Pediatric Neurology 100 (2019) 3–11

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Topical Review

From Clinical Trials to Clinical Practice: Practical Considerations for Gene Replacement Therapy in SMA Type 1

Samiah A. Al-Zaidy, MD [a, b], Jerry R. Mendell, MD [a, b, c, *]

[a] Department of Pediatrics, Ohio State University, Columbus, Ohio
[b] Center for Gene Therapy, Nationwide Children's Hospital, Columbus, Ohio
[c] Department of Neurology, Ohio State University, Columbus, Ohio

ARTICLE INFO

Article history:
Received 6 February 2019
Accepted 5 June 2019
Available online 13 June 2019

Keywords:
AVXS-101
Zolgensma
Onasemnogene abeparvovec
Gene replacement
Gene therapy
Practical considerations
SMA1
Spinal muscular atrophy

ABSTRACT

Spinal muscular atrophy is a devastating neurodegenerative autosomal recessive disease that results from *survival of motor neuron 1* (*SMN1*) gene mutation or deletion. Patients with spinal muscular atrophy type 1 utilizing supportive care, which focuses on symptom management, never sit unassisted, and 75% die or require permanent ventilation by age 13.6 months. Onasemnogene abeparvovec (Zolgensma, formerly AVXS-101) is a gene replacement therapy comprising an adeno-associated viral vector containing the human *SMN* gene under control of the chicken beta-actin promoter. This therapy addresses the genetic root cause of the disease by increasing functional SMN protein in motor neurons and preventing neuronal cell death, resulting in improved neuronal and muscular function as previously demonstrated in transgenic animal models. In an open-label, one-arm, dose-escalation phase 1 trial, systemic administration of onasemnogene abeparvovec via a one-time infusion over one hour demonstrated improved motor function and survival in all infants symptomatic for spinal muscular atrophy type 1. Of the 12 patients who received the proposed therapeutic dose, 11 achieved independent sitting, two achieved independent standing, and two are able to walk. Most of these 12 patients remained free of respiratory supportive care. The only treatment-related adverse event observed was transient asymptomatic transaminasemia that resolved with a short course of prednisolone treatment. This review discusses the biological rationale underlying gene replacement therapy for spinal muscular atrophy, describes the onasemnogene abeparvovec clinical trial experience, and provides expert recommendations as a reference for the real-world use of onasemnogene abeparvovec in clinical practice. As of May 24, 2019, the Food and Drug Administration approved onasemnogene abeparvovec, the first gene therapy approved to treat children younger than two years with spinal muscular atrophy.

Introduction

Spinal muscular atrophy (SMA) is a devastating neurodegenerative autosomal recessive disease that results from a defect in the survival of motor neuron (*SMN*) gene. In humans, the *SMN* gene is duplicated (*SMN1* and *SMN2*), and it is a mutation or deletion in *SMN1* that leads to a deficiency of SMN protein, required by motor neurons at high levels to survive.[1] Motor neuron loss is the primary driver of disease pathogenesis.[1] The clinical phenotype of SMA varies owing, in part, to the number of copies of the *SMN2* gene, which is nearly identical to *SMN1*, except for a point mutation in exon 7 that results in a limited amount of functional SMN protein that cannot fully compensate for the loss of *SMN1*. Patients with two copies of *SMN2* without a disease-modifying mutation have a 97% risk of developing SMA type 1 (SMA1),[2] characterized by the inability to achieve independent sitting.[3] However, infants with SMA with three copies of *SMN2* are still at risk of developing SMA1. Feldkotter et al. found that 19.7% of an SMA1 cohort carried three copies of *SMN2*, yet presented as typical patients with SMA1 (i.e., symptom onset at age less than six months and inability to sit unassisted). Natural history studies of patients with recently diagnosed SMA1 showed a rapidly progressive decline in motor abilities, with patients demonstrating the highest achieved motor function at the first visit.[3,4]

Conflicts of interest: Neither author has financial interest in Zolgensma.
Financial disclosure statement: Both authors are consultants to AveXis, Inc.
Funding source: This study was supported by AveXis, Inc., Bannockburn, IL, USA.
* Communications should be addressed to: Dr. Mendell; Research Institute at Nationwide Children's Hospital; 700 Children's Drive, Rm WA2016, Columbus, OH 43205.
E-mail address: jerry.mendell@nationwidechildrens.org (J.R. Mendell).

https://doi.org/10.1016/j.pediatrneurol.2019.06.007

Multisystem symptom management by a multidisciplinary team is critical for patients with SMA to ensure optimal outcomes, irrespective of the treatment.[5] The current clinical management guidelines for SMA focus on acute and chronic symptom management.[5,6] These recommendations highlight the optimal nonpharmacologic management considerations to improve quality of life and potentially reduce disease burden. Future updates to standard of care guidelines are likely to include approved disease-modifying treatments, nusinersen[7] and onasemnogene abeparvovec (Zolgensma, formerly AVXS-101), as well as other emerging therapies.[8,9] Recently, a working group composed of 15 SMA experts recommended that presymptomatic infants with SMA1 with two to three copies of *SMN2* should receive immediate treatment with disease-modifying therapies.[10]

Nusinersen, an antisense oligonucleotide (ASO) delivered intrathecally through lumbar puncture, is the first disease-modifying drug approved for the treatment of SMA. In a phase 3 randomized, double-blinded study infants with SMA1 treated with nusinersen demonstrated improved motor milestones and permanent ventilation-free survival compared with a control group (Fig 1).[11] To maintain elevated levels of functional SMN protein, nusinersen requires multiple loading doses followed by repeated administration for life.[12,13] Furthermore, safety monitoring is required (platelet count, prothrombin time or activated partial thromboplastin time, and quantitative spot urine protein testing).[13] In addition, postmarketing experience suggests that hydrocephalus and serious infections, including meningitis, have occurred as complications, although the clear association has not been established.[13]

