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Risdiplam in types 2 and 3 spinal muscular atrophy: A randomised, placebo-controlled, dose-finding trial followed by 24 months of treatment

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Abstract

Background and purpose: Spinal muscular atrophy (SMA) is caused by reduced levels of survival of motor neuron (SMN) protein due to deletions and/or mutations in the *SMN1* gene. Risdiplam is an orally administered molecule that modifies *SMN2* pre-mRNA splicing to increase functional SMN protein.

Methods: SUNFISH Part 1 was a dose-finding study conducted in 51 individuals with types 2 and 3 SMA aged 2–25 years. A dose-escalation method was used to identify the

appropriate dose for the subsequent pivotal Part 2. Individuals were randomized (2:1) to risdiplam or placebo at escalating dose levels for a minimum 12-week, double-blind, placebo-controlled period, followed by treatment for 24 months. The dose selection for Part 2 was based on safety, tolerability, pharmacokinetic, and pharmacodynamic data. Exploratory efficacy was also measured.

Results: There was no difference in safety findings for all assessed dose levels. A dose-dependent increase in blood SMN protein was observed; a median twofold increase was obtained within 4 weeks of treatment initiation at the highest dose level. The increase in SMN protein was sustained over 24 months of treatment. Exploratory efficacy showed improvement or stabilization in motor function. The pivotal dose selected for Part 2 was 5 mg for patients with a body weight ≥20 kg or 0.25 mg/kg for patients with a body weight <20 kg.

Conclusions: SUNFISH Part 1 demonstrated a twofold increase in SMN protein after treatment with risdiplam. The observed safety profile supported the initiation of the pivotal Part 2 study. The long-term efficacy and safety of risdiplam are being assessed with ongoing treatment.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease caused by reduced levels of survival of motor neuron (SMN) protein due to loss of the *SMN1* gene [1, 2]. Alternative splicing of *SMN2* excludes exon 7 from the majority of *SMN2* pre-mRNA transcripts, resulting in low levels of functional SMN protein that are unable to compensate for the loss of *SMN1* [1, 3, 4].

5q SMA is characterized by progressive loss of motor function and muscle weakness [2, 5, 6], and encompasses a broad spectrum of disease classified into five subtypes (types 0–4) determined by highest motor milestone achieved [7]. Type 2 SMA has symptom onset between 6 and 18 months of age; individuals achieve the ability to sit independently and may stand with assistance, but never walk independently [1, 8]. Type 3 SMA has symptom onset after 18 months of age; individuals are able to stand and walk independently, although this ability may be lost over time [1, 7].

There are three approved treatments for SMA: nusinersen (Spinraza), an intrathecally administered *SMN2* RNA-targeting antisense oligonucleotide indicated for the treatment of adult and paediatric patients with SMA [9, 10]; onasemnogene abeparvovec (Zolgensma), an intravenously administered adenovirus-associated vector-based gene therapy indicated for the treatment of patients with SMA aged <2 years (USA), or for patients with type 1 SMA or who have three or fewer *SMN2* copies (EU) [11, 12]; and risdiplam (Evrysdi), an orally administered small molecule *SMN2* splicing modifier [13], indicated for the treatment of patients of all ages (USA) [14], or aged ≥2 months with type 1, 2, or 3 SMA or one to four copies of the *SMN2* gene (EU) [15]. The safety and efficacy of risdiplam have been demonstrated in infants with type 1 SMA [16, 17] and in individuals with types 2 and 3 SMA [18].

SUNFISH [19] is a multicentre, randomized, double-blind, placebo-controlled, two-part, phase 2/3 study, designed to assess safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and efficacy of risdiplam in a broad population including children, teenagers, and adults aged 2–25 years with types 2 and 3 SMA. Part 1 was the dose-finding part of SUNFISH, assessing safety, tolerability, and PK and PD of different dose levels to select the risdiplam dose for Part 2. The confirmatory Part 2 investigates efficacy of risdiplam in individuals with type 2 and nonambulant type 3 SMA at the selected dose.

Here, we report data obtained in the dose-finding (Part 1) study in individuals with type 2 and ambulatory and nonambulatory type 3 SMA. The open-label extension of Part 1 is ongoing, with safety, tolerability, and PD data of risdiplam from >2 years of treatment now available. Exploratory efficacy is also reported.

METHODS

Study oversight

The SUNFISH study was approved by an ethics committee at each study site and was conducted in accordance with Good Clinical Practice guidelines, the World Medical Association Declaration of Helsinki, and regulations and procedures outlined in the study protocol. Written informed consent was provided by the patients or by their parents/caregivers. The sponsor, F. Hoffmann-La Roche, provided study drug, study management, medical monitoring, drug safety management and analysis, data management, statistical analysis, and PK and PD analysis. During the Part 1, dose-finding period, an internal monitoring committee (IMC) reviewed data on an

ongoing basis. An external independent data monitoring committee (iDMC) reviewed data to confirm the dose-selection decision of the IMC. After confirmation of the pivotal dose, responsibility of monitoring safety data was transferred to the iDMC. All authors attest to adherence to the protocol, accuracy of analysis, and complete reporting of adverse events (AEs). Further details of this trial can be found on ClinicalTrials.gov (NCT02908685) [19].

Patients

Enrolled patients were aged 2–25 years, with a genetically confirmed diagnosis and clinical symptoms of type 2 or type 3 SMA. Patients were excluded from study entry if they had received treatment with an *SMN2*-targeting antisense oligonucleotide, *SMN2* splicing modifier, or gene therapy. See Supplementary Appendix S1 for all inclusion/exclusion criteria.

Study design and outcomes

SUNFISH was the first clinical study of risdiplam in patients with SMA. The objective of SUNFISH Part 1 was to assess safety, tolerability, PK, and PD after administration of risdiplam to determine the pivotal dose to be used in Part 2.

