Treatment of infantile-onset spinal muscular atrophy with nusinersen: final report of a phase 2, open-label, multicentre, dose-escalation study

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Summary

Background Nusinersen showed a favourable benefit–risk profile in participants with infantile-onset spinal muscular atrophy at the interim analysis of a phase 2 clinical study. We present the study's final analysis, assessing the efficacy and safety of nusinersen over 3 years.

Methods This phase 2, open-label, multicentre, dose-escalation study was done in three university hospital sites in the USA and one in Canada. Infants aged between 3 weeks and 6 months with two or three *SMN2* gene copies and infantile-onset spinal muscular atrophy were eligible for inclusion. Eligible participants received multiple intrathecal loading doses of 6 mg equivalent nusinersen (cohort 1) or 12 mg dose equivalent (cohort 2), followed by maintenance doses of 12 mg equivalent nusinersen. The protocol amendment on Jan 25, 2016, changed the primary efficacy endpoint from safety and tolerability to reaching motor milestones, assessed using the Hammersmith Infant Neurological Examination section 2 (HINE-2) at the last study visit, in all participants who successfully completed the loading dose period and day 92 assessment. The statistical analysis plan was amended on Feb 10, 2016, to include additional analyses of the subgroup of participants with two *SMN2* copies. Adverse events were assessed in all participants who received at least one dose of study treatment. The study is registered at ClinicalTrials.gov (NCT01839656).

Findings Between May 3, 2013, and July 9, 2014, 20 symptomatic participants with infantile-onset spinal muscular atrophy (12 boys and 8 girls; median age at diagnosis 78 days [range 0–154]) were enrolled. Median time on study was 36·2 months (IQR 20·6–41·3). The primary endpoint of an incremental improvement in HINE-2 developmental motor milestones was reached by 12 (63%) of 19 evaluable participants. In the 13 participants with two *SMN2* copies treated with 12 mg nusinersen, the HINE-2 motor milestone total score increased steadily from a baseline mean of 1·46 (SD 0·52) to 11·86 (6·18) at day 1135, representing a clinically significant change of 10·43 (6·05). At study closure (Aug 21, 2017), 15 (75%) of 20 participants were alive. 101 serious adverse events were reported in 16 (80%) of 20 participants; all five deaths (one in cohort 1 and four in cohort 2) were likely to be related to spinal muscular atrophy disease progression.

Interpretation Our findings are consistent with other trials of nusinersen and show improved survival and attainment of motor milestones over 3 years in patients with infantile-onset spinal muscular atrophy, with a favourable safety profile.

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Introduction

Spinal muscular atrophy is an autosomal recessive neuromuscular disorder caused by deletions or mutations in the *SMN1* gene (*SMN2* is a nearly identical gene that produces only a small amount of full-length SMN protein).[1] The disease is characterised by degeneration of α-motor neurons in the spinal cord and brainstem, resulting in weakness and atrophy of skeletal muscles of the limbs and trunk, and of the bulbar and respiratory muscles.[2]

Nusinersen, onasemnogene abeparvovec, and risdiplam were identified as three new therapies that result in prolonged survival, enhanced motor milestone attainment, and better motor function in infants with onset of symptoms of spinal muscular atrophy by 6 months of age. Nusinersen is approved for individuals with spinal muscular atrophy of all ages in the European Union, USA, and other countries. Onasemnogene abeparvovec is approved for the treatment of patients with spinal muscular atrophy who are younger than 2 years in the USA and for the treatment of patients with spinal muscular atrophy type I or up to three *SMN2* copies and 21 kg in weight in European Union countries. Risdiplam has been approved in the USA for patients with spinal muscular atrophy age 2 months or older, and in the European Union for patients age 2 months or older; types 1, 2 and 3; and 1–4 copies of *SMN2*.

Research in context

Evidence before this study

We searched PubMed for research done in humans before and after 2013 using the search terms "((spinal muscular atrophy) AND (treatment) OR (standard of care))". At the time this trial was designed, the most recently published consensus statement (published in 2007) on the optimum management of infantile-onset spinal muscular atrophy emphasised supportive and palliative care. In reviewing the scientific evidence from January, 2007, to December, 2013, we found there were few studies of interventions for spinal muscular atrophy type I. In this period there was one phase 1 study of nusinersen in children with spinal muscular atrophy type II and type III, in which nusinersen showed a favourable benefit–risk profile. Additionally, eight phase 2 and phase 3 trials of systemic therapies for spinal muscular atrophy type II and type III were identified, but none of the interventions (L-carnitine or valproic acid, hydroxyurea, and phenylbutyrate) showed a survival or functional benefit.

Added value of this study

The end-of-study analysis of this open-label, multicentre, phase 2, dose-escalation study of intrathecal nusinersen in participants with infantile-onset spinal muscular atrophy shows that nusinersen improves attainment of developmental motor milestones, motor function, and neuromuscular electrophysiological activity, and prolongs the composite endpoint of overall and permanent ventilatory-free survival over a median observation period of 3 years. Clinical responses to nusinersen improved over the entire study period and were accompanied by rapid and sustained reductions in the plasma biomarker phosphorylated neurofilament heavy chain in a post-hoc analysis. The safety profile of nusinersen was consistent with that reported in the previous interim analysis and other nusinersen clinical trials.

Implications of all the available evidence

Our efficacy and safety data are consistent with a large phase 3, randomised, sham-controlled study of nusinersen in infantile-onset spinal muscular atrophy (ENDEAR; NCT02193074). The implication from the NURTURE phase 2 study (NCT02386553) of presymptomatic infants and trials in symptomatic infantile-onset spinal muscular atrophy, including the onasemnogene abeparvovec gene transfer study (NCT02122952), risdiplam *SMN2*-directed RNA splicing modifier study (NCT02913482), and this study, is that early treatment is associated with improved outcomes, emphasising the value of a timely diagnosis. Use of plasma phosphorylated neurofilament heavy chain as a responsive biomarker in spinal muscular atrophy is promising but requires further study.

