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DRUG EVALUATION

Onasemnogene abeparvovec for the treatment of spinal muscular atrophy

Hugh J. McMillan [a], Crystal M. Proud[b], Michelle A. Farrar[c,d], Ian E. Alexander[e,f], Francesco Muntoni[g,h] and Laurent Servais [i,j]

[a]Departments of Pediatrics, Neurology & Neurosurgery, Montreal Children's Hospital, McGill University Health Centre, Montreal, Canada; [b]Children's Hospital of the King's Daughters, Norfolk, VA, United States; [c]School of Clinical Medicine, UNSW Medicine and Health, UNSW Sydney, Sydney, NSW, Australia; [d]Department of Neurology, Sydney Children's Hospital Network, Sydney, NSW, Australia; [e]Gene Therapy Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney and Sydney Children's Hospitals Network, Westmead, NSW, Australia; [f]Discipline of Child and Adolescent Health, Sydney Medical School, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia; [g]The Dubowitz Neuromuscular Centre, University College London, Great Ormond Street Institute of Child Health London, UK; [h]NIHR Great Ormond Street Hospital Biomedical Research Centre, Great Ormond Street Institute of Child Health, University College London, & Great Ormond Street Hospital Trust, London, UK; [i]Department of Pediatrics, Centre Hospitalier Universitaire de Liège & Université de Liège, Liège, Belgium; [j]MDUK Oxford Neuromuscular Centre, University of Oxford, Oxford, UK

ABSTRACT

Introduction: Gene therapy for spinal muscular atrophy (SMA) represents a significant milestone in the treatment of neurologic diseases. SMA is a neurodegenerative disease that results in motor neuron loss because of mutations of the *survival motor neuron 1* gene, which directs survival motor neuron (SMN) protein production. Onasemnogene abeparvovec, a one-time gene replacement therapy, delivers a functional transgene to restore SMN protein expression. Onasemnogene abeparvovec has demonstrated improved survival and motor milestone achievements for presymptomatic infants and patients with SMA type 1.

Areas covered: This expert review describes the current state of gene therapy for SMA, reviews the mechanism of and clinical experience with onasemnogene abeparvovec, explains future efforts to expand applications of gene therapy for SMA, and provides context for developing gene therapy for other conditions.

Expert opinion: Onasemnogene abeparvovec has demonstrated efficacy in clinical trials and, because of this, is a valuable treatment option for patients with symptomatic infantile SMA and those identified by newborn screening. Gene therapy is still in its infancy, and challenges and uncertainties associated with transgene delivery must be addressed. With ongoing development of vector technology, more specific tissue tropism, reduced 'off-target' effects, and an enhanced safety profile will continue to evolve.

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1. Introduction

Gene therapy has opened the door to treatments for an increasing number of rare genetic diseases. New technologies and approaches to diagnosis and care allow clinicians, patients, and caregivers to anticipate novel, life-changing therapies for diseases that, until now, have received only supportive care. Spinal muscular atrophy (SMA) represents one such disease and its approved gene therapy, onasemnogene abeparvovec, has changed the treatment landscape of this devastating neurologic disease.

SMA is a neurodegenerative disease that results in motor neuron loss because of biallelic mutations of the *survival motor neuron 1* (*SMN1*) gene, leading to severe muscle weakness and atrophy. *SMN1* directs the production of the survival motor neuron (SMN) protein, which is essential for the development and maintenance of motor neurons [1,2]. SMA occurs in an estimated 1 in 10,000 live births and was, until recent advancements in treatment, a leading genetic cause of infant mortality [3–5]. The *survival motor neuron 2* (*SMN2*) gene functions as a partial backup gene to *SMN1*, and the severity of SMA correlates inversely with the polymorphic number of *SMN2* gene copies [6–8]. However, quantification of *SMN2* copies, and the clinical significance of this quantification, still requires standardization [9].

Traditionally, SMA phenotypes have been described according to age at symptom onset and maximum motor milestones achieved (Table 1) [4,10,11]. The wide phenotypic spectrum of SMA ranges from profound weakness evident at birth (type 0) to relatively mild symptoms for individuals with adult onset (type 4). Type 1 is the most common and is a very severe phenotype of SMA, with symptoms usually appearing during the first few months of life. Without treatment, patients with SMA type 1 never achieve independent sitting and usually do not survive beyond 2 years of age.

Until relatively recently, most studies of SMA focused on symptom management [12–14]. The advent of an antisense oligonucleotide (nusinersen) and a small-molecule drug (risdiplam) that affect *SMN2* gene splicing, as well as a gene

CONTACT Hugh J. McMillan hugh.mcmillan@mcgill.ca Departments of Pediatrics, Neurology & Neurosurgery, Montreal Children's Hospital, McGill University Health Centre, 1001 Decarie Blvd, Montreal, QC, H4A 3J1 Canada

