Results from a phase 1 study of nusinersen (ISIS-SMN~Rx~) in children with spinal muscular atrophy

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ABSTRACT

Objective: To examine safety, tolerability, pharmacokinetics, and preliminary clinical efficacy of intrathecal nusinersen (previously ISIS-SMN~Rx~), an antisense oligonucleotide designed to alter splicing of *SMN2* mRNA, in patients with childhood spinal muscular atrophy (SMA).

Methods: Nusinersen was delivered by intrathecal injection to medically stable patients with type 2 and type 3 SMA aged 2–14 years in an open-label phase 1 study and its long-term extension. Four ascending single-dose levels (1, 3, 6, and 9 mg) were examined in cohorts of 6–10 participants. Participants were monitored for safety and tolerability, and CSF and plasma pharmacokinetics were measured. Exploratory efficacy endpoints included the Hammersmith Functional Motor Scale Expanded (HFMSE) and Pediatric Quality of Life Inventory.

Results: A total of 28 participants enrolled in the study (n = 6 in first 3 dose cohorts; n = 10 in the 9-mg cohort). Intrathecal nusinersen was well-tolerated with no safety/tolerability concerns identified. Plasma and CSF drug levels were dose-dependent, consistent with preclinical data. Extended pharmacokinetics indicated a prolonged CSF drug half-life of 4–6 months after initial clearance. A significant increase in HFMSE scores was observed at the 9-mg dose at 3 months postdose (3.1 points; $p = 0.016$), which was further increased 9–14 months postdose (5.8 points; $p = 0.008$) during the extension study.

Conclusions: Results from this study support continued development of nusinersen for treatment of SMA.

Classification of evidence: This study provides Class IV evidence that in children with SMA, intrathecal nusinersen is not associated with safety or tolerability concerns. *Neurology*® 2016;86:890–897

GLOSSARY

AE = adverse event; **AUC** = area under the concentration time curve; **DSMB** = data and safety monitoring board; **HFMSE** = Hammersmith Functional Motor Scale Expanded; **LP** = lumbar puncture; **PedsQL** = Pediatric Quality of Life Inventory; **SMA** = spinal muscular atrophy; **SMN** = survival of motor neuron.

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized primarily by motor neuron degeneration, resulting in muscular atrophy and weakness involving limbs, and more variably, bulbar and respiratory muscles.[1,2] The natural history of SMA includes several major phenotypes that are characterized by age at onset and achieved motor abilities.[3] SMA is caused by deletions or loss-of-function mutations in the survival of motor neuron (*SMN1*) gene located on chromosome 5q13.[4] Humans also have a paralogous *SMN2* gene that has an identical coding sequence to *SMN1* but differs by 11 nucleotides.[5] One of the nucleotide changes is a C to T transition within exon 7, resulting in 80%–90% of the transcripts, excluding exon 7, producing a truncated protein that is rapidly degraded.[5] As a result, the limited amount of full-length protein produced is insufficient to compensate for loss of the *SMN1* gene, causing the SMA phenotype.[5]

Nusinersen (previously ISIS-SMN$_{Rx}$ and also known as ISIS 396443) is an antisense oligonucleotide designed to bind to the *SMN2* pre-mRNA and promote inclusion of exon 7 (figure 1).[6,7] In mouse models of SMA, nusinersen enhanced exon 7 inclusion, increased SMN protein production, and improved function.[6–9] Nusinersen is delivered by intrathecal injection as antisense oligonucleotide drugs do not cross an intact blood–brain barrier when delivered systemically.[10] This first-in-human, open-label, single-ascending dose study was designed to assess safety, tolerability, pharmacokinetics, and clinical effects of intrathecal nusinersen in patients with childhood SMA.

METHODS **Standard protocol approvals, registrations, and participant consents.** Written informed parental consent and assent was obtained for all participants as required. Studies were initiated after institutional review board approvals of the participating study centers and carried out in accordance with Good Clinical Practice guidelines. A data and safety monitoring board (DSMB) monitored the studies. Studies were registered with ClinicalTrials.gov (NCT01494701; NCT01780246).