This trial is the first study of nusinersen, an intrathecally administered 2′-O-methoxyethyl phosphorothioate-modified antisense oligonucleotide, in infantile-onset spinal muscular atrophy.[3] Most individuals with infantile-onset spinal muscular atrophy and two *SMN2* gene copies develop spinal muscular atrophy type I. In the absence of treatment to increase the quantity of full-length SMN protein in target motor neurons, three-quarters of infants with spinal muscular atrophy type I either die or require permanent ventilation by age 2 years.[4] The interim analysis of this trial showed that, after 2–32 months of follow-up, nusinersen treatment was generally well tolerated and was associated with progressive improvements in motor function and prolonged survival relative to a natural history cohort.[3] A modified nusinersen dosage regimen was used in the two registrational phase 3 trials of participants with infantile-onset spinal muscular atrophy (ENDEAR)[5] and later-onset spinal muscular atrophy (CHERISH),[6] and initially in an ongoing phase 2 study of infants likely to develop spinal muscular atrophy type I or II who initiated treatment during the presymptomatic stage (NURTURE).[7]

The Hammersmith Infant Neurological Examination section 2 (HINE-2) is a standardised tool that evaluates developmental milestones by assessing changes in head control, sitting, voluntary grasp, ability to kick, rolling, crawling, standing, and walking.[8] Although not developed specifically to measure neurological outcomes in spinal muscular atrophy, all three sections of HINE were used for safety evaluation, and HINE-2 was used specifically as the primary efficacy measure in this phase 2 study and the coprimary efficacy measure in the ENDEAR trial.[3,5] Interim data from this trial showed that using HINE-2 in a population with spinal muscular atrophy type I was feasible, and showed gains in motor function in parallel to the gains identified in the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) and electrophysical outcome measures (ie, compound muscle action potential [CMAP]) in two nerves.[3,9] Furthermore, HINE-2 can reliably capture partial or full attainment in three of the six fundamental WHO motor milestones expected in a typically developing child.[9,10]

The CHOP INTEND score is used frequently in research and clinical practice because of its sensitivity to detect changes in patients with spinal muscular atrophy type I and its reliability, tolerability, and short completion time.[11] However, CHOP INTEND was designed to measure motor function in infants with neuromuscular disease who were particularly weak; thus, improvements among healthier infants responding to treatment might not be captured because of a ceiling effect.

Here we present end-of-study efficacy and safety data for this phase 2 study on infantile-onset spinal muscular atrophy, which represents a median of 36·2 months of follow-up in all participants. New post-hoc data are presented on the plasma phosphorylated neurofilament heavy chain (pNF-H). Plasma pNF-H concentrations have been shown to decline substantially following initiation

of nusinersen treatment, and are being investigated as a biomarker of disease activity and treatment response.[7,12]

Methods

Study design and participants

This phase 2, open-label, multicentre, dose-escalation trial was done in three university hospital sites in the USA and one in Canada: Columbia University Irving Medical Center (New York, NY, USA), Stanford University School of Medicine (Standford, CA, USA), Nemours Children's Hospital (Orlando, FL, USA), and University of Toronto Hospital for Sick Children (Toronto, ON, Canada). The study protocol and population have been described previously.[3] Eligible participants were aged between 3 weeks and 7 months with genetically confirmed biallelic mutations in the *SMN1* gene, two or three *SMN2* gene copies, and symptom onset between age 3 weeks and 6 months. Bodyweight had to be higher than the fifth percentile for age, gestational age was 35–42 weeks, and birthweight was 2 kg or heavier. Additionally, participants had to be receiving adequate nutrition and hydration at study entry (in the opinion of the investigator) and their care had to be anticipated to meet standard of care guidelines.[13] The main exclusion criteria were hypoxaemia (oxygen saturation <96% when awake or asleep without ventilatory support), presence of infection requiring therapy during the screening period, and a history of brain or spinal cord disease that would interfere with the lumbar puncture procedure.[3]

Review boards at all participating institutions approved the study, which was done according to the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonisation. Written informed consent was obtained from parents or caregivers for all participants. The study was monitored on an ongoing basis by an independent data safety board (see appendix p 1).

Procedures

Nusinersen was diluted to 1·2 mg/mL (6 mg dose equivalent) or 2·4 mg/mL (12 mg dose equivalent) with artificial CSF. The individual dose was adjusted on the basis of age to reach a scaled dose equivalent (4–5 mL) from the participant's projected cerebrospinal fluid volume. The 12 mg dose equivalent is identical to that in ENDEAR[5] but differs from the US Food and Drug Administration-approved label (all patients receive a full 12 mg dose). Doses were administered intrathecally by lumbar puncture over 1–3 min. All participants received standard of care according to 2007 guidelines,[13] which were current at trial initiation, and the option of palliative care.[3]

Four participants were treated with 6 mg equivalent nusinersen on days 1 (predose evaluations and administration of the first dose), 15, and 85 (loading doses), followed by 12 mg equivalent doses on day 253 and then repeated every 4 months (cohort 1). 16 participants were then treated with 12 mg equivalent nusinersen at days 1, 15, 85 (loading doses), and 253, and repeated every 4 months (cohort 2).[3] Participants attended follow-up visits on days 16, 29, 86, 92, 169, 254, 337, and 442, then every 4 months, and families were contacted by telephone every 2–3 weeks to monitor safety and ventilation status. Samples to determine *SMN2* copy number and gene sequencing (Athena Diagnostics; Marlborough, MA, USA) were collected at day 85 and plasma samples were stored at –80°C for possible future analysis.[3] Plasma samples were collected at multiple study visits from day 1–1352. The total duration of study participation was approximately 3·7 years.

Outcomes

The protocol was amended on Jan 25, 2016, to change the primary efficacy endpoint from safety and tolerability to reaching motor milestones as evaluated by HINE-2. At this timepoint the study was of sufficient duration to assess the efficacy of nusinersen in this population. The statistical analysis plan was subsequently amended on Feb 10, 2016, to reflect this change, and to include analysis of the subgroup of participants with two *SMN2* copies. Following this protocol amendment, the primary endpoint was the proportion of participants who had improvement in motor milestones as evaluated by HINE-2.

Improvement in motor milestones was defined as evidence of at least one of the following three criteria: 1) an increase from baseline of 2 points or more, or ability to pincer grasp in the category of voluntary grasp; 2) an increase from baseline of 2 points or more in the ability to kick or ability to touch toes, or 3) an increase from baseline of 1 point in any of the remaining six categories (head control, rolling, sitting, crawling, standing, or walking).

