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ORIGINAL RESEARCH ARTICLE

Clinical Trial and Postmarketing Safety of Onasemnogene Abeparvovec Therapy

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Abstract

Introduction This is the first description of safety data for intravenous onasemnogene abeparvovec, the only approved systemically administered gene-replacement therapy for spinal muscular atrophy.

Objective We comprehensively assessed the safety of intravenous onasemnogene abeparvovec from preclinical studies, clinical studies, and postmarketing data.

Methods Single-dose toxicity studies were performed in neonatal mice and juvenile or neonatal cynomolgus nonhuman primates (NHPs). Data presented are from a composite of preclinical studies, seven clinical trials, and postmarketing sources (clinical trials, $n = 102$ patients; postmarketing surveillance, $n = 665$ reported adverse event [AE] cases). In clinical trials, safety was assessed through AE monitoring, vital-sign and cardiac assessments, laboratory evaluations, physical examinations, and concomitant medication use. AE reporting and available objective clinical data from postmarketing programs were evaluated.

Results The main target organs of toxicity in mice were the heart and liver. Dorsal root ganglia (DRG) inflammation was observed in NHPs. Patients exhibited no evidence of sensory neuropathy upon clinical examination. In clinical trials, 101/102 patients experienced at least one treatment-emergent AE. In total, 50 patients experienced serious AEs, including 11 considered treatment related. AEs consistent with hepatotoxicity resolved with prednisolone in clinical trials. Transient decreases in mean platelet count were detected but were without bleeding complications. Thrombotic microangiopathy (TMA) was observed in the postmarketing setting. No evidence of intracardiac thrombi was observed for NHPs or patients.

Conclusions Risks associated with onasemnogene abeparvovec can be anticipated, monitored, and managed. Hepatotoxicity events resolved with prednisolone. Thrombocytopenia was transient. TMA may require medical intervention. Important potential risks include cardiac AEs and DRG toxicity.

Key Points

We comprehensively describe the overall safety data from preclinical and clinical studies and postmarketing data. Safety risks included hepatotoxicity, transient thrombocytopenia, cardiac events, thrombotic microangiopathy, and ganglionopathy.

Risks associated with onasemnogene abeparvovec can be anticipated and monitored with diligent standard of care and sometimes require medical management.

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1 Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disease with an incidence of approximately 1:11,000 [1, 2]. Survival motor neuron 1 (*SMN1*) gene mutations result in functional SMN protein deficiency. The most severe forms of SMA are characterized by motor neuron degeneration and progressive loss of muscle function that culminates in death or permanent ventilation early in childhood. SMA is classified into four subtypes based on age at symptom onset and maximum motor function [3], with approximately 45–60% of historical cases classified as the most severe SMA type 1 [1]. Before the availability of disease-modifying treatments, SMA was the leading genetic cause of infant mortality [1].

Onasemnogene abeparvovec is a gene-replacement therapy delivering a functional human *SMN* transgene through nonreplicating, recombinant adeno-associated virus (AAV) serotype 9 (AAV9). Increased SMN protein expression following treatment prevents motor neuron death, leading to improved neuronal and muscular function.

Following intravenous onasemnogene abeparvovec, clinically meaningful efficacy outcomes have been reported [4, 5]. Important safety risks include hepatotoxicity [6], thrombocytopenia, cardiac events, thrombotic microangiopathy (TMA) [7], and ganglionopathy [8]. This report is the first comprehensive description of safety data through 12 November 2020 for all clinical and preclinical experience with intravenous onasemnogene abeparvovec, the only approved systemically administered gene therapy. Data presented here are from a composite of preclinical studies, clinical trials, and postmarketing sources. Postmarketing surveillance involves evaluation of data on safety from sources such as spontaneous (voluntary) reporting, social media, published literature, market research, and public databases. While every attempt was made to follow-up with the reporters to gain as much information as possible, this information was not obtained through rigorous prospective data collection. Therefore, these data, albeit important, may not always be comprehensive. Overall, this serves as a pivotal report of the safety profile observed thus far in this emerging class of therapeutic options for rare monogenic disorders.

2 Methods

2.1 Preclinical Studies

Five investigative studies evaluating single-dose toxicity and tolerability in neonatal mice and juvenile or neonatal cynomolgus nonhuman primates (NHPs) were undertaken using intravenous onasemnogene abeparvovec administration to mimic the drug's intended use and to assess safety to inform any potential safety risks that could be associated with onasemnogene abeparvovec use in humans. Principles of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) M3 (R2) *Guideline on Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals*, ICH S6 (R1) *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*, and US FDA guidance for industry were applied. Two mouse toxicology studies were performed in accordance with good laboratory practice guidelines at contract research organizations using a good manufacturing practices-compliant test article representative of the pivotal clinical trial and commercial drug product. All other preclinical studies did not use commercial drug product.