Study design. In this open-label, escalating-dose, phase 1 study, participants received a single intrathecal injection of 5 mL nusinersen following standard lumbar puncture (LP) techniques (over 1–3-minutes). Four dose levels (1, 3, 6, 9 mg) were evaluated in sequential groups: dose escalation was dependent on DSMB review of safety data through study day 8 for the preceding dose group. Participants were eligible to re-enroll in an open-label extension study at 9–14 months after their initial nusinersen dose to receive additional study drug; data included in this report include baseline evaluations for the follow-up study only.

Participants. Male and female patients aged 2–14 years with symptomatic SMA and documented *SMN1* homozygous gene deletion were eligible to participate. Participants were enrolled at 4 study centers: Columbia University, University of Utah, Boston Children's Hospital, and University of Texas Southwestern Medical School. Inclusion/exclusion criteria were designed to enroll patients who were medically stable and in whom safety concerns could be clearly assessed. Briefly, participants needed to be able to complete all study procedures, meet age-appropriate

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Figure 1 **Mechanism of action of nusinersen**
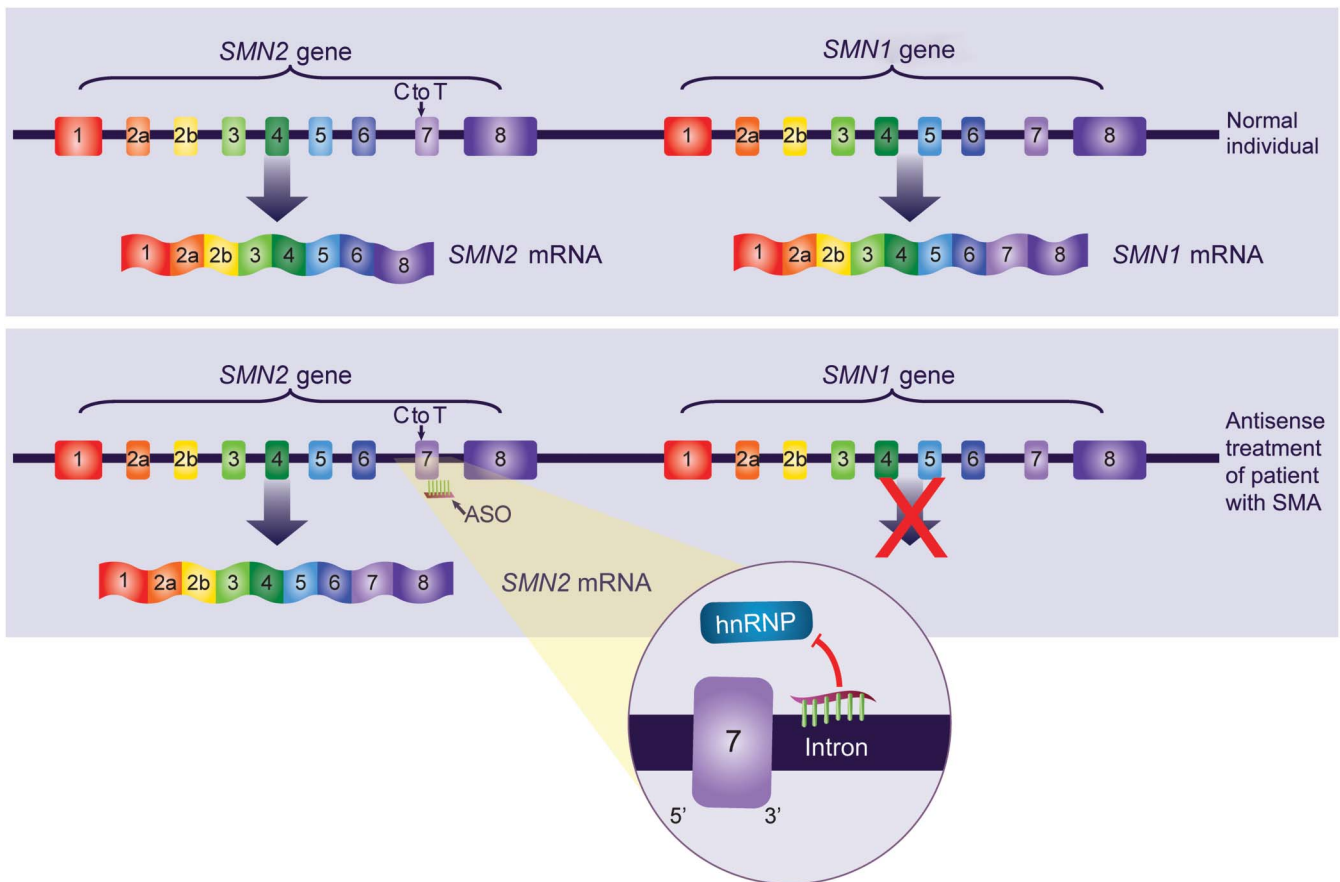
Nusinersen is a 2′-*O*-(2-methoxyethyl) modified ASO drug designed to target an hnRNP-A1/A2–dependent splicing silencer, ISS-N1, in intron 7 of the *SMN* pre-mRNA. Nusinersen displaces hnRNP proteins from the ISS-N1 site on the *SMN2* pre-mRNA, facilitating accurate splicing of *SMN2* transcripts (e.g., increasing the synthesis of transcripts containing exon 7) and resulting in increased production of full-length SMN protein. ASO = antisense oligonucleotide; hnRNP = heterogenous nuclear ribonucleoprotein; ISS = intronic splicing silencer; mRNA = messenger RNA; SMA = spinal muscular atrophy; SMN = survival of motor neuron.