Secondary endpoints included change from baseline in motor function, event-free survival, change from baseline in CMAPs, and safety. Motor function was measured using the CHOP INTEND, with higher scores on the 0–64 point scale representative of improved motor function.[14] A change of 4 points or more from baseline to the last available visit in the CHOP INTEND score was regarded as clinically significant.[5] Event-free survival was defined as the proportion alive and not requiring permanent ventilatory support (tracheostomy or the need for ≥16 h of ventilation per day continuously for ≥2 weeks in the absence of an acute reversible illness).[3] Neuromuscular electrophysiology was measured by the CMAP of the ulnar nerve (recorded from the abductor digiti minimi muscle) and the peroneal nerve (recorded from the tibialis anterior muscle). Improvement was defined as an increase in CMAP amplitude of greater than 0·5 mV from baseline to last available study visit. Growth parameters were an exploratory endpoint and investigation of plasma pNF-H concentration was a post-hoc analysis. Plasma pNF-H

See Online for appendix

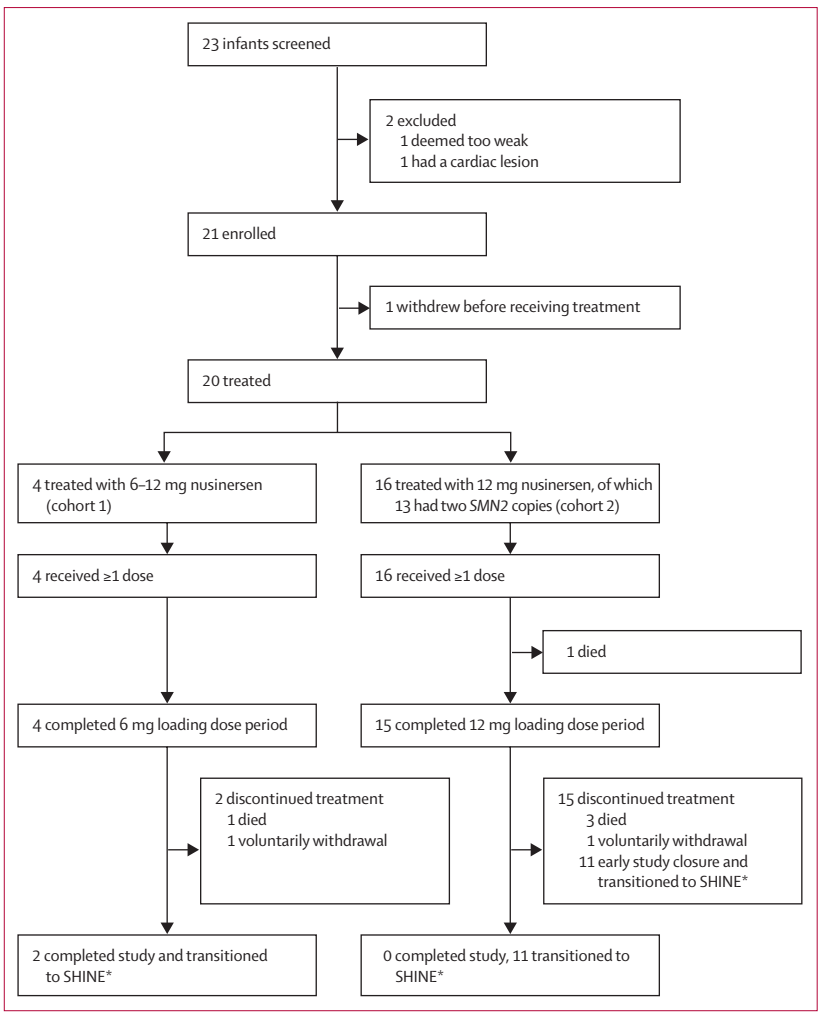


Figure 1: **Study profile**
Two participants, both in cohort 1, completed the study and entered SHINE after 162–170 days. Early study closure and transition to SHINE was the most common reason for treatment discontinuation (11 from cohort 2; 111–147 days between studies). 13 participants in the safety population were alive and participating (not withdrawn) at end-of-study assessments. *SHINE (NCT02594124) is an open-label extension study for patients with spinal muscular atrophy who previously participated in investigational studies of nusinersen.

concentrations were measured using an enzyme-linked lectin assay (ProteinSimple; San Jose, CA, USA).[12]

Statistical analysis

The primary efficacy analysis was done in the per-protocol efficacy evaluable population; this population was selected as it represents all participants who successfully completed the loading dose period and day 92 assessment. The proportion of participants who had improvement in motor milestones at their last available visit was evaluated by dose cohort in the safety population (received ≥1 dose of study treatment). For consistency with ENDEAR,[5] endpoints were evaluated in participants from cohort 2 with two *SMN2* gene copies.

The non-parametric Kaplan-Meier product threshold method was used to estimate the distribution of time to events. SAS version 9.4 was used for all key analyses.

Role of the funding source

This study was funded by Biogen and Ionis Pharmaceuticals. Employees of Biogen (RF, YL, DR-S, JW, and WF) and Ionis Pharmaceuticals (KMB [at the time of the study], ES, and CFB) contributed to the study design, analysis or interpretation of data, and writing of the report. The corresponding author had full access to all data from the study and had final responsibility for the content of the report and decision to submit for publication.

Results

20 infants with spinal muscular atrophy were enrolled between May 3, 2013, and July 9, 2014. Treatment course of these 20 participants is shown in figure 1 and the analysis populations in the appendix (p 9). The clinical cutoff date for the end-of-study analysis was Aug 21, 2017; the study closed early to allow participants to transition to the open-label SHINE extension study (NCT02594124). Two participants, both in cohort 1, completed the study and entered SHINE after 162–170 days. Early study closure and transition to SHINE was the most common reason for treatment discontinuation (11 from cohort 2; 111–147 days between studies). 13 participants in the safety population were alive and participating (not withdrawn) at end-of-study assessments.

Participant-related characteristics and spinal muscular atrophy symptoms at baseline are shown in table 1; additional baseline characteristics were reported previously.[3] Time on study ranged from 62 to 1429 days (median 1101 days [IQR 627–1254]), representing 50·6 participant-years (appendix p 2).