Article highlights

- Spinal muscular atrophy (SMA) is a neurodegenerative disease that results in motor neuron loss because of biallelic mutations of the *survival motor neuron 1* (*SMN1*) gene, leading to inadequate concentrations of survival motor neuron (SMN) protein, which is critical for motor neuron development and maintenance.
- SMA type 1 is the most severe and most common phenotype of SMA, with symptom manifestation within the first months of life. Without treatment, patients with SMA type 1 are not predicted to survive beyond 2 years of age.
- Onasemnogene abeparvovec is a one-time, intravenously administered gene replacement therapy approved for the treatment of SMA.
- Onasemnogene abeparvovec uses an adeno-associated viral (AAV) vector that offers long-term transgene expression and has low immunogenicity compared with other viral vectors and transgenes.
- Onasemnogene abeparvovec demonstrated clinical benefit in the Phase I START and Phase III STR1VE-US and STR1VE-EU trials, which included infants with SMA type 1, most of whom were aged <6 months. Overall, patients demonstrated longer survival, achievement of motor milestones, and improved motor function after administration of onasemnogene abeparvovec. Several clinical and real-world studies and disease registries are planned or underway that will evaluate onasemnogene abeparvovec in a broader SMA patient population, as well as clinical durability and long-term safety of onasemnogene abeparvovec.
- SMA is associated with enormous medical and societal costs. The availability of gene therapy has the potential to decrease direct and indirect costs, but the considerable cost of the gene therapy and the difference between results achieved when treatment is administered before symptom onset compared with after symptom onset must be considered in the balance of costs.
- New and emerging evidence will continue to shape the way onasemnogene abeparvovec is prescribed in routine clinical practice. The lessons learned from early interventions and new treatment paradigms in SMA will influence how future screening and treatment programs are implemented for other neurologic conditions.

replacement therapy (onasemnogene abeparvovec) that delivers a functional gene to restore expression of full-length SMN protein, has changed the model of SMA treatment [15]. Multidisciplinary care in combination with disease-modifying therapies remains imperative, necessitating an approach focused on each individual patient's clinical status and current needs, to optimize motor, respiratory, and bulbar function. Although these treatments do not offer a cure for SMA, they do offer substantial gains in motor function achievements and life expectancy. With the introduction of disease-modifying treatments, SMA phenotypes are evolving, and classification is increasingly described according to functional status (e.g. non-sitter, sitter, walker) [12,13]. Age, *SMN2* copy number, and baseline motor function are important determinants of outcomes [16,17].

Several consensus statements and documents to guide SMA treatment considerations are available [18–20], including recommendations specific for the use of onasemnogene abeparvovec [18,21]. These algorithms guide clinicians to provide the best possible support and improve outcomes for patients with SMA and, overall, underscore the importance of early treatment to moderate symptom development and progression. Specifically, because of the rapidly progressive nature of the disease, universal newborn screening (NBS) and early SMA treatment are critical to ameliorate irreversible motor neuron loss and maximize functional outcomes [22–28]. Prior to the availability of disease-modifying treatments for SMA, NBS for SMA was not widely accepted or implemented [29]. However, the availability of treatments that can be potentially administered before symptom onset has led to substantial gains in public and health care provider acceptance of NBS [22]. In addition, the rapid expansion of NBS for SMA will likely further impact disease classification, which will include infants treated presymptomatically stratified by *SMN2* copy number.

Regional differences in access to care impact treatment of patients with SMA. Disparities among countries have been reported in genetic testing and diagnosis; support such as cough assistance and ventilation, gastric and nasogastric tubes, and scoliosis surgery; loss of ambulation; and health-related quality of life and survival. These differences are attributed to dissimilarities in health care systems (public vs. private); financial limitations or constraints; availability (or lack) of specialty care; social or cultural attitudes toward chronic, life-limiting diseases [10]; and differences in the approvals of disease-modifying treatments by regulatory bodies around the world [22,30]. Treatment algorithms and clinical guidelines continue to evolve as new evidence emerges, more knowledge is gained, and clinician and patient/caregiver acceptance for SMA treatment grows.

The availability of gene therapy for SMA provides a new paradigm both for the treatment of SMA — moving from supportive care to interventional care — and for broader treatment of neurologic disorders using gene therapy. This expert review provides an overview of the current state of gene therapy for SMA, explains future efforts to expand applications of gene therapy for this condition, and provides context for developing gene therapy for other disease states.

2. Methods

We searched PubMed to explore the breadth of literature on AAV9-based gene therapy and its applications for SMA. We conducted two searches to capture literature released since publication of the pivotal Phase I START trial of onasemnogene abeparvovec (formerly AVXS-101) in 2017 through 1 November 2021. For the first search, terms included onasemnogene abeparvovec AND spinal muscular atrophy. For the second search, terms included adeno-associated virus serotype-9 OR AAV9 AND gene therapy. We also searched the reference lists of publications in the PubMed results to identify new and emerging research. Congress presentations, abstracts, and posters were also obtained by searching relevant congress web sites (including the American Academy of Neurology, the Academy of Managed Care Pharmacy, the British Paediatric Neurology Association, the European Paediatric Neurology Society, and SMA Europe). All publications were considered for this review, including those from industry, academia, and clinical practice, to ensure that an objective, independent review was conducted.

Table 1. **Types of spinal muscular atrophy** [4,10,11].

