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Original Article

Adeno-associated virus serotype 9 antibodies in patients screened for treatment with onasemnogene abeparvovec

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Spinal muscular atrophy is a progressive, recessively inherited monogenic neurologic disease, the genetic root cause of which is the absence of a functional *survival motor neuron 1* gene. Onasemnogene abeparvovec (formerly AVXS-101) is an adeno-associated virus serotype 9 vector-based gene therapy that delivers a fully functional copy of the human *survival motor neuron* gene. We report anti–adeno-associated virus serotype 9 antibody titers for patients with spinal muscular atrophy when they were screened for eligibility in the onasemnogene abeparvovec clinical trials (intravenous and intrathecal administration) and managed access programs (intravenous). Through December 31, 2019, 196 patients and 155 biologic mothers were screened for anti–adeno-associated virus serotype 9 binding antibodies with an enzyme-linked immunosorbent assay. Of these, 15 patients (7.7%) and 23 biologic mothers (14.8%) had titers >1:50 on their initial screening tests. Eleven patients (5.6%) had elevated titers on their final screening tests. The low percentage of patients with exclusionary antibody titers indicates that most infants with spinal muscular atrophy type 1 should be able to receive onasemnogene abeparvovec. Retesting may identify patients whose antibody titers later decrease to below the threshold for treatment, and retesting should be considered for patients with anti–adeno-associated virus serotype 9 antibody titers >1:50.

INTRODUCTION

The adeno-associated virus (AAV) serotype used as the delivery vector has potential implications for the safety and efficacy of any AAV-based gene therapy.[1] When an individual is exposed to endogenous AAV infections, an immune response to the AAV capsids can be mounted.[2,3] Consequently, a percentage of humans express neutralizing antibodies in the blood that could block gene transfer to cellular targets.[1,2] In addition, administration of recombinant AAVs (rAAVs) can induce antibodies that can neutralize the transduction of AAV gene therapies, activate the innate immune response, and trigger an adaptive immune response that includes a cellular response that may result in loss of transgene expression.[2] In healthy humans, the prevalence of anti-AAV antibodies can be greater for some AAV serotypes, e.g., anti-AAV serotype 1 (AAV1) and anti-AAV2, than for other serotypes such as AAV5, AAV6, AAV8, and AAV9.[3] In addition, most individuals seropositive for AAV5, AAV8, and AAV9 have low titers.[3] Intravenous (i.v.) injection of AAV9 has been proven to traverse the blood-brain barrier and efficiently transduce motor neurons in non-human primates.[4] As a result, AAV9 can serve as an effective vector for gene therapies for neurodegenerative diseases, as recently demonstrated in spinal muscular atrophy (SMA).[5] In addition, because motor neurons are post-mitotic and potentially long-lived, a single administration of AAV9 gene therapy may be sufficient for lifetime transgene expression.[1]

SMA is a progressive neurologic disease caused by decreased amounts of the ubiquitously expressed survival motor neuron (SMN) protein, which is required for survival of motor neurons.[1,6] The most severe cases, generally associated with two copies of the backup gene, *SMN2*, result in death or the need for permanent ventilation by 2 years of age if untreated.[5–10] In 2019, the US Food and Drug Administration (FDA) approved AAV9-based onasemnogene abeparvovec-xioi (Zolgensma; Novartis Gene Therapies, Inc., Bannockburn, IL, USA), administered i.v., for SMA in patients less than 2 years of age.[1,11] Onasemnogene abeparvovec (formerly AVXS-101) is a recombinant, self-complementary, AAV9 vector-based gene therapy that delivers a transgene encoding human SMN protein under the control of a cytomegalovirus enhancer/chicken-β-actin hybrid