In the per-protocol efficacy evaluable population, 12 (63%) of 19 participants met the primary endpoint by having an incremental improvement in HINE-2 developmental motor milestones at the last visit relative to baseline. Median time to reach a motor milestone in the total efficacy evaluable population was 11·6 months (95% CI 0·92–18·63). Eight (42%) of 19 participants had full head control at last visit, eight (42%) could sit (three could stable sit and five could pivot), seven (37%) could roll, two (11%) could crawl, four (21%) could stand (three with support and one unaided), and two (11%) could walk (one with support and one unaided). A developmental motor milestone was fully achieved by one (25%) of four participants in cohort 1 versus eight (53%) of 15 participants in cohort 2.

The proportion who had an improvement in motor milestone achievement was 12 (60%) of 20 participants in the safety population and nine (69%) of 13 participants in the cohort 2 subset with two *SMN2* copies; further details of the proportion of cohort 2 reaching motor milestones over time are shown in the appendix (p 4).

In the cohort 2 subset with two *SMN2* copies, the HINE-2 motor milestone total score increased steadily over time from a baseline mean of 1·46 (SD 0·52) to 11·86 (6·18) at day 1135, representing a clinically significant change of 10·43 (6·05; figure 2A). Median age at first dose was 129 days (range 37–216) in the subset with symptom onset at age 12 weeks or younger (spinal muscular atrophy type IB) and 215 (208–222) at age older than 12 weeks (SMA spinal muscular atrophy IC). Slightly greater improvements in motor milestone score over time were observed in participants with spinal muscular atrophy symptom onset at age 12 weeks or younger than in participants with symptom onset at age older than 12 weeks (appendix p 5). Further details on change in HINE-2 total score are shown in the appendix (pp 5–6). There was some variability in individual HINE-2 total scores, with milestones occasionally gained and lost between visits.

In the safety population, 11 (55%) of 20 participants met the motor milestone response criteria defined in ENDEAR (appendix p 2).[5] Of the 13 participants in cohort 2 with two *SMN2* copies, eight (62%) met the ENDEAR response criteria (appendix p 2).

In the safety population, mean CHOP INTEND total score increased from 29·7 (SD 10·5) at baseline to 48·3 (12·7) at the last study visit for the 13 surviving participants, a relative improvement of 17·3 points (12·2). Further details on change in CHOP INTEND scores are shown in the appendix (p 7). Figure 2B shows the average change in CHOP INTEND score over time for the 13 participants in cohort 2 with two *SMN2* copies. Eight (62%) of these 13 participants reached a CHOP INTEND score greater than 40 and six (46%) reached a score greater than 50; no participants reached a score greater than 60. The change over time in the overall group was similar to that in the subgroup of participants who were followed up for approximately 3 years (day 1135 visit), suggesting that the observed trend over time is not affected by the earliest participants enrolled in the trial or due to individuals dropping out of the study. Seven (54%) of the 13 participants in cohort 2 with two *SMN2* copies had an increase of 4 or more points from baseline in CHOP INTEND total score, the CHOP INTEND-responder definition used in ENDEAR.[5] An improvement of 4 points or more in the CHOP INTEND score at the last study visit was seen in eight (73%) of 11 participants with symptom onset at age 12 weeks or younger at first dose (median 129 days [range 37–216]) and one (50%) of two participants with symptom onset at age older than 12 weeks (215 days [208–222]).

At study closure, 15 (75%) of 20 participants who received nusinersen in the safety population were alive, although two participants had voluntarily withdrawn—one had withdrawn by month 8 (no reason stated) and one had withdrawn by month 37 (hardship travelling to study site). 11 (55%) of 20 participants were alive without the need for permanent ventilation; thus, a median time to death or permanent ventilation was not reached (figure 2C). None of these 11 participants received a tracheostomy. All 13 participants in cohort 2 with two *SMN2* copies required some ventilator use during the study; two were alive and met the endpoint of permanent ventilation. In this subset, ventilator use increased over time from 0 h per day at baseline to a mean of 5·2 h at day 92, 8·5 h at day 253, 12·5 h at day 379, 11·8 h at day 694, 11·5 h at day 757, 11·1 h at day 946, and 10·6 h at day 1072.

Among the 13 participants in cohort 2 with two *SMN2* copies, mean peroneal nerve CMAP amplitudes increased steadily over time, whereas mean ulnar nerve CMAP amplitudes plateaued after day 568 (figure 2D–E; appendix p 2).