2.2 Clinical Trials

All seven intravenous clinical studies (Table 1) involved a total of 102 patients as of 12 November 2020. All studies were performed in accordance with ethical principles in the Declaration of Helsinki and are consistent with ICH/good clinical practice and applicable regulatory requirements. All study protocols were approved by the institutional review boards or ethics committees, with appropriate informed consent obtained. Studies were registered with www.clinicaltrials.gov (NCT02122952 [START], NCT03306277 [STR1VE-US], NCT03505099 [SPR1NT], NCT03461289 [STR1VE-EU], NCT03837184 [STR1VE-AP], NCT03421977 [LT-001], and NCT04042025 [LT-002]) [4, 5, 9–12]. Eligibility requirements are described in page 3 of the electronic supplementary material (ESM).

2.3 Postmarketing Cases

Safety data (Argus Safety Database, an adverse event [AE] management system for safety monitoring) were monitored through voluntary reporting of AEs, literature sources, solicited venues, and social media and include 665 case reports with 2407 AEs. Of note, literature sources were monitored using LiSA (Literature Search Application), an assessment and tracking tool for published safety cases and potential safety signals. LiSA acts as a "clearing house" for collecting scientific and medical journals and articles, and extracting, filtering, and highlighting the relevant information. OVID was also used to search literature databases (e.g., MEDLINE and Embase) as part of standard safety signal detection. The information obtained from LiSA was entered into the Argus Safety Database. Multiple cases and/or events could be reported for one patient. Additional details were obtained upon follow-up from the reporting sources.

2.4 Safety Assessments

In clinical trials, safety was assessed through monitoring AE incidence and severity, vital-sign assessments, cardiac assessments, laboratory evaluations, physical examinations, and concomitant medication usage. AE reporting and objective clinical data were evaluated whenever available from postmarketing and early access programs. Data were further analyzed to characterize AEs of special interest (AESIs), including hepatotoxicity, thrombocytopenia, cardiac events, TMA, and symptoms suggestive of ganglionopathy (see page 3 in the ESM).

Table 1 Overview of clinical studies with onasemnogene abeparvovec following intravenous administration

Study; phase; country	Study design; administration route; treatment duration	Number of pts planned/ number enrolled	Healthy participants or diagnosis of pts	Safety population	SMA type (*SMN2* copy no.)	Length of follow-up	Study status; data included in submission (yes/no)
START; I; USA [4, 9]	Open-label, single-center; IV; single dose (cohort 1: single dose 6.7×10^{13} vg/kg; cohort 2: single dose 1.1×10^{14} vg/kg)	15/15	Pts with SMA type 1	Any pt who underwent gene-therapy infusion	1 (2)	24 months	Completed; yes
STR1VE-US; III; USA [5]	Open-label, single-arm, multicenter; IV; single dose 1.1×10^{14} vg/kg	Up to 20/22	Pts with SMA type 1 with one or two copies of *SMN2* aged < 6 months at time of gene-replacement therapy	All pts who underwent gene-therapy infusion	1 (1 or 2)	18 months	Completed; yes
SPR1NT; III; Australia, Belgium, Canada, Japan, UK, USA, Taiwan, Italy [10]	Open-label, single-arm, multicenter; IV; single dose 1.1×10^{14} vg/kg	≥ 26/30: ≥ 14 with two copies of *SMN2*/14; ≥ 12 with three copies of *SMN2*/15 (one pt with four copies of *SMN2*)	Presymptomatic pts with genetically confirmed SMA type 1 or 2 with two or three copies of *SMN2*, aged ≤ 6 weeks at time of gene-replacement therapy	All pts who received an IV infusion of onasemnogene abeparvovec	1, 2 (2, 3, 4)	18 months	Ongoing (enrollment completed on 8 Nov. 2019); yes
STR1VE-EU; III; Italy, UK, Belgium, France [11]	Open-label, single-arm, multicenter; IV; single dose 1.1×10^{14} vg/kg	Up to 30/33	Pts with SMA type 1 with one or two copies of *SMN2* aged < 6 months at time of gene-replacement therapy	All pts who received an IV infusion of onasemnogene abeparvovec	1 (1, 2)	18 months	Ongoing (enrollment completed on 21 May 2019); yes
STR1VE-AP; III; Japan, South Korea, Taiwan	Open-label, single-arm, multicenter; IV; single dose 1.1×10^{14} vg/kg	≥ 6/2	Pts with SMA type 1 with one or two copies of *SMN2* aged < 6 months at time of gene-replacement therapy	All pts who received an IV infusion of onasemnogene abeparvovec	1 (1, 2)	18 months	Ongoing (enrollment terminated for reasons unrelated to efficacy or safety); yes
LT-001[a]; long-term follow-up; USA [12]	Observational; NA; pts will be followed for up to 15 years	Up to 15 (13 actual)	Pts treated with onasemnogene abeparvovec in START	NA	1 (2)	5.2 years as of 11 June 2020	Ongoing; yes
LT-002[a]; long-term follow-up; USA, Canada, Italy, Belgium, Japan, Australia	Observational; NA; pts will be followed for up to 15 years from dosing in parent study	Approximately 308/32	Pts treated with onasemnogene abeparvovec in prior efficacy/safety studies	NA	1, 2, 3 (1, 2, 3, 4)	2.0 years as of 12 Nov. 2020	Ongoing; yes

