Safety and efficacy of gene replacement therapy for X-linked myotubular myopathy (ASPIRO): a multinational, open-label, dose-escalation trial

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Summary

Background X-linked myotubular myopathy is a rare, life-threatening, congenital muscle disease observed mostly in males, which is caused by mutations in *MTM1*. No therapies are approved for this disease. We aimed to assess the safety and efficacy of resamirigene bilparvovec, which is an adeno-associated viral vector serotype 8 delivering human *MTM1*.

Methods ASPIRO is an open-label, dose-escalation trial at seven academic medical centres in Canada, France, Germany, and the USA. We included boys younger than 5 years with X-linked myotubular myopathy who required mechanical ventilator support. The trial was initially in two parts. Part 1 was planned as a safety and dose-escalation phase in which participants were randomly allocated (2:1) to either the first dose level ($1·3\times10^{14}$ vector genomes [vg]/kg bodyweight) of resamirigene bilparvovec or delayed treatment, then, for later participants, to either a higher dose ($3·5\times10^{14}$ vg/kg bodyweight) of resamirigene bilparvovec or delayed treatment. Part 2 was intended to confirm the dose selected in part 1. Resamirigene bilparvovec was administered as a single intravenous infusion. An untreated control group comprised boys who participated in a run-in study (INCEPTUS; NCT02704273) or those in the delayed treatment cohort who did not receive any dose. The primary efficacy outcome was the change from baseline to week 24 in hours of daily ventilator support. After three unexpected deaths, dosing at the higher dose was stopped and the two-part feature of the study design was eliminated. Because of changes to the study design during its implementation, analyses were done on an as-treated basis and are deemed exploratory. All treated and control participants were included in the safety analysis. The trial is registered with ClinicalTrials.gov, NCT03199469. Outcomes are reported as of Feb 28, 2022. ASPIRO is currently paused while deaths in dosed participants are investigated.

Findings Between Aug 3, 2017 and June 1, 2021, 30 participants were screened for eligibility, of whom 26 were enrolled; six were allocated to the lower dose, 13 to the higher dose, and seven to delayed treatment. Of the seven children whose treatment was delayed, four later received the higher dose (n=17 total in the higher dose cohort), one received the lower dose (n=7 total in the lower dose cohort), and two received no dose and joined the control group (n=14 total, including 12 children from INCEPTUS). Median age at dosing or enrolment was 12·1 months (IQR 10·0–30·9; range 9·5–49·7) in the lower dose cohort, 31·1 months (16·0–64·7; 6·8–72·7) in the higher dose cohort, and 18·7 months (10·1–31·5; 5·9–39·3) in the control cohort. Median follow-up was 46·1 months (IQR 41·0–49·5; range 2·1–54·7) for lower dose participants, 27·6 months (24·6–29·1; 3·4–41·0) for higher dose participants, and 28·3 months (9·7–46·9; 5·7–32·7) for control participants. At week 24, lower dose participants had an estimated 77·7 percentage point (95% CI 40·22 to 115·24) greater reduction in least squares mean hours per day of ventilator support from baseline versus controls (p=0·0002), and higher dose participants had a 22·8 percentage point (6·15 to 39·37) greater reduction from baseline versus controls (p=0·0077). One participant in the lower dose cohort and three in the higher dose cohort died; at the time of death, all children had cholestatic liver failure following gene therapy (immediate causes of death were sepsis; hepatopathy, severe immune dysfunction, and pseudomonal sepsis; gastrointestinal haemorrhage; and septic shock). Three individuals in the control group died (haemorrhage presumed related to hepatic peliosis; aspiration pneumonia; and cardiopulmonary failure).

Interpretation Most children with X-linked myotubular myopathy who received *MTM1* gene replacement therapy had important improvements in ventilator dependence and motor function, with more than half of dosed participants achieving ventilator independence and some attaining the ability to walk independently. Investigations into the risk for underlying hepatobiliary disease in X-linked myotubular myopathy, and the need for monitoring of liver function before gene replacement therapy, are ongoing.

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Introduction

X-linked myotubular myopathy is a rare, life-threatening congenital myopathy caused by mutations in the *MTM1* gene, which encodes myotubularin. Myotubularin is required for the normal development, maturation, and functioning of skeletal muscle cells.[1] X-linked myotubular myopathy has an estimated incidence of one case in 40 000–50 000 live male births.[2] Approximately 80% of boys with X-linked myotubular myopathy experience severe muscle weakness and hypotonia at birth, leading to respiratory distress and chronic ventilator dependency (ie, typical presentation).[2,3] In around 20% of boys, a less common, milder phenotype, featuring independent ambulation and survival into adulthood independent of ventilatory support, has been reported.[4] In rare cases, female carriers might present with symptoms including progressive muscle weakness, most notably in adulthood.[4] Approximately half of male infants with the typical severe neonatal presentation die before age 18 months.[3,5] Those who survive are highly dependent on permanent (>16 h per day) invasive mechanical ventilation and gastrostomy tube feeding.[3,6,7] Most of these children are non-ambulatory and cannot sit unsupported, and the achievement of motor milestones is absent, transient, or substantially delayed.[7,8] Natural history studies have reported that respiratory function and motor function do not improve in the majority of individuals with X-linked myotubular myopathy who require mechanical ventilator support.[7,9]

At present, no approved therapies are available for X-linked myotubular myopathy. Patient management is mainly supportive, with a multidisciplinary approach focused on maximising functional abilities and minimising medical complications. Other therapeutic strategies are under development, having shown promise in preclinical disease models,[10,11] and tamoxifen is currently

Research in context

Evidence before this study

We searched PubMed for clinical trials, meta-analyses, and systematic reviews describing gene therapy for X-linked myotubular myopathy from Jan 1, 2000 to Aug 3, 2017 (the start date of the ASPIRO study), using the search terms ("X-linked myotubular myopathy" OR "XLMTM" OR "X-linked centronuclear myopathy" OR "myopathies, structural, congenital/drug therapy[MeSH terms]") AND "gene therapy", without language restrictions. No published reports relating to gene therapy or clinical trials of treatment for X-linked myotubular myopathy were identified. Review articles describing X-linked myotubular myopathy (among the broader classification of congenital myopathies) identified a need for treatment options and proposed that monogenic conditions such as congenital myopathies might be candidates for gene therapy. As of Oct 7, 2023, no approved treatments or disease-modifying therapies are available for X-linked myotubular myopathy, and patient management is mainly supportive, requiring a complicated multidisciplinary approach aimed at maximising functional abilities and minimising medical complications. Natural history studies of children with X-linked myotubular myopathy have reported high mortality within the first 2 years of life; extensive dependence on permanent (>16 h per day) invasive mechanical ventilation and gastrostomy tube feeding; and absent, transient, or substantially delayed achievement of motor milestones, such as walking without assistance. In these studies, respiratory and motor function did not improve spontaneously in patients with severe X-linked myotubular myopathy.

Added value of this study

This study is the first trial of a potential disease-modifying therapy (resamirigene bilparvovec, an investigational adeno-associated virus [AAV]-mediated gene replacement therapy delivering *MTM1* complementary DNA to muscle) for X-linked myotubular myopathy. Single administration of *MTM1* gene replacement therapy substantially reduced daily hours of ventilator dependence in participants with severe X-linked myotubular myopathy receiving mechanical ventilator support and resulted in the unexpected outcome of ventilator independence for some participants. Substantial improvements in motor function included acquisition of the ability to sit, stand, and walk independently, an exceedingly rare occurrence in this population. Four participants died after receiving gene therapy, all of whom had cholestatic liver failure at the time of death. This clinical trial revealed a previously unknown proclivity for cholestatic liver disease in some children with X-linked myotubular myopathy, highlighting how investigations into rare disease therapies can unveil new insights into the diseases themselves.

Implications of all the available evidence

The unprecedented findings of ventilator independence and acquisition of motor milestones in ASPIRO should be carefully weighed against the potential for fatal adverse events in the context of a life-threatening disease. The risk–benefit profile observed in this study will provide a foundation for future clinical trials in this patient population. The deaths of four dosed participants with evidence of pre-existing cholestasis highlights the need to better understand the role of hepatobiliary disease in the natural history of X-linked myotubular myopathy and the potential interaction of adeno-associated virus (AAV)-mediated gene therapy in this setting.

being studied in a phase 2 clinical trial.[12,13] Adeno-associated virus (AAV)-mediated gene replacement is another approach, which has shown clinical efficacy in other monogenic diseases such as spinal muscular atrophy and *RPE65*-mediated retinal dystrophy.[14,15]

Resamirigene bilparvovec (also known as AT132) is an investigational AAV serotype 8 (AAV8) vector designed to deliver full-length human *MTM1* complementary DNA to skeletal and cardiac muscle under the control of the muscle-specific desmin promoter and enhancer (appendix 1 p 2). In animal models of X-linked myotubular myopathy, a single administration of AAV vectors expressing *MTM1* complementary DNA early in disease progression led to reversal of the disease phenotype and persistence of treatment effect.[16–19]

The ASPIRO clinical trial aimed to evaluate the safety and efficacy of a single infusion of resamirigene bilparvovec at two dose levels in children with X-linked myotubular myopathy receiving mechanical ventilator support. ASPIRO used a unique study design to accommodate challenges related to conducting clinical trials in rare diseases, including the absence of validated trial endpoints and ethical concerns about masked randomisation of participants to gene therapy (video).