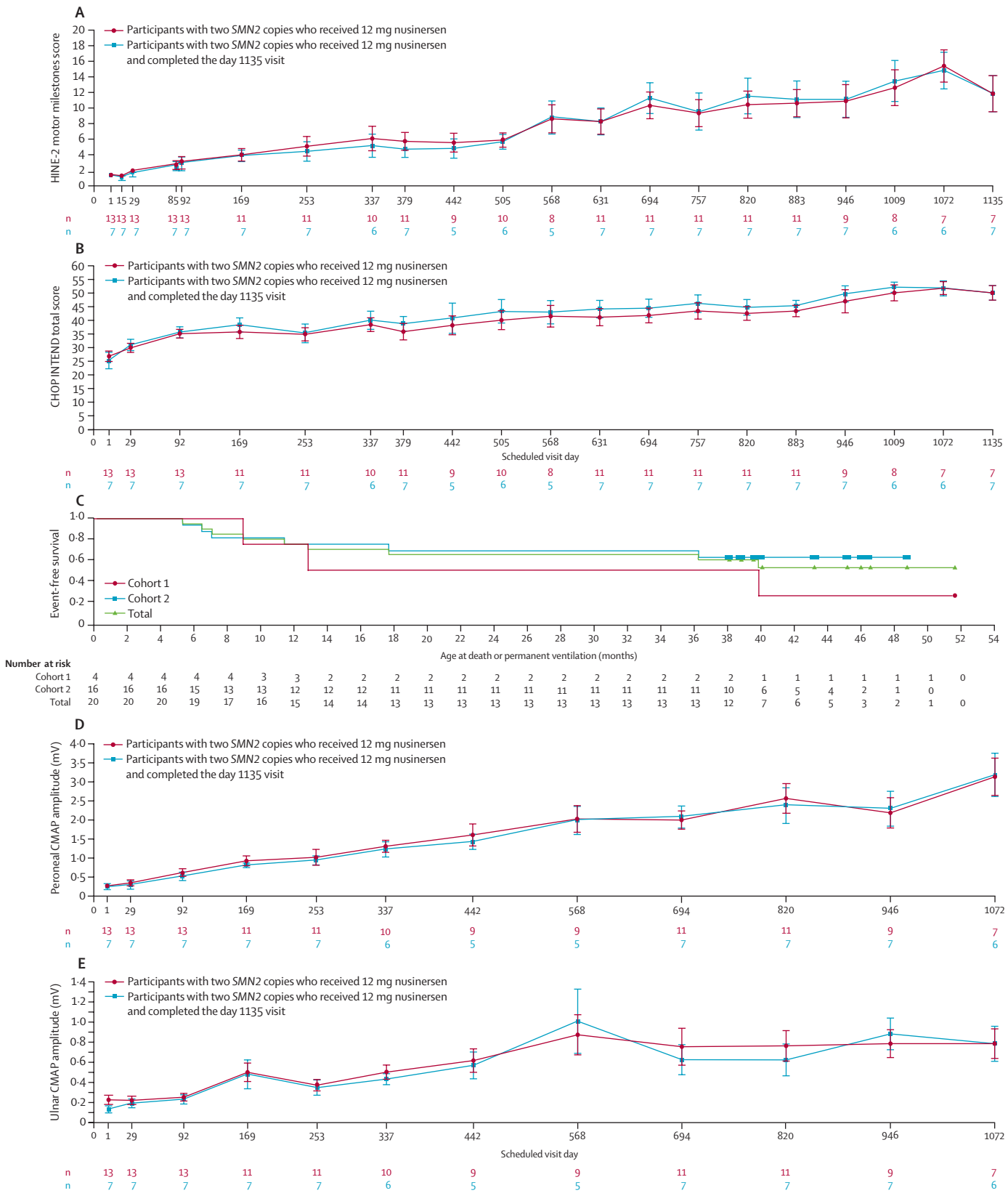
In the safety population, steady growth was observed in arm and chest circumference. Changes in growth parameters for participants in cohort 2 with two *SMN2* copies are shown in the appendix (pp 2 and 8). Correlation analyses between outcome measures are also shown in the appendix (pp 2–3 and 10).

In the safety population, 101 serious adverse events were reported in 16 (80%) of 20 participants (table 2). All five deaths that occurred during the study (one in cohort 1 and four in cohort 2) were due to serious adverse events that were likely related to spinal muscular atrophy disease progression (see appendix p 3). None of the

	Cohort 1, two *SMN2* copies (n=4)	Cohort 2, two *SMN2* copies (n=13)*	Cohort 2, three *SMN2* copies (n=2)*	Total (n=20)
Gender				
Male	3 (75%)	6 (46%)	2 (100%)	12 (60%)
Female	1 (25%)	7 (54%)	0	8 (40%)
Age at symptom onset (days)†	47 (19; 28–70)	56 (37; 21–133)	123 (45; 91–154)	60 (39; 21–154)
Age at diagnosis (days)†	74 (27; 42–105)	82 (51; 0–154)	70 (69; 21–119)	78 (45; 0–154)
Time from symptom onset to enrolment (days)	97 (50; 39–151)	75 (38; 15–130)	76 (62; 32–119)	81 (40; 15–151)
Age at enrolment (days)	145 (67; 67–207)	131 (62; 36–207)	198 (17; 186–210)	141 (60; 36–210)
HINE-2 score	2 (0·8; 1–3)	1 (0·5; 1–2)	8 (5·7; 4–12)	2 (2·4; 1–12)
CHOP INTEND score	27 (5·1; 22–34)	27 (6·9; 17–38)	53 (15·6; 42–64)	30 (10·5; 17–64)
Hypotonia	4 (100%)	13 (100%)	1 (50%)	19 (95%)
Developmental motor delay	3 (75%)	12 (92%)	1 (50%)	17 (85%)
Pneumonia or respiratory symptoms	3 (75%)	5 (38%)	0	9 (45%)
Limb weakness	4 (100%)	13 (100%)	1 (50%)	19 (95%)
Swallowing or feeding difficulties	3 (75%)	5 (38%)	0	9 (45%)
Other	0	0	1 (50%)‡	1 (5%)‡

Data are n (%) or mean (SD; range). Some data for the total cohort have been published in the interim report.[3] Motor function was measured using the CHOP INTEND, with higher scores on the 0–64 point scale representative of improved motor function.[3,4] A change of 4 points or more from baseline to the last available visit in the CHOP INTEND score was regarded as clinically significant. HINE-2 scores range from 0–26, with higher scores indicative of improved motor function.[8] CHOP INTEND=Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders. HINE-2=Hammersmith Infant Neurological Examination section 2. *Number of *SMN2* copies is unknown for one participant. †Diagnosis is possible by genetic testing before the onset of symptoms. ‡Areflexia.

Table 1: **Baseline characteristics**


A
HINE-2 motor milestones score
Participants with two SMN2 copies who received 12 mg nusinersen
Participants with two SMN2 copies who received 12 mg nusinersen and completed the day 1135 visit
B
CHOP INTEND total score
Scheduled visit day
C
Event-free survival
Cohort 1
Cohort 2
Total
Age at death or permanent ventilation (months)
Number at risk
Cohort 1 4 4 4 4 4 3 3 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1 1 1 0
Cohort 2 16 16 16 15 13 13 12 12 12 11 11 11 11 11 11 11 11 11 11 10 6 5 4 2 1 0
Total 20 20 20 19 17 16 15 14 14 13 13 13 13 13 13 13 13 13 13 12 7 6 5 3 2 1 0
D
Peroneal CMAP amplitude (mV)
E
Ulnar CMAP amplitude (mV)

serious adverse events or deaths were considered related to study treatment or lumbar puncture procedure. Data on ancillary medical procedures and mortality among participants in cohort 2 by *SMN2* copy number are shown in the appendix (p 11).