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Table 1. Study designs

Study	Phase	Study status	No. of centers	Patient age at dosing	Patient population	Onasemnogene abeparvovec dose	Route of administration
START (ClinicalTrials.gov: NCT021222952)	I	complete	1 (United States)	<9 months	biallelic *SMN1* mutations; 2 copies *SMN2*; disease onset between 0 and 6 months	6.7×10^{13} vg/kg i.v.; 2.0×10^{14} vg/kg[a]	i.v.
STR1VE-US (ClinicalTrials.gov: NCT03306277)	III	complete	12 (United States)	<6 months	biallelic *SMN1* mutations; two copies *SMN2* (excluding c.859G>C)	1.1×10^{14} vg/kg	i.v.
STR1VE-EU (ClinicalTrials.gov: NCT03461289)	III	complete	10 (Belgium, France, Italy, United Kingdom)	<6 months	biallelic *SMN1* mutations; two copies *SMN2* (excluding c.859G>C)	1.1×10^{14} vg/kg	i.v.
US MAPs (ClinicalTrials.gov: NCT03955679)[b]	N/A	terminated	N/A (United States)		genetic diagnosis of SMA		i.v.
SPR1NT (ClinicalTrials.gov: NCT03505099)	III	active, not recruiting	29 (United States, Europe, Japan, the Republic of Korea, Taiwan, Australia)	<6 weeks	biallelic *SMN1* deletion; two to four copies *SMN2*	1.1×10^{14} vg/kg	i.v.
STRONG (ClinicalTrials.gov: NCT03381729)	I	suspended (partial clinical hold)	11 (United States)	6–60 months	homozygous absence of *SMN1* exon 7; three copies *SMN2* (excluding c.859G>C); able to sit but not stand or walk independently	6.0×10^{13} vg	i.t.

i.t., intrathecal; i.v., intravenous; N/A, not applicable; SMA, spinal muscular atrophy; *SMN*, survival motor neuron gene; US MAP, managed access program in the United States; vg, vector genomes.
[a]Values based on a quantitative PCR assay. When the Digital Droplet PCR assay was used, as it was in the other clinical trials and the US MAPs, the 2.0×10^{14} vg/kg dose was equivalent to 1.1×10^{14} vg/kg.
[b]The US MAPs were an early access program and not a clinical trial, and they provided access to i.v. onasemnogene abeparvovec only.

promoter.[12] In two clinical trials completed to date, START and STR1VE-US, a one-time i.v. dose of onasemnogene abeparvovec improved motor function and survival in symptomatic infants with SMA type 1 with transient, manageable adverse events, mainly asymptomatic transaminase elevations (J.R. Mendell et al., 2020, Am. Acad. Neurol., conference; unpublished data).[1,5]

Given that anti-AAV9 antibody concentrations are important considerations for the safety and efficacy of AAV9-based therapies, inclusion criteria for the onasemnogene abeparvovec clinical trials and the managed access programs (MAPs) for single-patient investigational new drug (IND) requests from treating physicians in the United States (US MAPs) included a relatively low ($\leq$1:50) anti-AAV9 antibody titer.[1,5] The objective of this report was to describe anti-AAV9 antibody titers in SMA patients potentially eligible for either i.v. or intrathecally administered onasemnogene abeparvovec in clinical trials and the US MAPs (i.v. only).

RESULTS

Through December 31, 2019, 241 patients had been screened in the Novartis Gene Therapies clinical trial and US MAPs (Table 1), of whom 196 patients had anti-AAV9 antibody test results (Table 2). The median (range) age of the patients tested was 4.8 (0.2–58.1) months. Fifteen (7.7%) patients tested had anti-AAV9 antibody titers >1:50 at initial screening, of whom 11 (5.6%) had titers >1:50 on their final tests and were excluded from receiving onasemnogene abeparvovec. Individual patient antibody titers are presented in Table S1, and the percentage of patients with >1:50 antibody titer at initial and final status is presented in Table S2.