Prior to the start of SUNFISH, risdiplam had only been administered as a single dose to healthy adult volunteers [20]. Therefore, the safety and tolerability of multiple doses of risdiplam, and the PK and PD in the target population of patients with SMA aged 2–25 years had to be investigated in a careful dose-escalation design to ensure the safety of all study participants (Figure 1). Dose-finding

was performed in a dose-escalation manner to reach the protocol-specified target exposures based on PK data obtained from the enrolled patients. The first step was to reach an area under the plasma concentration time curve (AUC) of 700 ng·h/ml. Once the dose had been identified to reach this exposure, and this exposure was shown to be safe and well tolerated, the next step was to administer a dose targeting an AUC of 2000 ng·h/ml. SMN protein was measured in blood. The aim was to obtain a minimum twofold increase in SMN protein in blood versus baseline, as this increase was expected to be efficacious based on animal data [21]. Risdiplam exposure at the highest dose was not supposed to exceed the exposure cap of a mean AUC of 2000 ng·h/ml to maintain a safety margin versus the findings observed in the animal toxicology studies [21].

Risdiplam was administered as a solution orally at the assigned dose once daily. Patients were enrolled into five subsequent cohorts in a dose-escalation design based on age (2–11 and 12–25 years; Figure 1). At each dose level, patients were randomized (2:1) to receive either placebo or risdiplam in a double-blind manner for at least 12 weeks. Once all patients in a specific cohort had completed the 12-week double-blind treatment period, patients receiving placebo were switched to risdiplam (at the dose tested in their cohort) at their next scheduled visit, upon IMC review of all data. After the pivotal dosing regimen for Part 2 was chosen, all patients in Part 1 switched to the pivotal dose and continued treatment over 24 months.

Safety assessments included AE reporting, laboratory assessments, electrocardiography, anthropometric and physical examinations, and vital signs. Due to effects observed on the retina in preclinical studies in monkeys [13], ophthalmological assessments were performed every 2 months. Blood samples were obtained for the measurement of risdiplam concentrations in plasma (quantified by
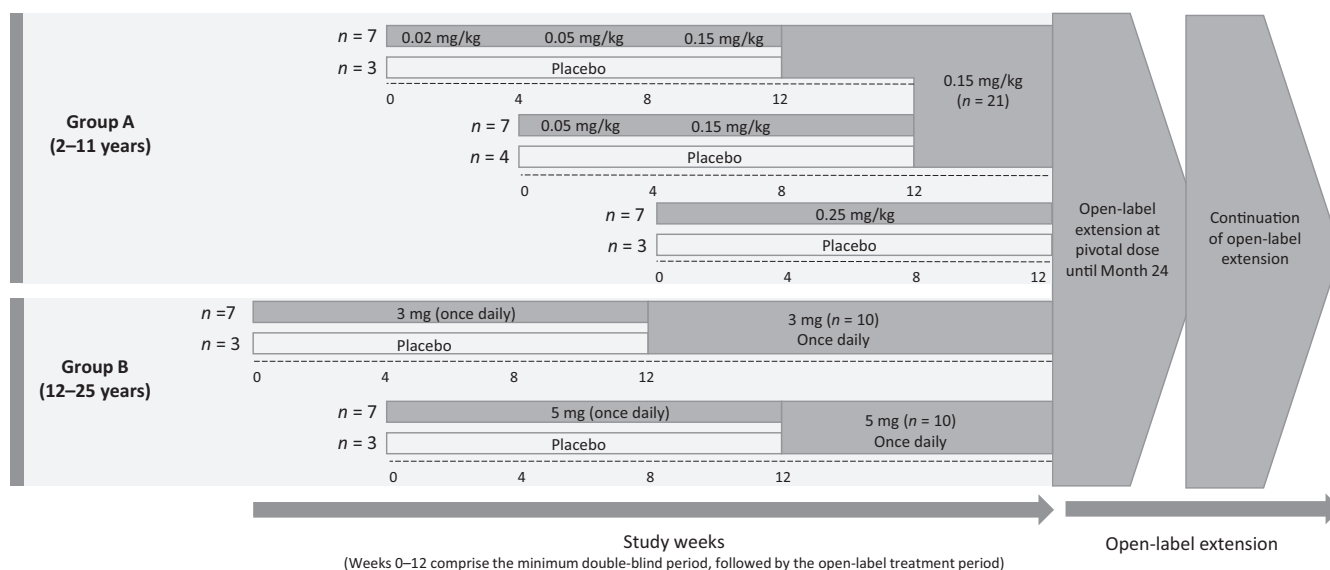
FIGURE 1 Study design. Week 0 indicates the recruitment start date for each cohort. The double-blind, placebo-controlled period occurred at minimum from Week 0 to Week 12 once all patients in a specific cohort had completed this and upon internal monitoring committee review of all data. The next cohort of patients in each age group was enrolled at a higher dose after pharmacokinetics, safety, and tolerability were confirmed after at least 4 weeks of treatment at the first dose level.

liquid chromatography–tandem mass spectrometry) and SMN protein in blood (quantified by an immunoassay developed on the Elecsys platform). See Supplementary Appendix S1 for the assessment schedule.

Exploratory efficacy endpoints included motor function (32-item Motor Function Measure [MFM32], Hammersmith Functional Motor Scale–Expanded [HFMSE], and Revised Upper Limb Module [RULM]) and respiratory function (forced vital capacity, forced expiratory volume in 1 s, peak cough flow, and sniff nasal inspiratory pressure).

Statistical methods

Per protocol, it was planned to enroll at least 36 patients, with the option to enroll up to 72 patients, to enable dose selection for Part 2. With 12 patients receiving active drug per dose/exposure level, this gave a 93% chance of detecting an AE in at least one patient, based on a true underlying AE rate of 20%. All safety, SMN protein, and efficacy results are summarized using descriptive statistics. Safety analyses include all data collected over at least 24 months up to the clinical cutoff date (CCOD; 15 January 2020). Safety analyses in the 12-week double-blind treatment period grouped by initial treatment and/or initial dose level were included to enable comparison between all assessed dose levels of risdiplam (CCOD: 9 January 2019). Safety results were summarized by the all-exposure-to-risdiplam period (total time after each individual received risdiplam at any dose level up to the CCOD) and by the first and second year of exposure. Exploratory efficacy analyses were conducted when all patients had been treated for 12 and 24 months; these results are summarized by the all-exposure-to-risdiplam period up to the CCOD for the overall population and by age group (2–11 and 12–25 years).