The most frequently reported serious adverse events concerned the respiratory system, consistent with events typically observed in children with spinal muscular atrophy type I. In cohort 2, the incidence of adverse events and serious adverse events tended to decrease over time. Incidence of adverse events and serious adverse events (including related hospitalisations) of the respiratory, thoracic, and mediastinal system organ class decreased from 69% (11 of 16 participants) and 31% (five of 16 participants) between days 1 and 90 to 20% (two of ten participants) and 10% (one of ten participants) between days 991 and 1080. Similarly, the incidence of adverse and serious adverse events of the gastrointestinal system decreased from 63% (ten of 16 participants) and 6% (one of 16 participants) between days 1 and 90 to 30% (three of ten participants) and 0% between days 991 and 1080.

Clinical laboratory results for cohort 2 did not reveal a specific pattern among participants who received nusinersen (appendix p 12). Three infants had transient shifts in platelet concentrations from normal (>150×10⁹ platelets per L) to low (132–145×10⁹ platelets per L) values (appendix p 3). None of the low platelet counts were considered clinically significant by the investigators nor reported as an adverse event. There were no bleeding events reported during these transient episodes of low platelet count. There were no clinically relevant changes related to nusinersen with respect to urinalysis or hepatic or renal function. No clinically significant changes from baseline were observed in coagulation test results.

Post-hoc analyses showed that the geometric mean plasma pNF-H concentration at baseline in the 13 participants from cohort 2 with two *SMN2* copies was 10020 pg/mL (95% CI 4774–21030). Nine (69%) of the 13 participants had plasma pNF-H concentrations of around 10000 pg/mL or more at baseline. Following administration of nusinersen 12 mg loading doses, pNF-H concentrations declined sharply by day 85 (3342 pg/mL [95% CI 2340–4773], n=13) and remained low throughout nusinersen maintenance treatment (day 1072: 622 pg/mL [331–1169], n=6). Concentrations in individual participants from cohort 2 are shown in figure 3. Plasma pNF-H concentrations in three cohort 1 participants with two *SMN2* copies were 9692 pg/mL (95% CI 5045–18 619) at baseline, 6296 pg/mL (1732–22 883, n=3) at day 85, and 951 pg/mL (24–37 569, n=2) at day 1072.

Discussion

The final analysis of this phase 2 study provides complete and longitudinal assessments of the clinical benefit of nusinersen in participants with symptomatic infantile-onset spinal muscular atrophy over a median follow-up of 36·2 months. The end-of-study efficacy and safety findings were consistent with the interim results.[3] Steady improvements in the attainment of developmental motor milestones and motor function continued through to

	Cohort 2 (n=16)		Total (n=20)	
	Events (n)	Participants (n, %)	Events (n)	Participants (n, %)
Any serious adverse event	86	13 (81%)	101	16 (80%)
Any adverse event	636	16 (100%)	802	20 (100%)
Upper respiratory tract infection	45	11 (69%)	47	12 (60%)
Nasopharyngitis	14	7 (44%)	15	8 (40%)
Pneumonia	18	7 (44%)	20	8 (40%)
Otitis media	8	6 (38%)	9	7 (35%)
Respiratory tract infection	11	4 (25%)	30	7 (35%)
Rhinovirus infection	9	7 (44%)	9	7 (35%)
Viral infection	7	5 (31%)	7	5 (25%)
Viral upper respiratory tract infection	4	4 (25%)	4	4 (20%)
Constipation	9	8 (50%)	10	9 (45%)
Vomiting	12	6 (38%)	15	8 (40%)
Diarrhoea	15	4 (25%)	18	6 (30%)
Gastro-oesophageal reflux	6	6 (38%)	6	6 (30%)
Pyrexia	46	14 (88%)	52	17 (85%)
Pain	4	4 (25%)	4	4 (20%)
Respiratory distress	13	7 (44%)	16	9 (45%)
Cough	11	5 (31%)	13	7 (35%)
Nasal congestion	8	6 (38%)	11	7 (35%)
Respiratory failure	8	7 (44%)	8	7 (35%)
Acute respiratory failure	9	4 (25%)	15	6 (30%)
Atelectasis	11	6 (38%)	11	6 (30%)
Increased upper airway secretion	7	5 (31%)	7	5 (25%)
Hypoxia	8	4 (25%)	8	4 (20%)
Joint contracture	19	7 (44%)	38	10 (50%)
Scoliosis	8	6 (38%)	11	8 (40%)
Rash	8	5 (31%)	12	6 (30%)

Treatment-emergent adverse events listed are those that occurred with a frequency of >20% in participants treated with 12 mg nusinersen.

Table 2: **Summary of adverse events in cohort 2 (nusinersen 12 mg equivalent) and in the entire safety population (nusinersen 6 mg and 12 mg equivalent)**

Figure 2: **Motor function and event-free survival**
(A) Mean (SE) total HINE-2 score over time for participants in cohort 2 (12 mg equivalent nusinersen) with two *SMN2* copies. Maximum possible score=26. (B) Mean (SE) CHOP INTEND scores over time for participants in cohort 2 (12 mg equivalent nusinersen) with two *SMN2* copies. Maximum possible score=64. (C) The probability of event-free survival (the proportion of infants who were alive without the use of permanent assisted ventilation) in the safety population (N=20). The median time to event was not reached. (D) Mean (SE) CMAP amplitude of the peroneal nerve over time for participants in cohort 2 (12 mg equivalent nusinersen) with two *SMN2* copies. (E) Mean (SE) CMAP amplitude of the ulnar nerve over time for participants in cohort 2 (12 mg equivalent nusinersen) with two *SMN2* copies. CHOP INTEND=Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders. CMAP=compound muscle action potential. HINE-2=Hammersmith Infant Neurological Exam section 2.