Onasemnogene abeparvovec is a one-time gene replacement therapy (GRT) consisting of an adeno-associated viral (AAV) vector containing the human *SMN* gene under control of the chicken beta-actin promoter[14]; it addresses the genetic root cause of the disease by providing a functional copy of the human *SMN* gene, thus increasing functional SMN protein in motor neurons, preventing neuronal cell death and halting disease progression. In an SMA transgenic mouse model, postnatal delivery of a one-time dose resulted in improved survival, motor function, and neuromuscular electrophysiology.[14] Based on promising preclinical results, an open-label, single-arm, dose escalation phase 1 trial, systemic administration of onasemnogene abeparvovec via a one-time intravenous infusion demonstrated improvements in motor milestones and survival in all infants symptomatic for SMA1 (Fig 2).[9] Of the 12 patients who received the proposed therapeutic dose, 11 achieved independent sitting and two achieved independent standing and walking at the final 24-month post-treatment visit.[9] After study completion, two additional patients achieved independent sitting (≥30 seconds) and two others achieved standing with support after enrollment in the long-term follow-up study.[9,15] In addition, seven of 10 patients (70%) who did not require supportive care at baseline remained free of respiratory supportive care. During this trial, this therapy demonstrated a favorable safety profile, with the only adverse event (AE) observed after onasemnogene abeparvovec GRT assessed as treatment-related being a transient asymptomatic elevation in liver enzymes (LEs) that was easily managed and resolved with prednisolone treatment.[9] These transient asymptomatic LE elevations have been observed in other intravenously administered AAV gene therapy clinical trials, and there were no clinically symptomatic acute immune-mediated responses reported in the onasemnogene abeparvovec trial or in other AAV trials.[9,16] The promising results of this phase 1 study of intravenous onasemnogene abeparvovec are currently being further evaluated in open-label, single-arm phase 3 trials in infants younger than six months with one or two copies of the *SMN2* gene in both the United States (ClinicalTrials.gov number NCT03306277 [STR1VE], and the European Union (ClinicalTrials.gov number NCT03461289 [STR1VE-EU]), both of which have completed enrollment of infants with SMA1. In addition, a phase 3 global study (SPR1NT) evaluating the efficacy of onasemnogene abeparvovec in presymptomatic neonatal patients with SMA (age six weeks or less at dosing) with multiple *SMN2* copies (two and three copies) is currently ongoing and enrolling patients (ClinicalTrials.gov number NCT03505099).

The previously published phase 1 results and preliminary results of the phase 3 (STR1VE) study of SMA1 continue to demonstrate promising results.[15,17] This review discusses the biological rationale underlying GRT for SMA, describes the clinical trial experience from the onasemnogene abeparvovec phase 1 clinical trial, and provides expert recommendations for the real-world use of onasemnogene abeparvovec as a reference for pediatric neurologists and other clinicians caring for and treating patients with SMA1.

Rationale for GRT in SMA

AAV-mediated GRT for monogenic diseases

The AAV vector platform is designed to deliver the desired transgene as a nonintegrating stable extranuclear episome, greatly reducing the risk of insertional mutagenesis reported in early retroviral gene therapy trials.[18] AAV serotypes, including AAV1, 2, 5, 6, 8, and 9, infect a broad range of proliferative and nonproliferative cells, with no pathogenicity in humans observed to date.[19] Although the proportion of the population that is positive for the anti-AAV9 antibody is smaller than for most other AAV serotypes[19] and children have low anti-AAV9 antibody titer frequencies, anti-AAV9 antibody levels are an important safety and efficacy

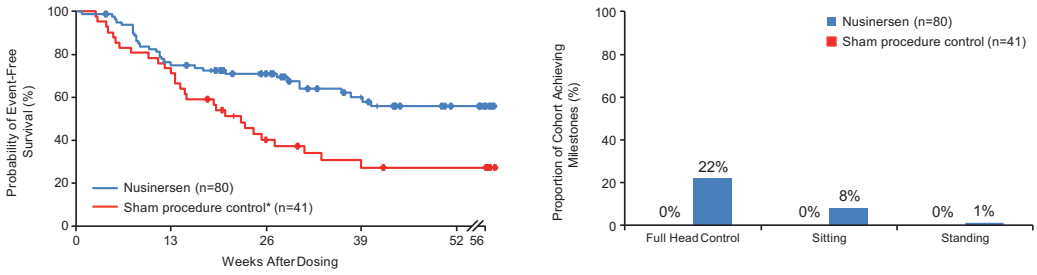

FIGURE 1. Phase 3 trial outcomes after nusinersen treatment in infants with SMA1.[11] Left panel: Probability of event-free survival in nusinersen-treated infants (n = 80) compared with the sham procedure control group (n = 41) as published by Finkel et al. in 2017 in the final analysis.[11] Event-free survival is survival independent of permanent ventilation, as defined as tracheostomy or ventilatory support for ≥16 hours per day for >21 continuous days in the absence of an acute reversible event. Right panel: At final analysis, motor milestones achieved by nusinersen-treated infants (n = 80) compared with SMA1 infants who underwent the sham procedure (control group; n = 41). SMA1, spinal muscular atrophy type 1. Reprinted with permission of the Massachusetts Medical Society.[11]

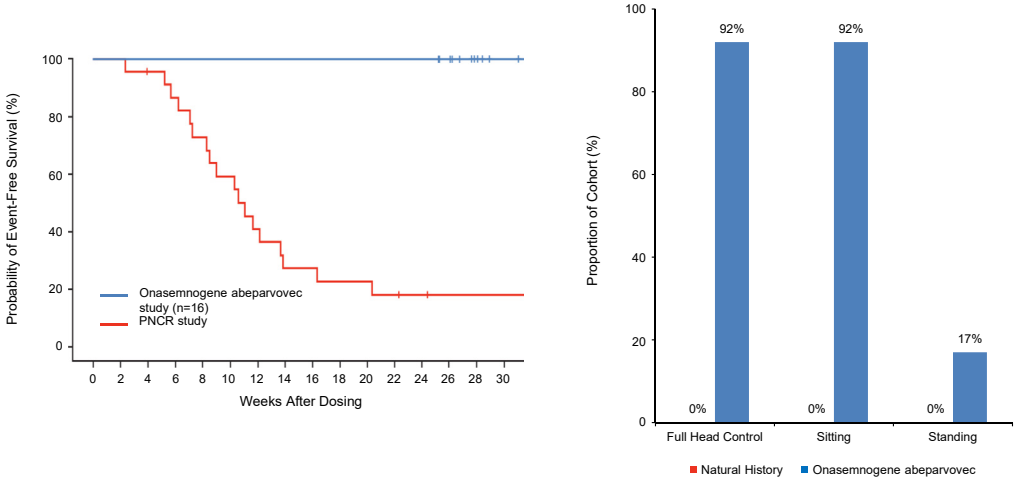

FIGURE 2. Phase 1 trial outcomes after onasemnogene abeparvovec gene replacement therapy in infants with SMA1. Left panel: Probability of event-free survival in SMA1 infants receiving the proposed therapeutic dose (n = 12) or as reported in a natural history cohort.[4] Event-free survival is survival independent of permanent ventilation, as defined as tracheostomy or ventilatory support for ≥16 hours per day for >14 continuous days in the absence of an acute reversible event. Right panel: Motor milestones achieved by infants treated with the proposed therapeutic dose of onasemnogene abeparvovec (n = 12) or as reported in a natural history cohort.[3] PNCR, Pediatric Neuromuscular Clinical Research Network; SMA1, spinal muscular atrophy type 1.

