Safety and efficacy of risdiplam in patients with Type 1 spinal muscular atrophy (FIREFISH part 2): secondary analyses from an open-label trial

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Word count: Abstract (words 534); Manuscript (3846 words)

Research in context panel:

Evidence before this study

We searched PubMed, on May 15th, 2022, for primary manuscripts on spinal muscular atrophy (SMA) using the search term 'spinal muscular atrophy'. We identified clinical trials using the search terms ‘nusinersen’ OR ‘onasemnogene abeparvovec’ OR ‘risdiplam’ in ‘Type 1 SMA’. The search was unbounded by year or language.

Our initial search identified twelve publications that evaluated the safety, efficacy, or both, of the above-mentioned therapies in infants with Type 1 SMA. One manuscript was published in Japanese and removed from our search. We manually searched the remaining studies for those conducting a follow-up visit after treatment initiation for either safety or efficacy endpoints: five publications reported on clinical efficacy and safety assessments following nusinersen treatment, over 6 months, 12 months, 24 months (one paper each), and two publications with follow-up over 3 years from treatment initiation. Four publications reported on onasemnogene abeparvovec treatment: one paper assessed motor function over 12 months from treatment initiation, two papers evaluated clinical efficacy and safety until 18 months of age at study visit or early termination, and one paper evaluated safety and efficacy assessments after a maximum follow-up of 6·2 years from treatment initiation.

Lastly, we identified two papers on FIREFISH (NCT02913482) that reported on risdiplam treatment: one publication presented safety and dose-finding data over 12 months (FIREFISH part 1) and one publication reported safety and clinical efficacy assessments over 12 months (the primary results from FIREFISH part 2).

Results from FIREFISH part 2 demonstrated efficacy and safety of risdiplam in infants with Type 1 SMA after 12 months of treatment. The primary endpoint of the FIREFISH study, the proportion of infants in part 2 sitting without support for ≥5 s at Month 12, was met (as assessed by item 22 of the Bayley Scales of Infant and Toddler Development, third edition gross motor subscale), and demonstrated a clinically meaningful benefit of risdiplam to infants with Type 1 SMA. The majority of infants in this study were alive, without permanent ventilation, and were able to feed orally after 12 months of risdiplam treatment. Furthermore, infants achieved clinically meaningful motor milestones and showed improvements in motor function compared with natural history cohorts.

Added value of this study

In this manuscript, we provide evidence for the safety and efficacy of risdiplam over 24 months of treatment in infants with Type 1 SMA. We found that infants continued to improve in motor functions (as assessed by the Bayley Scales of Infant and Toddler Development, third edition gross motor subscale, the Hammersmith Infant Neurological Examination, Section 2 and the Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders scale), and the majority of infants were alive without permanent ventilation. Most patients maintained the ability to swallow and feed orally from baseline to Month 24. Furthermore, the efficacy results observed at Month 12 were maintained at Month 24. To the best of our knowledge, FIREFISH is the only study of an approved orally administered treatment that demonstrated efficacy and safety in the most vulnerable group of patients with SMA – infants with Type 1.

Implications of all the evidence

Overall, the efficacy and safety of risdiplam treatment over 24 months in the FIREFISH study indicate that infants continued to benefit from treatment and demonstrated levels of motor function and motor development that deviate from the natural history cohorts of untreated infants with Type 1 SMA. The FIREFISH open-label extension phase will provide further evidence regarding long-term safety and efficacy over an additional 3 years of risdiplam treatment in infants with Type 1 SMA.

Background

Risdiplam is the first orally administered therapy that modifies the pre-mRNA splicing of the survival of motor neuron 2 (*SMN2*) gene approved for the treatment of spinal muscular atrophy (SMA). Risdiplam is distributed both systemically and in the central nervous system. FIREFISH (NCT02913482) is a multicentre, open-label, two-part study of risdiplam in infants with Type 1 SMA. The primary endpoint of FIREFISH part 2 demonstrated that infants attained the ability to sit without support for ≥5 s after 12 months of treatment. This study reports on the safety and efficacy of risdiplam in infants with Type 1 SMA over 24 months of treatment.

Methods

FIREFISH was conducted in 14 hospitals in ten countries across Europe, North and South America, and Asia. Eligible infants were aged 1–7 months at enrolment, with a genetically confirmed diagnosis of SMA, and two *SMN2* gene copies. Risdiplam was orally administered once daily at 0·2 mg/kg for infants ≥5 months and <2 years of age. Once an infant reached 2 years of age the dose was increased to 0.25 mg/kg. Infants <5 months old started at 0.04 or 0.08 mg/kg, and this starting dose was adjusted to 0.2 mg/kg as soon as possible once pharmacokinetic data was available for each infant. Herein we present the remainder of the secondary efficacy endpoints that were included in the statistical hierarchy at Month 24, these were: the ability to sit without support for ≥30 s, stand alone, and walk alone, as assessed by the Bayley Scales of Infant and Toddler Development, third edition gross motor subscale. Secondary endpoints in the statistical hierarchy at Month 24 were compared with a performance criterion of 5% that was defined based on the natural history of Type 1 SMA; the results were considered statistically significant if the lower limit of the two-sided 90% confidence interval (CI) was above the 5% threshold. FIREFISH is ongoing.

Findings

Forty-one infants were enrolled in FIREFISH part 2 between March 13, 2018 and November 19, 2018. After 24 months of treatment, 38 infants were ongoing in the study and 18 infants (44%, 90% CI 31–58) were able to sit without support for ≥30 s ($p<0·0001$), compared with the performance criterion derived from the natural history of untreated infants with Type 1 SMA. No infants could stand alone (0%, 90% CI 0–7) or walk alone (0%, 90% CI 0–7) after 24 months of treatment ($p=1·0$, both), compared with the performance criteria based on the natural history of Type 1 SMA. The most common serious adverse events were pneumonia in 16 infants (39%) and respiratory distress in three infants (7%).

Interpretation

Treatment with risdiplam over 24 months resulted in continual improvements in motor function and achievement of developmental motor milestones. The FIREFISH open-label extension phase will provide additional evidence regarding long-term safety and efficacy of risdiplam.