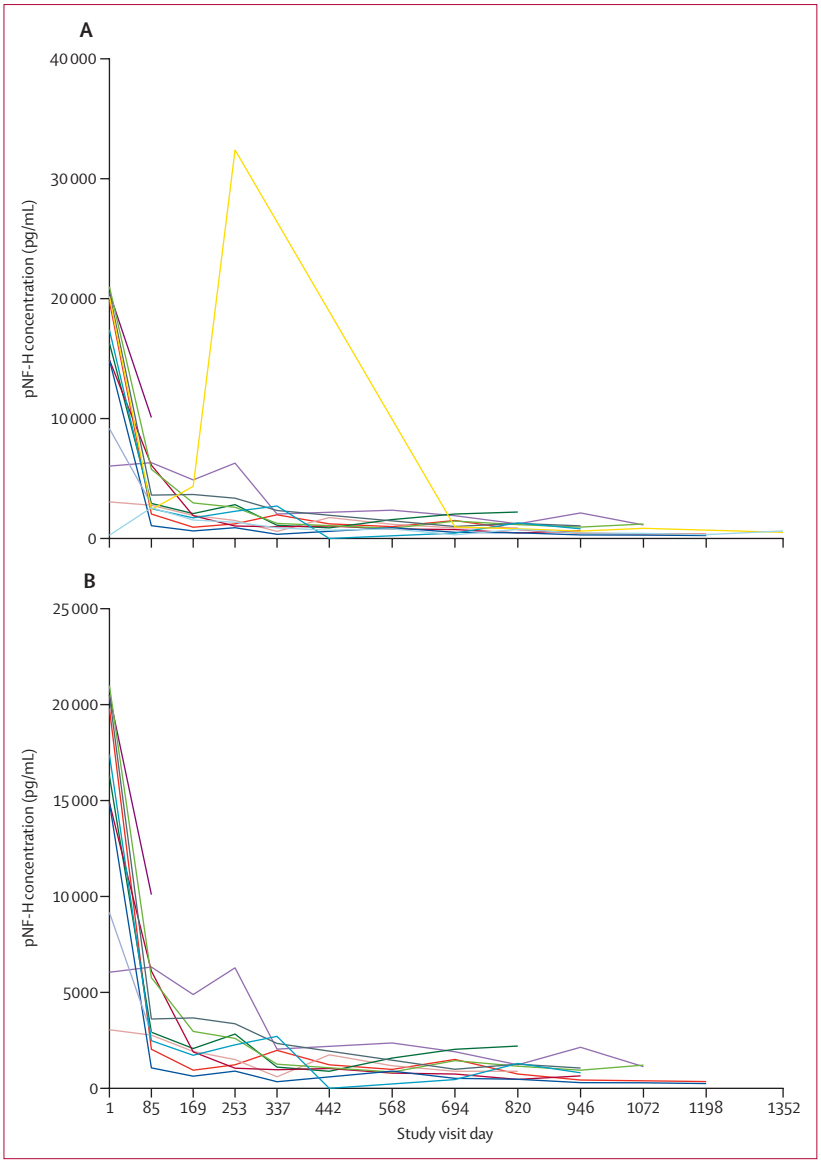


***Figure 3*: Post-hoc analyses of plasma concentrations of pNF-H over time**
(A) Individual pNF-H concentrations in all participants in cohort 2 with two *SMN2* copies. (B) Sensitivity analysis excluding two participants with results that were outliers in this subset. pNF-H=phosphorylated neurofilament heavy chain.

the final study visit and beyond age 2 years. The rapid, marked, and sustained reduction in pNF-H concentrations (post-hoc analyses) accompanying the clinical responses provides further biomarker evidence for the benefit of nusinersen, as increased pNF-H concentrations are associated with neurodegeneration.[15]

These efficacy data deviate from the natural history of infantile-onset spinal muscular atrophy.[4,5,16,17] The participants with two *SMN2* copies receiving nusinersen 12 mg equivalent had a mean HINE-2 motor milestone total score of 11·86 at the last study visit, which compares favourably with a mean of 0·28 in 75 untreated participants in an observational study.[16]

Mean CHOP INTEND total score increased by 17·3 points from baseline to last visit in this study, compared with the decrease observed among 17 untreated patients older than 6 months in a natural history study who were followed for up to 72 months: none of these 17 patients reached a score greater than 45 points.[4] Similarly, in the NeuroNEXT[18] cohort, CHOP INTEND scores decreased and the highest score was 33. Event-free survival in the study was also longer than that reported in the other published research among untreated infants with infantile-onset spinal muscular atrophy and two *SMN2* gene copies.[4,5] Peroneal and ulnar CMAP amplitudes remain low or worsen in untreated infantile-onset spinal muscular atrophy,[4,17] yet both increased among nusinersen-treated infants in the current study.

The subset analysis of cohort 2 with two *SMN2* copies provides an opportunity to compare outcomes with the active treatment arm of ENDEAR,[5] in which infants with two *SMN2* copies received the same recommended dosage regimen of nusinersen 12 mg equivalent.[19,20] Notably, the age at first dose was slightly younger in this study than in ENDEAR.[5] In the current study, as in ENDEAR, nusinersen-treated infants continued to improve on both the HINE-2 and CHOP INTEND along a similar trajectory.[5] This observation highlights the value of continual monitoring using different measures, as CHOP INTEND is sensitive to early response and the HINE-2 reflective of more sustained clinically meaningful benefit in terms of developmental motor milestones gained.

Although encouraging outcomes with nusinersen were observed in most infants with spinal muscular atrophy type I participating in the current trial and in ENDEAR,[5] it is noteworthy that restricted, predefined response criteria to a finite number of clinical tools were applied in the context of the trials. By extension, a minority of infants in both studies did not meet the response criteria using this restricted definition, despite experiencing symptom mitigation or small improvements in clinical outcomes. In clinical practice, it might be important to holistically assess the effect of treatment on infants with spinal muscular atrophy across multiple outcome measures to determine the effect of treatment and avoid terminating beneficial treatment. In this regard, additional research will be important to further characterise pNF-H and other biomarkers as potential pharmacodynamic indicators of treatment response and SMN protein restoration in spinal muscular atrophy.