SMA type	0	1	2	3	4
Approximate percentage of all SMA cases	<1%	45–60%	~20–30%	~15–20%	<1%
Typical *SMN2* copies present	1 copy	2–3 copies	3 copies	3–4 copies	≥4 copies
Age at symptom onset	Prenatal	0–6 months	6–18 months	>18 months	20–30 years
Symptoms and typical features	Profound hypotonia Facial muscle weakness Inability to suck/swallow Respiratory failure at birth	Limb weakness Respiratory distress Weak cry Poor feeding Inability to sit unsupported Bell-shaped chest	May sit unsupported Poor crawling Limb weakness more profound in lower than upper limbs Some limitation of head and neck control Inability to stand or walk independently Impaired swallowing Ventilatory insufficiency Scoliosis	May stand or be able to walk independently Unable to run, jump, or climb Abnormal gait, foot deformity Able to eat independently	Ambulatory Mild, slowly progressive limb weakness
Support needed	Respiratory support beginning at birth	Respiratory and nutritional support by 5–6 months of age	Ventilatory support, feeding tube, wheelchair	Wheelchair or braces, physical and occupational therapy	Walking aids
Survival without disease-modifying treatment	Weeks	<2 years	Reduced life expectancy	Does not impact life expectancy	Does not impact life expectancy

SMA: spinal muscular atrophy; *SMN2: survival motor neuron 2* gene.

3. Onasemnogene abeparvovec: overview and therapeutic mechanism

The preferred vector for a gene therapy targeting the central nervous system (CNS) is derived from adeno-associated virus (AAV), a dependent parvovirus. Recombinant AAV vectors are suitable for gene therapy because of their capacity to transduce both dividing and non-dividing cells and confer long-term transgene expression in non-dividing cells, primarily as a non-integrating episome [31–34]. In addition, AAV-based therapies have relatively low immunogenicity compared with other viral vectors, such as adenovirus. The importance of a patient's immune response to a viral vector was first demonstrated by a patient with ornithine transcarbamylase deficiency who died in 1999 after suffering complications from an adenovirus-mediated gene transfer [35]. This case highlighted the need to consider other less immunogenic viral vectors for gene therapy applications targeting genetic disease.

As the transgene is primarily maintained in an extrachromosomal episome that resides in the nucleus [36], the risk of insertional mutagenesis is less than that observed with other viral vectors, such as those derived from retroviruses in which integration is an obligate feature of the viral life cycle. However, while predominantly episomal, a small percentage of AAV vector genomes (or fragments thereof) do undergo genomic integration, and a large portion of the vector dose ends up in the liver and carries a small risk of insertional mutagenesis, which has been highlighted in several preclinical studies, but, to date, not in humans [37].

Recombinant AAVs have the same basic structure as wild-type AAVs, with an outer shell, or capsid, enclosing a single-stranded DNA genome, but the recombinant genome does not contain any AAV protein-coding sequences. Instead, the recombinant genome contains a therapeutic gene expression cassette [38,39] and retains only the flanking inverted terminal repeats, which are of viral origin and required for genome replication and packaging [39,40]. The removal of all viral coding sequences allows for a lesser risk of immunogenicity and cytotoxicity [39]. Another instrumental property of the AAV vector system is the ability to cross-package the recombinant viral genome into multiple different capsid types depending on the desired gene transfer properties conferred by the capsid. For example, the critical and unique property of the AAV9 capsid used in onasemnogene abeparvovec is the ability to cross the blood-brain barrier and transduce neurons.

One barrier to AAV administration is pre-existing anti-capsid humoral immunity driven by natural exposure to wild-type AAV, particularly AAV2, which is endemic — though nonpathogenic — in human populations and can lead to the production of antibodies that cross-react with the AAV9 capsid [41]. The prevalence of AAV-neutralizing antibodies is moderate at birth, decreases between 7 and 11 months of age, and progressively increases through childhood and adolescence. Overall, approximately 40–80% of adult humans have antibodies against AAV [42]. Patients who have a greater seroprevalence of anti-AAV antibodies may not be suitable candidates for AAV-based gene therapy [41–44]. Similarly, AAV vector exposure induces an immune response to the AAV capsid that currently precludes retreatment. Transgenes (or their protein products) can also stimulate immune responses, but such responses are unlikely for patients with SMA treated with onasemnogene abeparvovec because some SMN protein is produced by all patients as a result of paralogous *SMN2* genes. Systemic corticosteroids are administered before and for a period of a few months after gene therapy to attenuate the immune response, providing anti-inflammatory and immunosuppressive effects [41].

The AAV serotype used in onasemnogene abeparvovec, AAV9, was isolated from the human liver, which is one of two predominant sites of natural AAV infection (the other is the spleen) [39]. AAV9 differs from other serotypes because it crosses the blood-brain barrier, which allows for intravenous delivery to achieve widespread gene expression in the CNS. Thus, AAV9 is a promising therapeutic tool for CNS and neurologic disorders [45] and has already demonstrated success in treating SMA [46–50].

Onasemnogene abeparvovec is composed of an AAV9 capsid that carries a recombinant AAV genome encoding a therapeutic cassette that is flanked by AAV2 inverted terminal repeats [51]. One of the inverted terminal repeats carries a specific deletion that allows the formation of a self-complementary genome configuration that facilitates rapid expression of the transgene [51]. Sustained, ubiquitous SMN transgene expression from the therapeutic cassette is driven by a hybrid cytomegalovirus enhancer–chicken β-actin promoter.

Following intravenous administration, onasemnogene abeparvovec is widely distributed in the CNS and peripheral tissues. The AAV9 mechanism of targeting motor neurons after systemic delivery differs from most other forms of AAV gene therapy, which are not able to efficiently cross the blood-brain barrier and target the CNS [52].