In START, 16 patients were screened for antibodies, 3 (18.8%) had anti-AAV9 antibody titers >1:50 on their initial screening tests, and 1 (6.3%) had an elevated titer on the final screening test and was excluded from receiving onasemnogene abeparvovec. In STR1VE-US, 25 patients were screened for antibodies and none had anti-AAV9 antibody titers >1:50 at their initial screening tests. In STR1VE-EU, 40 patients were screened for antibodies, 6 (15.0%) had anti-AAV9 antibody titers >1:50 on their initial screening tests, and 5 (12.5%) had elevated titers on their final screening tests. In SPR1NT, biologic mothers were tested for anti-AAV9 antibody first, and infants whose mother's result was >1:50 subsequently required testing. Some physicians also elected to test mothers and infants simultaneously outside of this requirement. In all, 44 infants were screened for inclusion, and 41 biologic mothers were tested for anti-AAV9 antibodies in SPR1NT. Three biologic mothers (7.3%) had anti-AAV9 antibody titers >1:50, and physicians tested 14 patients for antibodies in this study. Two infants had anti-AAV9 antibody titers >1:50 on their initial screening tests, and both (14.3% of the 14 infants tested) also had elevated titers on their final screening tests and were excluded from the study. In STRONG, 37 patients were screened for antibodies, 3 (8.1%) had anti-AAV9 antibody titers >1:50 on their initial screening tests, and all 3 also had elevated titers on their final screening test. In the US MAPs, 64 patients were screened for antibodies, 1 (1.6%) had an anti-AAV9 antibody titer >1:50 at

Table 2. Anti-AAV9 antibody results

Study	Mothers with antibody tests (n)	Mothers with titers >1:50 at final screening test (n)	Mothers with titers >1:50 at final screening test (%)	Patients screened (n)	Patients with antibody tests[a] (n)	Median (range) age of patients tested for antibodies (months)	Patients with titers >1:50 at initial screening (n)	Patients with titers >1:50 at initial screening (%)	Patients with titers >1:50 at final screening test (excluded for AAV9) (n)	Patients excluded because of elevated titers (%)[b]	Patients excluded for other reasons (n)	Patients treated as of December 31, 2019 (n)
START	15	3	20	16	16	3.1 (0.6–7.4)	3	18.8	1	6.3	0	15
STR1VE-EU	40	10	25	40	40	3.3 (1.4–5.8)	6	15.0	5	12.5	2	33
STR1VE-US	24	3	13	26	25	3.1 (0.2–5.7)	0	0.0	0	0.0	4	22
US MAPs	–	–	–	77	64	6.1 (0.6–45.3)	1	1.6	0	0.0	20	57
SPR1NT	41	3	7	44	14	0.6 (0.3–1.2)	2	14.3	2	14.3[c]	12	30
STRONG	35	4	11	38	37	20.0 (6.2–58.1)	3	8.1	3	8.1	3	32
Total	155	23	14.8	241	196[d]	4.8 (0.2–58.1)	15	7.7	11	5.6	41	189

AAV9, adeno-associated virus serotype 9, US MAP, managed access program in the United States.
[a]Patients screened may have been excluded prior to antibody testing for other reasons.
[b]Variability in percentages across trials may result from sampling error caused by the limited number of patients in each trial.
[c]Two patients were excluded from the trial because of anti-AAV9 antibodies >1:50. Of the 44 patients screened for inclusion in SPR1NT, 41 biologic mothers were tested for anti-AAV9 antibody titers. Three biologic mothers had anti-AAV9 antibody titers >1:50. When their infants were tested, two had titers >1:50 and were excluded from the study, while one had titers ≤1:50 but was excluded from the study for another reason. Twelve additional patients were tested for anti-AAV9 antibodies, all of whom had titers ≤1:50; seven were enrolled in the study and five were excluded for other reasons. In addition, 30 patients were not tested directly for anti-AAV9 antibodies, 23 of whom were enrolled in the study and 7 of whom were excluded for reasons unrelated to anti-AAV9 antibodies.
[d]The 45 patients who were not tested for anti-AAV9 antibodies include 22 who were deemed ineligible for enrollment for reasons other than anti-AAV9 antibody titers and 23 patients enrolled in SPR1NT who were not tested because their biologic mothers had sufficiently low titers.

initial screening, and none had elevated titers on the final screening test.

