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

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Summary

Background Nusinersen is a 2′-O-methoxymethyl phosphorothioate-modified antisense drug being developed to treat spinal muscular atrophy. Nusinersen is specifically designed to alter splicing of SMN2 pre-mRNA and thus increase the amount of functional survival motor neuron (SMN) protein that is deficient in patients with spinal muscular atrophy.

Methods This open-label, phase 2, escalating dose clinical study assessed the safety and tolerability, pharmacokinetics, and clinical efficacy of multiple intrathecal doses of nusinersen (6 mg and 12 mg dose equivalents) in patients with infantile-onset spinal muscular atrophy. Eligible participants were of either gender aged between 3 weeks and 7 months old with onset of spinal muscular atrophy symptoms between 3 weeks and 6 months, who had SMN1 homozygous gene deletion or mutation. Safety assessments included adverse events, physical and neurological examinations, vital signs, clinical laboratory tests, cerebrospinal fluid laboratory tests, and electrocardiographs. Clinical efficacy assessments included event free survival, and change from baseline of two assessments of motor function: the motor milestones portion of the Hammersmith Infant Neuropathological Exam—Part 2 (HINE-2) and the Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) motor function test, and compound motor action potentials. Autopsy tissue was analysed for target engagement, drug concentrations, and pharmacological activity. HINE-2, CHOP-INTEND, and compound motor action potential were compared between baseline and last visit using the Wilcoxon signed-rank test. Age at death or permanent ventilation was compared with natural history using the log-rank test. The study is registered at ClinicalTrials.gov, number NCT01839656.

Findings 20 participants were enrolled between May 3, 2013, and July 9, 2014, and assessed through to an interim analysis done on Jan 26, 2016. All participants experienced adverse events, with 77 serious adverse events reported in 16 participants, all considered by study investigators not related or unlikely related to the study drug. In the 12 mg dose group, incremental achievements of motor milestones (p<0.001), improvements in CHOP-INTEND motor function scores (p=0.0013), and increased compound muscle action potential amplitude of the ulnar nerve (p=0.0103) and peroneal nerve (p<0.001), compared with baseline, were observed. Median age at death or permanent ventilation was not reached and the Kaplan-Meier survival curve diverged from a published natural history case series (p=0.0014). Analysis of autopsy tissue from patients exposed to nusinersen showed drug uptake into motor neurons throughout the spinal cord and neurons and other cell types in the brainstem and other brain regions, exposure at therapeutic concentrations, and increased SMN2 mRNA exon 7 inclusion and SMN protein concentrations in the spinal cord.

Interpretation Administration of multiple intrathecal doses of nusinersen showed acceptable safety and tolerability, pharmacology consistent with its intended mechanism of action, and encouraging clinical efficacy. Results informed the design of an ongoing, sham-controlled, phase 3 clinical study of nusinersen in infantile-onset spinal muscular atrophy.

Funding Ionis Pharmaceuticals, Inc and Biogen.

Introduction

Classic proximal 5q spinal muscular atrophy (OMIM 253300), a progressive motor neuron disorder, is the most common genetic cause of childhood mortality, having an incidence of about one in 11,000 livebirths. About 60% of patients with spinal muscular atrophy are born with the severe form, infantile-onset type I, developing profound limb and trunk weakness before 6 months of age, and failing to rollover or achieve independent sitting. There is a high disease burden with substantial morbidity and mortality from dysphagia, failure to thrive, hyperventilation, poor airway clearance due to weak cough, and lower respiratory tract infections.

Deletions or mutations in the Survival Motor Neuron 1 (SMN1) gene cause spinal muscular atrophy. Absence of the SMN1 gene results in reliance on a nearly identical gene, SMN2, which differs from SMN1 by 11 nucleotides. SMN2 has a c.840C→T substitution at an exon splice enhancer site that regulates exon 7 inclusion, so that only 10–25% of SMN2 transcripts contain exon 7 and generate full-length functional SMN protein. Although the role of SMN protein in motor neurons is incompletely understood and the concentration required for optimum functioning unknown, the phenotype of spinal muscular atrophy (type I, II, III, or IV) is largely related to the number of SMN2 gene copies present.
Antisense oligonucleotides provide a targeted strategy for treatment of spinal muscular atrophy by specifically binding to repressive sites within SMN2 exon 7 or the flanking introns, thus promoting exon 7 inclusion and increased production of functional SMN protein. Nusinersen (also known as ISIS 396443 and ISIS-SMNRx) is a uniformly modified 2'-O-methoxymethyl phosphorothioate antisense oligonucleotide being developed for the treatment of spinal muscular atrophy. We searched PubMed using the keywords “nusinersen”, “ISIS 396443”, “ISIS-SMNRx”, and “ASO 10–27” with no date restrictions. Of the six publications identified, two reported the results of the phase 1 study described below, three described preclinical results using nusinersen, and one was a review article on SMA therapeutics. A phase 1, short-term, single-dose clinical study of intrathecally delivered nusinersen was previously done in children with spinal muscular atrophy.