MFM data were compared with an external comparator, comprising of patients in the NatHis-SMA (NCT02391831) [5] study and patients in the placebo arm of the WN29836 study (NCT01302600) [22]. NatHis-SMA is a prospective, longitudinal study of untreated patients with types 2 and 3 SMA, aged 2–30 years, a similar patient population to that of SUNFISH [5, 6]. Study WN29836 was a randomized, double-blind, placebo-controlled, phase 2 study of the discontinued compound olesoxime, which enrolled patients with SMA aged 3–25 years [23]. To be included in the comparator, patients from these studies had to have MFM total scores available at baseline and Month 12 and/or Month 24. The inverse probability of treatment weighting approach was applied to weight patients in the external comparator according to key prognostic factors. A mixed models repeated measures analysis was performed to compare MFM results.

RESULTS

Patients

Fifty-one patients were enrolled from four countries (Italy [two sites], Germany, France, and Belgium [one site each]) in five subsequent cohorts. All patients completed the minimum 12-week, double-blind,

placebo-controlled period and were then treated with risdiplam at the dose assigned to their cohort. The placebo-controlled period ranged from 19.1 to 26.9 weeks, as all patients in a specific cohort were switched to risdiplam only when the last patient enrolled in the cohort completed 12 weeks and after IMC review of all data. Three cohorts of patients aged 2–11 years (Group A) were administered the following dose levels: 0.02, 0.05, 0.15, and 0.25 mg/kg. Two cohorts of patients aged 12–25 years (Group B) received 3 and 5 mg of risdiplam (Figure 1). Following selection of the pivotal dose for Part 2 (5 mg for patients with a body weight ≥20 kg, 0.25 mg/kg for patients weighing <20 kg), all patients switched to the pivotal dose; 50 patients completed treatment at this dose for 24 months. One patient withdrew consent for participation and was discontinued from the study at Day 287 (Figure S1).

Patient baseline characteristics are shown in Table 1. Median patient age at screening was 7 years (range = 2–24 years), with 8% (4/51) of patients aged ≥18 years. Part 1 included patients with types 2 (73%) and 3 SMA (27%); the majority (90%) had three *SMN2* copies. The proportion of patients with type 2 SMA was similar across patients aged 2–11 and 12–25 years. Patients displayed varied baseline motor function (sitter [77%], stander [18%], walker [18%]). Twelve percent of patients had severe scoliosis (a Cobb angle of >40°), with 53% of patients having already undergone scoliosis surgery before screening.

AEs

Placebo-controlled period

There was no numerical difference in safety findings for all assessed dose levels (Table 2). Ninety-three AEs were reported in 25 (71%) patients treated with risdiplam, and 27 AEs were reported in 11 (69%) patients treated with placebo. No serious AEs (SAEs) were reported in patients treated with risdiplam at any dose level. Treatment-related AEs (TRAEs) were reported in six (17%) patients treated with risdiplam and three (19%) patients treated with placebo.

The most common AEs in patients treated with risdiplam occurred in the system order classes "infections and infestations" (*n* = 12 patients), "general disorders and administration site conditions" (*n* = 9 patients), "gastrointestinal disorders" (*n* = 8 patients), "respiratory, thoracic, and mediastinal disorders" (*n* = 8 patients), and "skin and subcutaneous tissue disorders" (*n* = 7 patients).

All-exposure-to-risdiplam treatment period

The median duration of exposure to risdiplam was 31.9 months (range = 9.4–38.9 months). A total of 737 AEs were reported in 49 (96%) patients during this period (Table 3). The rate of overall AEs per 100 patient-years (100PY) continuously decreased over the 24-month treatment period from 880.54 AEs/100PY during the 0- to 6-month period to 463.05 AEs/100PY during the 18- to 24-month period.

TABLE 1 SUNFISH Part 1 patient baseline characteristics

Characteristic	Patients aged 2–11 years, n = 31	Patients aged 12–25 years, n = 20	All patients, N = 51
Age at screening, years, median (range)	5.0 (2–11)	14.5 (12–24)	7.0 (2–24)
Age at symptom onset, months, median (range)	13 (4–70)	16 (2–34)	14 (2–70)
Gender, female/male, n (%)	14 (45)/17 (55)	13 (65)/7 (35)	27 (53)/24 (47)
Disease duration, months, median (range)[a]	49.9 (21–127)	167.4 (133–275)	78.1 (21–275)
SMA type, n (%)			
Type 2	23 (74)	14 (70)	37 (73)
Type 3	8 (26)	6 (30)	14 (27)
SMN2 copy number, n (%)			
2	0	1 (5)	1 (2)
3	28 (90)	18 (90)	46 (90)
4	3 (10)	1 (5)	4 (8)
Functional status, n (%)			
Sitter[b]	31 (100)	8 (40)	39 (77)
Stander[c]	6 (19)	3 (15)	9 (18)
Walker[d]	6 (19)	3 (15)	9 (18)
BiPAP support <16 h per day			
Yes	8 (26)	4 (20)	12 (24)
Scoliosis, n (%)			
Yes [0 to ≥40° curvature]	13 (42)	16 (80)	29 (57)
Severe scoliosis [>40° curvature]	1 (3)	5 (25)	6 (12)
Previous SMA surgery, n (%)[e]			
Yes	2 (7)	7 (35)	9 (18)
Spinal fusion with segmental instrumentation	0	3 (15)	3 (6)
Baseline MFM32 total score, mean (SD)[f]	44.4 (11.9) [n = 24]	40.9 (18.2) [n = 20]	42.9 (15.0) [n = 44]

Note: Data cutoff: 15 January 2020.

Abbreviations: BiPAP, bilevel positive airway pressure; HFMSE, Hammersmith Functional Motor Scale–Expanded; MFM, Motor Function Measure; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

[a]Time between symptom onset and treatment initiation.

[b]"Sitting" is defined as a score ≥1 on Item 9 of the MFM scale, and "could not sit" is defined as a score <1 on Item 9 of the MFM.

[c]"Standing" is defined as a score ≥1 on Item 25 of the MFM, and "could not stand" is defined as a score <1 on Item 25 of the MFM.

[d]"Walking" is defined as a score ≥2 on Item 20 of the HFMSE (able to take >4 steps unaided), and "could not walk" is defined as a score <2 on Item 20 of the HFMSE (able to take ≤4 steps unaided).