consideration for AAV-mediated GRT studies. Other GRT clinical studies with intravenous administration of AAV vectors have used anti-AAV antibody cutoffs ranging from less than 1:1 neutralizing antibodies to less than 1:400 immunoglobulin G (IgG), and no dose-limiting toxicities have been reported in treated subjects. Hence, the safety of dosing patients with elevated antibodies remains an area of exploration and future study, and it may be safe and feasible to dose patients with higher antibody titers. Although AAV vectors are nonpathogenic, asymptomatic transient LE elevations have been reported in the majority of intravenously administered AAV GRT studies and have been managed with short regimens of prednisolone or prednisone.[20,21] These LE elevations have been attributed to CD8+ T cell-mediated immunity directed against transduced cells presenting AAV capsid antigens on the cell surface rather than against the delivered transgene.[22] In monogenic diseases wherein a protein is completely absent, GRT-mediated production and expression of the protein in the host cell can cause an immunogenic response and eventual reduction or elimination of the novel protein, suppressing the therapeutic benefit. In SMA, patients express some fraction of the functional protein via the *SMN2* gene; thus the potential for an immune response to the transgene is theoretically extremely low.

A key biological property of the onasemnogene abeparvovec vector is the AAV9 serotype, which has been shown to cross the blood-brain barrier, permitting the targeting of cells critical in the pathogenesis of SMA motor neurons.[14,23] As motor neurons are long lived, one-time administration of AAV9 GRT is thought to be sufficient for lifetime episomal transgene expression in the cell. Furthermore, SMA1 is rapidly progressive; thus, early delivery and onset of high transgene expression with minimal delay is critical to arrest further motor neuron loss. Along with self-complementary AAV technology, onasemnogene abeparvovec is designed with a hybrid cytomegalovirus enhancer—chicken beta-actin promoter—to drive high, sustained human *SMN* expression by increasing the onset of transgene translation and avoiding the rate-limiting step of cell-mediated second-strand synthesis typically required by recombinant AAV,[24] promoting rapid and efficient transduction.

The potential to affect SMA1 outcomes through GRT was first demonstrated in preclinical studies. Intravenous *SMN* transgene delivery with the AAV9 vector led to early and persistent transgene expression and corrected the phenotype in the murine model of severe SMA (SMNΔ7 murine model), increasing survival from the expected 15 days to more than 250 days when dosed early on postnatal day 1 and at doses of $\geq 2.0 \times 10^{14}$ vector genomes per kilogram.[14,25]

Clinical trial experience and recommendations for real-world use

In this and the following sections, we describe the safety precautions implemented for the first-in-human phase 1 onasemnogene abeparvovec clinical trial, some of which were taken out of extreme caution given the nature of a phase 1 safety study and the novelty of the intravenous administration of a viral vector therapy in a medically fragile young patient population. With the available safety data from this study and data forthcoming from ongoing pivotal studies, these extra precautions may not be necessary in future clinical practice. The recommendations presented are based on the authors' experience in this study and other GRT trials and their expertise in neuromuscular disorders and gene-mediated therapies, including the US Food and Drug Administration (FDA)-approved SMA treatment, nusinersen.

Safe product handling and risk mitigation

For the safety aspects of vector administration and vector shedding, all AAV serotypes are categorized by the National Institutes of Health guidelines as Risk Group 1, the lowest categorization, indicating that they are noninfectious and not associated with disease in healthy adult humans. Given this low-risk categorization, physical containment of the vector was handled at Biosafety Level 1. Standard microbiological practices were implemented in its handling, whereas specialized containment equipment was not required.[26]

Although the vector produced for GRT is replication incompetent by design owing to removal of original viral packaging and reproduction genes, parents and bedside health care providers were given guidance on proper handling of waste material generated from stool, urine, or saliva in the phase 1 study based on the National Institutes of Health guidelines on AAV vector risk group.

Guidance was also provided to patient families and caregivers regarding use of protective gloves when coming into direct contact with patient bodily fluids and waste, as well as good hand hygiene for four weeks after the injection. During the phase 1 trial, patient saliva, urine, and stool samples were collected through month 18 postdose on a monthly basis to gather information on vector shedding. Saliva, urine, and stool samples from five patients were analyzed by droplet digital polymerase chain reaction (S.A.A., personal communication). Onasemnogene abeparvovec concentrations in urine and saliva were lower on day one postdose (0.1% to 0.01% of initial concentration), after which they were not detectable. Onasemnogene abeparvovec was cleared from the body primarily in feces, with concentrations declining approximately 4 logs (1000-fold) over 30 days postdose. The next monthly sample, after 60 days postdose, showed onasemnogene abeparvovec below the detection limits. Together, these data demonstrate the rapid decline of shed vector quantities well below dosing concentrations in patients treated with onasemnogene abeparvovec, which is consistent with previous studies evaluating AAV biodistribution and shedding in humans.[27,28]

This practice also aimed to minimize the potential risk of seroconversion of an antibody-negative mother exposed to the AAV vector with the subsequent risk of future seropositive pregnancies. Whether limited exposure to a replication-incompetent vector in a caregiver (likely via fecal-oral transmission) is sufficient to generate an antibody response is not presently known.

Recommendation 1: Based on this clinical experience, we recommend vector handling by clinical staff according to Biosafety Level 1-directed research guidelines.[26]

Recommendation 2: Based on vector shedding data from the phase 1 trial, we also recommend advising caregivers to use protective gloves when coming into direct contact with patient feces and to use good hand hygiene for approximately 60 days after the injection. This recommendation is especially important for pregnant or potentially pregnant mothers and caregivers, who should use gloves for diaper changes in the first two months after treatment because risk of transfer of the AAV vector to a fetus remains largely unknown.

Preexisting neutralizing antibodies to naturally occurring AAV serotypes are common in most humans.[19,29] The antibody levels against the AAV9 serotype used in this trial are relatively low compared with other serotypes,[19] although they can potentially be passed from mother to child through breastfeeding.[30] Although secretory immunoglobulin A is the predominant antibody in human breast milk, its polymeric structure reduces the risk of entering the infant circulation through the intestinal mucosa.[30] However, passive transfer of the low levels of maternal breast milk IgG to the infant and the unknown risks of transfer of the anti-AAV9 IgG antibodies to enrolled subjects were taken into consideration during the phase 1 trial.[31,32] The aim of this safety study was to minimize the risks of reducing vector transduction of a single-dose therapy in an otherwise fatal disease; hence, a conservative approach was taken, in which breastfeeding was restricted before, during, and briefly after viral vector administration (i.e., 30 days).