Funding

F. Hoffmann-La Roche Ltd.

Introduction

Spinal muscular atrophy (SMA) is a neuromuscular disease caused by reduced levels of the survival of motor neuron (SMN) protein due to mutations in the *SMN1* gene.[1,2] Individuals with SMA retain at least one copy of the paralogous gene *SMN2* which produces low levels of functional SMN protein that are insufficient to fully compensate for the loss of *SMN1*.[3,4]

Type 1 SMA is a common and severe form of SMA (approximately 50%–70% of cases) with symptoms occurring by 6 months of age.[2] Untreated infants are unable to sit without support and have reduced life expectancy.[5,6] Most infants fail to achieve almost any motor milestones,[7] and experience progressive motor function decline, along with a decline in respiratory and swallowing functions.[5,8] To date, the Food and Drug Administration and the European Commission have approved three disease-modifying treatments with published clinical efficacy and safety assessments over at least 12 months: nusinersen, an intrathecally dosed *SMN2*-targeting antisense oligonucleotide;[9,10–15] onasemnogene abeparvovec, an intravenously administered adeno-associated virus vector-based gene replacement therapy;[16–21] and risdiplam, an orally administered small molecule.[22–25] Risdiplam selectively modifies splicing of *SMN2* pre-mRNA to increase the levels of functional SMN protein through promoting inclusion of exon 7 into the mRNA transcript, and is approved for the treatment of patients with SMA of all ages (US) or patients aged ≥2 months with a clinical diagnosis of Type 1, 2, or 3 SMA or with 1–4 *SMN2* copies (EU).[22,23]

The disease course of SMA is changing as disease-modifying therapies are becoming more widely available, with treated individuals living longer and demonstrating improved functional abilities (motor, respiratory, bulbar).[13,21,24] However, these treatments are not curative; patients can continue to experience motor disability and exhibit downstream consequences of reduced respiratory function, bulbar dysfunction, and speech impairment.[26,27] Long-term studies into the efficacy and safety of disease-modifying therapies are still warranted, whilst the efficacy and safety of combinations of approved disease-modifying therapies are still under investigation.[28-31]

FIREFISH (NCT02913482) is an ongoing, multicentre, open-label, two-part study of risdiplam in infants with Type 1 SMA. Part 1 assessed the safety, tolerability, pharmacokinetics, and pharmacodynamics of risdiplam at different dose levels.[25] In part 1, risdiplam treatment led to an increase in functional SMN protein in the blood. Part 2 assessed the efficacy and safety of risdiplam at the dose selected in part 1.[24] The primary endpoint, the proportion of infants able to sit without support for ≥5s after 12 months of treatment, as assessed by item 22 of the Bayley Scales of Infant and Toddler Development, third edition (BSID-III) gross motor subscale, was met by 12 (29%) infants; the percentage was significantly higher than the performance criterion of 5% defined based on natural history data ($p<0{\cdot}0001$); this motor milestone is never attained by untreated infants with Type 1 SMA. Here, we present safety and efficacy results of risdiplam treatment after 24 months for part 2 (clinical cut-off date [CCOD]: 12 November 2020).

Methods

Study design and participants

FIREFISH (NCT02913482) is an ongoing, multicentre, open-label, two-part study of risdiplam in infants with Type 1 SMA compared with untreated historical controls (part 2). In FREFISH part 2 infants were enrolled at 14 hospitals in ten countries across Europe, North and South America, and Asia. The clinical trial was conducted in accordance with the principles of the "Declaration of Helsinki", following Good Clinical Practice guidelines and was approved by an ethics committee at each site. Written informed consent was provided by the infant's legally authorised representative

at screening. All authors attest to adherence to the protocol, accuracy of analysis and complete reporting of adverse events (AEs). After dose selection in part 1, an external independent data monitoring committee reviewed safety data from both FIREFISH parts 1 and 2 on an ongoing basis.

Eligible infants were aged 1–7 months, with a genetically confirmed diagnosis of SMA, two *SMN2* gene copies, and a clinical profile consistent with Type 1 SMA, with onset of symptoms between 28 days and 3 months of age (inclusive). At the time of screening, infants were required to have received adequate nutrition and hydration (with or without gastrostomy). Infants were excluded if they required invasive ventilation or awake non-invasive ventilation, if they had experienced awake hypoxemia (oxygen saturation <95%) with or without ventilatory support, required tracheostomy, or had received concomitant or previous treatment with an *SMN2*-targeting antisense oligonucleotide, other *SMN2* splicing modifier, or gene therapy. Full inclusion and exclusion criteria can be found in the **appendix pp. 6**. Patients' demographic and clinical characteristics at baseline have been published previously.[24,25] Copies of the study protocol and statistical analysis plan are included in the **appendix pp. 27 and pp 155**.

Study procedures

As determined in part 1, risdiplam was orally administered once daily at 0·2 mg/kg for infants aged ≥5 months and <2 years of age. Infants <5 months of age started treatment at 0·04 or 0·08 mg/kg and the dose was adjusted to 0·2 mg/kg following review of initial pharmacokinetic data. The dose was increased to 0·25 mg/kg once an infant reached 2 years of age.[24,25] Risdiplam was administered with an oral syringe or through a feeding tube. Efficacy and safety assessments were conducted following the study protocol and schedule of assessments. Briefly, during the first 24 months of treatment, the following study assessments were performed on the days with site visits starting on week 1 (pre-risdiplam treatment): the CHOP-INTEND, respiratory plethysmography, level of respiratory support and nutritional checks were performed every 2 months (8/9 weeks), BSID-III, HINE-2 and compound muscle action potential, and the Infant/ toddler quality of life questionnaire – short form 47 item version every 4 months (17/18 weeks) and the swallowing assessment every 6 months (26 weeks). Laboratory assessments were scheduled every 4 months after Week 17 (Month 4); ECG, vital sign assessments and physical examinations (including anthropometric measurements) were performed every 2 months (8/9 weeks) and ophthalmology assessments were performed every 2 or 6 months. AEs and serious AEs (SAEs) were monitored through the entire study (screening through open-label extension or the study completion/early withdrawal visit and follow-up); see **appendix pp. 10** for the relevant methodology.

Outcomes

The primary endpoint of part 2 was the proportion of infants sitting without support for ≥5s at Month 12 (as assessed by item 22 of the BSID-III gross motor subscale).[24] The secondary endpoints in the statistical hierarchy at Month 12 were:[24] the proportion of infants who achieve a score ≥40 on the CHOP-INTEND (the scale ranges from 0–64, the higher the score the better the motor function); the proportion of infants who achieve a ≥4-point increase in CHOP-INTEND score from baseline; the proportion of motor milestone responders as assessed by the HINE-2 scale (**see appendix pp. 11** for the definition of motor milestone responder); proportion of infants who are alive without permanent ventilation (event-free survival).

Secondary endpoints included in the statistical hierarchy at Month 24 were assessed using selected items from the BSID-III gross motor subscale as follows: proportion of infants sitting without support for ≥30s (item 26), proportion of infants standing alone (item 40), and proportion of infants walking alone (item 42). These motor outcomes are clinically relevant for infants with Type 1 SMA and were pre-specified in the statistical analysis plan.