In a recent study of the biodistribution of onasemnogene abeparvovec in two symptomatic infants with SMA type 1 who died because of SMA-related complications, transduction was observed in multiple body systems [36]. Transgene copy numbers ranged from 0.04 vector genomes (vg)/cell in the thymus to 399.25 vg/cell in the liver. Distribution to skeletal muscle was also observed at 1.1–4.0 vg/cell in the diaphragm, 1.4 vg/cell in the quadriceps, 1.1–1.3 vg/cell in the psoas, and 2.5–3.3 vg/cell in the intercostal muscles. Distribution in spinal motor neurons ranged from 1.49–2.65 vg/cell. The degree of transduction in the liver was expected and consistent with studies of onasemnogene abeparvovec administration [53,54]. This AAV9 hepatotropism has important safety implications and illustrates the need for long-term follow-up. The increased delivery of vector genomes to hepatocytes could be associated with a greater theoretical risk of insertional mutagenesis and a greater potential lifetime risk of liver neoplasia.

SMN protein expression was also reported in the CNS and peripheral organs, including the brain (cortical and subcortical regions), choroid plexus, vascular structures, quadriceps, heart, liver, kidney, lungs, pancreas, spleen, thymus, stomach, large and small intestine, and inguinal lymph nodes [36]. SMN expression was greatest in the liver, with high expression also found in the heart, psoas, and diaphragm muscles.

The recommended dose of onasemnogene abeparvovec is 1.1×10^{14} vg per kilogram of body weight (vg/kg). Onasemnogene abeparvovec is administered as an intravenous infusion over 60 minutes. Treatment with systemic corticosteroids equivalent to oral prednisolone 1 mg/kg/day must be initiated 1 day before onasemnogene abeparvovec administration and continued for 30 days and then weaned over an additional 4 weeks [55]. If adverse events such as serum transaminase concentration elevations occur, increasing or continuing prednisolone dosing for a longer treatment period may be necessary [56].

4. Clinical experience with onasemnogene abeparvovec

The efficacy of onasemnogene abeparvovec in SMA type 1 has been established in clinical trials, and real-world clinical experience with onasemnogene abeparvovec is expanding rapidly, facilitated by SMA disease registries. As more patients receive onasemnogene abeparvovec, our knowledge of therapeutic effects, the overall risk of adverse events, and applications to larger patient populations is expanding.

4.1. Phase I trial of onasemnogene abeparvovec

The first clinical trial to demonstrate the benefit of onasemnogene abeparvovec, START (NCT02122952) [48], was a Phase I trial conducted in 2014 and 2015 at Nationwide Children's Hospital in Columbus, Ohio. START included 15 patients with SMA type 1, all of whom had two copies of *SMN2*. All patients received a single dose of onasemnogene abeparvovec. Three patients received a low dose (6.7×10^{13} vg/kg) and 12 received a high dose (2.0×10^{14} vg/kg [equivalent to the therapeutic dose of 1.1×10^{14} vg/kg according to the potency assay]). At the time of treatment, the ages of patients in the low-dose group ranged from 5.9 to 7.2 months (mean, 6.3 months), and the ages in the high-dose group ranged from 0.9 to 7.9 months (mean, 3.4 months). Prior to onasemnogene abeparvovec treatment, three patients in the low-dose group and five patients in the high-dose group required nutritional support, and three patients in the low-dose group and two patients in the high-dose group required ventilatory support. Safety, time until death or the need for permanent ventilatory assistance, and changes in scores on the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) scale of motor function were evaluated. The CHOP INTEND scale ranges from 0 to 64, with greater scores indicating better function. Historically, children with SMA type 1 are not predicted to achieve scores greater than 40 [57–59]. Endpoints were compared with a cohort of untreated patients from natural history studies [48].

Overall, treatment with onasemnogene abeparvovec resulted in longer survival, achievement of motor milestones, and improved motor function, especially for patients treated early and patients who had greater baseline function treated with the greater dose. At 20 months after administration, all 15 patients were alive and free from ventilatory support (a historic comparison cohort reported only 8% survival at the same time point). Of the 12 patients who received the high dose of onasemnogene abeparvovec, 11 sat unassisted, nine rolled over, 11 fed orally and could speak, and two walked independently. The high-dose group also achieved rapid and early increases in CHOP INTEND scores of 9.8 points from baseline at 1 month and 15.4 points at 3 months. CHOP INTEND scores declined in the historic cohort [48].

At the last follow-up 24 months after administration, the mean CHOP INTEND score in the high-dose group was 56.5, compared with 5.3 in the historic comparison cohort. All 12 infants in the high-dose START group achieved improvements in CHOP INTEND of ≥4 points, and 11 of 12 achieved a score ≥40.0. In addition, 11 patients sat unassisted for ≥5 seconds, 10 for

≥10 seconds, and nine for ≥30 seconds at 24 months of follow-up. Two patients could stand and walk independently. Importantly, these two patients were treated very early and had a baseline CHOP INTEND score of 50 [60]. This is far greater than the CHOP INTEND score threshold of 40 that patients with SMA type 1 typically do not reach without treatment, which once again demonstrates the importance of early intervention for optimal outcomes.

During the 24 months of follow-up, 53 serious adverse events were reported among 10 patients. Two of the events were related to the study treatment and involved asymptomatic transaminase elevations [60].