institutional guidelines for LP procedures, and have a life expectancy of >2 years per investigator judgement. Participants were excluded for respiratory insufficiency, hospitalization for surgery or pulmonary event within the past 2 months, active infection at screening, history of brain or spinal cord disease or bacterial meningitis, presence of implanted CSF drainage shunt, clinically significant laboratory abnormalities, any ongoing medical condition that would interfere with the conduct and assessments of the study, or treatment with another investigational drug ≤1 month of screening.

Study procedures. LP was performed under anesthesia/sedation per institutional guidelines. Before intrathecal injection of study drug, 5–6 mL of CSF was collected for analysis. Following injection, participants were observed for 24 hours. Follow-up visits were performed on days 8 and 29 for all participants and on day 85 for participants in the 6- and 9-mg dose groups. A second LP to collect CSF for safety and pharmacokinetics was performed on day 8 in the 1-, 3-, and 6-mg dose groups and on days 8 or 29 in the 9-mg dose group (n = 5 at each time point). Participants were assessed 9–14 months postdose at enrollment into the long-term extension study, using assessments identical to those employed in the single-dose study.

Safety assessments. Safety assessments included collection of adverse events (AEs), physical/neurologic examinations, vital signs, clinical laboratory tests (serum chemistry, hematology, urinalysis; analyzed centrally at PPD, Wilmington, NC), and ECGs. CSF safety laboratory tests (cell counts, protein, glucose) were assessed at 7 or 28 days and 9–14 months postdose (analyzed in local laboratories), and CSF cytokines (interleukin-6, tumor necrosis factor–α, monocyte chemotactic protein 1; Aushon BioSystems, Billerica, MA) at 7 or 28 days postdose. Plasma samples for participants in the 6- and 9-mg cohorts were collected on days 1, 8 or 29, and 85 and 9–14 months postdose for the analysis of anti–nusinersen antibodies using an immunogenicity assay validated to be specific for the detection of anti–nusinersen antibodies (Charles River Laboratories, Wilmington, MA).