Secondary endpoints not included in the statistical hierarchy at Month 24 were not adjusted for multiplicity, and thus no definitive conclusions can be drawn for these endpoints. The endpoints were: proportion of infants who achieve head control (defined as a score ≥3 for item 12 of the CHOP-INTEND); change from baseline in the total raw score of the BSID-III gross motor subscale; achievement of motor milestones as measured by the HINE-2 (milestones include head control, sitting, voluntary grasp, ability to kick, rolling, crawling, standing, and walking); proportion of motor milestone responders as assessed by the HINE-2 (see **appendix pp. 11** for definition); proportion of infants who are sitting without support for ≥5 s, as assessed by the BSID-III gross motor subscale; proportion of infants who are alive; proportion of infants who are alive without permanent ventilation (event-free survival); proportion of infants who are without permanent ventilation; proportion of infants who do not require invasive or non-invasive respiratory support; proportion of infants able to feed orally; and highest motor milestone achieved as assessed by six items of the BSID-III gross motor subscale. The BSID-III endpoint of the highest motor milestone achieved by an infant during the 24 months of treatment was calculated, per protocol, from among the following six milestones: head control (item 9 'controls head while upright for 15s'), rolling (item 14 'rolls from side to back'), sitting without support for 5s (item 22), crawling (item 30 'crawls on stomach'), standing (item 40 'stands alone'), and walking (item 42 'walks alone').
Safety assessments were incidence and severity of AEs, laboratory values, electrocardiogram (ECG), vital signs (body temperature, systolic and diastolic blood pressure, pulse rate, and respiratory rate), ophthalmological, physical and anthropometric examinations. SMN protein levels were measured for every patient from venous blood samples.

Additional methodological details (including statistical methodology, information on the hierarchical endpoint analysis, a full list of safety assessments, the study protocol and the statistical analysis plan, as well as the SMN protein data and the results of the exploratory efficacy endpoints at Month 24) are available in the **appendix pp. 8**.

Statistical analysis

To assess the efficacy of risdiplam treatment, a performance criterion was defined for the primary endpoint based on the well-established natural history of Type 1 SMA. For secondary endpoints at Month 12 included in the statistical hierarchy, performance criteria were based on the upper limit of the 90% confidence intervals (CIs) from the historical data on individuals who met each endpoint. CIs were calculated using the Clopper-Pearson method for the BSID-III, CHOP-INTEND and HINE-2 endpoints, and the complementary log-log transformation for the proportion of infants alive without permanent ventilation. For secondary endpoints at Month 24 included in the statistical hierarchy, performance criteria were based on the well-defined natural history of Type 1 SMA. Details of the pre-defined performance criteria are available in the **appendix table S4 pp. 21**. The study protocol and statistical analysis plan pre-specified the use of 90% CIs for proportions and to match the one-sided statistical tests employed for the hypotheses testing. Hypothesis testing was performed for endpoints with a pre-defined performance criterion. An exact binomial test was performed for the BSID-III, CHOP-INTEND and HINE-2 endpoints, and a z-test conducted for event-free survival.

The proportion of infants who were event free at Month 24 was estimated using Kaplan–Meier methodology. For the other endpoints, infants were classified as non-responders if they were unable to achieve a response, had not maintained a response achieved earlier at the time of the assessment, had withdrawn from the study, had died, or were missing an assessment at a visit. Missing scores or instances recorded as 'cannot test' for items on the CHOP-INTEND, BSID-III, and HINE-2 were assigned a score of 0.

Because the same enrolment criteria, safety and efficacy assessments, schedule, and dosing regimen were used, exploratory post-hoc safety and efficacy (at Month 12 and Month 24) analyses were

conducted with pooled data from the part 1 (high-dose cohort, n=17) and part 2 (N=41) populations. No formal hypothesis testing was performed for the pooled populations.

Role of the funding source

The funder of the study (F. Hoffmann-La Roche) provided study drug, study management, medical monitoring, drug safety management and analysis, data management, and statistical analysis. Some employees of F. Hoffmann-La Roche (Ksenija Gorni, Heidemarie Kletzl, and Paulo Fontoura) contributed to study conception and design. F. Hoffmann-La Roche had no role in data collection, which was performed by the clinical staff at each study site. All authors were involved in data interpretation, including employees of F. Hoffmann-La Roche. Medical writing and editorial support were funded by F. Hoffmann-La Roche.

Results

41 infants (n=22 [54%] female and n=19 [46%] male) were enrolled in FIREFISH part 2 between March 13, 2018 –and November 19, 2018 (**figure 1**). The majority of infants were White (22/41, 54%) or Asian (14/41, 34%); race was reported as unknown for five infants (12%). Five infants (12%) were of Hispanic or Latino ethnicity.

The median age at enrolment was 5·3 months (interquartile range [IQR]: 4·2–6·8) and the median disease duration (i.e., time between onset of symptoms and first treatment) was 3·4 months (IQR: 2·5–4·9). Median baseline CHOP-INTEND total score (22·0, IQR: 15·0–28·0) and HINE-2 score (1·0, IQR: 0·0–1·0) were low, as expected for this population. Most infants (n=35, 85%) fed orally at baseline, including infants who fed exclusively orally (n=33, 80%) and those who fed orally in combination with a feeding tube (n=2, 5%).

The primary and secondary endpoints included in the statistical hierarchy and assessed at Month 12 were met (p<0·0001 for all endpoints; **table 1**), and previously reported. The first secondary endpoint included in the statistical hierarchy at Month 24 was met, with 18 infants (44%, 90% CI 31–58) able to sit without support for ≥30s. This was significantly higher than the 5% performance criterion (p<0·0001). No infants (0%, 90% CI 0–7) could stand alone or walk alone after 24 months of treatment; these milestones were not statistically different from the pre-defined performance criterion of 5% (p=1·0).

At Month 24, further improvements were reported for the majority of endpoints included in the statistical hierarchy at Month 12 (**table 1**). An increase was observed in both the proportion of infants achieving sitting without support for ≥5s and of infants achieving a CHOP-INTEND score ≥40 points. The proportion of infants who achieved an increase of ≥4 points from baseline on the CHOP-INTEND was maintained at Month 24. Overall, three more infants were HINE-2 motor milestone responders at Month 24 versus Month 12 (**table 1**). Additionally, over 24 months of risdiplam treatment, the infants showed continued improvement in their mean change from baseline in CHOP-INTEND score (**appendix figure S1 pp. 22)**.

Up to the current CCOD, one additional infant required permanent ventilation after Month 12 (**figure 2**). Therefore, at Month 24, a total of 34 of 41 infants (83%, 90% CI 71–90) were event free versus 35 infants (85%, 90% CI 73–92) at Month 12 (**table 1**).

Similarly, a greater proportion of infants showed improvements in the other secondary endpoints at Month 24 versus Month 12 (not included in the statistical hierarchy). Seven more infants achieved head control (score ≥3 on item 12 of the CHOP-INTEND) at Month 24 (**table 2**).

Although no infants could walk or stand independently at Month 24, more infants achieved a higher motor milestone category in the HINE-2 compared with Month 12. For example, for the standing milestone, more infants achieved 'standing with support' (n=6 [15%] vs 2 [5%] at Month 12) (**figure 3**); for the walking milestone, one infant (2%) achieved 'cruising', while no infant achieved this milestone at Month 12 (**figure 3**). Furthermore, more infants were recorded as able to achieve the highest motor milestone category. For instance, more infants were able to 'pivot (rotate)' as recorded within the sitting milestone (n=12 [29%] vs 4 [10%] at Month 12), more infants were able to 'roll from supine to prone' (n=18 [44%] vs 4 [10%] at Month 12) (**figure 3**); two infants (5%) were recorded for the crawling milestone as able to 'crawl on their hands and knees' at Month 24 (**appendix figure S2 pp. 23**) while no infant attained this milestone at Month 12.
Following 24 months of risdiplam treatment, 38 of 41 infants (93%, 90% CI 82–97) were alive. Furthermore, 35 infants (85%, 90% CI 73–93) were able to feed orally at Month 24 versus 34 infants (83%, 90% CI 70–92) at Month 12. Moreover, at Month 24, eight infants (20%, 90% CI 10–33) did not require ventilatory support and 37 infants (90%, 90% CI 78–95) were without permanent ventilation compared with ten infants (24%, 90% CI 14–38) and 38 infants (92%, 90% CI 81–97) at Month 12, respectively (**table 1**).