Methods

Study design and participants

ASPIRO was designed as a multinational, open-label, dose-escalation, randomised trial done at seven academic medical centres in Canada, France, Germany (one centre each), and the USA (four centres). The original trial design consisted of two parts: a safety and dose-escalation phase (part 1) and a confirmatory phase (part 2; randomised, delayed-treatment concurrent control design). We planned to use the selected dose identified in part 1 for further study in part 2. The protocol was reviewed and approved by the institutional review boards of all participating institutions. An independent data and safety monitoring committee monitored the integrity and safety of the trial. Events and experience gained during the trial resulted in a series of protocol amendments. An overview of the study and changes to the study design are described in appendix 1 (pp 3–5) and three protocol documents (protocol version 1, protocol version 10, and a summary of protocol amendments; appendix 2). During the design of the study, we conducted patient and family meetings to better understand the humanistic impact of the disease and inform selection of relevant endpoints to measure in a clinical trial.

In the trial, we assessed two dose levels of resamirigene bilparvovec: $1·3\times10^{14}$ vector genomes (vg)/kg bodyweight (lower dose) and $3·5\times10^{14}$ vg/kg bodyweight (higher dose). After selection of the higher dose for part 2 of the study, three participants who received the higher dose died. The first of these deaths resulted in the US Food and Drug Administration (FDA) placing a clinical hold on the trial, on May 7, 2020. On Dec 23, 2020, the FDA lifted this clinical hold and allowed the trial to continue with dosing at the lower dose only, along with increased hepatic monitoring and the use of prophylactic or reactive therapies known to benefit children with chronic or recurring cholestasis due to causes other than X-linked myotubular myopathy or gene therapy. This decision eliminated the two-part feature of the study design and dosing of participants who had been randomly assigned to delayed treatment continued with the lower dose. The subsequent death of a participant who received the lower dose resulted in the FDA issuing a second clinical hold on the trial on Sept 3, 2021, which is still in effect.

See Online for appendix 1

Before ASPIRO, 34 children with X-linked myotubular myopathy were enrolled in a prospective run-in study (INCEPTUS; NCT02704273),[9] which was designed to inform the choice of clinically relevant endpoints to assess treatment efficacy in ASPIRO, as described in appendix 1 (p 4). INCEPTUS participants who met the eligibility criteria for ASPIRO (appendix 1 p 18) could enrol directly from INCEPTUS into ASPIRO. The eligibility criteria for enrolment in ASPIRO comprised boys younger than 5 years or those who participated in the INCEPTUS run-in study with a genetically confirmed diagnosis of X-linked myotubular myopathy and receiving mechanical ventilator support (protocol versions 1–4: mechanical ventilatory support ranging from non-invasive support during sleeping hours only to invasive mechanical ventilation for up to 24 h per day; protocol version 5 and later: invasive mechanical ventilatory support ranging from 20 h to 24 h per day). The age range of children from INCEPTUS (n=22) was 8·4–72·1 months at the time of enrolment in ASPIRO. Detailed inclusion and exclusion criteria are presented in appendix 1 (p 18). Written informed consent was obtained from participants' parents or legal guardians.

See Online for video

An ad-hoc untreated control group comprised retrospective data from boys who participated in INCEPTUS[9] but were not subsequently enrolled in ASPIRO, and participants in the delayed treatment cohort in ASPIRO who did not receive any dose of resamirigene bilparvovec. Given that INCEPTUS was a natural history study, no treatments were administered for X-linked myotubular myopathy and participants only received therapy to manage their care (eg, respiratory ventilation). The follow-up data from INCEPTUS were used as control data.

See Online for appendix 2

Randomisation and masking

The randomisation algorithms and schedules for part 1 and part 2 were programmed and maintained by Rho (Chapel Hill, NC, USA). Full details of the randomisation procedures are in appendix 1 (pp 3–4). Briefly, in the safety and dose-escalation phase (part 1), participants were randomly allocated (2:1) to either the lower dose level ($1·3\times10^{14}$ vg/kg) of resamirigene bilparvovec or delayed treatment, then, for later participants, to either the higher dose ($3·5\times10^{14}$ vg/kg) of resamirigene bilparvovec or delayed treatment. In part 2, the planned

randomisation ratio was 1:1 (active:control). No masking was done because the study was open label.

Procedures

Resamirigene bilparvovec was administered as a single dose by intravenous infusion. As is now common practice with systemically administered AAV therapies,[20] participants received prednisolone (1 mg/kg) once a day beginning 1 day before infusion to mitigate potential immune responses, including hepatic inflammation, which was observed in previous trials of gene therapy with AAV vectors.[21] The first three participants received this dose once a day for 4 weeks, followed by a 4-week taper. This period was extended to 8 weeks with an 8-week taper in the remaining participants, in response to increases in troponin I in one participant at 5 weeks and aminotransferases in another participant at 7 weeks after dosing. The delayed treatment control participants were followed up for 24 weeks before an optimal dose was determined; their eligibility was then confirmed with predose baseline procedures.

Motor skills were assessed using the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND), the Bayley III assessment, and the motor function measurement-20. Motor skills were assessed at baseline and weeks 2, 4, 8, 12, 16, 24, 36, and 48 after dosing (excepting Bayley III at weeks 2 and 8); controls did not have the week 2 and 8 assessments. CHOP INTEND scores range from 0 to 64 points, with higher scores indicating better motor function and the maximum score of 64 achieved by age 3–6 months in healthy children.[22,23] Results of the motor function measurement-20 are not reported herein, as it was only administered to children older than 2 years, making meaningful comparisons difficult. Measurements of maximal inspiratory pressure (MIP) were obtained at individual study sites and sent to a central reader to evaluate respiratory muscle strength, and were assessed at baseline and weeks 2, 4, 12, 16, 24, and 48 after dosing; controls were not assessed at week 2 but had an additional assessment at week 36. Ventilator dependence data were collected by e-diary (ie, very frequent reporting) for control participants and higher dose participants, and collected at discreet site visits for lower dose participants. The Ventilator Weaning and Discontinuation Assessment was added in protocol version 5; thus, prior to that, for lower dose and some higher dose participants, investigators had no standardised algorithm for initiating ventilator weaning.

Open muscle biopsy specimens were obtained at baseline (after randomisation but before 6 days prior to dosing) and were recommended to be taken from the left gastrocnemius, at post-dose week 24 from the right gastrocnemius, and at post-dose week 48 from the vastus lateralis, and processed for histopathological analysis. Muscle biopsies were used to assess the efficacy of resamirigene bilparvovec by measuring vector copy number, *MTM1* mRNA transcripts, myotubularin protein expression, and histopathology. We assessed vector biodistribution in muscle by quantitative PCR, myotubularin mRNA by RNA sequencing, and protein expression by western blot. Immunological assessments included measurement of anti-AAV8 neutralising antibodies (luciferase reporter assay), T-cell response (interferon-γ ELISpot assay), and anti-myotubularin antibodies (electrochemiluminescence assay) in serum, and immunohistochemical stains for inflammatory markers in muscle biopsy specimens. Additional details of these analyses and a summary of study assessments up to week 48 are provided in appendix 1 (pp 6–8, 19–20). The follow-up of dosed participants is ongoing and is planned for 10 years.

Outcomes

The INCEPTUS prospective run-in study[9] was designed to inform the choice of clinically relevant endpoints to assess treatment efficacy in ASPIRO because of an absence of validated endpoints for investigating treatments for X-linked myotubular myopathy. Details on how we chose outcome measures for the present analysis are in appendix 1 (pp 4–5).

The primary efficacy outcome measure was the change in hours of daily ventilator support from baseline to week 24. Secondary outcome measures included the changes from baseline to week 24 in: the percentage of participants achieving functionally independent sitting for at least 30 s (Bayley-III assessment item 26: sits without support, 30 seconds[24]); CHOP INTEND total score;[23] MIP (a measure of respiratory muscle strength); myotubularin expression in muscle biopsy (quantitative analysis); Assessment of Caregiver Experience with Neuromuscular Disease (ACEND) total score;[25] and Pediatric Quality of Life Inventory Neuromuscular Module (PedsQL-NM) score.[26] Other secondary outcomes were the time to reduction in required ventilator support to 16 h or less a day (participants who required invasive ventilation only); the number (%) of age-appropriate clinically relevant gross motor function milestones attained by 24 weeks (Bayley-III assessments[24]; reported at the participant level herein); the percentage of participants achieving full ventilator independence in the absence of acute illness by week 24 (as assessed by site pulmonologist); and overall survival. The FDA requested that assessment of primary and secondary outcomes at week 48 be added to the study protocol to evaluate durability of effects as well as safety. For both primary and secondary outcomes there was a 2-week visit window either side of each endpoint. The Euroqol 5D questionnaire (EQ-5D) was added as a secondary outcome in protocol version 5, but not analysed due to limited data, including missing baseline data for participants dosed before protocol 5. Specifically, the EQ-5D-Y Proxy version 1 was collected from eligible participants (ie, children aged ≥4 years), and the EQ-5D-5L was intended to assess caregiver state of health, but was

misunderstood by many caregivers and completed on the basis of their child's health.