START was the first study to demonstrate that a single dose of an AAV containing DNA coding for the SMN protein could offer clinical benefit with a favorable safety profile for patients with SMA type 1, and the long-term follow-up of the START study (LT-002; NCT04042025) supports the long-term safety and durable efficacy of onasemnogene abeparvovec [49]. The ongoing follow-up safety study includes 13 patients enrolled in START (three from the low-dose cohort and 10 from the high-dose cohort). More than 6.2 years after administration, all 10 patients in the high-dose cohort were alive and free from ventilatory support at the time of follow-up. All motor milestones achieved in START have been maintained, and two patients have achieved new milestones during long-term follow-up. Eight patients experienced serious adverse events, including acute respiratory failure, pneumonia, dehydration, respiratory distress, and bronchiolitis, but none led to study discontinuation or death, and none were deemed to be related to the study drug. This study provides evidence of the clinical durability and favorable safety of onasemnogene abeparvovec, although durability is difficult to ascertain in all patients because four patients in the high-dose cohort and three patients in the low-dose cohort also received nusinersen [49].

4.2. Phase III trials with onasemnogene abeparvovec

The open-label, single-arm, single-dose Phase III STR1VE trials built on the success of START by studying the safety and efficacy of the therapeutic dose of onasemnogene abeparvovec (a single intravenous infusion of 1.1×10^{14} vg/kg). Endpoints were again compared with patients from natural history data sets [47,50].

4.2.1. STR1VE

The first of two STR1VE trials was conducted at 12 hospitals in the United States from 2017 to 2019. STR1VE-US (NCT03306277) included 22 patients with SMA type 1 and two copies of *SMN2*. The co-primary endpoints were independent sitting for 30 seconds or longer (Bayley-III Scales of Infant and Toddler Development item #26) at 18 months of age and survival (absence of death or permanent ventilation). Secondary outcomes included ability to thrive (a composite endpoint including swallowing function, nutritional support requirements, and weight maintenance) and being free from ventilatory support at 18 months of age.

The patients' ages ranged from 0.5–5.9 months (mean, 3.7 months). The mean CHOP INTEND score at baseline was 32.0. At 14 months of age, 20 patients were alive and free from ventilatory support (compared with six patients in the natural history cohort). At 18 months of age, 13 patients achieved sitting for 30 seconds or longer (compared with zero patients in the natural history cohort). Nine patients maintained the ability to thrive and 18 were free from ventilatory support (compared with zero for either endpoint in the natural history cohort). CHOP INTEND scores increased rapidly, with mean increases from baseline reported as early as 1 month after administration. Mean increases in scores improved by 6.9 points at 1 month, 11.7 points at 3 months, and 14.6 points at 6 months [47].

All patients experienced at least one adverse event, and pyrexia was the most common. Three serious adverse events were possibly treatment-related, with two patients experiencing hepatic transaminase elevations and one patient experiencing hydrocephalus. Overall, STR1VE-US demonstrated clinically meaningful benefit for survival, motor milestone achievement, and motor function, and further supported the use of onasemnogene abeparvovec in symptomatic infants with SMA type 1 [47].

The second Phase III STR1VE trial was conducted at nine hospitals and universities in Italy, the United Kingdom, Belgium, and France from 2018 to 2020. Similar to STR1VE-US, STR1VE-EU (NCT03461289) included patients younger than 6 months of age with SMA type 1 and two copies of *SMN2* [50]. Compared with STR1VE-US, STR1VE-EU comprised a broader patient population that included some patients who were receiving nutritional and/or respiratory support at baseline. Patients who required non-invasive ventilatory support for at least 6 hours per day or feeding support were excluded from STR1VE-US but were eligible for STR1VE-EU. The primary endpoint was independent sitting for at least 10 seconds at any time up to and including 18 months of age, defined by the World Health Organization (WHO) Multicentre Growth Reference Study. The secondary endpoint was ventilation-free survival at 14 months of age. Ability to thrive (defined as weight greater than the third percentile, free of nutritional support, and normal swallowing function with thin or very thin liquids at 18 months of age) was assessed as an exploratory outcome.

A total of 33 patients were included in STR1VE-EU. The patients' ages ranged from 1.8 to 6.0 months (mean, 4.1 months), and the mean CHOP INTEND score at baseline was 27.9. Nine patients reported feeding support and nine reported ventilatory support at baseline. Five patients were receiving both feeding and ventilatory support. At 14 months of age, 31 patients were alive and free from ventilatory support. At 18 months of age, 14 patients achieved independent sitting for 10 seconds. Seven patients met the ability to thrive criteria. Patients achieved rapid and sustained increases in CHOP INTEND scores, with mean increases in scores of 6.0 points from baseline at 1 month, 10.3 points at 3 months, and 13.6 points at 6 months [50].

Thirty-two patients experienced at least one adverse event, and the most common was, again, pyrexia. Six serious drug-related adverse events were reported, including two cases of pyrexia,

three cases of increased hepatic transaminase concentrations, and one case of gastroenteritis. Overall, onasemnogene abeparvovec demonstrated efficacy even for patients with more severe disease than those included in studies to date, and no new safety signals were identified [50].