Recommendation 3: The restriction of breastfeeding during the phase 1 trial was largely based on the theoretical risks; however, data for the passive transfer of anti-AAV9 antibodies remain elusive. The decision to continue or briefly suspend breastfeeding should be made by the treating physician and parents, balancing the theoretical risk of potential interference of breast milk on vector transduction with the impact of short-term replacement of maternal breast milk with infant formula.

Patient selection

Patient inclusion and exclusion criteria were applied to select the most homogeneous SMA1 patient population for the clinical study of an investigational drug, such that differences in outcomes

TABLE 1.
Clinical Studies With Intravenously Administered AAV Gene Replacement Therapy

Clinical Study	Vector	Route/Doses	Weight kg	Anti-AAV Cutoff	Immune Responses Reported
SMA1, phase 1/2[9]	Onasemnogene abeparvovec, AAV9	Intravenous 6×10^{13}-2×10^{14} vg/kg	3.6-8.4	<1:50 IgG	Asymptomatic LE elevations, prednisolone regimen utilized
Hemophilia A, phase 1/2[35]	AAV5	Intravenous 6×10^{12}-6×10^{13} vg/kg	60-103	<1:1 NAb	Asymptomatic LE elevation in high-dose cohort, prednisolone regimen utilized in high-dose cohort
Hemophilia A, phase 1/2[36]	SPK-8011, rAAV	Intravenous 5×10^{11}-1×10^{12} vg/kg	Adults	<1:1 NAb	No sustained or unresolved LE elevations. One SAE for IV administration of steroids to treat asymptomatic immune response not resolved with oral steroids, which then resolved
Hemophilia B, phase 2[21]	AMT-060, AAV5	Intravenous 2×10^{13} vg/kg	71-96.0	<1:1 NAb	With more sensitive assay, three patients had positive anti-AAV5 NAb titers, and two were confirmed by additional assays. Highest NAb titer was 1:340. No patient had any clinically relevant T-cell immune response, and three experienced asymptomatic liver enzyme elevations treated with oral steroids
DMD, phase 1[37]	SGT-001, AAV9	Intravenous Dose 1-3	Children Teens	"Predetermined level"	Clinical hold based on unexpected adverse event in first patient dosed: fever, reduced platelet, and RBC count. Clinical hold released and trial continued. No coagulopathy signs or symptoms. No LE elevations reported
DMD, phase 1[38]	AAVrh74	Intravenous 2×10^{14} vg/kg	13.7-20.7	<1:400 IgG	Three patients had elevated GGT that resolved with steroid treatment within a week

Abbreviations:
AAV = Adeno-associated virus
DMD = Duchenne muscular dystrophy
GGT = Gamma-glutamyl transferase
IgG = Immunoglobulin G
IV = Intravenous
LE = Liver enzyme
NAb = Neutralizing antibody
RBC = Red blood cell
SAE = Serious adverse event
vg = Vector genome

against published natural history studies would be readily apparent. However, application of such stringent patient criteria for therapy may not be necessary if regulatory agencies allowed treatment of a broader patient population. All patients in the phase 1 study were symptomatic and were genetically confirmed to have biallelic deletions of *SMN1* and two *SMN2* copies.[9] In addition, all patients lacked the *SMN2* disease-modifying c.859G>C mutation in exon 7, which can increase the fraction of functional protein produced from *SMN2* and thus predict a milder phenotype.[33] Note that these genotype restrictions were applied to allow effective comparison with published data, not because onasemnogene abeparvovec would have less efficacy in patients with other genotypic profiles.

Patients were also selected based on age and symptom onset because a natural history study has demonstrated that infants experiencing symptoms at age less than six months never sat unassisted.[4] Although the onasemnogene abeparvovec clinical trial initially enrolled subjects younger than nine months (first nine patients), the inclusion criteria were subsequently modified to infants younger than six months because it quickly became apparent that patients dosed early in the disease progression had higher potential for better outcomes, which was consistent with preclinical murine studies.[14,34] Patients dosed early in age (less than three months) and early in disease progression (CHOP-INTEND [Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders] score greater than 40) achieved the ability to stand or walk.[15]

All infants with SMA1 in the study weighed between 3.6 and 8.4 kg at the time of gene transfer, and for this reason a maximum tolerated dose (i.e., maximum weight that can be safely treated) was not determined in this trial. The available safety data and clinical experience with intravenously administered onasemnogene abeparvovec GRT at the time of this review is less than 8.5 kg. However, it should be noted that other AAV-mediated GRT trials with systemic delivery have safely dosed children and adults at weights reviewed in Table 1.[39]

Patients were also selected based on AAV9 antibody titer because existing antibodies can increase the risk for immune response to GRT and potentially reduce transduction capacity, and thus the therapeutic benefit.[22] A conservative anti-AAV9 antibody titer of 1:50 was used, a caveat being that although a commercial antibody assay is newly available in the United States, there is no consensus on assay standardization or definitive antibody titer limits that could guide subject eligibility for such therapies.[40] Only one of 16 infants screened was excluded due to antibody elevations (>1:50 of anti-AAV9 IgG).[9] During screening, three patients had elevated anti-AAV9 antibody titers (>1:50). All three were retested within two weeks after the initial screening, at which time the antibody titers of two patients fell to 1:50, resulting in their enrollment into the trial (Table 2). Of note, the two patients with initially elevated anti-AAV9 antibody titers who were subsequently enrolled did not have higher LE elevations or lower milestone achievements than the rest of the cohort. Thus, slightly elevated antibody levels should be retested to potentially allow dosing with AAV carrying the *SMN* gene. Nusinersen and onasemnogene abeparvovec results suggest that dosing early in disease progression will yield the best outcomes in infants with SMA1, thus early dosing will certainly be encouraged. Moreover, newborn screening is making great strides in the United States, raising the potential to identify and treat newborns with SMA1. The treating physician should consider that neonatal liver maturity occurs in the first few weeks of life.

Recommendation 4: All infants treated with onasemnogene abeparvovec in this phase 1 study were aged 0.9 to 7.9 months. Although genetic and age constraints were put in place to maintain a homogeneous population, we believe patients with SMA1 outside of these study parameters could safely be dosed. FDA approval was for onasemnogene abeparvovec in the treatment of SMA up to age two years, including presymptomatic patients at diagnosis.