Adverse events were recorded from the time of obtaining informed consent and coded using the Medical Dictionary for Regulatory Activities, version 20.0. Relatedness to study treatment was assessed by the investigator as not related or related (reasonable possibility that the event might have been caused by the study drug). The severity of adverse events was assessed by the investigator according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 4.03). Serious adverse events were defined according to the International Conference on Harmonisation Topic E2A definitions and standards.

Statistical analysis

Because of the important and unexpected events that resulted in major changes to the study protocol and

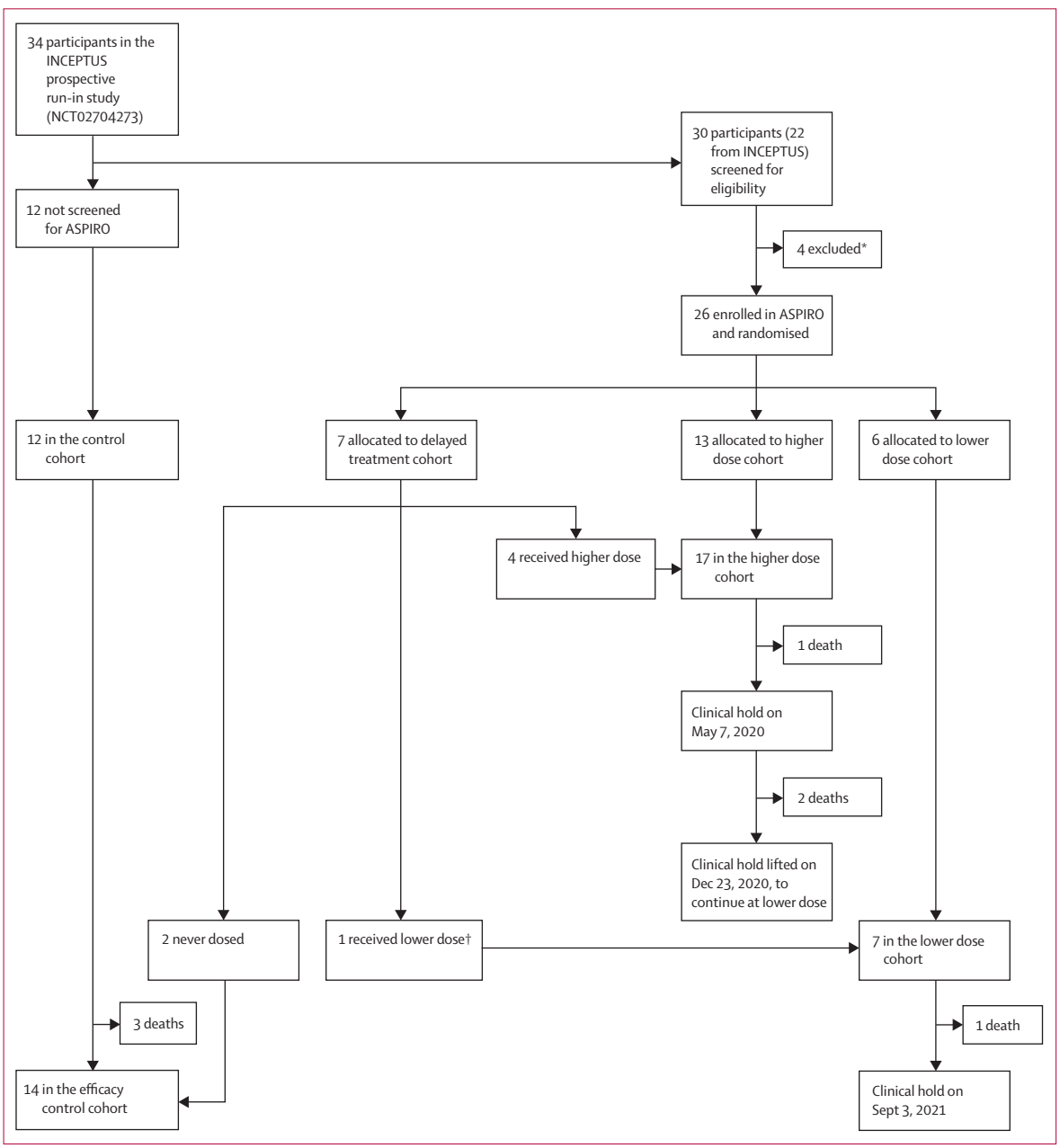

Figure 1: **CONSORT diagram of participant enrolment and dosing in the ASPIRO trial**
17 participants were dosed at $3{\cdot}5 \times 10^{14}$ vg/kg (higher dose) and seven participants were dosed at $1{\cdot}3 \times 10^{14}$ vg/kg (lower dose). vg/kg=vector genomes per kg bodyweight. *Three excluded participants had clinically significant underlying liver disease and the fourth was considered a screen failure and enrolment was terminated at the sponsor's request. †Participant was dosed after the first clinical hold was lifted.

	Lower dose cohort, $1·3×10^{14}$ vg/kg (n=7)	Higher dose cohort, $3·5×10^{14}$ vg/kg (n=17)	Control cohort (n=14)
Age at dosing, months*	12·1 (10·0–30·9)	31·1 (16·0–64·7)	18·7 (10·1–31·5)
Weight, kg	12·1 (4·9)	15·3 (5·5)	11·3 (2·4)
Race			
White	7 (100%)	11 (65%)	9 (64%)
Asian	0	1 (6%)	2 (14%)
Black or African American	0	4 (24%)	0
Not reported	0	1 (6%)	3 (21%)
Age at genetic diagnosis, months	3·2 (2·8–3·6)	1·7 (0·7–5·1)	2·7 (1·6–3·2)
Functional classification of *MTM1* variants by predicted consequence on protein function†			
Loss of function	2 (29%)	8 (47%)	7 (50%)
Partial loss of function	3 (43%)	7 (41%)	4 (29%)
In-frame exonic deletion	2 (29%)	1 (6%)	2 (14%)
Variant of unknown significance	0	1 (6%)	0
Missing†	0	0	1 (7%)
CHOP INTEND total score‡	34·6 (9·8)	30·7 (10·4)	33·5 (8·0)
Participants with invasive ventilation	6 (86%)	17 (100%)	9 (64%)
Participants with non-invasive ventilation§	1 (14%)	0	5 (36%)
Invasive ventilator dependence, hours per day	22·5 (2·7)	23·6 (0·9)	22·0 (3·0)
Non-invasive ventilator dependence§, hours per day	12·0 (NE)	NA	16·1 (4·9)
MIP, cmH_2O¶	28·6 (25·6–34·7)	25·2 (18·9–28·0)	34·2 (29·1–41·1)
Study duration, months	46·1 (41·0–49·5)	27·6 (24·6–29·1)	28·3 (9·7–46·9)
Participants who could sit independently for 30 s	1 (14%)	1 (6%)	1 (7%)

Data are median (IQR), mean (SD), or n (%). MIP=maximal inspiratory pressure. NA=not applicable. NE=not estimable. vg/kg=vector genomes per kg bodyweight. *Age for untreated control individuals given as age at informed consent in the INCEPTUS run-in study or the ASPIRO study, as applicable. †See appendix 1 (pp 21–22) for genotype data and potential mutation impact: all variants (including missing and variants of unknown significance) have been characterised in an ad-hoc analysis; however, the INCEPTUS database was locked at the time of analysis; hence, analysis was limited to ASPIRO participants. ‡Potential scores range from 0 to 64 points, with higher scores indicating better function.[23] §Includes bilevel positive airway pressure or continuous positive airway pressure. ¶Lower limit of the normal range for children aged 0–4 years is 80 cmH_2O.[27,28]

Table 1: **Demographic and clinical characteristics at baseline in dosed and control participants**

conduct of the trial (appendix 1 p 4), we did exploratory analyses to evaluate the ASPIRO data, rather than the planned analyses that were based on randomisation and the original sample size calculations. Results are reported as of the data cutoff of Feb 28, 2022, and analysed by dose level (lower dose and higher dose cohorts) versus the untreated control cohort. The analyses were done on an as-treated basis and we did not exclude any participants due to protocol deviations; thus, the analysis population closely mimics an intention-to-treat population. All treated and control participants were included in the safety analysis.