IV intravenous, *NA* not applicable, *pt(s)* patient(s), *SMA* spinal muscular atrophy, *SMN* survival motor neuron gene, *vg* vector genome

[a]Studies LT-001 and LT-002 are long-term follow-up studies. No additional gene-therapy treatment is administered to pts in these studies; however, pts are permitted to receive other SMA therapies

2.5 Statistical Analyses

These aggregate data, which include a smaller data set first described by Chand et al. [6], were analyzed using SAS version 9.4 software (SAS Institute), with all AEs coded using conventional Medical Dictionary for Regulatory Activities (MedDRA®) terms (version 23.1). To systematically search for events within AESI categories, we specified predefined standardized MedDRA® queries (SMQs). Frequency counts were programmatically calculated for all AEs. Criteria for deeming an AE as serious (SAE) are described in page 3 of the ESM. Vital-sign assessments, cardiac assessments, laboratory evaluations (chemistry, hematology, urinalysis, and immunology), and physical examinations were analyzed to evaluate for potential clinical significance by assessing changes over time and low, normal, and high shifts from baseline.

3 Results

3.1 Onasemnogene Abeparvovec-Related Toxicity in Preclinical Safety Studies

Table 2 summarizes key preclinical findings. In mice, the main target organs of toxicity were the heart and liver. Test article-related mortality was observed at doses $\geq 2.4 \times 10^{14}$ vector genome (vg)/kg, with the maximum tolerated dose at 1.5×10^{14} vg/kg based upon atrial thrombosis starting day 17, and through day 47 postdosing, and often considered the cause of death in unscheduled sacrifice mice. Thrombi were not observed in treated neonatal or juvenile NHPs.

Dorsal root ganglia (DRG) mononuclear cell inflammation sometimes associated with neuronal satellitosis or neuronal necrosis was observed 2 weeks after intrathecal administration in cynomolgus NHPs (data not shown) [8]. Similar findings were not observed in mice.

3.2 Onasemnogene Abeparvovec-Related Safety Signals for Patients

Demographics and baseline clinical characteristics of patients in the clinical studies are presented in Table 1 in the ESM. In total, 101 of 102 patients (99%) experienced at least one treatment-emergent AE (TEAE), and 58 patients (56.9%) experienced TEAEs considered related to treatment by the investigator. A total of 50 patients (49%) experienced an SAE, of which 11 (10.8%) were considered related to treatment (Table 3). None of the most frequently reported SAEs (pneumonia, respiratory distress, and upper respiratory tract infection) were considered treatment related by the investigators. The most frequently reported treatment-related SAEs were increased liver function test (LFT) findings and pyrexia (two of 99 for each; 2.0%), with fewer than two patients representing the other categories (Table 3).

In the postmarketing data set, 665 case reports containing 2407 AEs were identified, with 730 reported as SAEs. The most common events (≥ 50 reports) were pyrexia ($n = 185$ reports), vomiting ($n = 168$), aspartate aminotransferase (AST) increased ($n = 125$), alanine aminotransferase (ALT) increased ($n = 112$), hepatic enzyme increased ($n = 102$), platelet count decreased ($n = 61$), LFT results increased ($n = 57$), and thrombocytopenia ($n = 50$).

Although sporadic changes in vital-sign values were observed, none was persistent or deemed clinically meaningful. Blood pressure elevations were observed in association with prednisolone use. No clinically meaningful abnormal physical examination findings in the absence of AEs were recorded.

Deaths have been reported in children after onasemnogene abeparvovec administration (see pages 6–8 of the ESM). Specifically, three deaths were reported in the clinical trials as of 12 November 2020, including two patients in STR1VE-US [5] (one who died during the screening period and did not receive onasemnogene abeparvovec treatment) and one patient in STR1VE-EU [11]. Two additional deaths were reported in postmarketing data, which were assessed as not likely related to onasemnogene abeparvovec treatment but rather more likely because of underlying SMA.

3.3 Adverse Events of Special Interest

3.3.1 Hepatotoxicity

Data from clinical trials demonstrated elevations in aminotransferases without clinical manifestations. LFT elevations were initially observed at approximately day 7, nearly resolved at approximately day 14, with another transient increase at month 1, and returned to near baseline concentrations by month 2. Table 4 presents a summary of the AEs and relevant laboratory findings.