Pharmacokinetic and SMN protein assessments. Plasma pharmacokinetic specimens were collected on day 1 predose, at 1, 2, 4, 6, 8, 12, and 20 hours postdose, and on day 8 in the 6- and 9-mg cohorts. CSF pharmacokinetic specimens were collected on day 1 predose, days 8 and 29, and 9–14 months. Nusinersen concentrations were determined using a variation of the hybridization ELISA method[11] or an electrochemiluminescence method; both methods were validated (PPD). SMN protein concentration was determined using an Erenna Immunoassay System validated for human

CSF (Singulex, Inc., Alameda, CA). CSF elimination half-life was estimated for each individual using concentrations determined at 7 or 29 days and 9–14 months by using the following equation:

$$t_{1/2} = \frac{\ln(2)}{\ln\left(C_t/C_0\right)/(t_0 - t)},$$

where $t_{1/2}$ is half-life in days; C_t and C_0 are CSF concentrations at 9–14 months and 7 or 29 days, respectively; and $(t_0 - t)$ is time in days between measurements.

Clinical outcome measures. Exploratory clinical assessments included Hammersmith Functional Motor Scale Expanded (HFMSE)[12,13] performed at baseline, day 29, day 85 (6- and 9-mg cohorts), and at 9–14 months; and Pediatric Quality of Life Inventory (PedsQL) Measurement 4.0 Generic Core Scales and 3.0 Neuromuscular Module[14,15] at baseline, day 29, and day 85 (6- and 9-mg cohorts). For HFMSE, physical therapist evaluators were trained annually with a standardized procedure manual. Intraclass correlation coefficients for interrater reliability among all evaluators were very high (>0.951; computed using 1-way random effects analysis of variance models of 3 videotaped assessments).

Sample size and statistical analysis. Sample size was selected based upon prior experience with phase 1 single-dose studies of antisense oligonucleotide. There was no statistically based rationale for the number of participants chosen. All participants who received the single dose were included in safety, tolerability, pharmacokinetic, and efficacy analyses. Exploratory efficacy endpoints were assessed using Wilcoxon signed-rank test.

Classification of evidence. This study provides Class IV evidence that in children with SMA, a single dose of nusinersen up to 9 mg is not associated with safety or tolerability concerns on a follow-up of 9–14 months. A single dose of 9 mg nusinersen resulted in an increase in HFMSE scores in children with SMA; however, the study was open-label and results would need to be confirmed in a controlled trial.

RESULTS Participants. Six participants were enrolled in the 1-, 3-, and 6-mg dose groups, and 10 participants in the 9-mg group. All participants completed treatment and follow-up evaluations. Demographics/background disease characteristics are presented in table 1. Twenty-four of 28 participants in the phase 1 study were enrolled in the long-term

Table 1	Baseline characteristics of study participants				
	Total nusinersen (N = 28)	**1 mg nusinersen (n = 6)**	**3 mg nusinersen (n = 6)**	**6 mg nusinersen (n = 6)**	**9 mg nusinersen (n = 10)**
Age (min-max), y	6.1 (2-14)	7.7 (3-14)	5.3 (4-7)	6.0 (2-12)	5.8 (2-10)
Male, %	39	83	17	17	40
Caucasian, %	82	83	100	83	70
Type 2/3, n	15/13	2/4	3/3	3/3	7/3
Ambulatory, %	36	50	33	33	30
SMN2 copy no. 3/4/5, n	25/2/1	4/2/0	6/0/0	6/0/0	9/0/1

Abbreviation: SMN = survival of motor neuron.

extension study 9–14 months after initial dose and thus, their demographics were similar to those in the single-dose study.

Safety and tolerability. There were no serious AEs during the course of the study and no participant experienced an AE leading to discontinuation. Overall, 89% of participants reported AEs (table 2); most AEs were mild in severity, with only 5 participants reporting an event that was moderate in severity and none that was severe. The most frequently observed AEs by prevalence were headache (39.3% of participants), post-LP headache (21.4%), and back pain (17.9%). There were 2 AEs considered potentially related to the study drug: a mild paresthesia in the 1-mg group and a report of palpitations in the 3-mg group, both of which resolved spontaneously without sequelae. The incidence of post-LP headache was 10.9% (6 events reported for 55 LPs performed). No clinically significant changes in vital signs, neurologic or physical examinations, clinical laboratory tests, or ECGs were reported. There were no clinically significant changes in CSF safety laboratory tests or CSF cytokines. All samples were negative for the presence of anti–nusinersen antibodies, indicating that an immunogenic response to nusinersen was not observed at 9–14 months after a single intrathecal dose of nusinersen.