[e]Previous SMA surgeries include insertion of magnetically controlled growing rods, insertion of traditional growing rods, spinal fusion with segmental instrumentation, tendon release—knee, and rod adjustment surgery.

[f]Excludes seven patients who performed the MFM20 assessment at baseline.

Twenty-two TRAEs were reported in 12 (24%) patients. The most common TRAE was rash, which was reported in three patients (6%); all incidents resolved with ongoing treatment. All other TRAEs were reported by one patient each (See Supplementary Appendix S1 for all TRAEs). Most TRAEs resolved with ongoing treatment, except for headache in one patient and AEs of erythema, palmar erythema, and skin exfoliation in a second patient.

The incidence of SAEs was stable; 23 SAEs were reported in 15 patients (29%) over 31.9 months. The number of patients reporting SAEs was stable between the first and second year of treatment (six patients [12%] with SAEs in both years). The most common SAEs were pneumonia (n = 3 patients [6%]) and femur fracture (n = 2 patients [4%]). All other SAEs were reported by one patient each; no SAEs were reported as related to risdiplam treatment.

The majority of AEs were mild/moderate in severity. Fourteen grade 3–4 AEs were reported in nine (18%) patients. No grade 5 (fatal) AEs were reported. No AE led to dose modification of risdiplam; treatment interruptions were short term (n = 11 AEs, mean = 2.8 days, range = 1–14 days). Events all resolved and did not recur after reinitiation of risdiplam. No individual discontinued treatment or was withdrawn from the trial because of a TRAE.

TABLE 2 AEs and SAEs that occurred during the minimum 12-week, dose-finding, placebo-controlled period

Initial dose cohort	Group A patients, aged 2–11 years old, $n = 31$				Group B patients, aged 12–25 years old, $n = 20$		
	0.02 mg/ kg, $n = 7$	0.05 mg/ kg, $n = 7$	0.25 mg/ kg, $n = 7$	Placebo, $n = 10$	3 mg, $n = 7$	5 mg, $n = 7$	Placebo, $n = 6$
Total number of AEs	17	17	21	20	24	14	7
Patients with at least one, n (%)							
AE	4 (57)	5 (71)	6 (86)	8 (80)	4 (57)	6 (86)	3 (50)
SAE	0	0	0	1 (10)	0	0	0
TRAE	0	1 (14)	2 (29)	2 (20)	2 (29)	1 (14)	1 (17)
Related AE leading to withdrawal from treatment	0	0	0	0	0	0	0
Related AE leading to dose modification/ interruption	0	0	0	0	0	0	0
Grade 3–4 AE	0	0	0	0	0	0	0
AE with fatal outcome	0	0	0	0	0	0	0

Note: The exact length of the placebo-controlled period varied for each cohort (range = 19.1–26.9 weeks). A 12-week datacut was therefore chosen to allow comparison between the risdiplam and placebo groups. Data cutoff: 9 January 2019.

Abbreviations: AE, adverse event; SAE, serious AE; TRAE, treatment-related AE.

PK and SMN protein

The observed PK data for patients in Group A at the initially assigned risdiplam doses of 0.02 and 0.05 mg/kg were lower than expected. Therefore, the dose was increased by the IMC to 0.15 mg/kg to reach the predefined target exposure of approximately 700 ng·h/ml. The actual observed median exposure at 0.15 mg/kg was 822 ng·h/ml. The median estimated risdiplam AUC at 5 mg and 0.25 mg/kg was 1610 and 1450 ng·h/ml, respectively.

A dose-dependent increase in blood SMN protein was observed after 4 weeks of treatment. In Group A, a decrease of 7% (range = −29% to 38%) was observed in patients on placebo compared with increases of 9% (range = −19% to 47%), 51% (range = −3% to 129%), 67% (range = 20%–87%), and 96% (range = 17%–150%) in patients treated with 0.02, 0.05, 0.15, and 0.25 mg/kg of risdiplam, respectively. In Group B, placebo patients showed a median 4% decrease (range = −21% to 15%) compared with increases of 125% (range = 44%–152%) for 3 mg risdiplam and 151% (range = 49%–251%) for 5 mg. The observed twofold increase in SMN protein at the highest dose was maintained over 24 months, whereas there was no change over time in untreated patients in the NatHis-SMA study (Figure 2).

Risdiplam treatment at 5 mg and 0.25 mg/kg in Part 1 achieved the desired twofold increase in SMN protein in blood. PK simulations predicted a mean AUC of ≤2000 ng·h/ml for the dosing regimen of once-daily 5 mg for patients with a body weight ≥20 kg and 0.25 mg/ kg for patients with a body weight <20 kg. Therefore, this dosing regimen was selected as the pivotal dose of risdiplam to be tested in Part 2.

Exploratory efficacy measures

No efficacy evaluation was possible over the short 12-week double-blind treatment period; the focus for the dose-finding period was safety, PK, and PD data.

Patients showed improvement or stabilization in MFM32, RULM, and HFMSE total scores at Month 24 of risdiplam treatment. Greater improvements in motor function were observed in younger patients (Table 4). Patients achieved a mean increase from baseline of 2.7 (95% confidence interval [CI] = 1.2–4.2) in MFM32 total score ($n = 44$, excludes patients who performed the 20-item MFM [MFM20] at any time point), with an increase of 3.7 points (95% CI = 1.7–5.7) in patients aged 2–11 years and 1.5 points (95% CI = −0.9 to 3.9) in patients aged 12–25 years. Patients achieved a mean increase of 2.5 points (95% CI = 1.5–3.4) in RULM total score ($N = 51$), with an increase of 2.9 points (95% CI = 1.5–4.3) in patients aged 2–11 years and 1.7 points (95% CI = 0.6–2.9) in patients aged 12–25 years. Patients achieved an overall increase of 0.6 points (95% CI = −0.6 to 1.8) in HFMSE total score ($N = 51$), with an increase of 1.4 points (95% CI = −0.1 to 2.9) in patients aged 2–11 years and a decrease of 0.7 points (95% CI = 2.9–1.5) in patients aged 12–25 years (Table 4).