Methods

Study design and participants
This open-label, escalating dose phase 2 study was designed to assess the safety and tolerability, pharmacokinetics, and clinical efficacy of nusinersen in infants with spinal muscular atrophy. Eligible participants were of either gender between 3 weeks and 7 months old with onset of spinal muscular atrophy symptoms between 3 weeks and 6 months, who had SMN1 homozygous gene deletion or mutation, and who met additional eligibility criteria (appendix). The first four participants received loading doses of 6 mg equivalent nusinersen on days 1, 15, and 85, and then 12 mg equivalent doses on day 253 and every 4 months thereafter (6–12 mg group). The next 16 participants received 12 mg equivalent doses on the same schedule (12 mg group). Follow-up visits occurred on days 16, 29, 86, 92, 169, 254, 337, and 442, and then every 4 months. Participants also were monitored every 2–3 weeks by telephone for safety and ventilation status. SMN2 copy number and SMN2 gene sequencing were done on samples collected at day 85 (Athena Diagnostics, Marlborough, MA, USA).

The study was initiated after institutional review board approvals at the participating centres, in accordance with Good Clinical Practice guidelines. Written informed parental consent was obtained for all participants. An independent data safety monitoring board monitored the study.

Procedures
Nusinersen was diluted to a concentration of 1·2 mg/mL (6 mg dose equivalent) or 2·4 mg/mL (12 mg dose

Research in context

Evidence before this study
Spinal muscular atrophy is caused by deletions or mutations in the Survival Motor Neuron 1 (SMN1) gene and is the most common genetic cause of childhood mortality. Infantile-onset spinal muscular atrophy presents clinically as a severe, progressive motor neuron disease, resulting in generalised weakness and impaired feeding and breathing. Survival is dependent upon a small amount of normal SMN protein translated by the backup SMN2 gene, which, due to a splice site variant, usually excludes exon 7. Less than a quarter of these infants survive beyond 2 years of age without dependence upon ventilation support. There are no approved drug treatments for spinal muscular atrophy. Antisense oligonucleotides provide a targeted strategy for spinal muscular atrophy treatment by specifically binding to repressive sites within SMN2 exon 7 or the flanking introns, thus promoting exon 7 inclusion and increased production of functional SMN protein. Nusinersen was previously done in children with spinal muscular atrophy. The study was initiated after institutional review board approvals at the participating centres, in accordance with Good Clinical Practice guidelines. Written informed parental consent was obtained for all participants. An independent data safety monitoring board monitored the study.

Added value of this study
We report interim results of an ongoing open-label, phase 2, multiple-dose study of intrathecal nusinersen in patients with severe infantile-onset spinal muscular atrophy. We provide evidence that nusinersen has acceptable safety and tolerability when delivered by multiple intrathecal injections and shows promising clinical efficacy as evidenced by improvements in motor function, achievement of motor milestones, and permanent ventilation-free survival as compared with published natural history. Additionally, autopsies collected during the study indicate proof of target engagement and mechanism, as nusinersen altered SMN2 splicing, with an increase in full-length transcript that includes exon 7, and an increase in SMN protein in spinal cord motor neurons as compared with untreated infants with spinal muscular atrophy as control.

Implications of all the available evidence
Our study shows favourable safety and tolerability, pharmacokinetics, proof-of-concept pharmacodynamics, and a promising clinical response of intrathecal nusinersen. Results informed the design of an ongoing large phase 3, randomised, sham-controlled study of nusinersen in infantile-onset spinal muscular atrophy. More broadly, the mechanistic effects of nusinersen at the mRNA and protein level in participants of this study provides proof of principle for the use of antisense therapeutics in the treatment of neurological disorders. Finally, results from this study suggest treatments that increased SMN protein might provide clinical benefit to patients with spinal muscular atrophy.
equivalent) with artificial cerebrospinal fluid (CSF). The dose delivered was adjusted based on age such that each participant received a scaled dose equivalent (4–5 mL) based on their projected CSF volume. The dose regime used was designed, based on the spinal cord tissue half-life in preclinical and clinical studies, to provide a loading period of dosing over the first 3 months to achieve a target spinal cord drug concentration, and then, after 6 months, chronic dosing once every 4 months to sustain the tissue concentration. Intrathecal dosing was done via lumbar puncture with topical anaesthesia using standard techniques for infants. Sedation was not used in infants younger than 2 years, but some older children required sedation due to movements during the procedure. Injections were delivered over 1–3 min using a 22-gauge spinal anaesthesia needle. Before each injection, about 5 mL of CSF was collected for analyses.