Pharmacokinetic profile. Nusinersen concentrations were measurable over 24 hours postdose in plasma and 7 days postdose in CSF (figure 2), as well as 29 days postdose in CSF in the 9-mg group (2.33 ± 0.928 ng/mL). Nusinersen concentrations 7 days after dosing increased dose dependently but less than dose proportionally (3.7-fold) over the evaluated dose range. A substantial portion of intrathecally administered nusinersen rapidly transferred from the site of administration (CSF) into systemic circulation, with dose-dependent mean peak plasma levels observed within a few hours after dosing. After peaking, nusinersen plasma concentrations declined slowly ≤20 hours after intrathecal dosing, followed by a slower period of decline over the next 7 days. By 7 days postdose, concentrations in the 6-mg cohort had decreased to below the limit of quantification (1 ng/mL), and to <1% of the 20-hour postdose concentration in the 9-mg cohort. Dose-normalized total plasma exposure measures decreased ~3-fold over a ~3-fold increase in total body weight. A similar relationship between dose-normalized total plasma exposure and age was observed. No differences were observed between males and females for untransformed total plasma exposure (area under the concentration time curve from 0 to 20 hours [$AUC_{0–20h}$]) measures stratified by cohort or for dose-normalized total plasma exposure ($AUC_{0–20h}$) measures. At the 9- to 14-month evaluation, CSF nusinersen concentrations were still measurable. Apparent terminal half-life in CSF was estimated to be 132–166 days (mean ± SD days: 132 ± 42, 1-mg group; 135 ± 14.8, 3-mg group; 163 ± 26.5, 6-mg group; 177 ± 41.3, 9-mg group).

Analysis of CSF samples for SMN protein levels indicated no change in the 1- or 3-mg groups, but that SMN protein levels more than doubled at 9–14 months postdose compared with baseline in the 6- and 9-mg groups (6-mg group: baseline, 0.27 ± 0.03 pg/mL; 9–14 months, 0.56 ± 0.12 pg/mL; not statistically significant; 118% mean increase; 9-mg group: baseline, 0.31 ± 0.18 pg/mL;
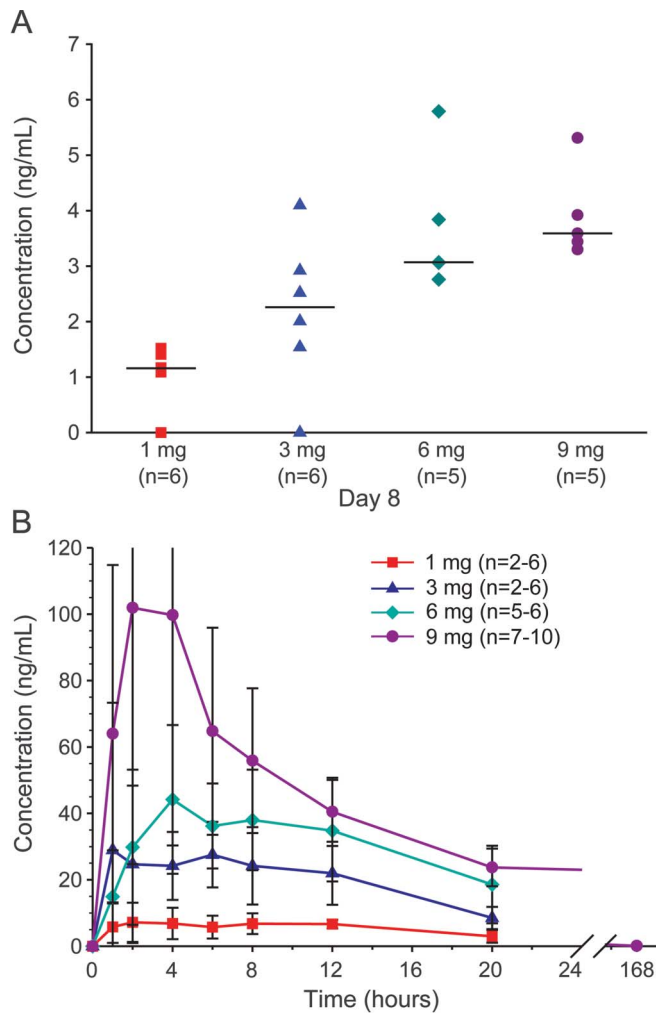
Adverse event term	Total nusinersen (N = 28)	1 mg nusinersen (n = 6)	3 mg nusinersen (n = 6)	6 mg nusinersen (n = 6)	9 mg nusinersen (n = 10)
Any serious adverse event	0	0	0	0	0
Any adverse event	89 (72)	100 (25)	67 (15)	100 (11)	90 (21)
Headache	39 (12)	67 (4)	0	50 (3)	40 (5)
Post-LP syndrome	21 (7)	17 (2)	33 (2)	33 (2)	10 (1)
Back pain	18 (7)	33 (2)	33 (4)	0	10 (1)
Pyrexia	14 (4)	17 (1)	0	17 (1)	20 (2)
Constipation	11 (3)	33 (2)	17 (1)	0	0
Upper respiratory tract infection	11 (5)	0	17 (1)	17 (1)	10 (3)
Nausea	11 (3)	17 (1)	33 (2)	0	0
Vomiting	11 (3)	17 (1)	33 (2)	0	0