At Month 24, a marked improvement ≥3 points from baseline in MFM32 total score was achieved in 66.7% (95% CI = 44.7–84.4) of patients aged 2–11 years and 47.4% (95% CI = 24.5–71.1) of patients aged 12–25 years. A marked improvement ≥2 points from baseline in HFMSE total score was achieved in 45.2% (95% CI = 27.3–64.0) of patients aged 2–11 years and 26.3% (95% CI = 9.2–51.2) of patients aged 12–25 years. A marked improvement ≥2 points from baseline

TABLE 3 AEs and SAEs that occurred during the all-exposure-to-risdiplam period

	Risdiplam, Months 0–12, N = 51[a]	Risdiplam, Months 12–24, N = 5[b]	All risdiplam, N = 51[c]
Total number of AEs	397	250	737
Patients with at least one AE, n (%)	47 (92)	34 (67)	49 (96)
Patients with at least one SAE, n (%)	6 (12)	6 (12)	15 (29)
Most frequently reported AEs, n (%)[d]			
Pyrexia	15 (29)	16 (31)	28 (55)
Cough	14 (28)	10 (20)	18 (35)
Vomiting	12 (24)	7 (14)	17 (33)
Upper respiratory tract infection	8 (16)	11 (22)	16 (31)
Nasopharyngitis	9 (18)	6 (12)	12 (24)
Oropharyngeal pain	9 (18)	2 (4)	11 (22)
Gastroenteritis	3 (6)	5 (10)	9 (18)
Headache	8 (16)	6 (12)	9 (18)
Most frequently reported SAEs, n (%)[e]			
Pneumonia	1 (2)	2 (4)	3 (6)
Femur fracture	0	0	2 (4)
Total number of deaths	0	0	0
Patients with at least one, n (%)			
TRAE	10 (20)	2 (4)	12 (24)
Related AE leading to withdrawal from treatment	0	0	0
Related AE leading to dose modification/ interruption	0	0	0
SAE leading to withdrawal from treatment	0	0	0
SAE leading to dose modification/ interruption	1 (2)	1 (2)	3 (6)
Treatment-related SAE	0	0	0
Grade 3–4 AE	5 (10)	3 (6)	9 (18)
AE with fatal outcome	0	0	0

Note: Data cutoff: 15 January 2020.

Abbreviations: AE, adverse event; SAE, serious AE; TRAE, treatment-related AE.

[a]Only includes AEs with onset date on or after the first dose of risdiplam at any dose level within the first 12 months of the study.

[b]Includes AEs with onset date from Month 12 to Month 24.

[c]Includes AEs with onset date on or after the first dose of risdiplam at any dose level in the all-exposure-to-risdiplam period.

[d]Events occurred in >15% of patients in the all-exposure-to-risdiplam period.

[e]Events occurred in >5% of patients in the all-exposure-to-risdiplam period.
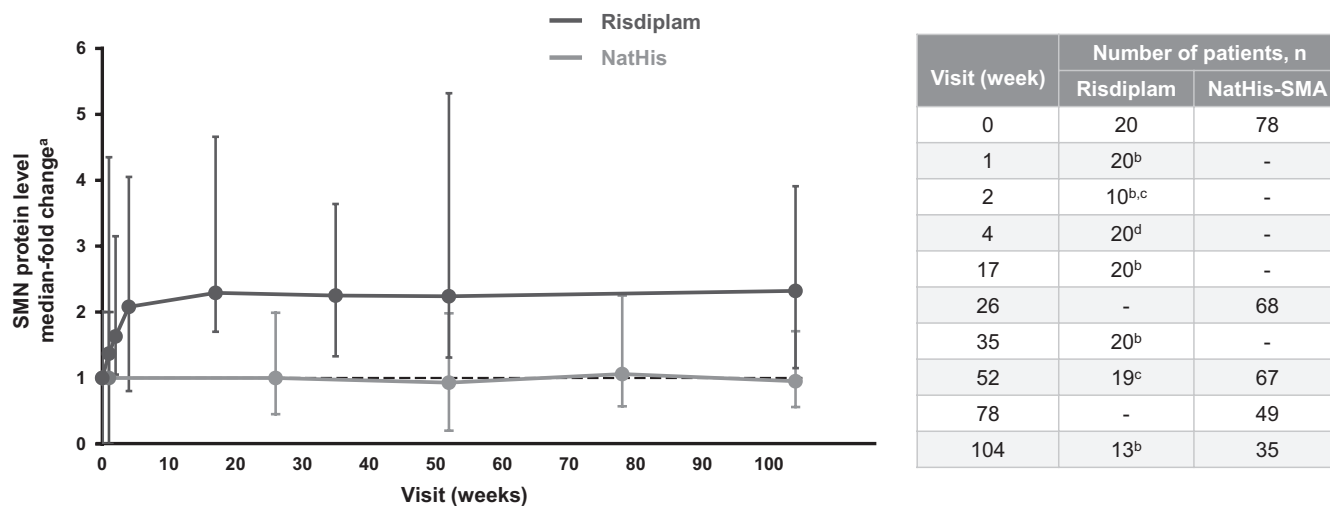
FIGURE 2 SMN protein (change from baseline) over 24 months of risdiplam treatment at the pivotal dose. [a]Data for patients who received the pivotal dose for SUNFISH Part 2 (5 mg for patients with a body weight ≥20 kg or 0.25 mg/kg for patients weighing <20 kg). Data cutoff: 15 January 2020. NatHis-SMA methods described in Chabanon, et al [6]. [b]Samples were taken prior to risdiplam dose. [c]Patients aged 12–25 years only due to limitations on the volume of blood that could be obtained from the 2- to 11-year-old population. [d]Samples were taken 4 h after receiving risdiplam. Error bars represent minimum–maximum values. NatHis, natural history; SMA, spinal muscular atrophy; SMN, survival of motor neuron.