A total of 155 biologic mothers had antibody tests. Of these, 23 (14.8%) had anti-AAV9 antibody titers >1:50 (Tables 1, S3, and S4). In the clinical trials, the percentages of mothers with anti-AAV9 antibody titers >1:50 ranged from 7% in SPR1NT to 25% in STR1VE-EU. Across all clinical trials, there were 127 mother-patient pairs, 98 (77.2%) of which had matching titer results at the initial screening test, while the remaining 29 pairs (26.4%) did not match (Table 3).

Of the 15 patients with anti-AAV9 antibody titers >1:50 on their initial screening tests, 12 (80%) were retested at least once before inclusion or not enrolling (Figure 1; Table S5). These 12 patients were ultimately tested a median of two times (range, 2–5). The mean time between tests ranged from as few as 3 days to 39 days (Table S5). The anti-AAV9 antibody titers of four patients fell to within thresholds following retesting to permit subsequent treatment with onasemnogene abeparvovec. The four patients ranged in age from 0.4 to 3.3 months at initial positive screening.

DISCUSSION

In clinical studies of onasemnogene abeparvovec, children with anti-AAV9 titers >1:50 were excluded because of the possibility that titers of anti-AAV9 antibodies that were >1:50 prior to dosing might impair efficacy or result in adverse events, although whether titers >1:50 interfere with the effectiveness or safety of onasemnogene abeparvovec in humans is unknown. The anti-AAV9 antibody enzyme-linked immunosorbent assay (ELISA) used in these studies measured total binding antibodies, not neutralizing antibodies. In the clinical protocols, children who had anti-AAV9 titers >1:50 at initial screening could be retested and would be eligible to receive onasemnogene abeparvovec if the anti-AAV9 antibody titer was ≤1:50 upon retesting.

Results from infants and children from clinical trials and US MAP enrollment through December 31, 2019, demonstrate that 7.7% of all patients under the age of 5 years had anti-AAV9 antibody titers >1:50 on the initial screening test, indicating that elevated anti-AAV9 antibody titers are relatively uncommon in infants and young children. Although there was a higher incidence of elevated titers in the STR1VE-EU data, commercial data from various regions, including Europe, the Middle East and Africa (EMEA), Asia-Pacific (APAC), and Latin America and Canada (LACan), indicate that the prevalence is relatively similar between regions at approximately 5% (data not shown). This is consistent with the data reported herein, as well as with another study that reported the prevalence of pre-existing antibodies specific for AAV9 in children 2–7 years of age as 6%.[13] Approximately 15% of biologic mothers tested had anti-AAV9 antibody titers >1:50, a lesser prevalence than total anti-AAV9 antibodies reported for

Table 3. Anti-AAV9 antibody titers in mother-patient pairs

Match	Study	n (%)	Median (range) age of patient at collection, months
No	START	2 (13.3)	1.95 (1.77–2.13)
	STRONG	10 (28.6)	27.60 (9.17–58.13)
	STR1VE-EU	10 (25.6)	4.15 (2.20–5.40)
	STR1VE-US	3 (12.5)	4.83 (2.03–4.83)
	SPR1NT	4 (28.6)	0.69 (0.53–0.82)
	All	29 (22.8)	4.83 (0.53–58.13)
Yes	START	13 (86.7)	3.83 (0.37–7.43)
	STRONG	25 (71.4)	19.83 (6.17–54.07)
	STR1VE-EU	29 (74.4)	2.70 (0.77–5.53)
	STR1VE-US	21 (87.5)	2.93 (0.23–5.67)
	SPR1NT	10 (71.4)	0.58 (0.26–1.22)
	All	98 (77.2)	3.80 (0.23–54.07)

Match indicates identical titers at initial screening test.

healthy adults (approximately 47%).[3,14] Collectively, these results suggest that in most infants with SMA type 1, exclusionary anti-AAV9 antibody titers are unlikely to preclude treatment with an AAV9-based gene therapy. Retesting in our studies demonstrated that anti-AAV9 antibody titer decreases are possible until at least 4 months of age. In the limited sample of older patients in our trial program, decreases in anti-AAV9 antibody titers were not detected.