Table 2 Adverse event summary by preferred term, % (number of events)[a]

Abbreviation: LP = lumbar puncture.
[a] Adverse events listed are those that occurred with a frequency >10% in nusinersen-treated participants (i.e., occurring in >2 participants).

Figure 2   CSF and plasma concentrations of nusinersen

A

B

Measured nusinersen concentrations for each dose group are shown. (A) CSF at 7 days postdose. (B) Plasma over 24 hours (all groups) or 7 days (6- and 9-mg groups) postdose (mean ± SEM). As anticipated, plasma levels were below the limit of detection of the assay at day 8 postdose.
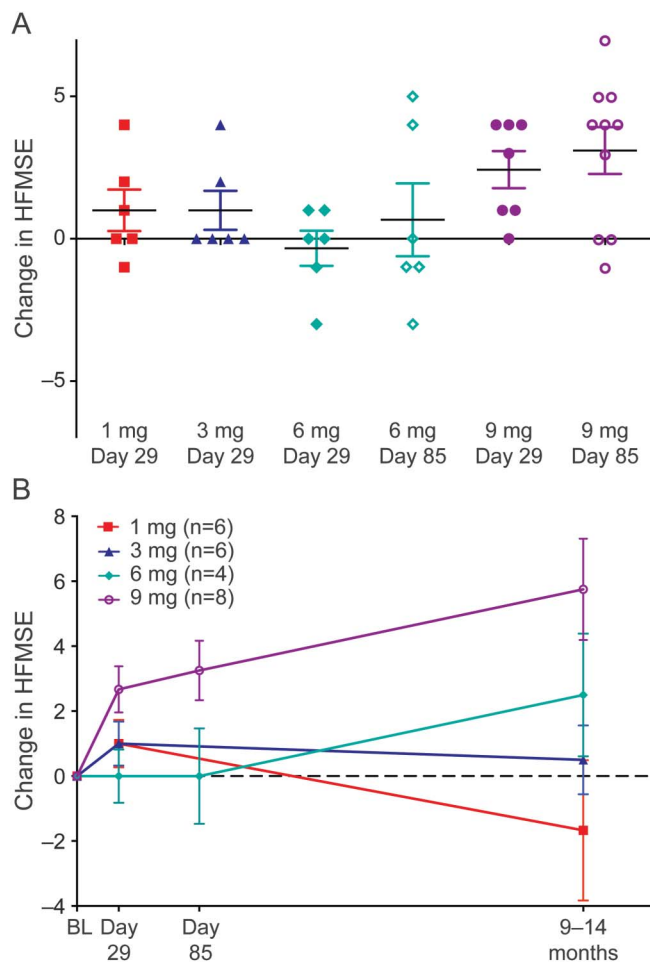
improvement was distributed broadly across the age range, with 3 children aged <5 years and 4 children aged ≥5 years showing improvement. At long-term follow-up, mean change in HFMSE score from baseline to 9–14 months was 5.8 points (32.8% increase; $p = 0.008$; n = 8). Six of 8 participants had an increase of ≥3 points. No participants in the 9-mg cohort declined in HFMSE score, and range of improvement was 1–14 points.