Under the missing-at-random assumption, the change from baseline over time in daily ventilation hours, CHOP INTEND total score, MIP, ACEND total score, and PedsQL-NM score were calculated and compared among the lower dose and higher dose cohorts and control group with a mixed model for repeated measures, with participant as a random effect and treatment, week, and treatment-by-week interaction as fixed effects; baseline measurement and its interaction with week were included as covariates. A heterogeneous, first-order, autoregressive covariance structure was used for changes in ventilation hours, ACEND total score, and PedsQL-NM score; a heterogeneous compound symmetry covariance structure was used for change in CHOP INTEND total score; and a Toeplitz covariance structure was used for change in MIP. Kenward-Roger approximation was used. This model assumed missingness at random without explicit imputation of missing data. Due to the exploratory nature of the study there was no adjustment for multiplicity. The means and 95% CIs for these outcomes were derived within the framework of these models. The point estimates and 95% CIs of treatment differences versus control were calculated to compare absolute changes in outcomes at weeks 24 and 48. Percentage change from baseline for ventilation hours per day was reported as least squares mean and 95% CI for each group, and differences between groups were reported as percentage point differences, at weeks 24 and 48.

For survival analysis, Kaplan-Meier estimates with log-rank tests were calculated. The probability of reaching a milestone in each of the dosed cohorts relative to that in the control group was estimated as relative risk (risk ratio) and associated 95% CI. For the binary outcomes of individual milestones reached and ventilator independence, the relative risk refers to the likelihood of achievement. SAS (version 9.4) was used for all analyses. Nominal p values are reported and compared to a two-sided nominal significance level of 0·05. The trial is registered with ClinicalTrials.gov, NCT03199469.

Role of the funding source

The funder manufactured the investigational drug and designed and managed the study. The funder had a role in data collection, data analysis, and data interpretation. The authors wrote the manuscript with the assistance of a medical writer funded by the sponsor.

Results

ASPIRO participants were enrolled between Aug 3, 2017, and June 1, 2021. 30 participants were screened for eligibility, of whom 26 were enrolled and randomised: seven were allocated to delayed treatment, six to the lower dose ($1·3×10^{14}$ vg/kg) and 13 to the higher dose ($3·5×10^{14}$ vg/kg; figure 1). Of the seven participants allocated to delayed treatment, one later received the lower dose, four the higher dose, and two did not receive any treatment. Thus, as of Feb 28, 2022, 24 participants had received resamirigene bilparvovec, seven at the lower dose and 17 at the higher dose. 14 participants (two allocated to delayed treatment in ASPIRO who did not receive resamirigene bilparvovec and 12 from

	Week 24			Week 48		
	Lower dose cohort, $1·3\times10^{14}$ vg/kg (n=7)	Higher dose cohort, $3·5\times10^{14}$ vg/kg (n=17)	Control cohort (n=14)	Lower dose cohort, $1·3\times10^{14}$ vg/kg (n=7)	Higher dose cohort, $3·5\times10^{14}$ vg/kg (n=17)	Control cohort (n=14)
Change from baseline in ventilation hours per day, mean (95% CI)	−13·61 (−20·24 to −6·99); n=6	−5·99 (−8·78 to −3·20); n=16	−0·19 (−2·62 to 2·23); n=13	−19·19 (−25·59 to −12·80); n=6	−14·16 (−19·26 to −9·06); n=13	−0·28 (−3·44 to 2·87); n=11
p value *vs* control	0·0015	0·033	..	<0·0001	<0·0001	..
Point estimate of treatment difference *vs* control (95% CI)	−12·53 (−19·95 to −5·10)	−4·17 (−7·99 to −0·35)	..	−19·67 (−25·18 to −14·17)	−13·05 (−16·41 to −9·68)	..
Participants achieving ventilator independence, n (%)*	2 (29%)	0	0	2 (29%)	5 (29%)	0
Time to reduction in required ventilator support to ≤16 h/day†, months, median (95% CI)	2·79 (0·95 to 3·71)	6·01 (3·32 to NE)	NE (0·03 to NE)	2·79 (0·95 to 3·71)	5·65 (3·32 to 9·92)	NE (0·03 to NE)
p value *vs* control (log rank; Wilcoxon)	0·020; 0·077	0·24; 0·45	..	0·020; 0·077	0·086; 0·23	..
Point estimate of treatment difference *vs* control (95% CI)	NE	NE	..	NE	NE	..
Change from baseline in MIP, cmH_2O, mean (95% CI)	52·59 (32·74 to 72·43); n=6	31·57 (15·48 to 47·66); n=11	−1·54 (−9·40 to 6·31); n=9	45·74 (14·58 to 76·91); n=4	47·81 (32·50 to 63·12); n=11	−4·11 (−10·72 to 2·50); n=9
p value *vs* control	<0·0001	0·0003	..	<0·0001	<0·0001	..
Point estimate of treatment difference *vs* control (95% CI)	52·64 (36·36 to 68·91)	27·06 (11·79 to 42·33)	..	54·56 (28·39 to 80·73)	52·50 (32·20 to 72·81)	..
Change from baseline in CHOP INTEND total score, mean (95% CI)	16·50 (6·37 to 26·63); n=6	15·64 (8·25 to 23·02); n=11	2·00 (−1·33 to 5·33); n=7	18·83 (10·02 to 27·65); n=6	20·25 (13·55 to 26·95); n=8	4·75 (−0·46 to 9·96); n=8
p value *vs* control	0·0005	0·0002	..	0·0009	0·0003	..
Point estimate of treatment difference *vs* control (95% CI)	13·01 (6·50 to 19·51)	14·90 (9·33 to 20·46)	..	16·13 (7·85 to 24·41)	15·79 (8·93 to 22·65)	..
Participants achieving functionally independent sitting for ≥30 s, n (%)‡§	5 (71%)	10 (59%)	4 (29%)	5 (71%)	13 (76%)	4 (29%)
Relative risk (95% CI) *vs* control	2·50 (0·96 to 6·47)	2·05 (0·82 to 5·15)	..	2·50 (0·96 to 6·47)	2·67 (1·12 to 6·38)	..
Change from baseline in ACEND total score, mean (95% CI)	25·77 (14·05 to 37·48); n=6	15·66 (10·52 to 20·81); n=14	−0·94 (−5·22 to 3·35); n=10	35·40 (24·90 to 45·89); n=6	19·58 (10·02 to 29·13); n=13	0·65 (−4·04 to 5·33); n=11
p value *vs* control	<0·0001	0·0002	..	<0·0001	0·0116	..
Point estimate of treatment difference *vs* control (95% CI)	24·76 (16·27 to 33·26)	12·51 (6·34 to 18·68)	..	32·21 (21·87 to 42·55)	11·33 (2·63 to 20·03)	..
Change from baseline in PedsQL-NM score, mean (95% CI)	13·78 (1·99 to 25·57); n=6	19·06 (10·95 to 27·17); n=11	−6·85 (−15·91 to 2·20); n=10	17·77 (7·42 to 28·12); n=6	18·89 (5·97 to 31·81); n=9	−7·64 (−14·90 to −0·38); n=11
p value *vs* control	0·0007	0·0001	..	0·0003	0·0023	..
Point estimate of treatment difference *vs* control (95% CI)	19·21 (8·42 to 30·01)	19·87 (10·13 to 29·61)	..	24·44 (12·17 to 36·71)	20·16 (8·19 to 32·14)	..

ACEND=Assessment of Caregiver Experience with Neuromuscular Disease. CHOP INTEND=Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders. MIP=maximal inspiratory pressure. NE=not estimable. PedsQL-NM=Pediatric Quality of Life Inventory Neuromuscular Module. vg/kg=vector genomes per kg bodyweight. *Relative risk values were not estimable. †Among participants requiring invasive ventilation. ‡Based on Bayley-III assessment item 26: sits without support, 30 seconds. §p values not calculated for this outcome.

Table 2: **Clinical efficacy outcomes at weeks 24 and 48 in dosed and control participants**