Of the 12 patients who had titers >1:50 on their initial screening test and were retested, 4 were found to have had titers $\leq$1:50 on retesting and were subsequently enrolled. Initial elevations in anti-AAV9 antibodies may have resulted from transplacental transfer of maternal antibodies, and titers may have decreased over time to an extent that permitted dosing in some patients, especially in younger patients who had passive maternal antibody transfer. Thus, retesting for anti-AAV9 antibody titers in young candidates for i.v. onasemnogene abeparvovec is appropriate, especially in consideration of the potentially life-altering beneficial effect of this treatment.

The four patients enrolled following retesting had their initial positive screening tests at <4 months of age (range, 0.4–3.3 months), suggesting that placental transfer of antibodies caused their initial positive tests. These placentally transferred antibodies, most of which are immunoglobulin (Ig)G, result in passive immunity to AAV9 in infants, and would be expected to be lost over time.[15,16] The half-life of transplacental IgG is approximately 6 weeks, with undetectable concentrations by 4–6 months.[15] Our findings that 77.2% mother-patient pairs had matching antibody titer results, that the patients in discordant pairs were generally older than those in matching pairs, and that the patients' titers were generally lesser than the mothers' titers are consistent with the possibility of gradually diminishing titers following placental antibody transfer. The limited number of patients retested was too small for the authors to draw definitive conclusions, and additional explanations are also plausible. For example, given the maturation of the children's adaptive immune systems, we could also expect the development of antibodies after an infection with wild-type AAV. Given the half-life of maternally inherited anti-AAV9 antibodies and the timing of retesting in patients who were ultimately enrolled in our studies, waiting 3–4 weeks between an initial anti-AAV9 antibody test and a retest may be appropriate.

Given the increasing prevalence of prenatal testing and newborn screening for SMA resulting in identification of neonates earlier in the course of their disease, placental antibody transfer may have a greater impact on eligibility of those patients for AAV9-based gene therapy treatment than in these studies of somewhat older infants and young children. However, the 14.8% prevalence of mothers with titers >1:50 implies that most infants up to 6 weeks of age will still be eligible for onasemnogene abeparvovec. In the SPR1NT clinical trial, infants were not required to have been tested for anti-AAV9 antibody titers if their biologic mothers had titers $\leq$1:50. Any delay in treatment intervention in neonates with two copies of *SMN2* could negatively impact prognosis. Thus, alternative therapies should be considered in these patients whose titers are >1:50, which would not preclude later treatment with AAV9-based gene therapy should antibody titers decrease.

Breast milk is another potential source of anti-AAV9 antibodies. However, despite high concentrations of IgA in breast milk, gut closure occurs precociously in humans, resulting in negligible transfer of intact IgA across the neonate/infant gut, and IgA antibodies do not enter circulation.[17] Instead, milk-derived IgA provides protection against enteric infections.[17] Because these IgA antibodies do not reach circulation, breastfeeding should not have a negative impact on the efficacy or safety of onasemnogene abeparvovec in infants.

Plasmapheresis may be useful in patients whose titers exceed the $\leq$1:50 limit. Preliminary clinical data demonstrate that this technique is effective at lowering antibody titers against AAV types 1, 2, 6, and 8 in seropositive patients.[18] However, for plasmapheresis to be successful, the plasma being replaced must have low titers of anti-AAV antibodies. Similarly, a recent study demonstrated that hemapheresis combined with AAV9 particles coupled to Sepharose beads can selectively deplete anti-AAV antibodies.[19] Whether plasmapheresis or hemapheresis is necessary in patients with antibody titers >1:50 is unclear. For either approach, multiple cycles of plasmapheresis or immunoadsorption may be required to lower the anti-AAV antibody titers and create a short window of opportunity for the administration of the AAV gene therapy before the titers of anti-AAV antibodies begin to rebound. We are not aware of such interventions having been attempted in patients with SMA to reduce high anti-AAV9 antibody titers prior to gene therapy.