A search of the postmarketing data set yielded 375 cases (695 hepatic events), which were allocated to one of four categories: (1) isolated LFT elevations (337 cases, 591 events); (2) clinical signs and symptoms (jaundice and ascites) and events of abnormal laboratory tests, indicating coagulopathy, hypoalbuminemia, and increased ammonia (14 cases, 42 events); (3) event terms under the hepatobiliary disorders system organ class (20 cases, 46 events); and (4) reported diagnosis of acute liver failure (four cases, 16 events; Table 2 in the ESM). Of note, one case may have contained multiple event terms based on the information provided by the reporter. Concomitant medications associated with the

Table 2 Summary of key safety findings from nonclinical studies

Heart-related findings in mice	Onasemnogene abeparvovec-related findings in the heart consisted of inflammation, edema, fibrosis, and features of scattered myocardial degeneration/regeneration in the ventricles of the heart These findings were present at all doses studied, were dose-related in severity, and demonstrated maturation from an early event predominated by inflammation, with gradual maturation of the reaction to yield fibrosis predominantly over the 12-week timeframe of the studies with evidence of partial reversibility The primary findings in the atrium of the heart were thrombosis and inflammation. These findings were dose-related and presented at doses $\geq 2.4 \times 10^{14}$ vg/kg Atrial thrombosis was often considered the cause of death in unscheduled sacrifice mice. Whenever present, this disorder was considered potentially life threatening and, as such, was the basis for defining the MTD at 1.5×10^{14} vg/kg in mice The translatability of the observed findings in mice to primates is not known at this time
Liver-related findings in mice	Onasemnogene abeparvovec-related liver findings were noted in mice at doses $\geq 1.5 \times 10^{14}$ vg/kg and consisted of dose-related hepatocellular hypertrophy/regeneration and, less frequently, individual cell hepatocellular necrosis and hepatocellular perinuclear vacuolization and, occasionally, increased numbers of Kupffer cells Findings in the liver were sometimes accompanied by modest liver enzyme increases Liver findings were partially reversible, demonstrating progressively reduced incidence/severity over time
DRG-related findings in NHPs	Inflammation of the DRG was noted during histopathologic evaluation of select tissues following intrathecal administration of onasemnogene abeparvovec at 3×10^{13} vg/animal alone and in combination with two iohexol-based contrast agents The inflammation was characterized by minimal to marked infiltration of mononuclear inflammatory cells, primarily lymphocytes, into the cervical, thoracic, lumbar, and sacral DRGs and associated nerves Minimal inflammation was associated with scattered infiltrates or small aggregates of mononuclear cells in the DRG, without evidence of neuronal necrosis. With mild to marked inflammation, aggregates to sheets of mononuclear cells were present, along with neuronal satellitosis, neuronal necrosis, or neuronal loss with rare mineralization. Inflammation was observed in ganglia from all examined levels, but incidence and severity were generally greater in the sacral DRG Moderate to marked inflammation was observed only in the sacral DRG in animals not administered corticosteroids Similar findings have been reported after administration of AAV9 vectors in monkeys and minipigs [13, 14]
Other toxicity-related information or data	Some mice affected with a form of SMA type 1 that were treated with the study vector developed localized vascular necrosis around the ear, which is called necrotic pinna. This is believed to be unrelated to the vector but likely related to an underlying defect that has been observed to occur in several SMA mouse models [33]

AAV9 adeno-associated virus serotype 9, *DRG* dorsal root ganglia, *MTD* maximum tolerated dose, *NHP* nonhuman primate, *SMA* spinal muscular atrophy, *vg* vector genome

potential for liver injury were also reported (concomitant acetaminophen, 18 cases; concomitant ranitidine, 19 cases).

3.3.2 Thrombocytopenia

Transient losses in mean platelet count were observed for patients at approximately day 7 postdosing. In clinical trials, two patients had AEs of thrombocytopenia associated with platelet values of $< 75 \times 10^9$/L, neither with bleeding concerns. One occurred in STR1VE-EU in conjunction with multiorgan failure in the setting of sepsis and likely disseminated intravascular coagulopathy. One was an isolated AE in STR1VE-US with a report of "platelet count decreased" (potentially clinically significant value of 74×10^9/L) without clinical signs or symptoms. Four additional patients had platelet-count values that met laboratory criteria for thrombocytopenia (platelet count of $< 75 \times 10^9$/L), but they were not reported as AEs. Nadir platelet values ranged from 4 to 74×10^9/L (days 2–63 postdosing, with four of six occurring $\leq$ 10 days postdosing). All thrombocytopenia events that were not attributable to other causes were transient and resolved without intervention or associated clinical sequelae.