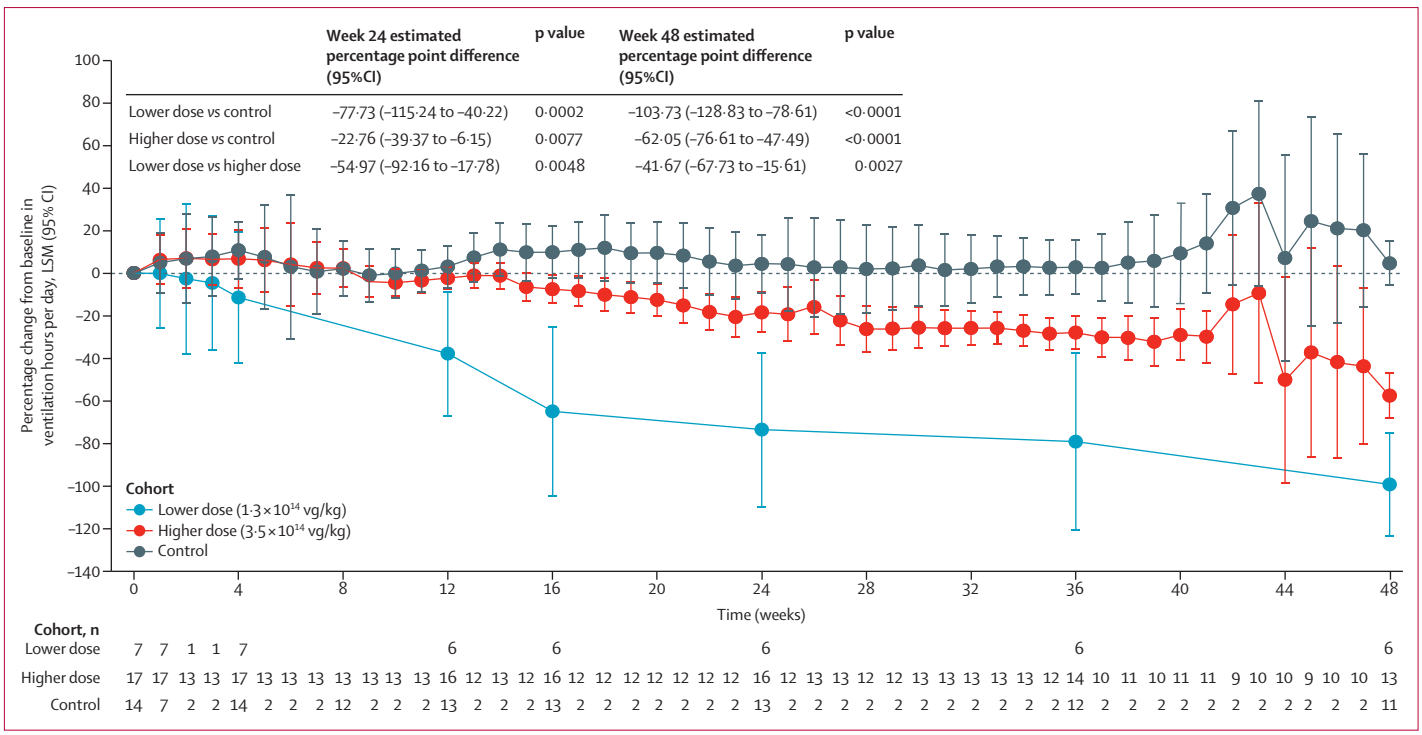

Figure 2: **Respiratory outcomes in dosed and control participants**
LSM percentage change from baseline in ventilation hours per day. Error bars indicate 95% CIs from a repeated measures analysis of covariance model. LSM=least squares mean. vg/kg=vector genomes per kg bodyweight.

INCEPTUS) were included in the untreated control cohort (figure 1).

The median age at dosing was 12·1 months (IQR 10·0–30·9; range 9·5–49·7) in the lower dose cohort and 31·1 months (16·0–64·7; 6·8–72·7) in the higher dose cohort. The median age at enrolment in the control cohort was 18·7 months (10·1–31·5; 5·9–39·3). Median follow-up was 46·1 months (IQR 41·0–49·5; range 2·1–54·7) for lower dose participants, 27·6 months (24·6–29·1; 3·4–41·0) for higher dose participants, and 28·3 months (9·7–46·9; 5·7–32·7) for control participants.

Participant characteristics at baseline were generally similar in the dosed and control groups, with some variations in age at genetic diagnosis and functional classifications of *MTM1* variants (table 1). 23 of 24 participants in the treatment groups, and nine of 14 participants in the control group were managed with invasive ventilation, for about 22–24 h per day at baseline. Median MIP values were notably lower than the lower limit of the normal range for children of similar age (80 cmH_2O for children aged 0–4 years). The mean baseline CHOP INTEND scores across all groups were approximately 50% lower than the maximum score of 64 expected in healthy children aged 3–6 months.[22,23] Only three participants (one each in the lower dose, higher dose, and control cohorts) were able to sit independently for 30 s at baseline. *MTM1* genotyping data are provided in appendix 1 (pp 21–22).

At 24 weeks after dosing, improvements were observed in daily hours of ventilation support among dosed participants compared with control individuals (table 2). The lower dose cohort had an estimated 77·7 percentage point (95% CI 40·22–115·24) greater reduction in least squares mean hours of ventilator support from baseline compared with control individuals (p=0·0002), and the higher dose cohort had an estimated 22·8 percentage point (6·15–39·37) greater reduction from baseline compared with controls (p=0·0077; figure 2). At 48 weeks after dosing, differences in the estimated reduction in daily hours of ventilation support from baseline between dosed participants and control individuals increased to 103·7 percentage points (78·61–128·83) for the lower dose cohort and 62·1 percentage points (47·49–76·61) for the higher dose cohort (p<0·0001 for both; figure 2). Ventilator independence was achieved by 16 dosed participants (six in the lower dose cohort and ten in the higher dose cohort) between 14 and 97 weeks (98–679 days) after dosing; however, one lower dose participant who had been decannulated subsequently required intermittent non-invasive ventilation due to respiratory illness. Age at ventilator removal ranged from 1·1 to 7·6 years. The percentages of patients achieving ventilator independence at weeks 24 and 48 are presented in table 2. Among dosed participants with ventilator independence, the time since tracheostomy before dosing ranged from 26·9 to 300·4 weeks (188–2103 days)

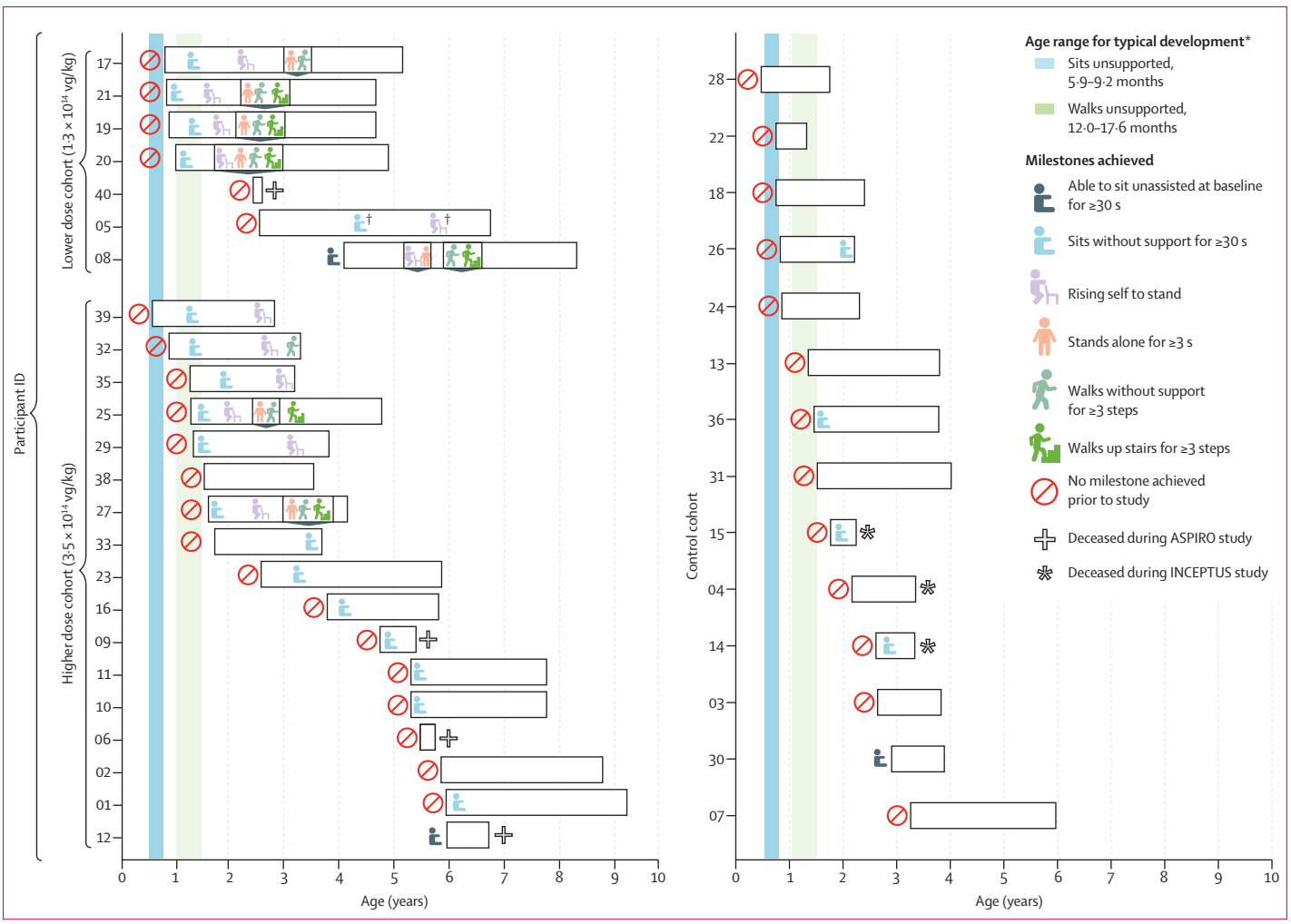

Figure 3: **Motor function outcomes in dosed and control participants**

Achievement of major motor milestones in individual dosed and control participants; when multiple symbols are contained within one box, the milestone occurred at the same visit. Milestones are based on Bayley-III assessments[24] (Bayley item 26: sits without support, 30 s; Bayley item 35: raises self to standing position; Bayley item 40: stands alone; Bayley item 42: walks alone; and Bayley item 47: walks upstairs). White boxes start at the age at dosing for dosed participants or the age at enrolment for control participants. The length of the box indicates the participant's time on study. The position of icons indicates age at motor milestone achievement. vg/kg=vector genomes per kg bodyweight. *Based on the WHO motor development study.[29] †Participant 05 reached the milestones of sitting independently and raising self to stand, but was not able to do either of these milestones at later assessments.

and the median time from dosing to ventilator independence was 73·6 weeks (95% CI 43·0–85·0). No control participants achieved ventilator independence.