TABLE 4 MFM32, RULM, and HFMSE change from baseline over 12 and 24 months

	MFM32			RULM			HFMSE		
	2–11 years, n = 24	12–25 years, n = 20	All patients, N = 44[a]	2–11 years, n = 31	12–25 years, n = 20	All patients, N = 51	2–11 years, n = 31	12–25 years, n = 20	All patients, N = 51
Month 12									
Mean	3.47	1.64	2.66	2.13	1.05	1.72	0.84	0.05	0.54
95% CI	1.88–5.06	−0.01 to 3.30	1.53–3.80	0.90–3.36	0.18–1.92	0.90–2.54	−0.62 to 2.29	−1.61 to 1.72	−0.53 to 1.61
Month 24									
Mean	3.69	1.54	2.74	2.94	1.74	2.48	1.39	−0.68	0.60
95% CI	1.72–5.66	−0.87 to 3.94	1.24–4.24	1.53–4.34	0.59–2.88	1.52–3.44	−0.11 to 2.89	2.87–1.50	−0.63 to 1.83

Note: Data cutoff: 15 January 2020.

Abbreviations: CI, confidence interval; HFMSE, Hammersmith Functional Motor Scale–Expanded; MFM, Motor Function Measure; RULM, Revised Upper Limb Module.

[a]Excludes patients who were assessed with the MFM20 (n = 7).

in RULM total score was achieved in 58.1% (95% CI = 39.1–75.5) of patients aged 2–11 years and 57.9% (95% CI = 33.5–79.8) of patients aged 12–25 years.

There was a least-square mean increase from baseline of 2.0 (95% CI = 0.3–3.7) in MFM total score in the SUNFISH Part 1 population compared with a decrease of 2.0 (95% CI = −3.4 to −0.3) in the external comparator, resulting in a treatment difference of 3.99 at Month 24 (95% CI = 2.34–5.65, p <0.0001; Figure 3). At Month 24, a greater proportion of patients treated with risdiplam achieved a marked improvement ≥3 points in MFM total score relative to the comparator (54.2% vs. 16.8%, p = 0.0015). A greater proportion of patients treated with risdiplam stabilized or improved in motor function relative to the comparator (defined as a change from baseline in MFM32 total score ≥0; 81.3% vs. 44.4%, p = 0.0016; Figure S2).

There were no clinically significant changes in respiratory function over 24 months (Table 5).

DISCUSSION

The objective of SUNFISH Part 1 was to assess the safety, tolerability, PK, and PD of risdiplam in patients with SMA for the first time, and to select the pivotal dose for Part 2, which was determined to
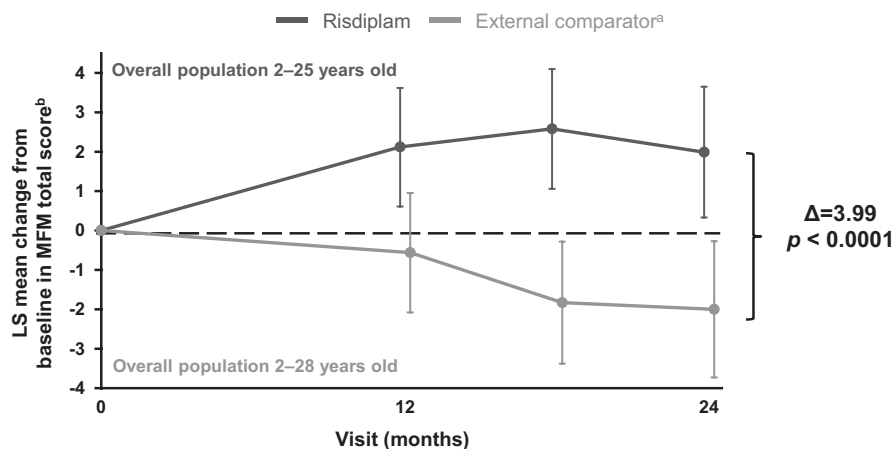
FIGURE 3 Motor Function Measure (MFM) total score change over 24 months compared with an external comparator. [a]External comparator data are comprised of data from Annoussamy et al [5], Bertini et al [23], and unpublished analyses of olesoxime phase 2 clinical trial data. [b]Error bars show ± 95% confidence interval. Weighted analysis of change from baseline is shown (mixed model repeated measures). Patients with baseline and at least one postbaseline time point at Month 12 or Month 24 with MFM total score are included in the analysis. MFM (derived) total score means that the MFM20 total score is used for all patients aged <6 years and the MFM32 total score is used for all patients aged ≥6 years. Both scales were transformed to 0%–100%. SUNFISH data cutoff: 15 January 2020. LS, least square.

TABLE 5 Change from baseline in percentage predicted values in respiratory measures at Month 24

Respiratory measures[a]	2–11 years, n = 31	12–25 years, n = 20	All patients, N = 51
FVC, mean (95% CI)	−6.42 (−16.29 to 3.46)	0.28 (−3.22 to 3.78)	−2.40 (−6.69 to 1.89)
FEV1, mean (95% CI)	−8.83 (−18.18 to 0.51)	1.17 (−2.33 to 4.67)	−2.83 (−7.19 to 1.52)
PCF, mean (95% CI)	6.25 (−6.34 to 18.84)	0.58 (−4.44 to 5.61)	3.42 (−2.93 to 9.77)
SNIP, mean (95% CI)	6.28 (1.22–11.33)	3.26 (−1.93 to 8.46)	5.08 (1.50–8.66)

Note: Data cutoff: 15 January 2020.

Abbreviations: CI, confidence interval; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; PCF, peak cough flow; SNIP, sniff nasal inspiratory pressure.

[a]Calculated as percentage predicted value.

be 5 mg for patients with a body weight ≥20 kg and 0.25 mg/kg for patients with a body weight <20 kg. This dosing regimen has now been approved for use in patients with SMA [14, 15].

In Part 1, there have been no treatment-related safety findings leading to withdrawal in any patients treated with risdiplam for up to 31.9 months. There was no difference in safety findings for all assessed dose levels.

Nonclinical findings in animal studies (at exposures higher than in SUNFISH) were not observed in patients receiving risdiplam [13]. Retinal findings observed in a nonclinical toxicology study in monkeys [13] have not been observed in any risdiplam-treated patient to date. Haematological nonclinical findings of risdiplam-associated bone marrow depression were not observed in any individuals [13]. Reported skin disorders were not suggestive of epithelial effects observed in nonclinical studies [13]. Preclinical studies in sexually mature rats and monkeys have shown spermatogenic arrest in certain stages of spermatogenesis, without evidence of damage to spermatogonia [13]. Although this effect was observed in nonresponder species used in toxicology studies for risdiplam, SMN protein deficiency itself appears to impair spermatogenesis in models of SMA [24]. Studies in rats and rabbits have shown adverse effects on foetal

development such as embryofoetal mortality in offspring [25]. The effect of risdiplam on male fertility and foetal development was not assessed in this study.