Postmarketing surveillance data provided 134 cases (148 thrombocytopenia events). In total, 19 unique cases indicated a bleeding event, including four TMA cases. One case report reflected distal lower extremity petechial rash and thrombocytopenia (platelet count 11×10^9/L), which were treated with two platelet transfusions and subsequently resolved. All other thrombocytopenia cases in the postmarketing surveillance data were reported as bleeding events

Table 3 Summary of related serious treatment-emergent adverse events by system organ class and preferred term for group 1 studies

System organ class; preferred term	START All ($N = 15$)	STR1VE-EU 1.1×10^{14} vg/kg ($N = 33$)	STR1VE-US 1.1×10^{14} vg/kg ($N = 22$)	SPR1NT 1.1×10^{14} vg/kg ($N = 30$)	STR1VE-AP 1.1×10^{14} vg/kg ($N = 2$)	Therapeutic IV dose[a] ($N = 99$)
Patients with at least one related serious TEAE	2 (13.3)	6 (18.2)	3 (13.6)	0	0	10 (10.1)
Investigations	2 (13.3)	1 (3.0)	2 (9.1)	0	0	4 (4.0)
Alanine aminotransferase increased	0	1 (3.0)	1 (4.5)	0	0	2 (2.0)
Aspartate aminotransferase increased	0	1 (3.0)	1 (4.5)	0	0	2 (2.0)
Transaminases increased	2 (13.3)	0	1 (4.5)	0	0	2 (2.0)
Coagulation test abnormal	0	1 (3.0)	0	0	0	1 (1.0)
General disorders and administration site conditions	0	2 (6.1)	0	0	0	2 (2.0)
Pyrexia	0	2 (6.1)	0	0	0	2 (2.0)
Infections and infestations	0	2 (6.1)	0	0	0	2 (2.0)
Gastroenteritis	0	1 (3.0)	0	0	0	1 (1.0)
Rhinovirus infection	0	1 (3.0)	0	0	0	1 (1.0)
Viral infection	0	1 (3.0)	0	0	0	1 (1.0)
Metabolism and nutrition disorders	0	2 (6.1)	0	0	0	2 (2.0)
Feeding disorder	0	1 (3.0)	0	0	0	1 (1.0)
Hypernatremia	0	1 (3.0)	0	0	0	1 (1.0)
Blood and lymphatic system disorders	0	1 (3.0)	0	0	0	1 (1.0)
Thrombocytopenia	0	1 (3.0)	0	0	0	1 (1.0)
Hepatobiliary disorders	0	1 (3.0)	0	0	0	1 (1.0)
Hypertransaminasemia	0	1 (3.0)	0	0	0	1 (1.0)
Nervous system disorders	0	0	1 (4.5)	0	0	1 (1.0)
Hydrocephalus	0	0	1 (4.5)	0	0	1 (1.0)

Data are presented n (%). Treatment-related adverse events are those that are considered possibly, probably, or definitely related to onasemnogene abeparvovec by the investigator

IV intravenous, *TEAE* treatment-emergent adverse event, *vg* vector genome

[a]Therapeutic IV dose includes patients who received the therapeutic dose in START and the 1.1×10^{14} vg/kg dose in STR1VE-EU, STR1VE-US, SPR1NT, and STR1VE-AP

related to isolated bruising at the injection site or platelets marginally low that were included in the broad SMQ for hemorrhage. These thrombocytopenia cases were not associated with bleeding, hematochezia, melena, prolonged prothrombin or international normalized ratio without bleeding sign, gastrointestinal bleed approximately 1 month after platelet normalized, or reported with a nonbleeding term.

3.3.3 Thrombotic Microangiopathy

No TMA was reported in clinical studies. Four cases were identified in the postmarketing data set. In all reported cases, TMA occurred within 6–11 days postdosing. Presenting features included vomiting, hypertension, oliguria/anuria, and/or edema. Laboratory data revealed elevated serum creatinine, proteinuria and/or hematuria, and hemolytic anemia (decreased hemoglobin with schistocytosis on peripheral blood smear). Two patients had TMA in the setting of recent infections. Medical interventions included one or more of the following: blood and/or platelet transfusions; plasmapheresis; systemic corticosteroids; and eculizumab, and/or supportive fluid, electrolyte, and hypertension management, with one patient requiring hemodialysis. All thrombocytopenia and anemia events resolved, and serum creatinine concentrations normalized.