To limit any assay variability, all of the ELISA tests used in the STR1VE-US and SPR1NT studies were performed in one laboratory using the same assay (CTL, USA). The STR1VE-EU assays were performed in another laboratory (Viroclinics, Rotterdam, the Netherlands),[20] but the methodologies used by the two laboratories were identical and are used for the selection of patients who are

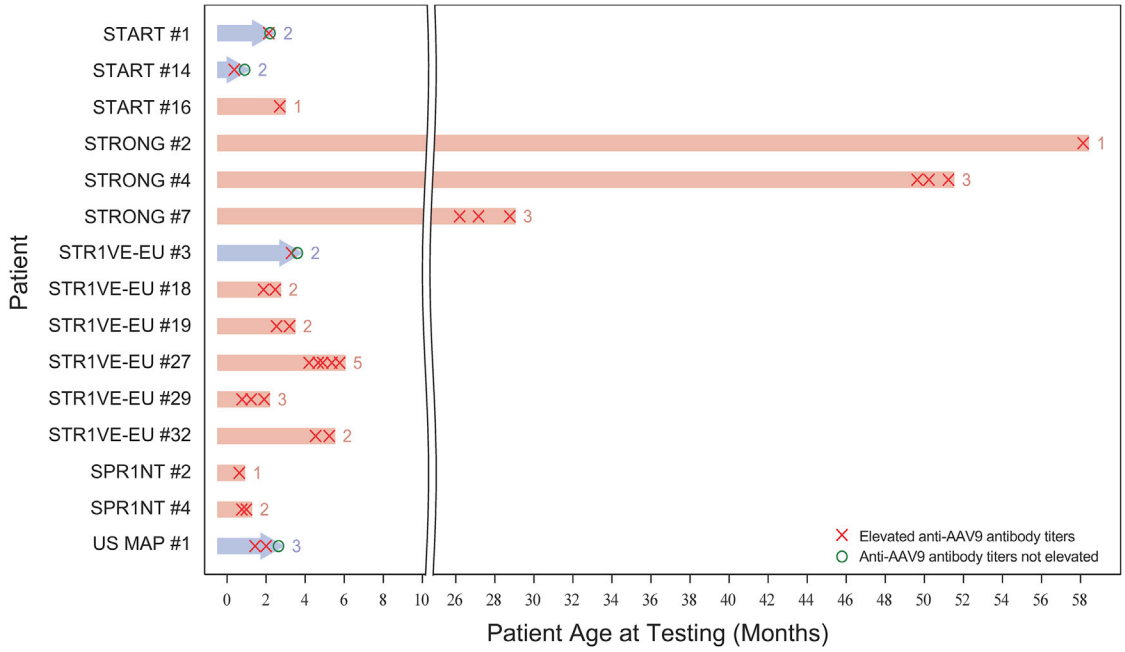

Figure 1. Testing frequency of patients with initially elevated anti-AAV9 antibody titers

Patients who were initially excluded on the basis of elevated titers were retested for anti-AAV9 antibodies, when possible. Each line includes the total number of tests performed, and symbols mark the age at which a test was elevated (red X) or not elevated (green O). Patients were permitted to retest until they met the age limit set within the enrollment criteria. AAV9, adeno-associated virus serotype 9.

eligible to be prescribed Zolgensma. START ELISA tests were performed at Nationwide Children's Hospital (Columbus, OH, USA). To reduce assay variability in current clinical practice, all anti-AAV9 antibody tests performed to determine eligibility for onasemnogene abeparvovec are centralized in two specialized laboratories in the United States, one laboratory in Japan, and one laboratory for the rest of world. The cutoffs and assays used by these laboratories may differ from those used in the clinical trials. In particular, Athena Diagnostics, one of the laboratories used for patients treated in clinical practice in the United States, defines patients with titers of <1:25 as being seronegative, whereas CTL and Viroclinics define patients with titers of $\leq$1:50 as being seronegative (i.e., in accordance with the criterion defined in the US package insert). The differences in these titers arise as a result of the different cut points used in the assays. Consequently, titers determined using the CTL/Viroclinics assays cannot be compared with titers determined using the Athena/MBL International assay.