Recommendation 5: Although clinical trial subjects were restricted to those with two copies of *SMN2* with no disease-modifying mutation to maintain a homogeneous population, there is no evidence to preclude newborns and infants with SMA identified through newborn screening with more or less than two copies of *SMN2* for treatment with onasemnogene abeparvovec. This was made clear in the recent FDA approval of onasemnogene abeparvovec recommending treatment for all patients with SMA aged less than two years, regardless of the *SMN2* copy number. This was also consistent with the published treatment algorithm[10] for immediate treatment for SMA diagnosed by newborn screening. The potential for overexpression of SMN protein in patients with SMA has shown no toxicities in mice,[14] and overexpression may actually protect motor neurons in a murine model of amyotrophic lateral sclerosis.[41] In addition, approximately 13.6% of the general population carries three or four copies of *SMN1* with no associated disease.[42] Thus, GRT in patients with more than two copies of *SMN2* is not expected to cause additional safety concerns.

Recommendation 6: In the phase 1 clinical trial, the protocol had no weight restrictions; however, given the enrolled subjects in our clinical trial, our experience indicates that infants with SMA1 weighing ≤8.5 kg can be safely dosed with intravenous onasemnogene abeparvovec at the therapeutic dose. The authors acknowledge the precedence for treating larger patients in other diseases (Table 1) and defer to regulatory guidelines that provided approval for patients with SMA aged less than two years, without any restrictions on weight, as well as the clinician's discretion in determining the feasibility and safety of treating individual subjects.

Recommendation 7: We recommend testing for the presence of anti-AAV9 antibodies before onasemnogene abeparvovec administration. The half-life of passively acquired IgG antibodies is 35 to 40 days.[43,44] Among infants, particularly neonates, antibody levels

TABLE 2.
Infants with SMA1 and Mothers With Antibody Levels >1:50 at Baseline in the Phase 1 Study

Patient	Anti-AAV9 Antibody Level				Highest Milestone Achieved
	Maternal		Patient		
	1st Test	Retesting	1st Test	Retesting	
12	1:200	1:400	1:100	1:50	Independent sitting for >30 seconds
13	1:100	NA	1:100	1:50	Independent sitting for >30 seconds
15	>1:800	NA	1:12.5	NA	Independent sitting for >30 seconds

Abbreviations:
AAV = Adeno-associated virus
NA = Not applicable
SMA1 = Spinal muscular atrophy type 1

likely reflect maternal placental transfer; thus, we recommend retesting of antibodies if the initial results are positive with an interval of no more than two weeks, because this may allow sufficient waning of modestly elevated titers to permit dosing. The waiting period should highly depend on SMA disease severity, stability of the CHOP-INTEND scores, and parental consent and should be assessed on an individual case basis.

Care before dosing

Standard of care for patients with SMA1 includes management of nutrition and ventilation[44] because most of them experience respiratory failure and dysphagia, necessitating interventions.[4] However, care management for these patients continues to vary across centers. In this trial, optimal management included gastrostomy tube placement with Nissen fundoplication for infants with evidence of inability to safely swallow and requiring nutritional support at enrollment. For these treated subjects, the tube feeding continued, albeit at a lower volume when the ability to swallow and resume oral feeding was achieved, to maintain optimal nutrition. Respiratory support needs were assessed before dosing and subsequently at every visit. Caregivers of children requiring respiratory support were able to adjust the level of noninvasive ventilatory support based on the child's day-to-day needs in coordination with the pulmonary team. Ventilatory assistance was provided according to consensus guidelines for care of SMA1.[44]

A prophylactic dose of prednisolone 1 mg/kg/day was administered orally or via gastrostomy tube 24 hours before onasemnogene abeparvovec gene delivery to subjects 2 through 15 and then continued for approximately 30 days after the observation of asymptomatic LE elevations in the first patient.[9] After institution of prophylactic prednisolone, 11 of 14 patients were dosed without significant LE elevations. The short course of prednisolone in GRT is a strategy to dampen or circumvent the expected immune response to the AAV viral capsid in the host liver cells. Minimal to no side effects of prednisolone were reported by caregivers.

Although the Centers for Disease Control and Prevention guidance recommends avoidance of live virus vaccinations only in cases of a substantial immunosuppressive corticosteroid dose ($\geq$2 weeks of daily receipt of 20 mg or 2 mg/kg body weight of prednisolone or equivalent),[45] given the prophylactic prednisolone treatment and the fragility of this patient population, a conservative approach to immunization was taken. No live vaccinations were administered during the phase 1 study while the infants were on steroids. Scheduled immunizations and seasonal vaccinations (including palivizumab prophylaxis) were given at least one week before dosing or after tapering prednisolone to minimize the risks of a vaccine-related AE that would confound the post-GRT safety and clinical assessments.[46]

Recommendation 8: We recommend employing SMA standards of care for nutrition and ventilation management before and after GRT.[5,6]

Recommendation 9: We recommend that all patients receive prophylactic oral prednisolone 24 hours before dosing. For those patients who need to travel for infusion, logistical considerations should be discussed with the treating physician and pertinent hospital staff.

Recommendation 10: We recommend modestly adjusting scheduled and seasonal vaccinations to accommodate the prednisolone taper (at least one week before dosing or after tapering prednisolone). Where avoiding vaccination while on steroids would represent an undue delay or interruption of the vaccination schedule, vaccination should continue at the discretion and judgment of the treating physician given (1) the importance of maintaining childhood vaccination in this population and (2) the literature indicating that vaccination while on steroid doses less than 2 mg/kg is safe and effective.[45]

Dosing and short-term monitoring

As the phase 1 study was the first-in-human investigational trial of onasemnogene abeparvovec in a young patient population, patient dosing was performed under sterile conditions in a pediatric intensive care unit patient room to permit continuous monitoring for any immediate acute reactions.[46] As a precautionary step, two intravenous catheters were inserted into two peripheral veins to minimize disruption of the infusion in the event the first infusion site failed.

Patient vital signs (body temperature, pulse rate, respiration rate, and blood pressure) and pulse oximetry were monitored continuously during the 60-minute infusion, and axillary temperature was taken before and after dosing. All treated SMA1 subjects, regardless of dose, remained clinically stable, and no acute reactions or abnormal changes in vital signs were observed in any of the 15 patients.[46]

An inpatient stay was required as part of the clinical research protocol, during which patient vital signs and pulse oximetry were monitored continuously throughout the first 24 hours after dosing. The following safety tests were also performed on days one and two after treatment before discharge: physical examination (weight and vital signs) and laboratory blood and urinary tests, including capillary blood gas. No abnormalities were observed in any of the patients.

Recommendation 11: Given the observed safety of the onasemnogene abeparvovec intravenous infusion and lack of safety signals during the 48-hour inpatient monitoring in the clinical trial, an outpatient setting of less than 24-hour observation is likely safe; infusion in the setting of the intensive care unit is not required for the procedure *per se*.