Improvements from baseline were observed in MIP and CHOP INTEND total score among dosed participants compared with control individuals at 24 and 48 weeks after dosing (table 2; appendix 1 pp 9–10). A higher percentage of dosed participants than control participants attained advanced motor milestones between baseline and last observation (figure 3; appendix 1 p 23). Time to reduction in invasive ventilator support to 16 h or less a day was about 2·8 months for lower dose participants, and 6·0 months for higher dose participants. Greater proportions of participants in both dose cohorts achieved functionally independent sitting for at least 30 s than in the control cohort, although the 95% CIs did not exclude 1. For dosed participants, change from baseline in ACEND and Peds-QL-NM scores showed improvements at weeks 24 and 48 compared with controls (table 2).

At 24 and 48 weeks after dosing, dose-dependent changes were observed in myotubularin protein expression (appendix 1 p 11), and in vector copy number and myotubularin mRNA expression (not shown). Muscle biopsy findings are described in appendix 1 (pp 11–13).

Results of Kaplan-Meier survival analysis are shown in appendix 1 (p 14). Three (21%) of 14 participants died in the control cohort, one (14%) of seven died in the lower dose cohort, and three (18%) of 17 died in the higher dose cohort by the data cutoff. Causes of death among control participants were: shock secondary to hepatic haemorrhage presumed to be related to hepatic peliosis;

***Panel 1:* Causes of death**

The immediate causes of death for the four participants who received resamirigene bilparvovec, as reported by investigators, were sepsis (participant 06); hepatopathy, severe immune dysfunction, and pseudomonal sepsis (participant 09); circulatory collapse due to gastrointestinal bleed (participant 12); and septic shock (participant 40). At dosing, these four participants were aged 5·6 years (15·8 kg), 4·8 years (weight 17·3 kg), 6·1 years (25·8 kg), and 2·5 years (14·7 kg), respectively. Beginning 1–4 weeks after dosing, all participants showed increases above the upper limit of normal range (>ULN) in direct and total bilirubin values (eventually peaking at 34–92×ULN and 21–54×ULN, respectively). Similar increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transferase values were also observed (peaking at 7–75×ULN, 7–67×ULN, and 1–11×ULN, respectively). All four dosed participants experienced progression to severe treatment-related decompensated liver injury, characterised by ascites, poor hepatic synthetic function, or both. All had progressed to cholestatic liver failure at the time of death. These four participants had evidence of cholestasis that pre-dated resamirigene bilparvovec dosing (appendix 1 p 17) but met the dosing eligibility criteria as defined by ALT and AST (≤5×ULN) at predose baseline and absence of imaging evidence of hepatic peliosis at the time of enrolment.

aspiration pneumonia with acute respiratory failure; and acute on chronic bronchopneumonia with evidence of cardiac dysfunction (cardiac hypertrophy) due to longstanding pulmonary disease. Among the four dosed participants who died after receiving gene therapy, all had cholestatic liver failure at the time of death, and all had ongoing serious treatment-emergent adverse events of hepatic and hepatobiliary disorders at the time of death. Further details about the immediate causes of death of the four dosed participants are in panel 1. Autopsy findings will be reported separately.

Five (25%) of the 20 surviving dosed participants were reported to have serious treatment-related hepatobiliary adverse events (panel 2). In all dosed study participants with liver-related serious adverse events, serological (ie, neutralising antibody titres to AAV8 and total antibody titres to myotubularin protein) and cellular (ie, interferon-γ response to AAV8 and myotubularin antigens) markers, complement factors, cytokines, and routine laboratory and histopathological assessments did not suggest that the serious adverse events were driven by immune responses, although data are insufficient to draw definitive conclusions. Notably, the four participants who had severe liver injury and fatal outcomes received prophylactic prednisolone and, in some cases, high-dose methylprednisolone and other immune-modulating therapies (eg, prolonged or increased dose of prednisolone, anakinra, tocilizumab, and ruxolitinib) with no apparent benefit.

Among the 24 dosed participants, the most common treatment-emergent adverse events reported were pyrexia (14 [58%]), increases in creatine phosphokinase (12 [50%]), and respiratory tract infections (upper, ten [42%]; viral upper, nine [38%]). A summary of severe, serious, and treatment-related adverse events is provided in table 3. Details of cardiac and haematological adverse events are in panel 2.

Serious adverse events reported in one or more participants are summarised in appendix 1 (pp 24–26). In the lower dose cohort, 24 serious adverse events were reported among five (71%) of seven participants, and seven events were deemed related to treatment. Of these seven events, three occurred in one participant and were secondary to liver failure, whereas the remaining four occurred in another participant and were thought to be associated with suspected clinical myocarditis. In the higher dose cohort, 58 serious adverse events occurred among 13 (76%) of 17 participants. Of these, 30 events occurring in nine participants were deemed related to treatment. In the control cohort, 43 serious adverse events were observed among 13 (93%) of 14 participants, including multiple respiratory infections and illnesses.

Detectable anti-AAV8 neutralising antibodies developed in all dosed participants starting at week 4 (not shown). After dosing, among 23 participants with available data, response to stimulation with a myotubularin peptide pool in the interferon-γ ELISpot assay (as a measure of T-cell response) was documented in six participants (participants 05, 08, and 17 in the lower dose cohort and participants 01, 02, and 23 in the higher dose cohort), all with predicted loss of function or in-frame exonic deletion mutations. Three participants had only negative assay responses after dosing (participant 06 with loss of function, participant 20 with in-frame exonic deletion, and participant 25 with partial loss of function mutation), and 14 participants had no evaluable post-dose data mainly due to indeterminate assay results (appendix 1 pp 15–16). Two participants with reactivity (05 and 23) had serious adverse events of myocarditis, and both received immunosuppressive treatment, including with T-cell modulating therapies, under the assumption of an anti-transgene cytotoxic T-cell reaction. In the remaining four participants with documented reactivity, the signal appeared transient and subsided without treatment. Detectable anti-myotubularin antibodies developed after dosing in 19 (83%) of 23 dosed participants (appendix 1 p 16), and the presence of antibodies was not associated with differences in clinical outcomes or findings on muscle biopsy nor did it correlate with age or dose used.

Discussion

In the ASPIRO trial, which was an open-label clinical trial of resamirigene bilparvovec, a gene therapy for X-linked myotubular myopathy, improvements were noted in ventilatory status and motor function, with unexpected attainment of ventilator independence and gross motor milestones. The deaths of four participants has led to a pause of the ASPIRO trial while the mechanism of the gene therapy-related hepatotoxicity is investigated. Of the four dosed participants who died (three who received the higher dose [$3·5\times10^{14}$ vg/kg] and one who received the lower dose [$1·3\times10^{14}$ vg/kg]), all had severe cholestatic liver injury with decompensation after AAV administration, and all had progressed to cholestatic liver failure

Panel 2: Hepatobiliary, cardiac, and haematological adverse events

Hepatobiliary serious adverse events

In addition to the four participant deaths associated with serious hepatobiliary treatment-related adverse events (panel 1), five participants (four who received the higher dose of resamirigene bilparvovec and one who received the lower dose) had non-fatal hepatobiliary serious adverse events that were deemed to be treatment related.

Participant 08 in the lower dose cohort was 4·1 years old and weighed 21·5 kg at dosing. He was not known to have abnormal liver-related tests pre-dating dosing but did show coarsened hepatic echotexture on predose ultrasounds. Starting approximately 6 weeks after dosing, he had a cholestatic episode with peak elevations in alanine aminotransferase (ALT; 87×upper limit of normal range [ULN]), aspartate aminotransferase (AST; 57×ULN), γ-glutamyl transferase (GGT; 5×ULN), total bilirubin (3×ULN), direct bilirubin (6×ULN), and serum bile acids (8×ULN). He received prednisolone and ursodeoxycholic acid. Although he later showed intermittent mild-to-moderate elevations in aminotransferases, he did not show evidence of hepatic sequelae.

Participant 01 in the higher dose cohort was dosed at 6·8 years of age and weighed 21·1 kg. Before dosing, he was known to have had occasional episodes of mild direct hyperbilirubinaemia. He had peak direct bilirubin and total bilirubin values of 32 times the ULN and ten times the ULN over a period of 10–17 weeks after receiving resamirigene bilparvovec, after which his bilirubin values decreased, and the event resolved without hepatic sequelae.

Participant 39 had no known liver laboratory or ultrasound abnormalities pre-dating dosing and received the higher dose at 0·6 years of age and weighed 6·9 kg. Beginning 1 week after dosing, he had abrupt elevations in ALT (peak 19×ULN) and AST values (peak 23×ULN) with normal bilirubin values, which subsequently resolved.