The median blood SMN protein concentration at Month 24 was 4·76 ng per millilitre (IQR: 4·11–5·62) with a median 1·95-fold change (IQR: 1·33–2·26) from baseline (**appendix figure S3 pp. 25**).

Up to the CCOD, a total of 356 AEs were reported in part 2 **(table 2**). A full list of AEs and SAEs can be found in **appendix table S2 pp. 16**. The most frequently reported AE was upper respiratory tract infection in 22 infants (54%). A total of 28 infants (68%) experienced 68 SAEs; the most frequently reported SAE was pneumonia in 16 infants (39%).
Seven infants (17%) experienced at least one AE that was considered to be related to risdiplam treatment by the Investigator. Treatment-related AEs included: rash maculo-papular, skin discolouration, and constipation, each in two infants (5%); eosinophilia, neutropenia, upper respiratory tract infection, decreased neutrophil count, and pulmonary hypertension each in one infant (2%). No infants left the study due to drug-related AEs.

One infant had an SAE (pneumonia event), unrelated to the study treatment, that required dose interruption. The incidence of SAE (pneumonia) per patient-year (PY) declined approximately three-fold between the first and second 12-month periods (from 38·85 events/100PY to 13·14 events/100PY).

A review of all available safety laboratory results, vital signs, ECGs, and ophthalmological assessments did not show any clinically significant adverse findings. No risdiplam-associated retinal/skin events observed in preclinical studies were observed in any patients up to the CCOD.[32,33] Results from the post-hoc pooled safety and efficacy analyses at Month 24 of FIREFISH part 1 (high-dose cohort) and part 2 are presented in **appendix pp. 13**.

Discussion

FIREFISH part 2 is an open-label study of risdiplam in infants with Type 1 SMA over a treatment period of 24 months (followed by a 36-month extension period). The primary analysis was performed after 12 months, and the primary endpoint was met.[24] The first secondary endpoint in the statistical hierarchy at Month 24, the proportion of infants sitting without support for ≥30s, was markedly different from the pre-defined performance criterion of 5% based on natural history data. Without treatment, children with Type 1 SMA are never able to sit without support,[7,34] and thus the ability to achieve sitting is an important motor milestone in treated Type 1 SMA.

All infants who met the primary endpoint at Month 12 continued to do so at Month 24. By Month 24, 13 more infants were able to sit without support for ≥5s and 11 more infants were able to sit

without support for ≥30s. Additionally, three more infants were classified as having a motor milestone response in the HINE-2 and eight more infants achieved a CHOP-INTEND score ≥40 points. The continuous benefit of risdiplam treatment is also reflected in the changes from baseline in the CHOP-INTEND total score and BSID-III gross motor subscale total score, demonstrating that motor ability continues to progress over 24 months. These findings demonstrate clinically meaningful gains in motor function and show a clear deviation from natural history data, where achievement of major motor milestones and a CHOP-INTEND score ≥40 points is rarely observed.[5,8]

After 24 months of treatment, infants showed continued improvement in motor function and in attaining motor milestones, demonstrating a continuum of developmental gains from Month 12. Despite this progress, no infants achieved independent standing or walking, as assessed by the BSID-III gross motor subscale**.** This may be related to the age at disease onset, age at treatment initiation (treatment initiated one day after enrolment; median age at enrolment: 5.3 months [IQR]: 4·2–6·8), and disease severity when patients started treatment (median disease duration [defined as time from symptom onset to first dose]: 3·4 months [IQR: 2·5–4·9]). Longer treatment might lead to achievement of some or part of these milestones; a possibility supported by the greater proportion of infants achieving higher responses in the sitting, standing, and walking categories in the HINE-2 at Month 24 versus Month 12.

Most infants maintained the ability to swallow and feed orally after 24 months of risdiplam treatment. This is markedly different from the results of the US Paediatric Neuromuscular Clinical Research Network natural history study, where infants with Type 1 SMA typically required nutritional support or combined ventilatory and feeding support by 11 months of age.[8] Event-free survival time was greatly improved in infants treated with risdiplam compared with natural history. In FIREFISH part 2, three infants experienced fatal respiratory complications characteristic of Type 1 SMA which occurred early in the study (within the first 3 months of treatment). Between the CCOD of the primary analysis and this CCOD there have been no additional deaths, and only one additional infant required permanent ventilation between Month 12 and Month 24.

Most AEs reported up to the CCOD (12 November 2020) were consistent with results from the previous CCODs for part 1 and part 2 of the study. No risdiplam-related AEs led to withdrawal or discontinuation of treatment. The SAE incidence rate of pneumonia declined in the second year of treatment. Ophthalmological monitoring did not reveal any findings suggestive of risdiplam effects previously observed in the preclinical study.[32] SMN protein levels were stable over time and were consistent with the results reported for the FIREFISH part 1 study,[25] and at Month 12 in FIREFISH part 2.[24]

Based on non-clinical studies in pubertal and adult rats and monkeys, male sperm cell division may be arrested while on treatment thus possibly affecting male fertility.[32] These effects are expected to be reversible upon discontinuation of treatment.[32] To date, there is no clinical evidence suggesting that risdiplam causes male fertility issues in humans.[22, 23]

Post-hoc analysis of the pooled efficacy results including data from the dose-finding part 1 of the study were consistent with the results from FIREFISH part 2 (**see appendix table S1 pp. 14**), demonstrating that in a larger cohort of infants with Type 1 SMA, prolonged treatment with risdiplam was associated with a clinically meaningful improvement in survival, motor function, and developmental milestones compared with natural history.

Despite the COVID-19 pandemic, at-home oral treatment with risdiplam was unaffected. The impact of the pandemic on the study was small and occurred due to hospital and/or pandemic-imposed movement restrictions which resulted in patients missing scheduled study assessments. Despite this, the reported deviations did not affect the conclusions and interpretation of the safety data or cumulative study results. All infants had an on-site visit at Month 24.

There were some limitations to this study, particularly the use of natural history data to derive performance criteria for achieving the clinical endpoints. Specifically, these were: differences in baseline characteristics between the natural history and FIREFISH cohorts, the relatively small sample size of historical cohorts, and the potential for unconscious selection bias associated with the use of historical cohorts. Despite these limitations, it is noteworthy that the primary and secondary endpoints are based on objective assessments and the results are clearly differentiated from available natural history data.