A slight improvement for the PedsQL Generic Core Scales was observed compared with baseline in the 9-mg group at day 85 (mean percent change: 9.8% [patient] and 8.4% [parent], not statistically significant). Similarly, a slight improvement in the PedsQL Neuromuscular Model was observed compared with baseline in the 9-mg group at day 85, with the change being greater for the patient compared with the parent report (mean percent change: 17.7% [patient] and 4.6% [parent], not statistically significant). No meaningful changes were observed in the other dose groups.

DISCUSSION Nusinersen was well-tolerated when given as a single intrathecal bolus injection to children with SMA and no safety concerns were identified. No serious AEs or discontinuations due to AEs were reported. The most commonly reported AEs were headache, post-LP headache, and backache; however, none of these events was considered to be related to nusinersen. No clinically significant abnormalities were noted on neurologic examinations or laboratory safety assessments, including CSF safety assessments at 7 or 28 days and 9–14 months postdose. These results are consistent with previous but limited clinical studies on direct CNS delivery of antisense oligonucleotide drugs, which appeared to be similarly well-tolerated.[16,17] In this study, the LP injection procedure itself was well-tolerated and was shown to be feasible in children with SMA. Incidence rate of post-LP headache overall was 10.9%, which is consistent with the expected post-LP headache rate of ~10% in children from published literature,[18–20] further taking into account that patients in the study underwent 2 LPs (except for 1 patient who had only 1 LP) and that patients with SMA often have scoliosis or spinal deformities, making LPs more challenging. Eighteen of the children with SMA underwent 3 repeated LPs, with no increase in AEs or tolerability issues reported, further supporting the feasibility of this approach. The presence of hardware from scoliosis surgery in some patients with SMA, however, may limit the feasibility of intrathecal injections in the broader population of patients with SMA.

CSF and plasma drug concentrations of nusinersen were measurable and dose-dependent. No readily

9–14 months, 0.59 ± 0.22 pg/mL; $p = 0.06$; 161% mean increase).

Clinical outcomes. There were no significant changes in HFMSE assessments (figure 3) from baseline at day 29, day 85, or 9–14 months postdose in the 1-, 3-, or 6-mg groups (mean change: 1-mg group, +1.0 point at day 29 and −1.7 point at 9–14 months; 3-mg group, +1.0 point at day 29 and +0.5 point at 9–14 months; 6-mg group, +0.7 point at day 85 and +2.5 at 9–14 months). In contrast, the 9-mg group demonstrated improved HFMSE scores from baseline at day 85 (mean increase of 3.1 points or 17.6% increase; $p = 0.016$), with 7/10 (70%) participants exhibiting an increase of 3–7 points. Observed improvements in HFMSE scores of ≥3 points were equally distributed by severity; 5/7 type 2 participants and 2/3 type 3 participants showed this level of improvement. Similarly, HFMSE score

(A) Individual change in HFMSE score from baseline for all participants. (B) Mean change in HFMSE score from baseline in participants followed through 9–14 months postdose. Bars represent mean ± SEM for each dose group. BL = baseline.

monkeys (139 ± 54 days)[22] and was confirmed in humans by the observed CSF half-life of between 4–6 months in children with SMA. This observation is critical in that it provides support for infrequent administration of nusinersen for maintenance of drug levels (i.e., once every 4–6 months) following drug loading of the target tissue.