Outcomes

Safety assessments included adverse events, physical and neurological examinations, vital signs, clinical laboratory tests (serum chemistry, haematology, and urinalysis), CSF laboratory tests (cell counts, protein, and glucose), and electrocardiograms. Clinical efficacy assessments included change from baselines of two assessments of motor function: the motor milestones portion of the Hammersmith Infant Neuropathological Exam—Part 2 (HINE-2), a general categorical measure of infant developmental motor milestones done by a paediatric neurologist, and the Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) motor function test, a validated 16 item scale (0–64 points) designed specifically to capture motor function in infants with spinal muscular atrophy. Physical therapist evaluators were trained annually in the CHOP-INTEND using standardised procedure manual and videotaped assessments to establish and maintain strong inter-rater reliability. Compound muscle action potentials (CMAPs) were recorded for stimulation of the ulnar nerve (recording from tibialis anterior), and change from baseline values were analysed. Survival, tracheostomy placement, and daily ventilation use were collected to assess the endpoint of age at death or permanent ventilation (defined as tracheostomy or ≥16 h ventilation per day continuously for at least 2 weeks in the absence of ventilation). Exclusions, however, were done via lumbar puncture with topical anaesthesia using standard techniques for infants. Sedation was not used in infants younger than 2 years, but some older children required sedation due to movements during the procedure. Injections were delivered over 1–3 min using a 22-gauge spinal anaesthesia needle. Before each injection, about 5 mL of CSF was collected for analyses.

Table 1: Demographics and baseline clinical characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>6–12 mg group (n=4)</th>
<th>12 mg group (n=16)</th>
<th>Total (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (75%)</td>
<td>9 (56%)</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (25%)</td>
<td>7 (44%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>3 (75%)</td>
<td>13 (81%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>Black</td>
<td>0</td>
<td>1 (6%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>1 (6%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Multiple Race</td>
<td>1 (25%)</td>
<td>0</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>1 (6%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>7 (5.2–8.9)</td>
<td>6.7 (5.1–9.3)</td>
<td>6.8 (5.1–9.3)</td>
</tr>
<tr>
<td><strong>SMN2 copy number</strong></td>
<td>2/3 (unknown)</td>
<td>4/0/0</td>
<td>13/2/1</td>
</tr>
<tr>
<td><strong>Age at symptom onset (days)</strong></td>
<td>47 (28–70)</td>
<td>63 (21–154)</td>
<td>60 (21–154)</td>
</tr>
<tr>
<td><strong>Age at diagnosis (days)</strong></td>
<td>74 (42–105)</td>
<td>80 (0–154)</td>
<td>78 (0–154)</td>
</tr>
<tr>
<td><strong>Symptom onset to enrolment (days)</strong></td>
<td>97 (39–151)</td>
<td>77 (35–130)</td>
<td>81 (35–151)</td>
</tr>
<tr>
<td><strong>Age at enrolment (days)</strong></td>
<td>145 (67–207)</td>
<td>140 (36–210)</td>
<td>141 (36–210)</td>
</tr>
<tr>
<td><strong>CHOP-INTEND score</strong></td>
<td>27 (22–34)</td>
<td>30 (17–64)</td>
<td>30 (17–64)</td>
</tr>
<tr>
<td><strong>HINE-2 score</strong></td>
<td>2 (1–3)</td>
<td>2 (1–12)</td>
<td>2 (1–12)</td>
</tr>
<tr>
<td><strong>On Bi-PAP at entry</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Gastrostomy tube at entry</strong></td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Ulnar CMAP amplitude (mV)</strong></td>
<td>0.37 (0.20–0.60)</td>
<td>0.53 (0.3–2.0)</td>
<td>0.50 (0.3–2.0)</td>
</tr>
<tr>
<td><strong>Peroneal CMAP amplitude (mV)</strong></td>
<td>0.67 (0.30–1.40)</td>
<td>0.52 (0.2–2.70)</td>
<td>0.55 (0.2–2.70)</td>
</tr>
</tbody>
</table>

Data are n (%), mean (range), or n, unless otherwise stated. Bi-PAP=bi-level positive airway pressure. CHOP-INTEND=Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders. CMAP=compound muscle action potential. HINE-2=Hammersmith Infant Neurological Exam—Part 2. SMN2=Survival Motor Neuron 2.

Statistical analysis

The sample size was selected to examine the safety and tolerability of nusinersen. All participants were included in the safety and pharmacokinetic analyses. The per-protocol efficacy analysis included all participants who completed the loading doses and day 92 assessment. An additional participant with three SMN2 gene copies was excluded from the CHOP-INTEND analysis due to a ceiling effect, having a baseline CHOP-INTEND score of 64, the maximum achievable. Excluded participants were in the 12 mg dose group (efficacy evaluable, n=14).