Participant 38 had a history of cholestasis before study enrolment and received the higher dose at 1·5 years of age and weighed 9·4 kg. Beginning at 14 weeks, he had elevations in ALT (peak 20×ULN) and AST values (peak 6×ULN) with normal bilirubin values, which subsequently resolved. At 58 weeks after dosing, he developed recurrent similar elevations in ALT and AST and elevations in direct and total bilirubin (values unknown). Diagnostic liver biopsy was consistent with intrahepatic cholestasis, and serum bile acid levels were elevated (value unknown). At last report (18 months after dosing), all levels had normalised.

Participant 29 in the higher dose group was 1·3 years old and 11·0 kg when he received the higher dose and was noted to have mild-to-moderate elevations in liver transaminases pre-dating dosing. Beginning approximately 4 weeks after dosing, he had a cholestatic episode with peak elevations in ALT (42×ULN), AST (21×ULN), GGT (19×ULN), total bilirubin (4×ULN), direct bilirubin (11×ULN), and serum bile acids (4×ULN) which subsequently resolved. He has shown no residual hepatic sequelae, although following normalisation has intermittently had mild elevations in hepatic markers.

Cardiac adverse events

Troponin I was elevated in four higher dose participants, two lower dose participants, and no controls, indicating a potential for cardiac stress or cardiomyopathy.

One participant from each dose cohort (participant 23 in the higher dose cohort and participant 05 in the lower dose cohort) was considered to have probable myocarditis. These participants had *MTM1* mutations that were expected to produce no myotubularin protein, although neither participant was among those with the highest anti-myotubularin antibody titres in the study. Both participants were treated with pulsed methylprednisolone and sirolimus, with mycophenolate mofetil and intravenous immunoglobulin additionally given to participant 05. In both patients, myocarditis was reported to have resolved (within approximately 4 months for participant 23 and 10 months for participant 05) and serial echocardiograms continued to show normal myocardial function, although participant 23 continued on sirolimus after resolution of symptoms.

Haematological adverse events

Transient thrombocytopenia within the first 2 weeks of dosing was reported in eight participants who received the higher dose, and the events of thrombocytopenia were deemed possibly or probably related to treatment in all eight participants. In participant 23, thrombocytopenia was categorised as a serious adverse event. Analyses of samples taken during the first month of dosing showed non-specific antibody binding to glycoproteins (GpIIb/IIIa, GpIb/IX, GpIV, HLA class I, and GpIa/IIa) in different platelet populations.

at the time of death. Despite the safety concerns, resamirigene bilparvovec is the first potential therapy to be tested in a clinical trial for this serious disease with no current treatment options, and ASPIRO is an important milestone towards successful systemic gene therapy for a congenital myopathy.

Based on existing natural history data, and as observed in the control group, individuals with X-linked myotubular myopathy receiving long-term mechanical ventilation are highly unlikely to wean from the ventilator.[30] In a study of 33 ventilated patients who were followed up prospectively for 1 year, none achieved ventilator independence or had meaningful reduction in time using the ventilator.[7] The ventilator independence achieved by some participants who received resamirigene bilparvovec, thus, represents a very rare spontaneous occurrence in the natural course of this disease. In the ASPIRO trial, children ranged in age from 1·1 to 7·6 years at ventilator removal and had

	Lower dose cohort, $1{\cdot}3\times10^{14}$ vg/kg (n=7)	Higher dose cohort, $3{\cdot}5\times10^{14}$ vg/kg (n=17)	Control cohort (n=14)
Any TEAE	7 (100%)	17 (100%)	14 (100%)
Treatment-related TEAE	6 (86%)	17 (100%)	0
Severe TEAE (grade ≥3)	7 (100%)	12 (71%)	13 (93%)
Serious TEAE	5 (71%)	13 (76%)	13 (93%)
Treatment-related serious TEAE	2 (29%)	9 (53%)	0
TEAE leading to study discontinuation	1 (14%)	1 (6%)	2 (14%)
TEAE resulting in death	1 (14%)	3 (18%)	3 (21%)

TEAE=treatment-emergent adverse event.

Table 3: **Summary of TEAEs in dosed and control participants**

originally undergone tracheostomy between 26·9 and 300·4 weeks before dosing, showing that even patients who have been on invasive ventilation for several years can attain ventilator independence with treatment. As respiratory comorbidities are the primary cause of death for children with X-linked myotubular myopathy,[6] ventilator independence is likely to improve survival and overall quality of life by reducing risk of aspiration pneumonia, hospitalisation, and dependence on caregivers.

Although dosed participants showed notable improvements on respiratory parameters, untreated control participants did not improve spontaneously, as also observed in natural history studies.[7,8] We caution against comparing the changes in daily ventilator support between lower dose and higher dose participants (figure 2), because lower dose participants had longer follow-up and higher dose participants were weaned from ventilation gradually using a conservative algorithm (weaning algorithm to be reported separately). Similarly, the marked improvements in MIP occurred more quickly in lower-dose participants versus higher dose participants (appendix 1 p 9). It is important to note that as soon as participants achieved ventilator independence and reached an MIP of 80 cmH_2O on two separate assessments, MIP testing was stopped to minimise the testing burden (which is arduous and distressing for children, their families, and investigators). Because all six surviving lower dose participants achieved ventilator independence (one lower dose participant subsequently required intermittent ventilation due to respiratory illness), there were fewer MIP measurements in the lower dose cohort.

Dosed participants had rapid increases in mean CHOP INTEND scores from baseline (appendix 1 p 10), with some participants reaching the ceiling of the scale (64 points). This finding is in contrast to mean CHOP INTEND scores for control participants, or from participants in previous natural history studies, who had scores approximately 50% lower than expected in healthy children aged 3–6 months.[23] A treatment effect was also observed in participants who had long disease durations and multiple disease-related medical comorbidities. Generally, study participants who received resamirigene bilparvovec achieved and subsequently maintained crucial motor milestones, which are unprecedented in patients with X-linked myotubular myopathy with such an extensive disease burden. This includes five of seven lower dose participants and three of 17 higher dose participants walking independently at last assessment.

Dosed participants had improvements in muscle pathology at week 24 (organelle localisation and myofibre size) that paralleled their clinical improvements. Of note, the elevated proportion of myofibres with internal or central nuclei, which is a defining pathological feature of X-linked myotubular myopathy, was not altered in post-dose muscle biopsy samples, despite the observed clinical improvements. Additionally, although exogenous *MTM1* expression was quite variable, dosed participants showed clinically significant improvements, including ventilator independence and independent ambulation. We speculate that low levels of transgene expression within myofibres might be sufficient for clear histopathological and functional improvements, as some participants with among the lowest levels of protein expression became independently ambulatory and ventilator independent. Histopathological analyses of dosed participants from ASPIRO will be reported in a separate manuscript.

The deaths of four participants who received resamirigene bilparvovec have prompted re-examination of hepatobiliary disease in the natural history of X-linked myotubular myopathy and consideration of the potential interaction of AAV-mediated therapy in this setting. All four participants showed elevations in total and direct bilirubin levels (>ULN) beginning 1–4 weeks after dosing, progressing to severe decompensated liver dysfunction characterised by ascites or reduced hepatic synthetic function (or both), which might have been causative of the fatal events (panel 1). These four deceased participants who had hepatic and hepatobiliary serious adverse events had different mutation types (appendix 1 pp 21–22) but had clinical evidence consistent with cholestasis pre-dating resamirigene bilparvovec dosing, including intermittent hyperbilirubinaemia, cholestatic hepatitis, and hepatic ultrasounds showing increased echogenicity. This toxicity is different from the non-cholestatic, hepatocellular hepatoxicity documented for approved gene therapies for haemophilia A (valoctocogene roxaparvovec [$6{\cdot}0\times10^{13}$ vg/kg]),[31] haemophilia B (etranacogene dezaparvovec [$2{\cdot}0\times10^{13}$ vg/kg]),[32] and spinal muscular atrophy (onasemnogene abeparvovec [$1{\cdot}1\times10^{14}$ vg/kg]).[33] Additionally, the progressive liver disease that occurred after dosing did not respond to immune suppression, including (in some instances) intense multiagent therapy. This lack of response to immune suppression contrasts with other AAV-mediated liver toxicity reported in several AAV trials in haemophilia, Duchenne muscular dystrophy, and spinal muscular atrophy.[34] Of note, among surviving participants at both dose levels, liver laboratory abnormalities were frequently observed in both the

predose and post-dose setting, including abnormalities documented or suspected to be related to intrahepatic cholestasis.