The natural history of types 2 and 3 SMA involves progression of disease and continued loss of function [5, 26, 27]. In natural history cohorts, MFM32 scores declined significantly by an average of 3.03 (SD = 3.77) over 24 months [5]. RULM scores decreased by an average of 0.41 (SD = 2.93) over 12 months; improvements were mainly seen in children aged <5 years [27]. HFMSE scores decreased by an average of 0.54 (95% CI = −1.45 to 0.36) over 24 months [26].

Although the focus of this study was on safety, PK, and PD for dose selection, exploratory efficacy analyses demonstrated improvements in MFM32 and RULM total scores and stabilization in HFMSE total scores after 24 months of treatment. Due to the lack of a placebo group for 24 months, MFM total score was compared with a weighted external comparator; there was a statistically significant and clinically relevant difference of 3.99 points between risdiplam and the comparator. Three points is considered a marked improvement and represents gaining a new function or improvement in several functions, suggesting that there was a meaningful improvement in motor function after 24 months of treatment [28].

Previous natural history studies have shown a decrease in HFMSE scores over 24 months, highlighting the importance of stabilization in motor function [26]. Patient surveys have shown that stabilization is an important treatment goal for patients with SMA [29] and is considered to be a positive outcome in this population. In line with other studies that have shown an inverse correlation between disease duration and efficacy [1, 30], younger patients showed greater improvements in all exploratory efficacy endpoints. Due to the absence of a control group, these results must be interpreted with caution. The results in SUNFISH cannot be compared with CHERISH, a double-blind, placebo-controlled study of nusinersen, as CHERISH had stricter inclusion/exclusion criteria that included patients aged 2–12 years who were able to sit independently but never had the ability to walk independently [1].

Limitations of this study include the small sample size and the lack of 24-month placebo data. As the aim of Part 1 was to select the dose of risdiplam and assess PK, PD, and safety, the study was not designed for efficacy evaluation. The weighted external comparator was therefore constructed to provide context to the exploratory MFM results at Month 24. To minimize bias, more than one external control group was included in the analysis, and selected control groups were as similar as possible to the SUNFISH population.

SUNFISH Part 1 demonstrated a positive safety profile in patients with types 2 and 3 SMA, further supporting the study of risdiplam in this population. Treatment resulted in sustained increase of blood SMN protein levels. Patients in Part 1 entered a 3-year extension period for regular monitoring of safety, tolerability, and efficacy. The long-term efficacy of risdiplam is being assessed in patients in Part 2.

AUTHOR CONTRIBUTIONS

methodology (equal); project administration (supporting); resources (supporting); software (supporting); supervision (equal); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Carmen Martin:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (equal); project administration (supporting); resources (supporting); software (supporting); supervision (equal); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Tammy McIver:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (equal); methodology (equal); project administration (supporting); resources (supporting); software (supporting); supervision (equal); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Renata S. Scalco:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (equal); project administration (supporting); resources (supporting); software (supporting); supervision (equal); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Wai Yin Yeung:** Conceptualization (equal); data curation (supporting); formal analysis (lead); funding acquisition (lead); investigation (supporting); methodology (equal); project administration (supporting); resources (supporting); software (supporting); supervision (equal); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Laurent Servais:** Conceptualization (equal); data curation (supporting); formal analysis (supporting); investigation (lead); methodology (equal); project administration (supporting); supervision (lead); validation (supporting); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal).

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CONFLICT OF INTEREST

E.M. reports nonfinancial support from F. Hoffmann-La Roche during the conduct of the study; and personal fees from F. Hoffmann-La Roche, Biogen, Novartis, and Scholar Rock outside the submitted work. G.B. reports consulting fees and payment/honoraria from F. Hoffmann-La Roche, Biogen, and Novartis Gene Therapies. He has served on safety monitoring boards (SABs)/advisory boards for and received equipment from F. Hoffmann-La Roche. O.B.-T. reports nonfinancial support from Minoryx outside the submitted work. L.D.W. reports nonfinancial support and personal fees from F. Hoffmann-La Roche during the conduct of the study; and personal fees and grants from Novartis and Biogen outside the submitted work. N.G. has served on SABs/advisory boards for F. Hoffmann-La Roche and Novartis. She has received grants from Biogen and payment/honoraria from Biogen and F. Hoffmann-La Roche. J.K. reports grants from F. Hoffmann-La Roche during the conduct of the study; and grants and personal fees from F. Hoffmann-La Roche, Biogen, and Novartis, and personal fees from Scholar Rock and Pfizer outside the submitted work. R.M. reports nonfinancial support from F. Hoffmann-La Roche during the conduct of the study. He also reports personal fees from F. Hoffmann-La Roche, Novartis Gene Therapies, and Biogen outside the submitted work. E.S.M. reports that she has served on advisory boards for F. Hoffmann-La Roche. A.P. reports grants/research support from Biogen and Novartis Gene Therapies and has served on an advisory board for Novartis Gene Therapies. M.C.P. has received payment for presentations from F. Hoffmann-La Roche, and support for attending meetings and/or travel from F. Hoffmann-La Roche and Biogen. C.V. has received grants, consulting fees, honoraria, and support for attending meetings and/or travel from and has served on advisory boards for F. Hoffmann-La Roche, Biogen, and Novartis Gene Therapies. S.B.-W., M.G., K.G., J.H., H.K., C.M., T.M., R.S.S., and W.Y.Y. report that they are current employees and stockholders in F. Hoffmann-La Roche. L.S. reports investigator-initiated trial, consultancy, and lecture fees from F. Hoffmann-La Roche, Biogen, and Novartis, and consultancy fees from Scholar Rock.

DATA AVAILABILITY STATEMENT

For eligible studies, qualified researchers may request access to individual patient level clinical data through a data request platform. At the time of writing this request platform is Vivli https://vivli.org/ourmember/roche/. For up-to-date details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see: go.roche.com/date_sharing Anonymized records for individual patients across more than one data source external to Roche cannot, and should not, be linked due to a potential increase in risk of patient re-identification.