Treatment with risdiplam over 24 months in the FIREFISH part 2 study resulted in extended survival, continued improvements in motor function, and achievement of motor milestones. These findings demonstrate meaningful gains in motor function from Month 12, confirming that longer-term treatment with risdiplam benefited patients with Type 1 SMA.

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Contributors

KR, KG, HK, PF, and BTD contributed to the study conception and design. Data were collected by RM, MMB, KR, LS, HX, EZ, GB, CB, JWD, ND, AK, EM, DV, YW, BTD. Analysis and interpretation were performed by all authors. MEK, BJ and RM accessed and verified the data. All authors had full access to all of the study data on request. All authors reviewed and edited drafts of the manuscript and approved the final submitted manuscript. All authors attest to the integrity of the data and had final responsibility for the decision to submit the manuscript. Ana Rondelli, PhD, of Nucleus Global, wrote the first draft of the manuscript based on an outline agreed by all authors and provided medical writing assistance with subsequent drafts. Medical editorial support was provided by Megan Speakman of Nucleus Global.

Declaration of interests

RM has received consulting fees from Biogen, F Hoffmann-La Roche, and Novartis Gene Therapies, and speaker honoraria from the last two companies named. RM reports that he received travel/ meeting attendance support and fees for serving on advisory boards from F Hoffmann-La Roche, Novartis Gene Therapies, and Biogen. MMB has received speaker honoraria and payment for serving on advisory boards for F Hoffmann-La Roche, Sanofi, Novartis, Biogen, and UCB. KR reports she has

received consulting fees from F Hoffmann-La Roche, Biogen, and Novartis; she has received support from Biogen for attending meetings; she has received speaker honoraria and has served on advisory boards for F Hoffmann-La Roche and Biogen. LS reports grants from F Hoffmann-La Roche, Biogen, and Novartis, and consultancy fees from F Hoffmann-La Roche, Biogen, Novartis, BioHaven Pharmaceuticals, and Scholar Rock. He has received speaker fees from and served on advisory boards for F Hoffmann-La Roche, Biogen, and Novartis. He reports he holds the position of secretary at the World Muscle Society. HX declared no competing interests. EZ reports grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPEP), The Brazilian National Council for Scientific and Technological development (CNPq), Sarepta Therapeutics, F Hoffmann-La Roche, Biogen, and Novartis. He has received consulting fees from F Hoffmann-La Roche, Novartis, Biogen, Sanofi, Astellas, and Sarepta Therapeutics. EZ received speaker honoraria from and served on advisory boards for F Hoffmann-La Roche, Novartis, Biogen, Sanofi, Astellas. He has received travel and meeting attendance support from F Hoffmann-La Roche, Novartis, Biogen, Sanofi, and Sarepta Therapeutics. GB reports that he has received consulting fees and speaker honoraria from Biogen, F Hoffmann-La Roche, and Novartis Gene Therapy. GB has received fees for serving on advisory boards and has received equipment for indirect calorimetry to University College London from F Hoffmann-La Roche. CB reports he has received grants or contracts from Biogen, Novartis, and F Hoffmann-La Roche. He has served on advisory boards for Sarepta Therapeutics, Novartis, and Biogen; he has received support from Sarepta Therapeutics and Biogen for attending meetings. JWD has received research grants from Biogen, Cytokinetics, Ionis Pharmaceuticals, Novartis Gene Therapies, F Hoffmann-La Roche, and Scholar Rock. He reports that he has received consulting fees from Shift Therapeutics and that he served on advisory boards for Biogen, Cytokinetics, Epirium Bio; Ionis Pharmaceuticals, Novartis Gene Therapies, F Hoffmann-La Roche /Genentech, and Scholar Rock. ND reports he has received support from Hoffmann-La Roche for provision of FIREFISH and SUNFISH clinical trials study materials, paid to the institution (Ghent University Hospital [US Gent]). He reports that he has received consulting fees for the SUNFISH trial advisory board, has received support for attending meetings and/or travel, and for serving on advisory boards from F Hoffmann-La Roche. AK has received consulting fees for serving on advisory boards for AveXis, Novartis Gene Therapies, Biogen and Hoffmann-La Roche. She has received speaker honoraria from F Hoffmann-La Roche and Biogen. AK reports that The Swiss-Reg-NMD receives unconditional financial support from PTC Therapeutics, Sarepta Therapeutics, Pfizer, and F Hoffmann-La Roche and research grants from Novartis Gene Therapies, Biogen, and the Swiss Foundation for Research on Muscle Diseases (FSRMM). EM has received grants or contracts from Biogen. He has received speaker honoraria for lectures from Biogen, F Hoffmann-La Roche, Novartis, AveXis. EM reports he has served on advisory boards for Biogen, F Hoffmann-La Roche, Scholar Rock, Novartis, AveXis, and Cytokinetics. DV reports grants from PTC Therapeutics, F Hoffmann-La Roche, Novartis, Biogen, NS Pharma, Sarepta Therapeutics, and Pfizer. He has received consulting fees from F Hoffmann-La Roche, Novartis, AveXis, and Biogen; he has received speaker honoraria for lectures from PTC Therapeutics, F Hoffmann-La Roche, Janssen and Novartis. DV has served on advisory boards for AveXis, Biogen, Novartis, and F Hoffmann-La Roche. YW reports she has received payment from F Hoffmann-La Roche for the FIREFISH part 2 trial to support the study according to agreement and has received grants or contracts from UCB and Biogen for clinical trial support. BJ reports she has received grants or contracts, support for attending meetings and/or travel, and holds royalties/ licenses from F Hoffmann-La Roche. HK has received grants or contracts and support for attending meetings and/or travel from F Hoffmann-La Roche. BD reports grants from F Hoffmann-La Roche during the conduct of the study; grants from PTC Therapeutics, Fibrogen, AveXis, Genentech, Ionis Pharmaceuticals, Biogen, and Summit. He reports research support from NIH/NINDA, Slaney Family Fun for SMA, Spinal Muscular Atrophy Foundation, and Working on Walking Fund. He served on advisory boards

for AveXis, Vertex, Genentech, F Hoffmann-La Roche, Sanofi/Genzyme, and Biogen; he reports his role as a member of the DSMB for Amicus Inc, outside of the submitted work. AD, MEK, KG, BJ, HK, EG, PF report that they are current employees and stockholders in F Hoffmann-La Roche

Data sharing

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 here: https://go.roche.com/data_sharing. Anonymised 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.

Acknowledgements

The authors would like to thank the Spinal Muscular Atrophy Foundation and PTC Therapeutics for their collaboration and all of the patients and families who participated in the risdiplam program, the patient groups, and the clinical trial sites and staff around the world for their ongoing partnership and support. The authors would also like to thank Lutz Mueller for his expert advice on the manuscript.

The authors thank Ana Rondelli, PhD, of Nucleus Global for providing medical writing support and Megan Speakman of Nucleus Global for providing medical editing support, which was funded by F. Hoffmann-La Roche Ltd, Basel, Switzerland in accordance with Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3). The FIREFISH study was funded by F. Hoffmann-La Roche Ltd and was conducted in collaboration with the SMA Foundation and PTC Therapeutics. The funder of the study (F. Hoffmann-La Roche) provided study drug, study management, medical monitoring, drug safety management and analysis, data management, and statistical analysis.

