.

^aParticipant was placed with gastrointestinal tube at Day 85. ^bParticipant had two severe AEs (acute/chronic respiratory failure and a chronic respiratory failure) < 2 months prior to Day 183. Both events were unrelated to the study drug and were resolved.

AE = adverse event; *SMN2* = *survival motor neuron 2*.

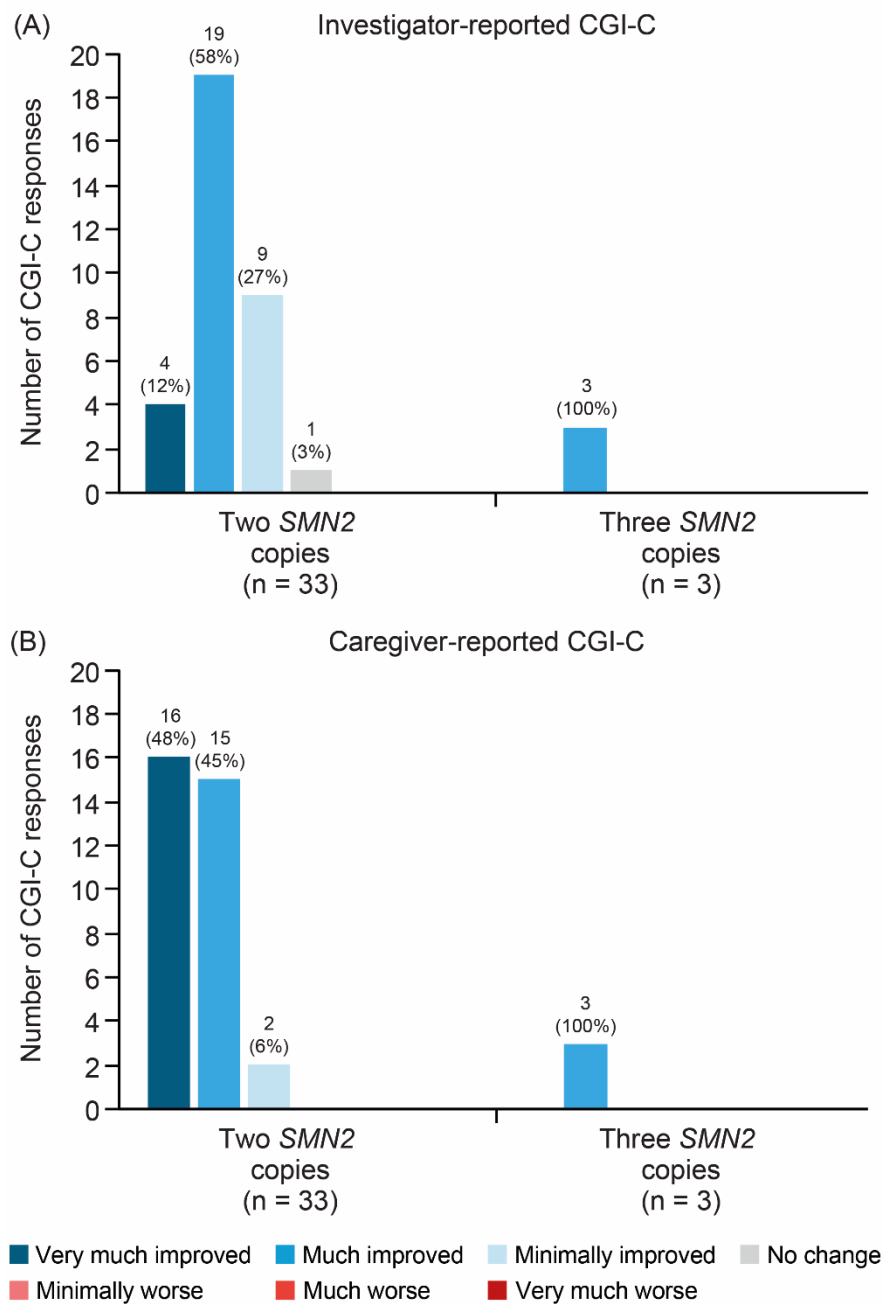


Figure 9. Proportion of CGI-C responders per (A) investigator and (B) caregiver assessment at Day 302.

CGI-C was assessed by both the investigator and caregiver using a 7-point scale, ranging from ‘very much improved’ to ‘very much worsened’, to rate overall improvement since study enrollment.

CGI-C = Clinical Global Impression of Change; *SMN2* = *survival motor neuron 2*.