HINE-2, CHOP-INTEND, and CMAP were compared between baseline and last visit following treatment using Wilcoxon signed-rank test. Age at death or permanent ventilation was estimated using a Kaplan-Meier survival curve and compared with natural history using the log-rank test. SMN protein staining intensity in neurons of treated infants with spinal muscular
Results

20 infants with spinal muscular atrophy were enrolled between May 3, 2013, and July 9, 2014. Demographic and baseline characteristics are summarised in table 1. SMN2 copy number and gene sequencing was done on 19 infants: 17 of 19 participants had two copies of the SMN2 gene and two patients three copies (both in the 12 mg dose group). No sequence variants in the SMN2 gene were reported. One infant died before the day 85 assessment when samples for SMN2 gene copy analysis were collected. Two screen failures occurred (one, a hypoxic-bradycardic event requiring intubation during screening; one cardiac abnormality) and one additional participant terminated from the study before dosing due to respiratory failure, secondary to an acute infection. We report interim results until Jan 26, 2016, which was about 18 months since the last participant was enrolled. At this time, the 6–12 mg group had been followed for 9–32 months and received four to nine doses of nusinersen, whereas the 12 mg group had been followed for 2–27 months and received two to eight doses.

Standard intrathecal injections of nusinersen resulted in no safety concerns. All participants experienced adverse events (570 events in total) during the study (table 2), with most being mild (359 events [63%]) or moderate (153 events [27%]) in severity. There were 77 serious adverse events reported in 16 participants, all considered by study investigators not related or unlikely related to the study drug, with the most common being respiratory distress or failure or respiratory infections, which are commonplace in infants with spinal muscular atrophy. No changes in neurological examination findings, laboratory assessments, vital signs, electrocardiogram parameters, or CSF safety parameters that were considered clinically significant and related to nusinersen were reported, with the exception of one mild event of transient, asymptomatic neutropenia and one mild event of vomiting, which were both considered possibly related to nusinersen by the investigators.

Incremental improvements in developmental motor milestones on the HINE-2 were observed for 16 of 19 participants (one of four participants in the 6–12 mg group; 15 of 15 participants in the 12 mg group) at the last visit compared with baseline (figure 1A, appendix). Change in HINE-2 score from baseline to last visit was significant for both cohorts combined (p=0·0002) and for participants in the 12 mg dose group (p=0·0001). Improvements of two or more levels per motor milestone category on at least one category were observed in 13 participants and were most often observed for grasping (13 participants), ability to kick (nine participants), and sitting (eight participants), but were also evident for head control (six participants), rolling (six participants), standing (five participants), crawling (two participants), and walking (two participants; figure 1B, appendix).

Motor function, assessed using the CHOP-INTEND, showed a mean increase of 11·5 points from baseline to 30

Table 2: Adverse event summary

<table>
<thead>
<tr>
<th>Nusinersen dosing</th>
<th>Days from last dose to autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mg on days 1, 15, 85; 12 mg on day 253</td>
<td>12</td>
</tr>
<tr>
<td>12 mg on days 1, 15, 85</td>
<td>36</td>
</tr>
<tr>
<td>12 mg on days 1, 15, 85</td>
<td>78</td>
</tr>
</tbody>
</table>

Table 3: Demographics and drug exposure for the three autopsy participants treated with nusinersen

<table>
<thead>
<tr>
<th>Age at death (days)</th>
<th>Gender</th>
<th>SMN2 copy number</th>
<th>Nusinersen dosing</th>
<th>Days from last dose to autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 385</td>
<td>Male</td>
<td>2</td>
<td>6 mg on days 1, 15, 85; 12 mg on day 253</td>
<td>12</td>
</tr>
<tr>
<td>2 157</td>
<td>Male</td>
<td>2</td>
<td>12 mg on days 1, 15, 85</td>
<td>36</td>
</tr>
<tr>
<td>3 342</td>
<td>Female</td>
<td>2</td>
<td>12 mg on days 1, 15, 85</td>
<td>78</td>
</tr>
</tbody>
</table>

Role of the funding source

The study was funded by Ionis Pharmaceuticals, Inc and Biogen. Employees of Ionis Pharmaceuticals, Inc (MY, FR, GH, ES, DAN, SX, CFB, and KMB) contributed to the study design, analysis and interpretation of data, and writing of the report. The corresponding author (RSF) had full access to all the data from the study and had final responsibility for the content of the report and decision to submit for publication.

Articles

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Figure 3: Percentage of participants with improvements of two or more levels per motor milestone on the HINE-2 from baseline to last visit compared with baseline, or at last visit. Participants are grouped by nusinersen dose and stratified by gender. Significant p=0·002 for both cohorts combined (p<0·0001 for the 12 mg group).
Figure 1: Clinical effects in infants with spinal muscular atrophy

(A) Change from baseline in the motor milestones as assessed by the Hammersmith Infant Neurological Exam – Part 2 (HINE-2) for participants in the 12 mg dose group. Red line=mean score. Dashed blue line=participants who reached the endpoint of death or permanent ventilation. Green line=participants with three copies of the SMN2 gene.