Figures and Tables

Table 1 Baseline characteristics

	Risdiplam (N=41)
Age at enrolment — months, median (IQR)	5.3 (4·2–6·8)
Gender — no. (%)	
Female	22 (54)
Male	19 (46)
Race — no. (%)	
Asian	14 (34)
White	22 (54)
Unknown	5 (12)
Ethnicity — no. (%)	
Hispanic or Latino	5 (12)
Not Hispanic or Latino	36 (88)
Age at onset of symptoms — months, median (IQR)	1.5 (1·0–2·0)
Disease duration — months, median (IQR)*	3.4 (2·5–4·9)
≤3 months, no. (%)	14 (34)
>3 months, no. (%)	27 (66)
CHOP-INTEND score — median (IQR)[†]	22.0 (15·0–28·0)
HINE-2 score — median (IQR)[†]	1.0 (0·0–1·0)
Able to swallow — no. (%)	39 (95)[‡]
Able to feed orally at baseline	35 (85)
Feeding Route — no. (%)	
Fed exclusively orally	33 (80)
Fed exclusively via a feeding tube	4 (10)
Fed via a combination oral and feeding tube	2 (5)
No pulmonary care — no. (%)[§]	29 (71)

*The time between onset of symptoms and first treatment. [†]All infants had undergone the CHOP-INTEND and HINE-2 assessments at baseline. No infants were missing any items with the CHOP-INTEND baseline assessment. One item, for the baseline HINE-2 score (walking item, which would be expected to be 0) was missing for one infant and was imputed to 0. [‡]One infant was fed exclusively via tube at baseline due to inadequate weight gain, the ability to swallow had not been assessed following enrolment into the study. [§]Defined as no ventilation support or airway clearance.

CHOP-INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; HINE-2, Hammersmith Infant Neurological Examination, Section 2; IQR, interquartile range.

Table 2 Primary and secondary efficacy endpoints at Month 12 and Month 24 from FIREFISH part 2

Endpoint	Month 12 All infants (N=41) n (%) [90% CI]*	Performance criterion (%)	p value†	Month 24 All infants (N=41) n (%) [90% CI]‡	Performance criterion (%)	p value†
Primary endpoint:						
Sitting without support for ≥5 secs§	12 (29) [18–43]	5	<0·0001	25 (61) [47–74]	..	..
Secondary endpoints:						
CHOP-INTEND						
Score ≥40	23 (56) [42–69]	17	<0·0001	31 (76) [62–86]	..	..
Increase of ≥4 points from baseline	37 (90) [79–97]	17	<0·0001	37 (90) [79–97]	..	..
HINE-2 motor milestone responder‖	32 (78) [65–88]	12	<0·0001	35 (85) [73–93]	..	..
Event-free survival¶	35 (85) [73–92]	42	<0·0001	34 (83) [71–90]	..	..
Sitting without support for ≥30 secs**	7 (17) [8–30]	..	..	18 (44) [31–58]	5	<0·0001
Standing alone††	0 [0–7]	..	..	0 [0–7]	5	1§§
Walking alone‡‡	0 [0–7]	..	..	0 [0–7]	5	1§§
Secondary endpoints not in the statistical hierarchy at Month 12 and Month 24:						
Head control (item 12 of the CHOP-INTEND)‖‖	22 (54) [40–67]	..	..	29 (71) [57, 82]	..	..
Change from baseline in the total raw score of the BSID-III gross motor subscale; median	7·0 (IQR: 2·0–11·0)¶¶	..	..	14·5 (IQR: 8·0–18·0)¶¶	..	..
Highest motor milestone achieved out of six motor milestones assessed by the BSID-III gross motor subscale***			..		..	..
Controls head upright for 15 seconds (item 9)	0†††	..	..	0‡‡‡	..	..
Rolls from side to back (item 14)	23 (56)†††	..	..	12 (29)‡‡‡	..	..
Sits without support for ≥5 seconds (item 22)	12 (29)	..	..	25 (61)	..	..
Crawls on stomach (item 30)§§§	0	..	..	0	..	..
Stands alone (item 40)	0	..	..	0	..	..
Walks alone (item 42)	0	..	..	0	..	..
Alive‖‖‖	38 (93) [82–97]	..	..	38 (93) [82–97]	..	..
Without permanent ventilation‖‖‖	38 (92) [81–97]	..	..	37 (90) [78–95]	..	..
Without invasive or non-invasive respiratory support	10 (24) [14–38]	..	..	8 (20) [10–33]	..	..
Able to feed orally	34 (83) [70–92]¶¶¶	..	..	35 (85) [73–93]****	..	..

*CCOD Month 12: 14 November 2019. †p values are for the comparison of the proportion of infants with the performance criterion from historical data for each endpoint. Hypothesis testing was performed hierarchically, at one-sided 5% significance level per endpoint, if p≤0·05 for previous endpoints in the hierarchy. Where data are presented without a p value, the outcomes were not part of the statistical hierarchy at the respective timepoint (Month 12 or Month 24); CHOP INTEND secondary endpoints that were part of the statistical hierarchy at month 12 were also not prespecified for analysis at month 24. For a list of endpoints for which a performance criterion was defined together with the resources used please refer to **appendix table S4 pp. 21**. ‡CCOD Month 24: 12 November 2020. §As assessed by item 22 of the BSID-III gross motor subscale. ‖Infants were classed as a responder if more motor milestones showed improvement than showed worsening. Improvement was defined as a ≥2-point increase in ability to kick (or maximal score) or a ≥1-point increase in head control, rolling, sitting, crawling, standing, or walking. Worsening was defined as a ≥2-point decrease in ability to kick (or lowest score) or a ≥1-point decrease in head control, rolling, sitting, crawling, standing, or walking. ¶Defined as alive with no permanent ventilation (i.e. no tracheostomy or BiPAP for ≥16 hours per day