Although preliminary and requiring confirmation by a larger controlled study, improvement in patients' motor function as evidenced by increases in HFMSE score was observed following a single intrathecal dose of 9 mg nusinersen, with a 3.1-point mean increase from baseline at 3 months and a 5.8-point mean increase at 9–14 months. This improvement in motor function is not expected to occur as part of the disease process, as the predicted natural history trajectory of the HFMSE in type 2 and type 3 patients with SMA indicates stability over 1 year[23] and a small decline over 2 years (−1 point).[24] Though trials in patients with SMA evaluating other investigational therapies have ultimately been unsuccessful and several previous open-label trials have shown strong placebo effects, variations on the HFMSE have been previously used as the primary outcome measures in SMA clinical studies.[25,26] It has been projected, including as an assumption in previous trials, that a change of 3 points over a 6-month period would constitute a clinically meaningful change in this endpoint,[26] and thus, the 5.8-point increase observed at 9–14 months in the 9-mg group in this study clearly falls within the meaningful range. Consistent with the intended biological mechanism of nusinersen, an increase in CSF SMN protein concentrations correlated well with the improvement in motor function. In addition, the study findings reaffirm the sensitivity of the HFMSE to detect change in functional motor outcome and inform the length of time that is required to observe improvement in motor function after treatment.

Nusinersen dose levels for this first-in-human study were selected based on preclinical pharmacokinetic observations from monkey studies and consideration of the target tissue concentration anticipated for drug pharmacology from SMA transgenic mouse models.[22] As nonhuman primates do not express the *SMN2* gene, it was not possible to measure pharmacology in that animal species. Based upon pharmacology and pharmacokinetic results in *SMN2* transgenic mice, the target tissue concentration to produce 50%–90% *SMN2* exon 7 inclusion was estimated to be between 1 and 10 µg/g in spinal cord tissues.[10] The lowest dose selected for this clinical study (1 mg) was predicted to achieve the low end of this range, while the highest dose (9 mg) was predicted to achieve spinal cord tissue concentrations at the upper end of this range. However, no dose-limiting safety issues were identified in this study,

apparent correlations were observed between age or total body weight and CSF concentrations, consistent with literature reports indicating that CSF volume is similar in all children aged >2 years,[21] and suggesting that fixed doses are appropriate in this pediatric population, with no dose scaling necessary. CSF and plasma drug levels were reasonably consistent with predicted values (within 2- to 3-fold) based on preclinical (monkey to human) CSF volume scaling. Dose-dependent mean peak plasma levels were observed within a few hours after dosing and then typically declined slowly ≤20 hours after intrathecal dosing. The slow decline may be because of CSF turnover and transfer of any remaining drug not taken up into CNS tissues from CSF into systemic circulation, coupled with the expected rapid and extensive distribution of systemically available drug from the plasma to systemic tissues. A consistent finding of a long drug half-life in CNS tissue for nusinersen has been observed in mice (145–191 days) and

suggesting that doses higher than the 9 mg tested in this study should be considered.

The favorable risk-benefit profile from this first-in-human clinical study of nusinersen in children with SMA provides encouragement for further development of nusinersen for the treatment of SMA, for which no therapeutic options currently exist. Ongoing multiple-dose studies, which include incorporation of higher doses, are currently being conducted in both infants and children with SMA.

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AAN Guideline Recommends Removal of Player if Concussion Suspected

Athletes who are suspected of having a concussion should be removed from the game immediately and not be returned until assessed by a licensed health care professional trained in diagnosing and managing concussion. That is one of the recommendations of the American Academy of Neurology's highly accessed evidence-based guideline for evaluating and managing athletes with concussion.

Share this information with patients, families, coaches, athletic trainers, and colleagues. Visit *AAN.com/concussion* for all your concussion resources:

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- Download the slide presentation
- Review a clinical example
- Download the Academy's convenient mobile app, **Concussion Quick Check**, to quickly help coaches and athletic trainers recognize the signs of concussion.

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