(B) Changes in individual milestone categories as assessed by the HINE-2 for the 12 mg dose group.

(C) Changes in Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) infant motor function test for individual participants in the 12 mg dose group. Solid black line=Pediatric Neuromuscular Clinical Research (PNCR) natural history comparison for infantile spinal muscular atrophy. Red line=mean score. Dashed blue lines=participants that reached endpoint of death or permanent ventilation. Green line=participants with three copies of SMN2.

(D) Kaplan-Meier curves for participants with infantile-onset spinal muscular atrophy and two SMN2 gene copies: nusinersen-treated versus untreated infants with spinal muscular atrophy from the PNCR natural history study (log-rank test, p=0.0014). (E and F) Change from baseline in peroneal (E) and ulnar (F) nerve compound muscle action potential (CMAP) negative peak amplitude for individual participants in the 12 mg dose group. Red line=mean score. Dashed blue lines=participants that reached endpoint of death or permanent ventilation. Green line=participants with three copies of SMN2.
Figure 2: Drug pharmacokinetics in infant plasma, cerebrospinal fluid, and spinal cord
(A) Plasma concentrations of nusinersen in infants with spinal muscular atrophy from day 1 for the first 24 h after the first 6 mg or 12 mg equivalent intrathecal dose of nusinersen, measured with an electrochemiluminescence assay. (B) Concentrations of nusinersen determined in different autopsy tissues taken from the three infants with spinal muscular atrophy who died during the course of the study (table S). The tissues were analysed for nusinersen concentration using a hybridisation enzyme-linked immunosorbent assay method. (C) Distribution of nusinersen in spinal cord and brain tissues obtained from infant 2 treated with nusinersen. Tissue sections were stained with an antibody raised to phosphorothioate oligonucleotides and visualised using a peroxidase-conjugated anti-rabbit antibody as previously described. Slides were counterstained with haematoxylin (blue). The brown staining in large neurons (red arrows) and, to a lesser extent, in smaller neurons and non-neuronal cells in the area, indicates the presence of nusinersen within these cells. ASO=antisense oligonucleotide.

last visit overall (p=0.0080; n=18), with 14 of 18 infants having an improvement (figure 1C, appendix). In the 12 mg group, 12 of 14 participants had an increase from baseline to last visit (mean increase 15.2 points; p=0.0013), compared with a natural history case series of infants with type I spinal muscular atrophy, in which a
Figure 3: Drug pharmacodynamics in infant spinal cord samples

(A) Amount of full-length and truncated (missing exon 7, Δ7) SMN2 transcripts in thoracic spinal cord tissues determined by radioactive reverse transcription polymerase chain reaction (RT-PCR) assay. Tissues from three infants without spinal muscular atrophy (non-SMA) and four infants with spinal muscular atrophy (SMA) also were analysed to determine the percent of full-length SMN2 transcripts in untreated infants. The numerical values under each lane represent the percentage of full-length SMN2 transcript in the sample.

(B) SMN2 splicing analysis of spinal cord and brain regions by radioactive RT-PCR. RNA was isolated from brain and spinal cord tissue and analysed for full-length SMN2 transcript by radioactive RT-PCR as described in the Methods.

(C) Thoracic spinal cord tissue from two participants with infantile-onset spinal muscular atrophy and three nusinersen-exposed participants with spinal muscular atrophy stained for presence of SMN protein and detected using immunohistochemistry as described in the Methods. The staining was done in batch for these samples to allow comparison. SMN protein (brown) was readily observed in large neurons, which have location, morphology, and size consistent with motor neurons. IgG=immunoglobulin G.
mean decline of 1.27 points per year (95% CI 0.21–2.33) was observed (figure 1C). A score greater than 40, a value rarely observed for symptomatic infants with type I spinal muscular atrophy with two SMN2 gene copies, was observed in none of the 13 infants with two SMN2 gene copies at baseline and increased to seven of 13 participants at last visit in the 12 mg group.