Cumulative data over the approximate 3 year study period did not reveal any notable changes in the nusinersen safety profile previously reported in the interim analysis and other clinical studies.[3,19,20] Most serious adverse events were consistent with events typically observed in children with spinal muscular atrophy type I. There was no evidence of any clinically relevant changes related to nusinersen with respect to hepatic or renal function or urinalysis, and the

three episodes of mild thrombocytopenia were transient (two of which had occurred in the setting of acute illnesses). Nevertheless, monitoring platelet count, prothrombin time, activated partial thromboplastin time, and testing quantitative spot urine protein is recommended at baseline and before each nusinersen dose, if clinically indicated or as required according to local regulations or standard of care.[19,20]

Post-hoc analyses of pNF-H concentrations in the study population are consistent with those previously reported using data from ENDEAR.[5] Both studies showed higher plasma pNF-H concentrations at baseline than those observed in individuals without spinal muscular atrophy, and a rapid decline with treatment during the loading phase, followed by stabilisation across later timepoints. The median pNF-H concentration in six individuals without spinal muscular atrophy aged younger than 1 year was previously reported as 1510 pg/mL (range 579–7030) using the same assay, and 124·5 pg/mL (below the threshold of quantification to 395 pg/mL) in those aged 1–18 years (n=28).[12] In this study, pNF-H concentrations decreased by around one order of magnitude in participants with two *SMN2* copies, regardless of nusinersen dose, although the loading dose decline was steeper in participants who received 12 mg versus 6 mg nusinersen. This rapid decline and stabilisation might reflect reduction of pNF-H release from SMN-deficient neurons due to increased full-length SMN protein production and attenuation of the degenerative disease process. Further investigation is warranted to determine the potential of pNF-H as a response biomarker for SMN-targeted therapies.

The nusinersen clinical pharmacology and proof of pharmacology shown in this study informed the dosage regimen used in ENDEAR,[5] CHERISH,[6] and NURTURE.[7] Positive mortality and motor function data observed in these studies led to the approval of 12 mg nusinersen administered as four loading doses (the first three administered at 14-day intervals and the fourth administered 30 or 35 days after the third dose) followed by a maintenance dose of 12 mg once every 4 months thereafter.[19,20] Nusinersen is an approved disease-modifying drug available across the spinal muscular atrophy phenotypic spectrum. Data on infantile-onset and presymptomatic spinal muscular atrophy from this and the NURTURE[7] study suggest that efficacy is enhanced when treatment is initiated soon after or before symptom onset. Findings from infantile-onset patients in the phase 1 and 2 studies of onasemnogene abeparvovec and part one of a phase 2/3 study of risdiplam also support the need for early treatment.[21–23]

In conclusion, the final phase 2 trial data show that clinical response to nusinersen in the treatment of infantile-onset spinal muscular atrophy is durable and that repeated intrathecal administration is well tolerated and feasible in this population. There is a need to continue data collection in spinal muscular atrophy clinical trials until end of study to ascertain response to treatment using clinical and biochemical assessments. In this trial, we observed a continued benefit and favourable risk–benefit profile during a median of 36·2 months of treatment and observation, and long-term pharmacodynamic effects on plasma pNF-H concentrations. Infants enrolled in this study had the opportunity to enrol in SHINE, an ongoing open-label extension study, in which long-term efficacy and safety data for nusinersen continue to be collected (NCT02594124).

Contributors

RSF, CAC, JV, JWD, JM, and DCD contributed to the trial design, data collection, interpretation of data, and writing of this study. KMB, RF, YL, DR-S, ES, CFB, JW, and WF contributed to the study design, analysis or interpretation of data, and writing of this study. RF and YL did the statistical analyses. RSF, RF, and WF accessed and verified the data.

Declaration of interests

RSF reports grants and advisor fees from Biogen and Ionis Pharmaceuticals during CS3A, ENDEAR and CHERISH; grants from AveXis, Cytokinetics, Roche, and Scholar Rock; and royalty payments from Children's Hospital of Philadelphia for licensing fees obtained for use of the CHOP INTEND motor function scale. RSF is also advisor to AveXis, Novartis, and Genentech–Roche, on the data safety monitoring board for the AveXis AVXS-101 phase 1 gene transfer study and Roche Moonfish phase 1b study, and is an advisor for non-profit organisations: CureSMA, EveryLife Foundation, n-Lorem Foundation, SMA Europe, SMA Foundation, and SMA Reach. CAC reports grants from AveXis, Biogen, Ionis Pharmaceuticals, Roche, and National Institutes of Health and is on advisory boards of spinal muscular atrophy studies for AveXis, Biogen, Cytokinetics, Genentech, Ionis Pharmaceuticals, and Roche. JV reports grants and advisor fees from Biogen and Ionis Pharmaceuticals during CS3A and ENDEAR, and a grant from CSL Behring. JWD reports grants from AMO Pharma, Audentes, Biogen, Ionis Pharmaceuticals, Novartis Gene Therapies, Pfizer, Roche–Genentech, Sanofi–Genzyme, Sarepta, and Scholar Rock, and is a consultant for Affinia, AMO Pharma, Avidity, Biogen, Ionis Pharmaceuticals, Kate Therapeutics, Novartis Gene Therapies, Pfizer, Roche–Genentech, Sarepta, Scholar Rock, and Shift Pharmaceuticals; and has patents licensed to Athena Diagnostics for genetic testing of myotonic dystrophy type 2 (US patent 7442782) and spinocerebellar ataxia type 5 (US patent 7527931). JM reports research support from Eunice Kennedy Shriver National Institute for Child Health and Human Development (1K01HD084690-01A1) and Muscular Dystrophy Association (575870 and 629259), is on advisory boards for Biogen, Cytokinetics, Roche, Scholar Rock, and SMA Foundation, and is a consultant for Biogen and Ionis Pharmaceuticals. DCD reports clinical trial funding from Biogen, Mallinckrodt, PTC, Sarepta, Scholar Rock, and Ultragenyx and grants from Hope for Children Research Foundation, National Institutes of Health, SMA Foundation, and US Department of Defense. DCD is also an advisor for AveXis, Biogen, Cytokinetics, Ionis Pharmaceuticals, Metafora, Roche, Sanofi, Sarepta, and SMA Foundation. KMB was an employee of Ionis Pharmaceuticals during the design and conduct of this study and is currently an employee of Locana. KMB is an advisor to Myotonic Dystrophy Foundation and SMA Foundation and has issued patents (US patents 9926559 and 8980853) concerning nusinersen. RF, YL, and JW are employees of and hold stock options in Biogen. DR-S and WF are former employees of and held stock options in Biogen. ES is an employee of Ionis Pharmaceuticals. CFB is an employee of Ionis Pharmaceuticals and has issued patents (US patents 9926559 and 8980853) concerning nusinersen.

Data sharing

Results from this study and the study protocol and statistical analysis plan are available on ClinicalTrials.gov (https://clinicaltrials.gov/ct2/show/results/NCT01839656). Requests for additional data supporting this manuscript should be submitted to the Biogen Clinical Data Request Portal (www.biogenclinicaldatarequest.com).

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