Recommendation 12: We recommend close monitoring of patient vital signs during the 60-minute infusion and insertion of intravenous catheters into two peripheral veins before infusion in the event of failure of the first. Upon completion of the infusion, a brief period of a few hours of observation and a repeat clinical examination postinfusion are recommended before being discharged home. Unless clinically warranted for the individual patient, repeated laboratory tests are not warranted before discharge until day seven as discussed in the next section.

Long-term monitoring (30 to 60 days)

In accordance with a phase 1 safety study protocol, the following assessments were performed weekly for the first month after infusion: comprehensive physical examination, including pulmonary evaluation, vital signs, pulse oximetry, capillary blood gas, glucose, creatine kinase, creatinine/blood urea nitrogen, alkaline phosphatase, amylase, and urinalysis. No abnormalities were observed in any of the patients.

Laboratory tests assessing LE and liver function were also completed, including aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase, total and direct bilirubin, albumin and total protein measurements, as well as the coagulation profile (partial thromboplastin time and international normalized ratio). A transient asymptomatic rise in AST and ALT levels was observed in some subjects as early as one week post-dose; however, clinically significant elevations were mostly observed at weeks 3 and 4 post-treatment, which is consistent with other AAV gene therapy trials.[20,21] In total, four of 15 patients had asymptomatic LE elevations ($n = 2$, $<10 \times$ upper limit of normal [ULN] and $n = 2$, $>10 \times$ ULN). In all cases, treatment-related serious

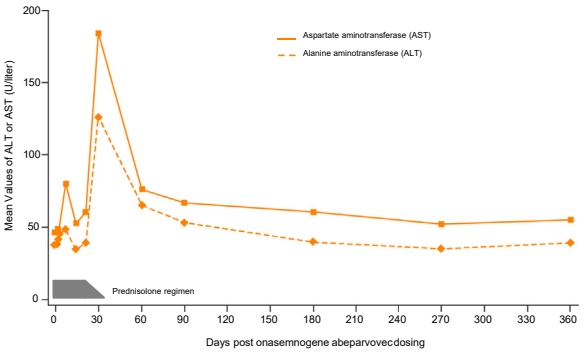

FIGURE 3. Liver enzyme levels after onasemnogene abeparvovec gene replacement therapy. Mean alanine transaminase (ALT) and aspartate transaminase (AST) levels in infants with SMA1 in cohort 2 over time after onasemnogene abeparvovec therapy are shown. Patients were started on prophylactic prednisolone (corticosteroid) (approximately 1 mg/kg/day) 24 hours before the gene transfer. Treatment continued for approximately 30 days with the following guidelines for tapering: If AST and ALT exceeded 120 U/L, prednisolone was maintained until enzymes fell below this level. Discrepancies from these precise recommendations were at the discretion of the investigator based on potential safety issues for participating patients in the phase 1 study. SMA1, spinal muscular atrophy type 1. The color version of this figure is available in the online edition.

adverse events and AEs were limited to asymptomatic transient elevated LEs, which resolved with prednisolone treatment (Fig 3). Of note, the asymptomatic transaminasemia experienced by some infants in this trial did not impact or correlate with their clinical response to onasemnogene abeparvovec therapy, as assessed by CHOP-INTEND.

In the onasemnogene abeparvovec clinical study, patients with greater than 1:50 anti-AAV9 antibody titers were excluded from enrollment. This cutoff was a decision by the FDA based on prior gene therapy trials.[47] As expected, after gene delivery, all patients had increased anti-AAV9 antibodies throughout the course of the study compared with their baseline levels. The antibody titers reached levels beyond the limits of assay quantification (>1:819,200) in 11 subjects. Antibody response was rapid with titers >1:1600 by one to two weeks from dosing, and anti-AAV9 antibody titers remained elevated (>1:50,000) in all patients at the last reported time point (range, 12 to 24 months). However, there was no correlation between antibody development and treatment outcomes or LE elevations. In addition, no patient developed anti-SMN antibodies.

The T-cell response was determined with an interferon-γ enzyme-linked immune absorbent spot assay, which demonstrated increases in immune response to AAV9 as early as week one after dosing; however, no immune response to SMN was detected. Interestingly, the immune response measured by the enzyme-linked immune absorbent spot assay did not reliably predict LE elevations; thus, it was not used to inform prednisolone regimen tapering.

In addition, platelet decreases that reached a nadir one week after dosing were noted in a few patients in the phase 1 study, although they were not clinically significant. Additional platelet decreases have been reported in the onasemnogene abeparvovec phase 3 study elsewhere, and regulatory review will determine if additional platelet monitoring will be recommended based on the entire clinical program.[48]

Recommendation 13: The follow-up related to gene therapy is limited to platelets and LEs as discussed in this section. Additional testing that could be directly related to the disease is deferred to the treating physician. Because asymptomatic LE elevations are an expected AE of intravenous AAV9-mediated GRT, AST/ALT levels should be monitored weekly for at least 30 days after administration. Liver function (clinical exam, AST/ALT levels, total bilirubin, prothrombin time) should be monitored weekly for the first month after administration and every other week for the second and third months, until results are unremarkable (normal clinical exam, total bilirubin, prothrombin results, and ALT/AST levels below 2 × ULN). LE elevations should be monitored weekly until they fall within the normal range. Regarding possible platelet decrease, weekly monitoring is recommended for the first 30 days.

Recommendation 14: Oral prednisolone (1 mg/kg) should be administered daily to each patient 24 hours before gene delivery, continuing for approximately 30 days, after which it should be tapered over 30 days for patients whose LE values are both below 2 × ULN. If AST/ALT levels exceed 3 × ULN after 30 days, prednisolone should be maintained until levels fall below 2 × ULN, after which tapering can begin. If AST/ALT levels continue to rise, the prednisolone dosage should be adjusted to achieve suppression.

Recommendation 15: For those patients who need to travel to an institution to receive GRT, AST/ALT levels and prednisolone dosing adjustments can be monitored remotely. Remote monitoring should be arranged between the child's primary care physician and the physician administering the dose before dosing.