As of the date of analysis, a median age of death or permanent ventilation had not been reached as most participants were surviving without permanent ventilation. A conservative analysis in which the median age at the endpoint or the data cutoff date (censoring) was calculated resulted in a censored median of 24.7 months in the 6–12 mg group and 25.2 months in the 12 mg group (which started enrolment about 5 months later). Of the seven participants who met the endpoint, one participant in the 6–12 mg group progressed to more than 16 h per day on bi-level positive airway pressure (Bi-PAP) at 8.7 months of age and one participant died from accidental asphyxia at 12.6 months. In the 12 mg group, one participant died from spinal muscular atrophy disease progression at 6.9 months, two participants died from progression of disease secondary to a recent pulmonary infection at 5.1 months and 11.2 months, and two participants had a tracheostomy at 6.3 months and 17.4 months. A log-rank test was done comparing infants with spinal muscular atrophy with two copies of SMN2 from this study (n=17) to infants with spinal muscular atrophy with two copies of SMN2 from the Pediatric Neuromuscular Clinical Research (PNCR) natural history case series (n=23; appendix).1 Given all the caveats associated with comparison to a natural history case series, the log-rank test indicates a differentiation in age at death or permanent ventilation (p=0.0034; figure 1D).

Electrophysiological assessment using CMAP showed that in the 12 mg group, all participants exhibited an increase in peroneal CMAP and 12 of 15 patients in ulnar CMAP at last visit compared with baseline (figure 1E, F). Statistically significant increases in CMAP amplitude were observed for both nerves, with a mean increase of 74.2% (p<0.0001) or 1.56 mV in the peroneal CMAP amplitude and a mean increase of 37.7% (p=0.0103) or 0.62 mV in the ulnar CMAP amplitude.

CSF and plasma pharmacokinetics indicated that nusinersen cleared from the CSF into systemic circulation, consistent with normal CSF turnover, with dose dependent mean peak plasma concentrations observed about 1 h after dosing and declining over 24 h (figure 2A). CSF drug concentrations were still quantifiable 15–168 days after dosing, indicating prolonged exposure of the CSF and CNS tissues to nusinersen (appendix). Pharmacokinetic and pharmacodynamic analyses were also done on spinal cord and brain tissues collected for three infants who died during the study (table 3, figure 2B, C). Drug concentrations were greater than 10 µg/g (figure 2B), a concentration predicted to produce pharmacological effects, in all areas of the spinal cord. Immunohistochemical staining for nusinersen confirmed localisation in neurons, vascular endothelial cells, and glial cells throughout the CNS, with neurons staining more intensely than other cell types (figure 2C, appendix). Nusinersen was also identified in peripheral tissues such as liver and kidney, consistent with clearance from the CSF into the systemic circulation (appendix).

Analysis of thoracic spinal cord tissues from untreated infants with spinal muscular atrophy or infants with no disease showed that 15–26% of the SMN2 transcripts contained exon 7 (figure 3A, B). By contrast, 50–69% of the SMN2 transcripts contained exon 7 in thoracic cord tissues from infants with spinal muscular atrophy exposed to nusinersen (figure 3A, B). This corresponded to a 2-6 times increase in full-length SMN2 transcripts compared with untreated infants with spinal muscular atrophy (appendix). Similar levels were also observed in multiple brain regions of infants with spinal muscular atrophy exposed to nusinersen (figure 3B, appendix). Infants exposed to nusinersen had an apparent increase in SMN protein staining in thoracic cord motor neurons compared with untreated infants with spinal muscular atrophy (figure 3C). Image analysis of thoracic spinal cord anterior horn indicated a 63.7% increase in SMN protein staining intensity in neurons of treated infants with spinal muscular atrophy compared with untreated infants with spinal muscular atrophy (mean optical density nusinersen-treated 0.1981 [SD 0.049], n=188 neurons analysed; untreated 0.1210 [0.023], n=122 neurons analysed; p<0.0001; appendix).

Discussion
In this study, multiple intrathecal doses of the antisense drug nusinersen were well tolerated in infants with spinal muscular atrophy, with no safety concerns identified for up to nine doses given over 32 months of treatment. Adverse events were generally consistent with those observed in fragile infants with type I spinal muscular atrophy. Similarly, nusinersen was well tolerated in older children aged 2–15 years with type II or type III spinal muscular atrophy. Additionally, the intrathecal injections were successful and well tolerated in infants with spinal muscular atrophy, similar to the experience with intrathecal injections in children with spinal muscular atrophy. This study is the first to give an antisense drug intrathecally to infants and thus adds to the growing body of clinical safety and tolerability data with this novel treatment modality.

Examination of postmortem tissue indicated that intrathecal nusinersen is distributed broadly throughout the spinal cord and brain, including target motor neurons, and that drug concentrations are above those predicted to produce the intended pharmacology. Pharmacology consistent with the intended mechanism of action of nusinersen was observed in infants with spinal muscular atrophy treated with nusinersen, via an increase in the amount of full-length SMN2 mRNA
transcript and a qualitative increase in SMN protein compared with untreated infants with spinal muscular atrophy. These data provide confirmation that the drug reaches target tissues and generates the desired molecular response of promoting inclusion of exon 7 in the transcript and are the first direct evidence for antisense pharmacology in the human CNS.