4.2.2. SPR1NT

SPR1NT (NCT03505099) was an open-label, single-arm, Phase III study evaluating the use of onasemnogene abeparvovec in presymptomatic infants younger than 6 weeks of age at risk of SMA type 1 with two or three copies of *SMN2* [61]. In all, 29 patients were enrolled in SPR1NT. Of these, 14 patients had two copies of *SMN2* and 15 patients had three copies of *SMN2*. Early results demonstrated that both patient groups (two and three copies of *SMN2*) survived and reached age-appropriate milestones. All patients in both groups were alive and free from permanent ventilation at the end of study for two- and three-copy patients [61–63]. At 18 months of age, all 14 patients in the two-copy group achieved the primary efficacy endpoint of sitting without support for at least 30 seconds, including 11 who achieved this milestone within the WHO normal developmental window [61,63]. All patients in the two-copy group achieved CHOP INTEND scores ≥58 during at least one follow-up visit [61]. All 15 patients in the three-copy group achieved the primary efficacy endpoint of standing without support, confirmed by independent video review. Fourteen of 15 did so within the WHO normal developmental window (≤514 days) [64]. Fourteen of 15 three-copy patients walked independently for at least five steps at any visit up to 24 months of age and 11 of 14 achieved this motor milestone within the WHO normal developmental window (≤534 days) [64]. No infant enrolled in SPR1NT experienced a serious adverse event that was considered treatment-related by the investigator, and no new safety signals were identified [61,62]. Overall, onasemnogene abeparvovec demonstrated clinical efficacy for children younger than 6 weeks of age with presymptomatic SMA.

4.3. SMA registries

Several registries of patients with SMA have been established to gather real-world data about the long-term safety and efficacy of SMA treatments.

4.3.1. The RESTORE registry

The RESTORE registry (NCT04174157) is a prospective, multicenter, multinational observational registry evaluating SMA history and treatment; pulmonary, nutritional, and motor milestones; health care resource utilization; work productivity; activity impairment; adverse events; health-related quality of life; caregiver burden; and survival [65–67]. Patients with genetically confirmed SMA are being enrolled during a period of 5 years, which began in September 2018. These patients will be followed for 15 years or until death [68].

Presentations at the 2022 American Academy of Neurology Annual Meeting reported interim results of RESTORE. Preliminary analyses as of 23 November 2021 (the most recent data cut) were conducted to evaluate outcomes for patients identified by NBS compared with clinical diagnosis of SMA and the use of onasemnogene abeparvovec for patients with SMA aged at least 6 months at infusion.

To compare NBS with clinical diagnosis, patients in the RESTORE registry from the United States with ≤2 copies of *SMN2* were stratified according to either clinical diagnosis of SMA type 1 based on SMA symptoms or prenatal screening/NBS. Each group was then limited to patients who had a follow-up period of at least 16 months. In all, 25 were identified as at risk for SMA type 1 based on prenatal screening or NBS and 70 were identified via clinical diagnosis. Patients identified via prenatal/NBS were diagnosed and received disease-modifying treatment significantly earlier than clinically diagnosed patients (0.8 vs. 3.7 months and 1.6 vs. 5.1 months, respectively [$p<0.0001$ for both]) [66].

Patients identified via prenatal/NBS generally achieved motor milestones at earlier ages than clinically diagnosed patients. The median age for achieving independent sitting for 30 seconds was 13.7 months in the prenatal/NBS group and 21.8 months in the clinically diagnosed group. Four patients (two in each group) achieved the milestone of walking independently. The patients in the prenatal/NBS group achieved this milestone at 24.7 months, and the patients in the clinically diagnosed group achieved it at 32.2 months. Most patients in both groups achieved increases of ≥4 points on CHOP INTEND (100% in the prenatal/NBS group and 80.1% in the clinically diagnosed group). Monthly increases in CHOP INTEND scores were similar at 1.2 points in the prenatal/NBS group and 1.0 in the clinically diagnosed group. Clinically diagnosed patients received more than one SMA treatment more often than patients identified via prenatal/NBS (70.0% vs. 60.0% [$p=0.3730$]) [66].

To analyze the use of onasemnogene abeparvovec in a population older than those patients included in clinical trials, patients who received onasemnogene abeparvovec when they were at least 6 months of age were considered [67]. In all, 145 patients were identified from the RESTORE registry. Of these, 67 received onasemnogene abeparvovec between 6 and 12 months of age, 67 between 12 and 24 months, and 11 at age 24 months or older. Ninety-six patients were diagnosed with SMA type 1, and 11 children were presymptomatic. Forty-six patients had available CHOP INTEND scores, including 19 patients who received onasemnogene abeparvovec between 6 and 12 months of age and who achieved a score increase of ≥4 points and 14 patients who received onasemnogene abeparvovec between 12 and 24 months of age and achieved the same increased score. The adverse event profile among these patients was consistent with the overall adverse event reporting for onasemnogene abeparvovec, and no new safety signals were observed [67]. Together, these early findings from RESTORE enhance the understanding of how onasemnogene abeparvovec is used in the real world and demonstrates clinical benefit for presymptomatic children and patients older than 6 months.

4.3.2. Canadian Neuromuscular Disease Registry

The Canadian Neuromuscular Disease Registry (CNDR), established in 2011, is a longitudinal, prospective, observational study of patients with neuromuscular disease in Canada [69]. A subset of this registry focuses on SMA and will evaluate the real-world safety and effectiveness of novel therapies. As of

March 2020, 250 patients were enrolled across 37 clinics [70]. One recent abstract from the CNDR reported the patterns of switching among disease-modifying therapies for SMA. Of 217 patients in the CNDR with available data, 44 had SMA type 1. Of these, seven patients received nusinersen and then switched to onasemnogene abeparvovec [71]. Long-term outcomes and follow-up data are forthcoming.