The concentration of pre-existing anti-AAV antibody titers that precludes gene expression is currently unknown. The 1:50 antibody titer threshold used in the clinical trials was purposefully selected as a conservative approach for these early clinical trials.[1] No consensus currently exists on assay standardization or definitive antibody titer limits to guide patient eligibility.[1] This limit was chosen for all clinical studies of onasemnogene abeparvovec based on discussions with the FDA[1] and previous gene therapy trials, including a randomized controlled trial examining the intramuscular delivery of rAAV1.tMCK.hSGCA in patients with α-sarcoglycan deficiency.[21,22] In this study, one patient (patient 6) who did not respond (low-level gene expression and transgene copy numbers per nucleus) had pre-existing AAV1 neutralizing antibody titers of 1:1,600.[22] By day 7 following dosing, the antibody titer had risen to >1:102,400 and was sustained to at least 6 months after dosing. T cell immunity was also observed in this patient as early as day 2 using an enzyme-linked immunospot (ELISpot) assay.[22] The early humoral and T cell responses to the AAV1 capsid observed in this patient was indicative of pre-existing immunity and differed from those observed for other patients in this trial. However, the limits set for anti-AAV antibody in other clinical trials administering i.v. AAV vectors range from <1:1 for neutralizing antibodies to <1:400 for binding antibodies.[1] Nevertheless, the efficacy and safety of onasemnogene abeparvovec in patients with elevated titers remain unknown.

MATERIALS AND METHODS

This analysis includes data from all clinical trials in the onasemnogene abeparvovec program, as well as the US MAPs. The clinical trials were conducted in accordance with the Declaration of Helsinki, the International Council for Harmonisation/Good Clinical Practice guidelines, and applicable regulatory requirements, including those relating to informed consent and the protection of human patients in biomedical research. The study protocols and the informed consent form were approved by Institutional Review Boards at each site. The clinical trials were registered on ClinicalTrials.gov; a few of these clinical trials were also registered on EudraCT and/or JapicCTI.

Parent(s)/legal guardian(s) of patients provided signed and dated informed consent before the patient could undergo any study procedures. Biologic mothers of patients also gave informed consent to screen for their own circulating antibodies to AAV9.

Study designs

Study design information for onasemnogene abeparvovec clinical trials and the US MAPs are summarized in Table 1. All clinical trials were open-label studies, uncontrolled studies in which anti-AAV9 binding antibody titers >1:50 as determined by ELISA were exclusionary.

Per the START, STR1VE-US, STR1VE-EU, and STRONG protocols, a potential participant demonstrating an anti-AAV9 antibody titer >1:50 could receive retesting within 30 days of the initial screening test. The patient would be eligible to participate if the anti-AAV9 antibody titer was $\leq$1:50 upon retesting. The biologic mother also had blood drawn at screening. The specialized laboratories received serum samples for screening of anti-AAV9 antibodies.

According to the SPR1NT protocol, biologic mothers were to be tested for anti-AAV9 antibody first. If the biologic mother had anti-AAV9 titers >1:50, or the biologic mother was not available for testing, then the patient gave a 1-mL sample for baseline anti-AAV9 antibody titer testing. Patients were required to have an anti-AAV9 antibody titer $\leq$1:50 as determined by ELISA. A gene therapy candidate demonstrating anti-AAV9 antibody titer >1:50 could be retested within 30 days of initial screening and be eligible to participate if the anti-AAV9 antibody titer was $\leq$1:50, provided the patient was still <6 weeks of age at the time of dosing.