A promising clinical response to nusinersen in most, but not all, infants with spinal muscular atrophy was observed in all three categories examined: achievement of motor milestones and motor function, survival or permanent ventilation independence, and neuromuscular electrophysiology. Participants in the 12 mg dose group showed incremental achievement of motor milestones and improved motor function scores, as compared with an expected decline based on natural history, suggestive of a favourable drug effect. Some of these infants developed the ability to sit independently and roll over independently and improved in head control, kicking, hand function, and standing and walking, changes that are beyond the motor repertoire expected for infants with type I spinal muscular atrophy according to natural history reports. Although the improvements observed have not been reported to occur in type I spinal muscular atrophy infants, it should be noted that treatment with nusinersen has not restored normal age-appropriate function. Additionally, the peroneal and ulnar nerve CMAP amplitude increased over time, indicating an increase in electrically excitable muscle. These observations are unlike the natural history data, in which the ulnar CMAP is of uniformly low amplitude in symptomatic infants at the time of diagnosis and does not change over time. The improved functional performance of infants treated with nusinersen and the increases in CMAP might represent improved function of remaining motor neurons with neuronal sprouting and re-innervation, or improved neuromuscular transmission at the neuromuscular junction.

Survival, including the surrogate of avoiding the need for permanent ventilation, was divergent as compared with natural history cohorts. It is important in this context to also consider the standard of care because it has been shown that this will have an effect in infants with spinal muscular atrophy. In this phase 2 study, the standard of care guidelines from 2007 were used as a benchmark during the course of the study, but parents were allowed to pursue a palliative care approach. This same approach of allowing both palliative and proactive care (gastrostomy tube and noninvasive ventilation support) was used in the PNCR (US) and German natural history case series. In the PNCR case series, recently diagnosed patients with two SMN2 gene copies had a median age at reaching a similar endpoint of 10–5 months, whereas it was 6–5 months in the German study. In the data reported here overall and for participants with two SMN2 gene copies, a median endpoint has not yet been reached but the Kaplan-Meier curves diverge from the natural history, suggesting a drug effect. However, it is important that these results be interpreted cautiously, as this was a relatively small open-label study.

Overall, this phase 2 open-label study of intrathecal nusinersen in symptomatic infantile-onset spinal muscular atrophy has shown favourable safety and tolerability, pharmacokinetics, proof-of-concept pharmacodynamics, and an encouraging clinical response. Limitations of this study include the small number of participants, the relatively short duration of follow-up (2–32 months), and the open-label design. Additionally, comparison with natural history case series should be interpreted cautiously. Thus, a large phase 3, randomised, double-blind, sham-controlled study of nusinersen in infantile-onset spinal muscular atrophy is ongoing (NCT02193074). As no safety concerns were identified in this study, the phase 3 study has incorporated a more frequent dosing regimen, especially in the first few months of treatment, when infants with spinal muscular atrophy progress rapidly and are particularly vulnerable. Pre-symptomatic treatment might provide an even greater clinical response; this hypothesis is being examined in an ongoing phase 2 study (NCT02386553). As patients participating in the study still have active disease, combinations of intrathecal nusinersen with other drugs, including drugs that target peripheral tissues, warrant additional investigations. More broadly, the novel evidence for nusinersen mechanistic effects at the mRNA and protein level in participants of this study provides a proof of principle for the use of antisense therapeutics in the treatment of neurological disorders.

Contributors RSF, CAC, JV, JWD, JM, DCDV, DAN, and KMB designed the trial. RSF, CAC, JV, JWD, JM, and DCDV did the trial. MY and ES monitored safety. FR, GH, and DAN did the pharmacokinetic and pharmacodynamic analyses. SX and KMB did the analysis of the clinical and safety data. RSF, CFB, and KMB interpreted the data and wrote the manuscript. All authors critically revised and approved the final manuscript.

Declaration of interests RSF reports grants and personal fees from Ionis Pharmaceuticals during the conduct of the study; and grants and advisor fees from Biogen and Roche, outside the submitted work. RSF serves in an advisory capacity to the non-profit organisations, the SMA Foundation, Cure SMA, SMA Reach (UK), and SMA Europe, and also serves on the data safety monitoring board for the AveXis gene transfer study. CAC reports serving as a consultant for Roche Pharmaceuticals, AveXis, and Novartis, and receives grants from the SMA Foundation and the US Department of Defense. JWD reports serving as a consultant for Biogen, grants from Biogen and Ionis Pharmaceuticals, during the conduct of the study; grants from Ionis Pharmaceuticals, Cytokinetics, SMA Foundation, Roche, and Cytekinetics, outside the submitted work; serves as a consultant to AveXis, Cytokinetics, and Ionis Pharmaceuticals; and serves on the advisory board for SMA Foundation. JM reports serving as a consultant for Ionis Pharmaceuticals, and serving on advisory boards for Roche Pharmaceuticals and Biogen. DCDV reports serving as a consultant for, and receiving grants from, Ionis Pharmaceuticals, Biogen, Roche, Ultradynex, and Sarepta; serves as an advisor to the SMA Foundation, Hope for Children Research Foundation, AveXis, and Glut1 Deficiency Foundation; receives grants from Milestones for Children, Will Foundation, National Institutes of Health, US Department of Defense, and Glut1 Deficiency Foundation; and serves on the data safety monitoring board for Cytokinetics.
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Dawn of a new therapeutic era for spinal muscular atrophy