3.3.4 Cardiac Adverse Events

In clinical trials, 17 patients (16.7%) had at least one cardiac AE. Most were associated with transient tachycardia or bradycardia ($n = 7$; 6.9%). None were reflective of intracardiac thrombi. No thrombi were reported in available human autopsy data from two patients; however, myocardial inflammation was present in the setting of multiorgan

Table 4 Hepatotoxicity adverse events and laboratory findings

Hepatotoxicity	START (N = 15)	STR1VE-EU (N = 33)	STR1VE-US (N = 22)	SPR1NT (N = 30)	STR1VE-AP (N = 2)	Total (N = 102)
Reported AEs	4 (26.7)	18 (54.5)	7 (31.8)	8 (26.7)[a]	0	37 (36.3)
Elevations in LFT results (not reported as AEs)[b]	10 (66.7)	12 (36.4)	13 (59.1)	19 (63.3)	1	54 (52.9)
Postdosing elevations in LFT results[b]	15 (100)	29 (87.9)	20 (90.9)	26 (86.7)	0	90 (90.0)
Elevations in LFT results at baseline (prior to dosing)[b]	9 (60.0)	22 (66.7)	5 (22.7)	20 (66.7)	0	56 (54.9)

AEs adverse events, *LFT* liver function test, *ULN* upper limit of normal

[a]One of these events did not have laboratory abnormalities that were reported

[b]LFTs included analysis of aspartate aminotransferase, alanine aminotransferase, and bilirubin; all were < 2 × ULN

system failure caused by sepsis. No AEs of depressed cardiac function were reported. Baseline left ventricular ejection fraction (LVEF) was normal for all but two patients who had borderline low values (50.8 and 55.7%). Two additional patients each had a single post-treatment occurrence of LVEF values that were slightly below normal but not clinically significant (51.0 and 55.4%). The mean (±standard deviation [SD]) LVEF was 65.1% (± 5.19) at baseline and 68.9% (± 5.91) at 6 months postdosing, with similar values at 12 and 18 months.

Of the 16 patients with troponin I elevations, two had postdosing elevations and three had baseline elevations, which worsened in one patient without associated clinical signs or symptoms. Two patients had elevations decrease from baseline elevations, two of those untreated (screen failures) had elevations, and one patient exhibited an aberrant value.

In the postmarketing data set, asymptomatic troponin I elevations were reported; none were associated with clinical events other than a 15-month-old child with elevated troponin I at the time of death (2 h after resuscitation). Two postmarketing fatal cardiac arrests precipitated by respiratory failure were reported.

3.3.5 Ganglionopathy

No AEs suggestive of ganglionopathy were reported, and a review of documented neurologic physical examination data in clinical studies demonstrated no findings consistent with ganglionopathy.

4 Discussion

This report is the first comprehensive description of overall safety data from more than 800 patients treated with onasemnogene abeparvovec and represents the first report of overall safety associated with systemic AAV-mediated gene-replacement therapy. Safety risks included hepatotoxicity, thrombocytopenia, cardiac events, TMA, and ganglionopathy. Table 5 includes recommended mitigation strategies for each outlined onasemnogene abeparvovec-related risk.

Hepatotoxicity observed in pivotal mouse toxicology studies is consistent with cell-mediated immunity to the vector capsid. Kupffer cell transduction can also elicit an immune response [13]. Onasemnogene abeparvovec-related liver findings in mice were sometimes accompanied by modest liver enzyme increases with attenuation over time. Similarly, liver transaminase elevations were observed with other AAV gene therapies for humans (e.g., hemophilia B) [14]. One patient with SMA with acute liver injury presented with AEs consistent with hepatotoxicity. Clinical laboratory values indicated liver transaminase elevations and acute liver injury in some cases. In SMA clinical trials, these AEs were clinically asymptomatic and resolved with prednisolone. In two patients, liver failure was resolved with transient high-dosage steroid administration [15]. All patients received prophylactic prednisolone, but dosing and duration were variable. Based on the current evidence, recommendations regarding optimal prednisolone dosing should be individualized. In general, tapering of prednisolone should not commence until AST/ALT concentrations are less than two times the upper limit of normal [6]. Of note, nearly 55% of patients had LFT elevations at baseline, which is consistent with those reported previously [6]. Although this suggests that patients with SMA may have underlying hepatic abnormalities at baseline, neither the prevalence in patients with SMA nor the relationship to SMA are known at this time.

While decreased platelet counts were reported in some mouse studies, they were not always considered treatment related. In clinical studies and postmarketing data, platelet-count values that met laboratory criteria for thrombocytopenia were transient and without clinical sequelae, except for

one patient who developed late-onset thrombocytopenia (63 days postdosing) in the setting of multiorgan system failure because of multiple concurrent infections. The mechanism for thrombocytopenia remains unknown.

TMA represents a newly identified risk in postmarketing surveillance. Presenting features included vomiting, hypertension, oliguria/anuria, and/or edema. Two patients had infections before developing TMA. Thus, waiting for illness to resolve prior to treatment with onasemnogene abeparvovec was recently recommended [16]. Encapsulated bacteria can trigger TMA because the capsular polysaccharide is a virulence factor, enabling immune evasion. Because any source of immune activation can potentially trigger TMA, it was noteworthy that both patients with preceding infections were vaccinated within 2–3 weeks of dosing. As such, concurrent immune system activation (e.g., infection, vaccination) was identified as a potential contributing factor in some cases. The mechanism of postdosing TMA is unknown but might be linked to complement activation [17]. Early recognition of TMA is critical to implementing effective treatment. Patients may be at an elevated risk if they have concurrent infections or other immune triggers. Further clinical investigation should be prompted when clinical signs/symptoms emerge [7]. All four patients with TMA had elevated serum creatinine that normalized. However, creatinine concentrations in patients with SMA must be interpreted with care because they may be reduced secondary to diminished muscle mass.