4.3.3. SMArtCARE Registry

The SMArtCARE registry collects long-term, real-world data on the treatment of SMA in Germany, Austria, and Switzerland [72]. More than half of a subset of 76 patients were older and heavier at the time of onasemnogene abeparvovec administration (mean age, 16.8 months; mean weight, 9.1 kg) than patients in clinical trials. Fifty-eight patients had been treated with nusinersen. Of 60 patients with available data, 49 achieved improvements in CHOP INTEND scores of ≥4 points. CHOP INTEND scores increased significantly for patients younger than 24 months at the time of onasemnogene abeparvovec administration but not for those who were older than 24 months. CHOP INTEND scores also increased significantly for the 45 patients with available data who had received nusinersen. Notably, respiratory and bulbar function improved for patients with respiratory insufficiency or dysphagia despite nusinersen treatment [56].

Fifty-six patients experienced treatment-related adverse events, including eight serious adverse events. Overall, liver enzyme elevations were greater for patients who were older and heavier at the time of onasemnogene abeparvovec infusion [56]. These findings strengthen support for careful patient selection and comprehensive monitoring for potential liver dysfunction for older patients treated with onasemnogene abeparvovec.

Overall, this report adds to the evidence of a positive benefit–risk ratio of onasemnogene abeparvovec treatment for patients with SMA and extends the findings to older and heavier patients as well as to those treated with nusinersen. However, differentiating benefits related to onasemnogene abeparvovec or combination effects compared with the same potential results with nusinersen monotherapy over a longer observation period is difficult. Future research is needed to clarify these results.

4.4. Adverse events

Onasemnogene abeparvovec has been associated with specific adverse events, which can be managed with close anticipatory surveillance. Although some adverse events may be related to total viral vector dose (and, thus, body weight), further research is needed to clarify risk factors for specific adverse effects [73,74]. Patient selection is important in preventing and/or mitigating these events. Ensuring that the patient has had no recent as well as no active infection and evaluating for any signs of pre-existing liver dysfunction is necessary to optimize safety and determine eligibility for treatment.

4.4.1. Hepatotoxicity

Hepatotoxicity has been observed following onasemnogene abeparvovec administration [53]. Hepatotoxicity is especially concerning with high vector-dose systemic AAV regimens and deserves expanded attention. A recent study reported evidence of hepatotoxicity in 100 patients with SMA who had received onasemnogene abeparvovec [53]. The mean age of the patients at the time of onasemnogene abeparvovec administration was 2.9 months. Ninety patients had elevated serum transaminase concentrations (either alanine aminotransferase [ALT] or aspartate aminotransferase [AST]) and/or bilirubin. The elevations began as early as Week 1 and peaked approximately 1 month after administration. Thirty-four patients had at least one hepatotoxic adverse event. Sixty-one (61%) patients, including children with SMA who were presymptomatic, had elevated serum transaminase concentrations prior to onasemnogene abeparvovec administration. This reflects the early findings of the SPR1NT trial, in which 23 of 30 patients tested had elevated bilirubin and/or transaminase concentrations prior to administration of onasemnogene abeparvovec. Liver function for these patients normalized by the end of the study [53].

The early elevation in transaminases is sufficient to cause liver dysfunction that impacts the biosynthetic functions of the liver for approximately 2% of patients, evidenced by increased international normalized ratios [53]. However, the mechanism of the early increases in hepatic transaminases, while still unclear, is likely different from mechanisms involved in later increases. One report highlighted more severe hepatic dysfunction for two patients that occurred between 3 and 8 weeks after onasemnogene abeparvovec. The patients experienced increased ALT, AST, and bilirubin, which resolved with high-dose corticosteroid and supportive therapy, and both patients demonstrated clinical recovery. One patient demonstrated evidence of hepatic fibrosis on liver biopsy [75]. Further research is needed to better understand the mechanism and consequences of liver function test elevations and hepatic dysfunction.

Hepatic cytolysis observed for patients treated with onasemnogene abeparvovec is different than cholestasis observed in another neuromuscular condition, X-linked myotubular myopathy (XLMTM), treated by gene therapy. Three male patients in the ASPIRO trial received 3×10^{14} vg/kg of the AAV-based gene therapy and developed cholestasis and liver failure within 3 to 4 weeks after administration. Two patients experienced fatal liver dysfunction and the third was reported to have ongoing liver dysfunction. Compared with other patients who received AAV-based gene therapies, these three patients were older, heavier, and displayed evidence of pre-existing liver disease [76,77]. Increasing evidence suggests that hepatobiliary disease is a common comorbidity with XLMTM, which may present treatment challenges for children with this disorder [78], and indeed a fourth child recently developed fatal hepatic complication despite receiving a lower AAV8 viral load (1.3×10^{14} vg/kg) [79].

Unlike gene therapy with onasemnogene abeparvovec, gene therapy administered for XLMTM uses an AAV8 capsid. In addition, subclinical cholestasis has been established for patients who died following gene therapy in the XLMTM trial. The hepatotoxicity of onasemnogene abeparvovec is

evidenced by elevated AST and ALT, which demonstrates cytolysis. The hepatotoxicity in XLMTM patients was evidenced by elevated bilirubin, which demonstrates cholestasis and liver failure. In addition, the immune systems of patients with XLMTM are naïve to the missing endogenous protein, which is not the case in SMA, in which patients produce residual SMN protein. This difference could contribute to adverse events [80]. Furthermore, hepatotoxicity was not observed in murine or canine models of AAV8-based treatment in XLMTM [81,82], which highlights the importance of careful and well-conducted studies in human populations. However, these outcomes reinforce the importance of understanding the biodistribution of AAV-based gene therapy and the consequences that greater concentrations of distribution to the liver may confer.