When the ASPIRO study began, pre-existing cholestatic liver disease in children with X-linked myotubular myopathy was largely unrecognised. The primary liver disease known to associate with X-linked myotubular myopathy (and the focus of the liver-related eligibility criteria) is hepatic peliosis—a rare, well described, life-threatening vascular condition characterised by multiple, randomly distributed, blood-filled cavities throughout the liver.[7,35] A non-specific cholestatic tendency, reported primarily as jaundice, cholelithiasis, and pruritus, has been described in 7–17% of patients,[3,7,8,35] but the nature, extent, and pathophysiology were not well characterised and, unlike peliosis, were not known to be associated with morbidity or mortality in X-linked myotubular myopathy.[36] In INCEPTUS (the lead-in natural history study to ASPIRO), eight (24%) of 34 participants were found to have histories of hepatobiliary disease at enrolment; during that study, 12 (35%) of 34 participants had at least one episode of elevated total or direct bilirubin, including two with bilirubin above 5 times the ULN.[9] Hepatobiliary disease associated with the natural history of X-linked myotubular myopathy has been described in case series and reports published since ASPIRO was paused.[36–39] Furthermore, in July, 2022, the UNITE-CNM study of an antisense oligonucleotide directed against DNM2 pre-mRNA in patients with centronuclear myopathy, which is caused by mutations in *DNM2* or *MTM1*, was stopped due to liver enzyme elevations in several study participants who were receiving the lowest dose expected to have potential clinical efficacy.[40] Despite frequent hepatic laboratory abnormalities, patients with X-linked myotubular myopathy do not routinely undergo diagnostic liver biopsies due to the low frequency of symptomatic disease and the risk for life-threatening haemorrhage. However, this recently improved understanding of cholestatic disease indicates that consideration should be given to increased monitoring for cholestasis in patients with X-linked myotubular myopathy, in particular to include testing of serum bile acids.

Most participants in both the dosed and control groups had serious adverse events. Three control participants died of causes similar to those observed in the natural history of the disease (eg, hepatic complications and respiratory and cardiac sequelae).[6–9] Given that control participants were younger on average than dosed participants, it is possible that they had more severe disease. However, dosed and control participants had similar baseline ventilator dependence, MIP impairment, and CHOP INTEND scores indicating severe myopathy across all three cohorts. With the exception of the deaths related to hepatobiliary disease, most adverse events in dosed participants were consistent with those reported in patients who received other AAV-based gene therapy products,[14,41,42] including transient thrombocytopenia and troponin elevation.[33]

This first-in-human clinical trial in a paediatric disease with high mortality has important design considerations and limitations that can inform future trials of AAV gene replacement therapy. An open-label trial design with a delayed-treatment control was deemed appropriate for ASPIRO, because a double-blind, placebo-controlled trial would increase the burden for a vulnerable participant population, requiring sham muscle biopsies, double-dummy drug administration, use of placebo prednisolone for up to 16 weeks, and an intense visit schedule, often requiring considerable travel. The number of participants was small, and all were infants or young children and dependent on invasive ventilation, indicating a pressing need for improved treatments. Importantly, although the study design included some randomisation elements, the allocation to the lower dose, higher dose, and undosed control groups was not based on randomisation. Additionally, the small number of undosed participants precluded efforts to define a subset of controls who would more closely match the dosed participants with respect to potential confounding factors. Due to the limited randomisation and matching, the reported results should be interpreted with caution.

Because of the deaths of four dosed participants after developing severe cholestatic liver disease, investigations are ongoing into cholestatic predilection in individuals with X-linked myotubular myopathy and the mechanisms by which resamirigene bilparvovec exacerbates this state, so that the risk might be reduced for patients treated with gene therapy in the future. Such investigations include careful analysis of the clinical data and efforts to model X-linked myotubular myopathy-related cholestasis to better understand its exacerbation by resamirigene bilparvovec.[43] Nevertheless, data from the ASPIRO trial to date support potential clinical efficacy and histopathological improvements as proof of target engagement in muscles with resamirigene bilparvovec treatment, and highlight the potential for this therapy to provide transformative clinical improvements for this rare, severe, and often fatal paediatric neuromuscular disease.

Contributors

SP, SR, MWL, RJG, MM, and WM conceptualised and designed the study. AB-B and FMa developed the vectors. JC, JL, FV, and MWL curated and analysed data. CH and MN designed the statistical plan and analysis. ESJ managed the project. PBS, NLK, JJD, WM-F, CGB, AMS, LS, BKS, FMu, AB, ARF, DNS, SN, LNA, AHB, MKC, TD, RJG, MJ, VMacB, and MWL led the investigation and collected data. All authors evaluated and interpreted the outputs from the formal data analysis. PBS and SR wrote the first draft with support from ESJ. All authors were involved in critical review and revision of the manuscript. PBS, NLK, JJD, WM-F, CGB, BKS, WM, JC, FV, and MWL accessed and verified the data reported in the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

PBS has received funding and provision of study materials from Astellas Gene Therapies (formerly Audentes Therapeutics) to support clinical trial investigations relating to the present manuscript; has received research grants or contracts from Biogen, Novartis Gene Therapies, Pfizer, PTC Therapeutics, Reveragen, Sanofi, Sarepta, and Solid Biosciences; has received consulting fees for advisory board participation

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JJD has received research grants or contracts from Astellas Gene Therapies to his institute as a study site for the ASPIRO clinical trial and for preclinical studies; and has received an honorarium for a sponsored symposium and support for travel to an international meeting to present data from Astellas Gene Therapies. WM-F has received support for study materials and study personnel relating to the present manuscript from Astellas Gene Therapies; consulting fees from Sarepta, PTC Therapeutics, Novartis, and Roche; personal compensation from Novartis and Biogen and institutional funding from Roche, for lectures; and has served on scientific advisory boards for Deutsche Gesellschaft für Muskelkranke and Glykogenosis. CGB has received research grants, contracts, or travel support for various invited lectures at academic meetings from Noelia Foundation, Muscular Dystrophy UK, and Cure CMD; holds a patent for *COL6A1* intron 11 pseudoexon skipping technologies unrelated to the present manuscript; has participated (without fees) in advisory boards for Solid Biosciences (IGNITE trial), Rocket Pharma, and Nationwide Children's Hospital; and is Chair of the Scientific Advisory Board of the MDUK Oxford Neuromuscular Centre. LS has received consulting fees and honoraria for lectures from Astellas Gene Therapies; and is coordinating investigator of the European NatHis-CNM study, funded by Dynacure. BKS has received institutional research grants or contracts for her institution to serve as an INCEPTUS and ASPIRO study site from Astellas Gene Therapies. AB reports institutional grants or contracts from PTC Therapeutics; has received payments or honoraria from Roche, Biogen, and Pfizer; and has participated in advisory boards at Roche and Pfizer. ARF has been a member of an independent data monitoring committee for a different clinical trial for *MTM1*-related myopathy and *DNM2*-related myopathy (the trial was terminated early). LNA has received grants or contracts from Astellas Gene Therapies via her institution to provide training and quality control services supporting the ASPIRO clinical trial programme. 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AB-B has received consulting fees from Astellas Gene Therapies and research funding from the Myotubular Trust for preclinical work related to the present manuscript; and holds a patent on systemic gene replacement therapy for treatment of X-linked myotubular myopathy. MKC has received consulting fees and institutional research funding supporting preclinical experiments for a US investigational new drug application relating to the gene therapy in the present manuscript; holds a patent for a systemic gene replacement therapy for treatment of X-linked myotubular myopathy; and has received option payments from Wake Forest University for the patent. TD has received consulting fees from Astellas Gene Therapies for study training on CHOP INTEND measurement in ASPIRO. RJG reports limited consulting fees from Astellas Gene Therapies for work on the ASPIRO study design and clinical outcome measures. JC is an employee of Astellas Gene Therapies. 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MWL has received research funding from Astellas Gene Therapies to his academic institution (Medical College of Wisconsin) and to his company (Diverge Translational Science Laboratory) for work related to the present manuscript; has received research grants or contracts to his academic institution from Solid Biosciences, Kate Therapeutics, Taysha Therapeutics, Ultragenyx, and Prothelia; has received consulting fees from Astellas Gene Therapies, Encoded Therapeutics, Modis Therapeutics, Lacerta Therapeutics, AGADA Biosciences, Dynacure, Affinia, Voyager, BioMarin, Locanabio, and Vertex Pharmaceuticals; has received speaker fees and reimbursement for travel related to sponsored research from Astellas Gene Therapies; has received personal fees for scientific advisory board participation for Astellas Gene Therapies and Solid Biosciences; and his institution has received payment from Taysha Therapeutics for his advisory board participation. MWL is currently Chief Executive Officer, founder, and owner of Diverge Translational Science Laboratory, which continues to work under contracts from many gene therapy companies including Astellas Gene Therapies, Solid Biosciences, Rocket Pharma, Kate Therapeutics, Carbon Biosciences, Dynacure, Nationwide Children's Hospital, Taysha Gene Therapies, and Ultragenyx. SP was an employee at Astellas Gene Therapies from February, 2014, to June, 2019, and was the senior physician overseeing the study relating to the present manuscript. SR reports holding stock in Astellas Gene Therapies and was formerly employed by Astellas Gene Therapies. All other authors declare no competing interests.

Data sharing

Researchers may request access to anonymised participant-level data, trial-level data, and protocols from clinical trials sponsored by Astellas Gene Therapies at medinfo.us@astellas.com. For the Astellas criteria on data sharing see https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Astellas.aspx.

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