Considerations for combination therapy

Although there are no data supporting combination treatments for available SMA therapies, caregivers or physicians may choose to explore all therapeutic possibilities. Controlled studies are required to show clinical benefit because the mechanisms of both drugs in

TABLE 3.
Evidence of AAV-Delivered Transgene Durability in Various Clinical Programs

Disease	Disease Pathogenesis	Primary Target	Transgene Persistence
Parkinson disease	Complex	Neurons Nondividing Long-lived	Nonhuman primates: 15 y[49,*,†] Humans: 4 y[50,†]
Hemophilia B	Monogenic	Liver Nondividing Regenerative	Nonhuman primates: 5 y[51,*,†] Dogs: 8 y[52,*]; 8 y[52,†] Humans: 4 y[53,*]; 10 y[54,†]
Inherited retinal disease	Monogenic	Retinal pigment epithelial cells Nondividing Long-lived	Dogs: 10 y[55,*] Humans: 3 y[56,*]
Spinal muscular atrophy	Monogenic	Motor neurons Nondividing Long-lived	Mice: >250 days[14,*] Humans: 4 y[9,*]

Abbreviation:
AAV = Adeno-associated virus
* Persistence of treatment effect.
† Transgene persistence determined by presence in tissues.

combination treatment aim to increase functional SMN protein levels; however, for those that may pursue this avenue, we provide a few considerations based on experience with both drugs. onasemnogene abeparvovec and nusinersen have different mechanisms of action. Thus, although both treatments are designed to increase functional SMN protein levels, they are not expected to interact, because the nusinersen ASO binds the *SMN2* mRNA transcript that includes a portion of an intron sequence. The *SMN* transgene does not contain any introns, so its translation should not be affected by nusinersen. However, because thrombocytopenia has been reported as an AE in association with ASOs, caution is required when onasemnogene abeparvovec treatment is considered.

Conclusion

Onasemnogene abeparvovec GRT for patients with SMA1 appears to be promising, and because motor neurons are long-lived, a single administration of AAV9 GRT may be sufficient for continual transgene expression in SMA. Early signs of durable clinical effect of AAV GRT in this and other gene therapy programs are accumulating (Table 3). The recommendations provided here are intended to aid treating physicians and are based on the currently available clinical trial data. Onasemnogene abeparvovec is the first gene therapy approved by FDA to treat children younger than two years with SMA.

Acknowledgments

Medical writing assistance was provided by Marjet Heitzer, PhD, and Jessica Cardenas, PhD, of Cadent Medical Communications, LLC, a Syneos Health group company, and supported by AveXis, Inc.

Role of the funding source: The sponsor, AveXis, Inc., coordinated data management, statistical analyses, and report writing.

Addendum: Onasemnogene abeparvovec received FDA approval in May 2019 as a gene therapy that replaces survival motor neuron 1 (*SMN1*) gene in individuals with SMA. This is the first approval for intravenous delivery of gene therapy in patients with SMA who are under the age of two at the time of dosing.

References

1. Burghes AH, Beattie CE. Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick? Nat Rev Neurosci. 2009;10:597–609.
2. Feldkotter M, Schwarzer V, Wirth R, Wienker TF, Wirth B. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. Am J Hum Genet. 2002;70:358–368.
3. De Sanctis R, Coratti G, Pasternak A, et al. Developmental milestones in type I spinal muscular atrophy. Neuromuscul Disord. 2016;26:754–759.
4. Finkel RS, McDermott MP, Kaufmann P, et al. Observational study of spinal muscular atrophy type I and implications for clinical trials. Neurology. 2014;83:810–817.
5. Finkel RS, Mercuri E, Meyer OH, et al. Diagnosis and management of spinal muscular atrophy: part 2: pulmonary and acute care; medications, supplements and immunizations; other organ systems; and ethics. Neuromuscul Disord. 2018;28:197–207.
6. Mercuri E, Finkel RS, Muntoni F, et al. Diagnosis and management of spinal muscular atrophy: part 1: recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. Neuromuscul Disord. 2018;28:103–115.
7. Food and Drug Administration. FDA Approves First Drug for Spinal Muscular Atrophy [Press Release]. Silver Spring, MD: US Food and Drug Administration; 2016. Available at: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements /ucm534611.htm. Accessed March 26, 2018.
8. Baranello G, Kletzl H, Cleary Y, et al. RG7916 Significantly Increases SMN Protein in SMA Type 1 Babies. 2018. Paper Presented at: American Academy of Neurology Annual Meeting; Los Angeles, CA; April 21-27.
9. Mendell JR, Al-Zaidy S, Shell R, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med. 2017;377:1713–1722.
10. Glascock J, Sampson J, Haidet-Phillips A, et al. Treatment algorithm for infants diagnosed with spinal muscular atrophy through newborn screening. J Neuromuscul Dis. 2018;5:145–158.
11. Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus sham control in infantile-onset spinal muscular atrophy. N Engl J Med. 2017;377:1723–1732.
12. Luu KT, Norris DA, Gunawan R, Henry S, Geary R, Wang Y. Population pharmacokinetics of nusinersen in the cerebral spinal fluid and plasma of pediatric patients with spinal muscular atrophy following intrathecal administrations. J Clin Pharmacol. 2017;57:1031–1041.
13. Spinraza (Nusinersen). Cambridge, MA: Biogen Inc.; 2018 [package insert].
14. Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat Biotechnol. 2010;28:271–274.
15. Mendell JR, Shell R, Lehman R, et al. Gene-replacement therapy in spinal muscular atrophy type 1: Long-term follow-up from the Onsemnogene Abeparvovec-xioi Phase 1/2a Clinical Trial. Anaheim, CA: Presented at the 2019 CureSMA Annual Conference; 2019.
16. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood. 2013;122:23–36.
17. Day JW, Chiriboga CA, Crawford TO, et al. Onasemnogene Abeparvovec-xioi Gene-Replacement Therapy for Spinal Muscular Atrophy Type 1: Phase 3 Study (STR1VE) Update. Anaheim, CA: Presented at the 2019 CureSMA Annual Conference; 2019.
18. Wang J, Xie J, Lu H, et al. Existence of transient functional double-stranded DNA intermediates during recombinant AAV transduction. Proc Natl Acad Sci U S A. 2007;104:13104–13119.
19. Boutin S, Monteilhet V, Veron P, et al. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. Hum Gene Ther. 2010;21:704–712.
20. George LA, Sullivan SK, Giermasz A, et al. Hemophilia B gene therapy with a high-specific-activity factor IX variant. N Engl J Med. 2017;377:2215–2227.
21. Miesbach W, Meijer K, Coppens M, et al. Gene therapy with adeno-associated virus vector 5-human factor IX in adults with hemophilia B. Blood. 2018;131:1022–1031.
22. Colella P, Ronzitti G, Mingozzi F. Emerging issues in AAV-mediated in vivo gene therapy. Mol Ther Methods Clin Dev. 2018;8:87–104.
23. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol. 2009;27:59–65.
24. McCarty DM, Monahan PE, Samulski RJ. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. Gene Ther. 2001;8:1248–1254.
25. Foust K, Meyer K, Likhite S, et al. Extensive Dose Ranging Study of AVXS-101 in the Severe D7 SMA Mouse Model. 2017. Paper Presented at: ASGCT Annual Meeting; Washington, DC; May 10-13.
26. National Institutes of Health. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. 78. Bethesda, MD: National Institutes of Health, Office of Biotechnology Activities; 2016:66751–66766.
27. Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-factor IX and limitations imposed by the host immune response. Nat Med. 2006;12:342–347.
28. Salmon F, Grosios K, Petry H. Safety profile of recombinant adeno-associated viral vectors: focus on alipogene tiparvovec (Glybera(R)). Expert Rev Clin Pharmacol. 2014;7:53–65.
29. Leborgne C, Latournerie V, Boutin S, et al. Prevalence and long-term monitoring of humoral immunity against adeno-associated virus in Duchenne muscular dystrophy patients [e-pub ahead of print] Cell Immunol. 2018. https://doi.org/10.1016/j.cellimm.2018.03.004.
30. Van de Perre P. Transfer of antibody via mother's milk. Vaccine. 2003;21:3374–3376.
31. Shah U, Dickinson BL, Blumberg RS, Simister NE, Lencer WI, Walker WA. Distribution of the IgG Fc receptor, FcRn, in the human fetal intestine. Pediatr Res. 2003;53:295–301.
32. Yoshida M, Claypool SM, Wagner JS, et al. Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. Immunity. 2004;20:769–783.
33. Prior TW, Krainer AR, Hua Y, et al. A positive modifier of spinal muscular atrophy in the *SMN2* gene. Am J Hum Genet. 2009;85:408–413.
34. Robbins KL, Glascock JJ, Osman EY, Miller MR, Lorson CL. Defining the therapeutic window in a severe animal model of spinal muscular atrophy. Hum Mol Genet. 2014;23:4559–4568.
35. Rangarajan S, Walsh L, Lester W, et al. AAV5-factor VIII gene transfer in severe hemophilia A. N Engl J Med. 2017;377:2519–2530.
36. High KA, George LA, Eyster ME, et al. A phase 1/2 trial of investigational SPK-8011 in hemophilia A demonstrates durable expression and prevention of bleeds. Blood. 2018;132(suppl 1):487.
37. Duan D. Systemic AAV micro-dystrophin gene therapy for Duchenne muscular dystrophy. Mol Ther. 2018;26:2337–2356.
38. Sarepta Therapeutics Announces that at the 23rd International Congress of the World Muscle Society, Jerry Mendell, M.D., Presented Positive Updated Results from the Four Children Dosed in the Gene Therapy Micro-dystrophin Trial to Treat Patients with Duchenne Muscular Dystrophy [Press Release]. Cambridge, MA: Sarepta Therapeutics; 2018. Available at: http://investorrelations.sarepta.