Preclinical murine data identified the heart as a target organ of toxicity with histopathologic evidence of inflammation and intracardiac thrombi. Onasemnogene abeparvovec-related mortality in mice was associated with atrial thrombosis at a dose of $\geq 2.4 \times 10^{14}$ vg/kg. However, no evidence of intracardiac thrombi or echocardiogram findings with evidence of clinical pathology was observed in primates or humans. No patterns suggestive of clinical toxicity were identified as part of the clinical trial program, which included troponin I, electrocardiogram, and echocardiogram assessments. Other AAV9 vectors have been used for cardiac transgene expression without reports of cardiac toxicity [18,

Table 5 Risk mitigation strategies

Risk	Risk mitigation strategy
Hepatotoxicity	Patients should have liver function tests conducted at baseline and on a regular basis following onasemnogene abeparvovec infusion [34]. Patients with ALT/AST/total bilirubin > 2 × ULN should not be dosed unless elevated bilirubin is associated with neonatal jaundice Patients should be treated with prednisolone before and after onasemnogene abeparvovec infusion [34]. Pretreatment with oral prednisolone should be given 24 h prior to infusion with onasemnogene abeparvovec at a dosage of 1 mg/kg/day. An equivalent dosage of another corticosteroid may be used at the discretion of the treating physician AST/ALT/bilirubin should be assessed weekly for 30 days and every 2 weeks for an additional 60 days post administration of onasemnogene abeparvovec through the end of the corticosteroid taper, or longer if needed. Tapering of prednisolone should not be considered until AST/ALT are < 2 × ULN [34] Consider consultation with a pediatric gastroenterologist/hepatologist preemptively prior to dosing if necessary and/or if elevations in aminotransferases occur [6] If oral prednisolone is not tolerated (monitor patient for vomiting), consider IV administration of corticosteroids Potentially hepatotoxic medications should be avoided when possible [6]
Thrombocytopenia	Platelet counts should be obtained before onasemnogene abeparvovec infusion and monitored on a regular basis afterwards: weekly for the first month and every other week for the second and third months until platelet counts return to baseline [34]
TMA	Early recognition of TMA is imperative, as TMA may require such therapeutic interventions as plasmapheresis, dialysis, or pharmacotherapy to lessen associated morbidity and mortality [7] TMA is a clinical diagnosis: signs and symptoms may include vomiting, pallor, petechiae, purpura, oliguria, pitting edema, hypertension, and seizures. While routine platelet-count monitoring is recommended at baseline and regular intervals postdosing [34], clinical suspicion and increased awareness should guide the clinician in the event that additional evaluation is warranted If thrombocytopenia is present and there is a clinical suspicion of TMA, further evaluation, including hemoglobin and testing for hemolysis and renal dysfunction (including urinalysis), also should be obtained [7] Consult a pediatric nephrologist/pediatric hematologist/pediatric intensivist immediately in suspected cases for further evaluation and management
Cardiac events	Troponin I should be obtained at baseline and monitored for at least 3 months following onasemnogene abeparvovec infusion or until concentrations return to within normal reference range for patients with SMA [34] Should elevations in troponin occur, the patient should be evaluated for signs or symptoms of cardiac dysfunction, with a pediatric cardiologist consulted, if necessary
DRG cell inflammation	A detailed neurologic examination should be conducted. If abnormalities are detected, further evaluation should be undertaken as clinically warranted

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *DRG* dorsal root ganglion, *IV* intravenous, *SMA* spinal muscular atrophy, *TMA* thrombotic microangiopathy, *ULN* upper limit of normal

19]. In addition, the significance of troponin elevations in children remains unclear [20].