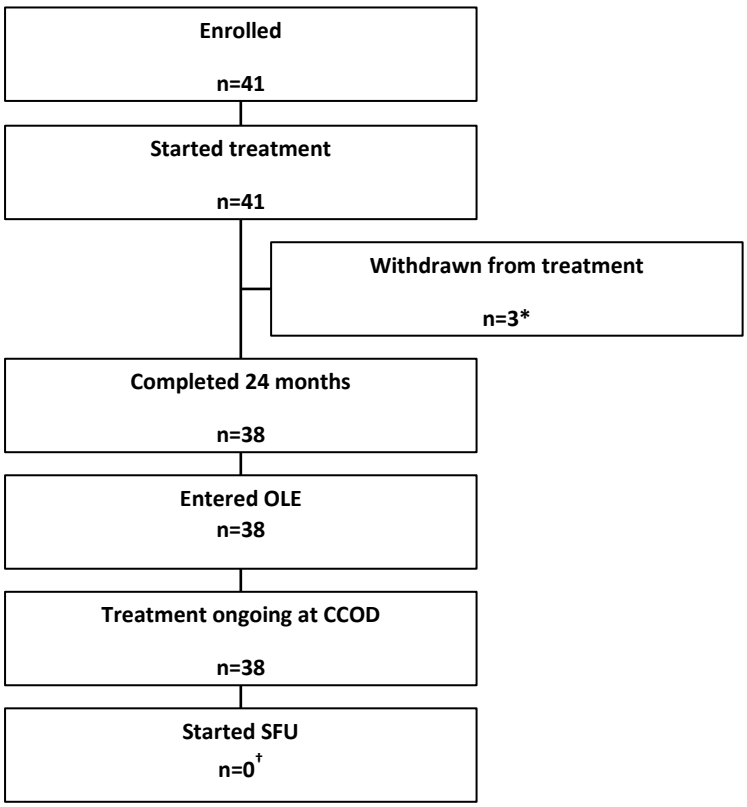
continuously for >21 consecutive days or continuous intubation for >21 consecutive days, in the absence of, or following the resolution of, an acute reversible event). The proportion of infants alive without permanent ventilation was estimated using Kaplan-Meier methodology. **As assessed by item 26 of the BSID-III gross motor subscale. ††As assessed by item 40 of the BSID-III gross motor subscale. ‡‡As assessed by item 42 of the BSID-III gross motor subscale. §§p value was not significant; the hierarchy was broken at the standing endpoint. ||||Defined as a score ≥3, patients maintain head upright for >15 seconds while sitting with trunk erect and support at the shoulders.
¶¶n=38 infants with data for this endpoint. ***Six infants (15%) did not achieve any of the six milestones by Month 12 and four infants (10%) did not achieve any of the six milestones by Month 24. However, because this endpoint includes only the six items, it does not reflect the overall highest milestones achieved by infants on the BSID-III gross motor subscale. †††Note, at Month 12, 18 infants (44%) were able to 'control head upright for 15 seconds' (item 9) and 34 infants (83%) were able to 'roll from side to back' (item 14), as assessed by the BSID-III gross motor subscale. ‡‡‡At Month 24, 30 infants (73%) were able to 'control head upright for 15 seconds' (item 9), and 35 infants (85%) were able to 'roll from side to back' (item 14), as assessed by the BSID-III gross motor subscale. §§§The infants who achieved the 'crawls on hands and knees' motor milestone at Month 24 (**appendix figure S2 pp. 23**) were assessed using the HINE-2 scale. This is different from the 'crawling' reported herein, which was assessed using item 30 (crawling on stomach) of the BSID-III gross motor subscale. ||||||The proportion of infants alive and the proportion of infants without permanent ventilation were estimated using Kaplan-Meier methodology. ¶¶¶Includes 68% (28 of 41) of infants who were able to feed exclusively orally, and 15% (6 of 41) who were fed orally in combination with a feeding tube. ****Includes 71% (29 of 41) of infants who were fed exclusively orally, 7% (3 of 41) of infants who were fed exclusively via a feeding tube, and 15% (6 of 41) of infants who were fed orally in combination with a feeding tube.
BiPAP=Bilevel Positive Airway Pressure; BSID-III=Bayley Scales of Infant and Toddler Development, third edition; CCOD=clinical cut-off date; CHOP-INTEND=Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CI=confidence interval; IQR=interquartile range.

Table 2. AEs* in FIREFISH part 2

	All infants (N=41)
Patients with at least one AE, n (%)	41 (100)
Total number of AEs	356
Total number of deaths, n (%)	3 (7)
Total number of patients with at least one AE, n (%)	
AE with fatal outcome†	3 (7)
SAE	28 (68)
SAE leading to withdrawal from treatment	0
SAE leading to dose modification/interruption	1 (2)
Treatment-related SAE	0
AE leading to withdrawal from treatment	0
AE leading to dose modification/interruption	2 (5)
Treatment-related AE	7 (17)
Related AE leading to withdrawal from treatment	0
Related AE leading to dose modification/interruption	0
Grade 3–5 AE	25 (61)
Most common AEs, ≥5 patients, n (%)	
Upper respiratory tract infection	22 (54)
Pneumonia	19 (46)
Pyrexia	18 (44)
Constipation	12 (29)
Nasopharyngitis	7 (17)
Bronchitis	6 (15)
Diarrhoea	6 (15)
Rhinitis	5 (12)
Most common SAEs, ≥2 patients, n (%)	
Pneumonia	16 (39)
Respiratory distress	3 (7)
Other‡	2 (5)

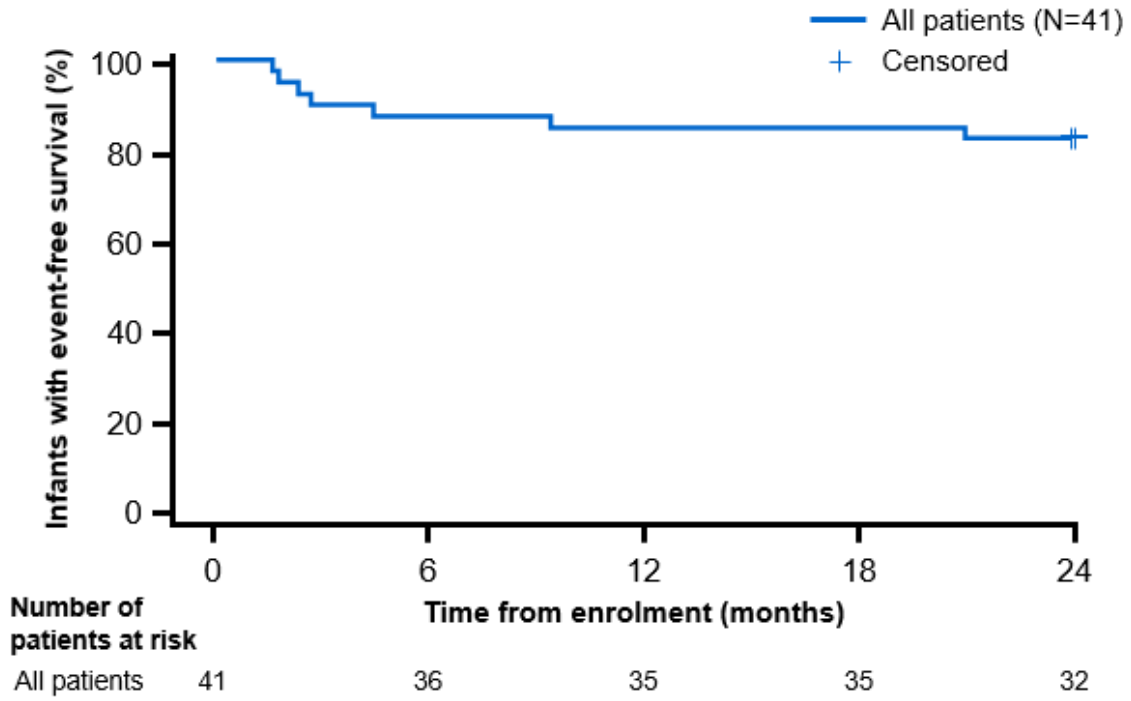
*Safety data up to the CCOD of 12 November 2020. †Fatal events were reported in three infants: (1) Pneumonia with fatal outcome on Study Day 51 in male infant aged 4·4 months at first dose; (2) Acute respiratory failure on Study Day 68 in male infant aged 6·9 months at first dose, related to Type 1 SMA and medical history or concurrent illness (thoracic cage deformity, probably an infection); (3) Pneumonia with fatal outcome on Study Day 79 in male infant aged 4·6 months at first dose. Events reported as unrelated to risdiplam and secondary to SMA-related respiratory complications. ‡Other SAEs include acute respiratory failure, aspiration, bronchiolitis, dehydration, hypotonia, and respiratory failure.
Medical Dictionary for Regulatory Activities (MedDRA 23·1)-preferred terms were used to classify the events. For frequency counts by preferred term, multiple occurrences of the same AE or SAE in an infant are counted once. For the "Total number of events" rows, multiple occurrences of the same AE or SAE in an infant are counted separately. Includes AEs or SAEs with onset from the first dose of study drug up to the CCOD. See **appendix table S2 pp. 16** for a full list of AEs and SAEs. AE=adverse event; CCOD=clinical cut-off date; SAE= serious AE; SMA=spinal muscular atrophy.