In START, STR1VE-US, STR1VE-EU, and SPR1NT, mothers who tested positive for antibodies to AAV9 were asked to refrain from breastfeeding until 30 days post-dose. Patients consuming banked breast milk from donor sources that could not be tested for anti-AAV9 antibodies were to be transitioned to formula prior to participation.

MAPs provide access to treatment for patients with a serious or life-threatening disease or condition, with no comparable or satisfactory alternative therapy available to monitor or treat the disease or condition. Eligible patients with a genetic diagnosis of SMA were treated i.v. in either the single-patient INDs, or later in the US cohort protocol MAP. The US MAPs terminated upon approval of onasemnogene abeparvovec by the FDA on May 24, 2019.. In the US MAPs, anti-AAV9 antibody titers >1:50 as determined by ELISA were exclusionary.

Anti-AAV9 antibody screening: ELISA

The standard procedure used to estimate circulating antibody titers (total IgG) to AAV9 capsid in human blood was an AAV9-binding ELISA. Serum samples were collected, shipped to the specialized laboratories, serially diluted (1:12.5 to 1:400 dilution), and then assayed for the presence of circulating antibodies to AAV9. In the assay used for this screening, empty AAV9 capsid was pre-coated onto 96 well plates, then serially diluted human plasma (or serum) was applied to the pre-coated wells to detect antibodies for AAV9. Unbound material was washed from the well and a peroxidase-conjugated secondary antibody was added, followed by a wash to remove any unbound antibody-enzyme reagent. Finally, a substrate solution was added to the wells, and color development, which was stopped by the addition of 1 N hydrochloric acid, occurred in proportion to the amount of anti-AAV9 antibody bound to the antigen. Optical density of the color was measured using a plate reader, and the endpoint titer was calculated based on the reciprocal value of the last dilution to yield a signal significantly above assay background. ELISAs for the STR1VE-US and SPR1NT studies were performed at CTL (USA), and assays for STR1VE-EU were performed at Viroclinics (the Netherlands), using identical methodology. ELISA for the START study was performed at Nationwide Children's Hospital (Columbus, OH, USA). ELISAs were performed in triplicate, with a negative and positive control. The ELISA assays used in the clinical trials were aligned between laboratories in terms of accuracy, intermediate precision, linearity, repeatability, specificity, and stability.

Statistical analysis

Data are described descriptively. No comparative analyses were conducted.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtm.2021.02.014.

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AUTHOR CONTRIBUTIONS

J.W.D., R.S.F., E.M., K.J.S., and J.R.M. were involved in the investigation and reviewed and edited the manuscript. M.M. provided data curation and formal analysis, visualization, and validation of the data. R.v.O. was involved in the investigation and formal analysis of the data and reviewed and edited the manuscript. S.T.-W. reviewed and edited the manuscript. All co-authors vouch for the data and analysis and decided to publish the data.

DECLARATION OF INTERESTS

J.W.D. reports financial relationships with Novartis Gene Therapies, Inc., Affinia Therapeutics, Biogen, Cytokinetics, Ionis, Pfizer, Roche, and Sarepta, and royalties from Athena Diagnostics. R.S.F. has served as an advisor to Novartis Gene Therapies, Inc., Ionis, Biogen, and Roche, and received licensing fees from the Children's Hospital of

Philadelphia related to development of the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders scale. E.M. has received personal compensation for clinical trial consulting and serving on scientific advisory boards from Novartis Gene Therapies, Inc. K.J.S. has received personal compensation from Novartis Gene Therapies, Inc., for serving as an advisory board member, and from Biogen for serving as a visiting professor; and has received research support from Novartis Gene Therapies, Inc., and Biogen-sponsored clinical trials. M.M. is an employee of Novartis Gene Therapies, Inc. R.v.O. and S.T.-W. are employees of Novartis Gene Therapies, Inc., and own Novartis stock or other equities. J.R.M. declares no competing interests.

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