Patients had no overt clinical symptoms/signs of sensory neuropathy suggesting DRG inflammation as reported by the investigators. The preclinical onasemnogene abeparvovec-related DRG findings were comparable with those observed with several other intrathecal- or intravenous-administered AAV gene therapies using similar or assorted capsids and transgenes in NHPs and appear to be an AAV class effect [21, 22]. The DRG microscopic findings were generally asymptomatic, with onset within weeks of dose administration. These findings do not indicate progressive courses, but they do display morphologic features of expected tissue remodeling [22]. The mechanism and translational relevance for humans is uncertain. Identification of ganglionopathies in children is often challenging. Several histologic studies for humans demonstrated degeneration in sensory neurons, DRG, and motor neurons in various diseases, including SMA type 1 [23]. The utility of electrophysiology in children with SMA appears variable. Small studies have reported normal sensory examinations and electrophysiology in patients with SMA type 2 or 3 [24]. Another analysis of 106 patients with SMA demonstrated that 32.0% (16/50) of those with SMA type 1, 15.8% (6/38) of those with SMA type 2, and 22.2% (4/18) of those with SMA type 3 had absent sensory nerve action potentials [25]. Interpreting sensory nerve action potential data for SMA related to the diagnosis of an inflammatory ganglionopathy was not straightforward as electrophysiologic findings are highly influenced by extremity temperature, electrode size, and electrode pressure [26] and are prone to errors in distance measurement, stimulation, and recording. These challenges limited the ability to delineate the true prevalence of sensory nerve abnormalities in SMA.

Deaths have been reported for children after onasemnogene abeparvovec administration (see pages 6–8 in the ESM). The natural history of absolute survival probabilities for children with SMA type 1 at ages 1, 2, 4, and 10 years was 40%, 25%, 6%, and 0%, respectively [27]. The mortality rate postdosing was two of 102 (1.9%) patients in the clinical trials and two of 600 (0.3%) events outside of clinical trials; deaths were considered unrelated to treatment by the investigator and sponsor. Although survival of children with SMA type 1 has improved during the past 3 decades because of improved respiratory and nutritional management, per the standard-of-care guidelines [28, 29], the mean age of death (±SD) was 10.4–48 months (3.4–4.0) [30], with some patients surviving for up to 24 years [31]. With more aggressive ventilatory and nutritional support, median survival time was 24.0 months for those born between 1995 and 2006 [30]. Furthermore, survival probability at age 12 months was 79.3% ($n = 78$). Thus, recognizing the potential comorbidities that these medically complex children face and ensuring that each patient is in the optimal state of health before and at the time of dosing (i.e., no signs/symptoms of infection) is critical.

Long-term follow-up analysis of patients in the START study (median time since dosing of 5.2 years [range 4.6–6.2] as of 11 June 2020) has not identified new safety signals [12]. However, as recommended in a recent European ad hoc consensus statement [32], further studies analyzing the long-term effects of gene therapy for SMA are necessary.

The present study is limited by the inherent nature of reporting postmarketing data because reporting is spontaneous (voluntary) and also includes sources such as social media, published literature, market research, and public databases. Therefore, these data may not be complete or capture details such as alternate etiologies, potential confounders, and time to resolution of AEs, if applicable.

5 Conclusion

This comprehensive integrated assessment of safety information indicates that, although the risks of onasemnogene abeparvovec administration might be serious if not anticipated and recognized early, they can be managed through diligent standard of care, sometimes requiring medical intervention.

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Declarations

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Conflicts of interest John Day, Jerry Mendell, Eugenio Mercuri, Richard Finkel, and Kevin A. Strauss were study investigators in onasemnogene abeparvovec clinical trials. Aaron Kleyn, Sitra Tauscher-Wisniewski, Francis Fonyuy Tukov, Sandra P. Reyna, and Deepa H. Chand are employees of Novartis Gene Therapies, Inc., the manufacturer of onasemnogene abeparvovec.

Availability of data and material Novartis is committed to sharing clinical trial data with external researchers and has been doing so voluntarily since 2014. Novartis was the third member to join ClinicalStudyDataRequest.com (CSDR), which is the first data-sharing consortium of clinical study sponsors and funders. CSDR is a leader

in the data-sharing community inspired to drive scientific innovation and improve medical care by facilitating access to patient-level data from clinical studies (https://www.novartisclinicaltrials.com/TrialConnectWeb/voluntarydataviewmore.nov). Novartis is committed to sharing, upon requests from qualified external researchers and subsequent approval by an independent review panel based upon scientific merit, anonymized patient-level and study-level clinical trial data, and redacted clinical study reports, for medicines and indications approved in the United States and Europe after the respective study is accepted for publication. All data provided are anonymized to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations. This trial data availability is according to the criteria and process described at http://www.clinicalstudydatarequest.com.

Code Availability Not Applicable.

Author contributions DHC and FFT, both of Novartis Gene Therapies, Inc., developed the first draft of the manuscript and conducted the data analysis. All authors reviewed the manuscript for important intellectual content and approved the final version for submission. DHC had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors had full access to all the data in the study and accept responsibility to submit for publication. All authors reviewed and approved the final version of the manuscript.

Ethics approval All study protocols were approved by the institutional review boards or ethics committees, with appropriate informed consent obtained. Studies were registered with www.clinicaltrials.gov (NCT02122952 [START], NCT03306277 [STR1VE-US], NCT03505099 [SPR1NT], NCT03461289 [STR1VE-EU], NCT03837184 [STR1VE-AP], NCT03421977 [LT-001], and NCT04042025 [LT-002]).

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