com/news-releases/news-release-details/sarepta-therapeutics-announces-23rd-international-congress-world. Accessed January 15, 2019.
39. Duan D. Micro-dystrophin gene therapy goes systemic in Duchenne muscular dystrophy patients. Hum Gene Ther. 2018;29:733–736.
40. AAV9 antibody test, Athena Diagnostics website test code 1181. Available at: https://www.athenadiagnostics.com/view-full-catalog/a/aav9-antibody-test. Accessed February 1, 2019.
41. Turner BJ, Alfazema N, Sheean RK, et al. Overexpression of survival motor neuron improves neuromuscular function and motor neuron survival in mutant SOD1 mice. Neurobiol Aging. 2014;35:906–915.
42. Cusin V, Clermont O, Gerard B, Chantereau D, Elion J. Prevalence of SMN1 deletion and duplication in carrier and normal populations: implication for genetic counselling. J Med Genet. 2003;40:e39.
43. Sato H, Albrecht P, Reynolds DW, Stagno S, Ennis FA. Transfer of measles, mumps, and rubella antibodies from mother to infant. Its effect on measles, mumps, and rubella immunization. Am J Dis Child. 1979;133:1240–1243.
44. Wang CH, Finkel RS, Bertini ES, et al. Consensus statement for standard of care in spinal muscular atrophy. J Child Neurol. 2007;22:1027–1049.
45. Kroger AT, Duchin J, Vázquez M. General best practice guidelines for immunization. Best practices guidance of the Advisory Committee on Immunization Practices (ACIP). Available at: https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/downloads/general-recs.pdf; 2017. Accessed January 30, 2019.
46. Robinson CL, Bernstein H, Romero JR, Szilagyi P. Advisory Committee on Immunization Practices Recommended Immunization Schedule for Children and Adolescents Aged 18 Years or Younger - United States, 2019. MMWR Morb Mortal Wkly Rep. 2019;68:112–114.
47. Mendell JR, Rodino-Klapac LR, Rosales XQ, et al. Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. Ann Neurol. 2010;68:629–638.
48. Day JW, Chiriboga CA, Darras BT, et al. AVXS-101 Gene-Replacement Therapy (GRT) for Spinal Muscular Atrophy Type 1 (SMA1): Pivotal Study (STR1VE) Update. 2019. Paper Presented at: Muscular Dystrophy Association (MDA) Clinical and Scientific Conference; Orlando, FL, USA; April 13-17.
49. Sehara Y, Fujimoto Ki, Ikeguchi K, et al. Persistent expression of dopamine-synthesizing enzymes 15 years after gene transfer in a primate model of Parkinson's disease. Hum Gene Ther Clin Dev. 2017;28:74–79.
50. Bartus RT, Kordower JH, Johnson Jr EM, et al. Post-mortem assessment of the short and long-term effects of the trophic factor neurturin in patients with alpha-synucleinopathies. Neurobiol Dis. 2015;78:162–171.
51. Nathwani AC, Rosales C, McIntosh J, et al. Long-term safety and efficacy following systemic administration of a self-complementary AAV vector encoding human FIX pseudotyped with serotype 5 and 8 capsid proteins. Mol Ther. 2011;19:876–885.
52. Niemeyer GP, Herzog RW, Mount J, et al. Long-term correction of inhibitor-prone hemophilia B dogs treated with liver-directed AAV2-mediated factor IX gene therapy. Blood. 2009;113:797–806.
53. Monahan PE. Gene therapy in an era of emerging treatment options for hemophilia B. J Thromb Haemost. 2015;13(suppl 1):S151–S160.
54. Buchlis G, Podsakoff GM, Radu A, et al. Factor IX expression in skeletal muscle of a severe hemophilia B patient 10 years after AAV-mediated gene transfer. Blood. 2012;119:3038–3041.
55. Cideciyan AV, Jacobson SG, Beltran WA, et al. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. Proc Natl Acad Sci U S A. 2013;110:E517–E525.
56. Bennett J, Wellman J, Marshall KA, et al. Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by RPE65 mutations: a follow-on phase 1 trial. Lancet. 2016;388:661–672.