The original studies reporting defects in the survival of motor neuron 1 (SMN1) gene as the underlying cause of spinal muscular atrophy were published more than 20 years ago.1 At the time, the identification of spinal muscular atrophy as an essentially mono-genetic disorder was heralded as a major research breakthrough that would lead to the rapid development of new therapies. Yet, despite over two decades of intensive basic and pre-clinical research, no approved treatment options are currently available. Against this background, the open-label, phase 2 clinical study by Richard S Finkel and colleagues in The Lancet is a major milestone on the journey towards a viable therapy.

Mutations in the SMN1 gene render it incapable of generating full-length survival motor neuron (SMN) protein.2 The continuing presence of low concentrations of SMN protein in patients with spinal muscular atrophy results from the expression of a near-identical SMN2 gene, the copy number of which affects disease severity.3 This backup SMN2 gene has a C to T substitution at an exon splice enhancer site that regulates exon 7 inclusion. As a result, less than 25% of SMN2 transcripts contain exon 7 and are capable of producing full-length SMN protein. However, the retained presence of an SMN2 gene in patients offers an opportunity for the development of therapies aimed at increasing concentrations of functional SMN protein, a strategy found to have potential in pre-clinical animal studies.3,6

Finkel and colleagues7 report on the delivery of nusinersen, a 2′-O-methoxyethyl phosphorothioate-modified antisense drug designed to alter splicing of SMN2 pre-mRNA and subsequently increase concentrations of SMN protein. Although data from a phase 1 trial of nusinersen in patients with less severe forms of spinal muscular atrophy have already been published,7 this study is the first robust demonstration of safety and tolerability, as well as a positive pharmacokinetic profile, for nusinersen after multiple intrathecal doses in infants with the most severe form of spinal muscular atrophy (type I). Most importantly, the study confirmed uptake of nusinersen into motor neurons throughout the spinal cord, as well as other neuronal populations throughout the nervous system, leading to increased SMN2 mRNA exon 7 inclusion and SMN protein concentrations. This establishes a crucial proof-of-principle that it is possible to target SMN2 to raise SMN protein concentrations across a range of affected cell types in patients with spinal muscular atrophy, without major adverse consequences.

Finkel and colleagues’ also present preliminary evidence suggesting that nusinersen can deliver incremental improvements in motor function for patients with severe forms of spinal muscular atrophy. Although these findings need to be interpreted cautiously in the context of the limitations of a small, open-label, interventional trial, they should generate substantial encouragement that raising SMN protein concentrations could be of therapeutic benefit to patients with spinal muscular atrophy. Conclusions cannot yet be drawn concerning the potential ability of nusinersen to affect broader aspects of the spinal muscular atrophy phenotype, such as a requirement for permanent ventilation or age of death. This is largely due to the relatively small number of patients enrolled in the study and the need to draw quantitative comparisons with an unrelated, natural history case series. Indeed, it should be noted that promising clinical responses were not uniformly observed across all infants enrolled on the study. However, the overall direction is encouraging, with future studies (including phase 3 trials) likely to generate the additional clinical data required to gain a full appreciation of the potential therapeutic benefits of nusinersen treatment.

As predicted from pre-clinical animal studies, the current study indicates that restoration of SMN protein can modify disease severity, but does not represent a complete cure. Thus, ongoing efforts to develop a second generation of therapies for spinal muscular atrophy are likely to be key for developing fully effective treatments applicable to patients with all subtypes of the condition. These include efforts to restore SMN protein earlier in disease progression (during a crucial therapeutic or developmental time-window),8 to facilitate systemic delivery of therapies throughout a range of additional peripheral tissues and organs,9,10 and to target additional SMN-independent pathways.11,12 However, the promise of nusinersen shown by Finkel and colleagues is a first step forward, indeed a new dawn, in developing safe and effective therapy options for spinal muscular atrophy that are urgently required.
Comment

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I am Chair of the Scientific Advisory Board of the SMA Trust and serve on scientific and clinical advisory boards for SMA Europe and Association Française contre les Myopathies. I am named on a patent application submitted by the University of Edinburgh for the use of β-catenin inhibitors for the treatment of spinal muscular atrophy.