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REFERENCES

1. Mercuri E, Darras BT, Chiriboga CA, et al. Nusinersen versus sham control in later-onset spinal muscular atrophy. *N Engl J Med.* 2018;378:625-635.
2. Lefebvre S, Burglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell.* 1995;80(1):155-165.
3. Singh RN, Howell MD, Ottesen EW, Singh NN. Diverse role of survival motor neuron protein. *Biochim Biophys Acta Gene Regul Mech.* 2017;1860(3):299-315.
4. Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc Natl Acad Sci USA.* 1999;96(11):6307-6311.

5. Annoussamy M, Seferian A, Daron A, et al. Natural history of type 2 and 3 spinal muscular atrophy: 2-year NatHis-SMA study. *Ann Clin Transl Neurol*. 2021;8(2):359-373.

6. Chabanon A, Seferian AM, Daron A, et al. Prospective and longitudinal natural history study of patients with type 2 and 3 spinal muscular atrophy: baseline data NatHis-SMA study. *PLoS One*. 2018;13(7):e0201004.

7. Mercuri E, Bertini E, Iannaccone ST. Childhood spinal muscular atrophy: controversies and challenges. *Lancet Neurol*. 2012;11(5):443-452.

8. Mercuri E, Lucibello S, Pera MC, et al. Long-term progression in type II spinal muscular atrophy: a retrospective observational study. *Neurology*. 2019;93(13):e1241-e1247.

9. Biogen Inc. SPINRAZA® (nusinersen) US prescribing information; 2016. Accessed November, 2021. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/209531lbl.pdf

10. Biogen Inc. SPINRAZA® (nusinersen) EMA prescribing information; 2017. Accessed November, 2021. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/004312/WC500229704.pdf

11. AveXis Inc. ZOLGENSMA® (onasemnogene abeparvovec-xioi) US prescribing information; 2019. Accessed November, 2021. https://www.fda.gov/media/126109/download

12. European Medicines Agency. ZOLGENSMA® (onasemnogene abeparvovec-xioi); 2020. Acrcessed November, 2021. https://www.ema.europa.eu/en/medicines/human/EPAR/zolgensma

13. Ratni H, Ebeling M, Baird J, et al. Discovery of risdiplam, a selective survival of motor neuron-2 *(SMN2)* gene splicing modifier for the treatment of spinal muscular atrophy (SMA). *J Med Chem*. 2018;61(15):6501-6517.

14. Evrysdi (risdiplam). US prescribing information; 2021. Accessed November, 2021. https://www.gene.com/download/pdf/evrysdi_prescribing.pdf

15. European Medicines Agency. EVRYSDI™ summary of product characteristics; 2021. Accessed November, 2021. https://www.ema.europa.eu/en/documents/product-information/evrysdi-epar-product-information_en.pdf

16. Baranello G, Darras BT, Day JW, et al. Risdiplam in type 1 spinal muscular atrophy. *N Engl J Med*. 2021;384(10):915-923.

17. Darras BT, Masson R, Mazurkiewicz-Bełdzińska M, et al. Risdiplam-treated infants with type 1 spinal muscular atrophy versus historical controls. *N Engl J Med*. 2021;385(5):427-435.

18. Mercuri E, Deconinck N, Mazzone E, et al. Safety and efficacy of once-daily risdiplam in type 2 and non-ambulant type 3 spinal muscular atrophy: a phase 3, double-blind, randomised, placebo-controlled trial. *Lancet Neurol*. 2021;21(1):42-52.

19. ClinicalTrials.gov. NCT02908685: A study to investigate the safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of RO7034067 in Type 2 and 3 spinal muscular atrophy participants (SUNFISH); 2020. Accessed July, 2021. https://clinicaltrials.gov/ct2/show/NCT02908685

20. Sturm S, Gunther A, Jaber B, et al. A phase 1 healthy male volunteer single escalating dose study of the pharmacokinetics and pharmacodynamics of risdiplam (RG7916, RO7034067), a *SMN2* splicing modifier. *Br J Clin Pharmacol*. 2018;85(1):181-193.

21. Ratni H, Karp GM, Weetall M, et al. Specific correction of alternative survival motor neuron 2 splicing by small molecules: discovery of a potential novel medicine to treat spinal muscular atrophy. *J Med Chem*. 2016;59(13):6086-6100.

22. Muntoni F, Bertini E, Comi G, et al. Long-term follow-up of patients with type 2 and non-ambulant type 3 spinal muscular atrophy (SMA) treated with olesoxime in the OLEOS trial. *Neuromuscul Disord*. 2020;30(12):959-969.

23. Bertini E, Dessaud E, Mercuri E, et al. Safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 spinal muscular atrophy: a randomized, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol*. 2017;16(7):513-522.

24. Ottesen EW, Howell MD, Singh NN, Seo J, Whitley EM, Singh RN. Severe impairment of male reproductive organ development in a low SMN expressing mouse model of spinal muscular atrophy. *Sci Rep*. 2016;6:20193.

25. Genentech. EVRYSDI (risdiplam) US prescribing information; 2020. Accessed July, 2021. https://www.gene.com/download/pdf/evrysdi_prescribing.pdf

26. Kaufmann P, McDermott MP, Darras BT, et al. Prospective cohort study of spinal muscular atrophy types 2 and 3. *Neurology*. 2012;79(18):1889-1897.

27. Pera MC, Coratti G, Mazzone ES, et al. Revised upper limb module for spinal muscular atrophy: 12 month changes. *Muscle Nerve*. 2019;59(4):426-430.

28. Duong T, Braid J, Staunton H, et al. Understanding the relationship between the 32-item motor function measure and daily activities from an individual with spinal muscular atrophy and their caregivers' perspective: a two-part study. *BMC Neurol*. 2021;21(1):143.

29. Gusset N, Stalens C, Stumpe E, et al. Understanding European patient expectations towards current therapeutic development in spinal muscular atrophy. *Neuromuscul Disord*. 2021;31(5):419-430.

30. Dangouloff T, Servais L. Clinical evidence supporting early treatment of patients with spinal muscular atrophy: current perspectives. *Ther Clin Risk Manage*. 2019;15:1153-1161.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.