Supplementary Appendix

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Additional methodological details

Full eligibility criteria

CS2

CS2 inclusion criteria included: signed informed consent of parent(s) or guardian(s); a genetic diagnosis of 5q-linked spinal muscular atrophy (SMA) due to homozygous gene deletion or compound heterozygote deletion/mutation of survival motor neuron 1 (*SMN1)*; clinical signs or symptoms attributable to SMA; males and females aged 2 to 15 years; ability to complete all study procedures and parent/guardian has adequate psychosocial support; estimated life expectancy >2 years from screening; meets age-appropriate institutional criteria for use of anesthesia/sedation at screening if anesthesia/sedation is to be used; and for participants who have reached reproductive maturity, females must have a negative pregnancy test at screening and be abstinent or use adequate birth control for the duration of the study and males must be abstinent for the duration of the study.

CS2 exclusion criteria included: respiratory insufficiency (the medical necessity for invasive or noninvasive ventilation during a 24-hour period); the medical necessity for a gastric feeding tube for the majority of feeds; previous scoliosis surgery that would interfere with the lumbar puncture (LP) procedure; hospitalization for surgery or a pulmonary event within 2 months of screening or planned during the study; presence of an untreated or inadequately treated infection requiring systemic treatment during screening; history of brain or spinal cord disease or abnormalities by MRI or CT scan that would interfere with the LP procedure or CSF circulation; presence of an implanted CSF drainage shunt or CNS catheter; history of bacterial meningitis; dosing with nusinersen in cohorts 2, 3, or 4 of CS1 or in CS10; any clinically significant abnormality in hematology or clinical chemistry parameters that would make the child unsuitable for inclusion; treatment with another investigational drug, biological agent, or device within 1 month of screening or 5 half-lives of the study agent (whichever is longer); treatment with valproate or hydroxyurea within 3 months of screening; history of gene therapy or cell

transplantation; and presence of a medical condition that would interfere with the infant's ability to participate in the study as assessed by the site investigator.

CS12

CS12 inclusion criteria included: signed informed consent of parent(s) or guardian(s); satisfactory completion of dosing and all study visits in CS2 or CS10 with an acceptable safety profile; ability to complete all study procedures and parent/guardian has adequate psychosocial support; estimated life expectancy >2 years from screening; meets age-appropriate institutional criteria for use of anesthesia/sedation at screening if anesthesia/sedation is to be used; and for participants who have reached reproductive maturity, females must have a negative pregnancy test at screening and be abstinent or use adequate birth control for the duration of the study and males must be abstinent for the duration of the study.

CS12 exclusion criteria included: any new or worsening condition that could interfere with study enrollment, participation, or completion; dosing with nusinersen in CS2 or CS10 within 180 days or >396 days from screening; hospitalization for surgery or a pulmonary event within 2 months of screening or planned during the study; presence of an untreated or inadequately treated infection requiring systemic treatment; any clinically significant abnormality in hematology or clinical chemistry parameters or EKG that would make the child unsuitable for inclusion; treatment with another investigational drug, biological agent, or device within 1 month of screening or 5 half-lives of the study agent (whichever is longer); and history of gene therapy or cell transplantation.

Study assessments

The Hammersmith Functional Motor Scale–Expanded (HFMSE) is a valid and reliable measure of motor function in SMA.[1-3] The items assessed in the HFMSE represent gross motor function; each item is scored from 0 to 2, with a score of 0 indicating no response or inability to perform

the movement, 1 indicating partial response or performance of the movement with modification, and 2 indicating full response or performance of the movement without modification.[4] HFMSE scores range from 0 to 66 points, with higher scores indicating greater motor function.[4] Score increases or decreases ≥3 points are considered clinically meaningful.[5]

The Upper Limb Module (ULM) is a 9-item test specifically designed to assess upper limb function in nonambulant individuals with SMA, including young children and those with severe lower limb contractures in whom assessment of lower limb functional changes is limited.[6] The items assessed in the ULM resemble activities of daily living; each item is scored from 0 to 2 for a maximum total score of 18, with higher scores indicating increased upper limb function. Score increases ≥2 points are considered clinically meaningful.[7] The ULM was assessed only in nonambulant participants.

The Six-Minute Walk Test (6MWT) evaluates the functional capacity of ambulatory patients by measuring the distance a person can walk in 6 minutes. 6MWT is valid and reliable in patients with SMA, with results correlating with other SMA outcome measures such as the HFMSE.[8,9] Distance increases ≥30 meters are considered clinically meaningful.[9] The 6MWT was only evaluated in ambulant participants who could walk without support.

Compound muscle action potential (CMAP) is an electrophysiologic measure that assesses the integrity of the peripheral nerve, the neuromuscular junction, and the resulting muscle action potential response. Maximum CMAP is achieved by incremental stimulation of the peripheral nerve of a target muscle(s) until a maximum amplitude and area are reached. Motor unit number estimation (MUNE) is an electrophysiologic measure used to estimate the number of motor units innervating a muscle. Maximum ulnar CMAP and MUNE values have been demonstrated to correlate with outcome measures of disease severity in patients with SMA and have been proposed as potential surrogates of disease progression or therapeutic effect.[10,11] The CMAP technique used in this study has been previously published.[12] The incremental

multipoint MUNE technique used in this study has been validated in an amyotrophic lateral sclerosis population and successfully implemented in multicenter trials.[13]

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