Figure 1. Patient disposition



*Three patients died from SMA-related respiratory complications that occurred while on treatment.
†Patients who completed or discontinued from the study were to complete the safety follow-up period. By the CCOD: 12 November 2020, there were no patients who had started safety follow-up.
CCOD=clinical cut-off date; OLE=open-label extension; SFU= safety follow-up; SMA=spinal muscular atrophy.

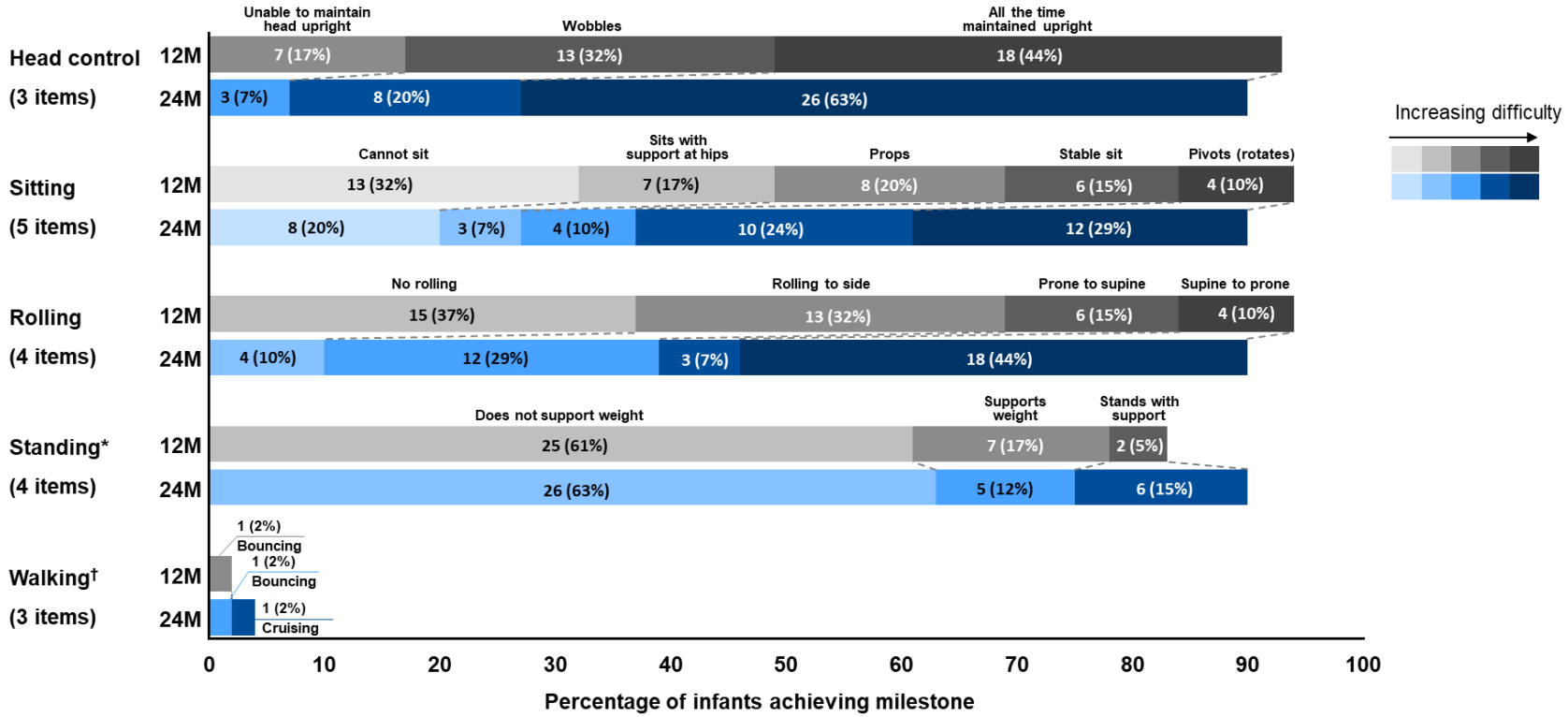
Figure 2. Event-free survival after 24 months of risdiplam treatment, from FIREFISH part 2



Event-free survival, a secondary endpoint in FIREFISH, is defined as alive with no permanent ventilation (i.e. no tracheostomy or BiPAP for ≥16 hours per day continuously for >21 consecutive days or continuous intubation for >21 consecutive days, in the absence of, or following the resolution of, an acute reversible event). Two patients attended the 24-month visit early, and therefore had not yet reached 24 months from enrolment as of the CCOD (12 November 2020), at which point the infants' data were censored (plus sign). The median time to death or permanent ventilation was not estimable as few patients had an event.

BiPAP=Bilevel Positive Airway Pressure; CCOD=clinical cut-off date.

Figure 3. Secondary efficacy endpoint: HINE-2 motor milestones at Month 12 and Month 24



Three infants had died within the first 3 months following enrolment. *For the 'standing' milestone, no infants were recorded as 'standing unaided' at Month 12 and Month 24. †For the walking milestone, no infants achieved 'cruising (walks holding on)' at Month 12 and 'walking independently' at Month 12 and Month 24. One infant for each of the following milestones was recorded as 'cannot test/not done': 'head control', 'sitting', and 'rolling' at Month 24; and for the 'standing' milestone, four infants at Month 12 and one infant at Month 24 were recorded as 'cannot test/not done'. For the 'walking' milestone, 37 (90%) infants at Month 12 and 36 (88%) infants at Month 24, were recorded as 'cannot test/not done'. For each motor milestone category, the values shown are in the format number of motor milestone responders (n) and percentage (%). CCOD: 14 November 2019 (Month 12); CCOD: 12 November 2020 (Month 24). CCOD=clinical cut-off date; HINE-2=Hammersmith Infant Neurological Examination, Section 2.