4.4.2. Hematologic abnormalities

Thrombocytopenia and thrombotic microangiopathy (TMA) were reported after onasemnogene abeparvovec administration [73,83–85]. In clinical trials and post-marketing safety analyses, thrombocytopenia was reported as transient in most cases and often resolved without intervention [84]. In a study of eight patients who received nusinersen followed by onasemnogene abeparvovec, six experienced thrombocytopenia. The lowest blood counts were observed between Days 6 and 8 after onasemnogene abeparvovec administration. No evidence of cutaneous, mucosal, or other bleeding was observed, and blood counts normalized within a few weeks after treatment [73]. Thrombocytopenia is a feature of TMA and should be monitored following onasemnogene abeparvovec administration [83].

No cases of TMA were reported in clinical studies [84], but as of July 2020, three cases of TMA following onasemnogene abeparvovec administration had been reported. Two were reported in the RESTORE registry, and one was reported by a managed access program in Australia. Two of these cases occurred in patients who had received nusinersen. TMA developed within 1 week of onasemnogene abeparvovec infusion. All three infants recovered, one with supportive care and two with therapies such as plasmapheresis, corticosteroids, and/or blood transfusions. TMA is thought to be an immune-mediated reaction or a dose-related toxicity. Concurrent or recent infections are often related to the development of TMA, but more investigation is required to confirm risk factors for TMA [83].

4.4.3. Animal data

Dorsal root ganglia (DRG) inflammation has been observed in nonhuman primates that received a different AAV9-based gene therapy product via the intrathecal route. However, these findings were not observed in mice [84].

Clinical evaluations of human patients treated with onasemnogene abeparvovec (intrathecal or intravenous) have not demonstrated evidence of sensory symptoms consistent with DRG inflammation, such as sensory neuronopathy [84]. However, DRG damage has been reported following intrathecal administration of an AAV-based gene therapy for amyotrophic lateral sclerosis targeting defects in the *SOD1* gene in one patient [86,87]. Together, these observations suggest that DRG findings may be a class effect of AAV therapies, though the mechanisms of damage are not defined. Electrophysiologic tests may be considered as part of onasemnogene abeparvovec monitoring, because signs and symptoms of sensory nerve abnormalities are difficult to assess for younger patients [84].

SMN overexpression associated with neuro-inflammation and innate immune response was demonstrated in a murine model, with SMNΔ7 mice that received an AAV9-SMN viral vector. Long-term overexpression of SMN led to motor dysfunction and neurodegeneration [88]. To date, however, no evidence of SMN overexpression or clinical signs of such overexpression in humans has been presented [88,89]. The clinical significance of these animal studies is yet to be fully determined.

4.4.4. Adverse events with other gene therapies

Other AAV-based gene therapies are associated with adverse events that have not been observed with onasemnogene abeparvovec [90]. For example, complement activation has been observed following AAV-based therapies for Duchenne muscular dystrophy (DMD), a genetic disease that results in progressive muscle wasting [91,92]. This immune-mediated reaction promotes inflammation and damage to cells such as red blood cells, platelets, and endothelial cells. This cell damage can further activate the complement system and establish a feedback loop of severe hematologic injury, organ damage, and bleeding, which, if untreated, may be fatal [91]. In a Phase Ib trial of six patients, fordadistrogene movaparvovec (an AAV9-based gene therapy for DMD) was associated with atypical hemolytic uremic syndrome-like complement activation in one patient. This patient suffered acute kidney injury, hemolysis, and decreased platelet count, but symptoms resolved and renal function returned to normal within 15 days because of prompt treatment with intermittent hemodialysis and administration of a complement inhibitor [46]. This prompted changes to the study protocol, with closer monitoring and greater doses of glucocorticoids post infusion. The death of a non-ambulant young man from cardiogenic shock was reported in December 2021 and led to a hold on the clinical development of fordadistrogene movaparvovec [93].

The immunogenicity of AAV-based therapy requires continued investigation. The design and monitoring of future therapies should consider the potential for target cell destruction by T cells directed against non-self epitopes encoded by therapeutic transgenes [94]. Consequently, some DMD gene therapy clinical trials have excluded specific dystrophin mutations to minimize the number of sequence differences between the defective self-gene and the therapeutic transgene, as well as potential T-cell immunity [95]. A muscle–specific promoter may minimize the risk of immune-related toxicity in non-muscle tissues [91,92].

5. Health economics

SMA is associated with substantial medical and societal costs [23]. Costs include inpatient, outpatient, and emergency care; prescription and over-the-counter medications; medical

devices and mobility aids such as wheelchairs, walkers, orthotics, and feeding products; respiratory and ventilatory assistance; copayments; and home and transportation modifications. Indirect costs related to SMA include loss of productivity (by patient or caregiver); time spent caregiving; caregiver strain such as sleep problems, injury related to moving the patient in their care, anxiety, and changes to employment status; and premature death [96–98]. Quantifying the total costs of SMA is difficult, but one review reported that the average annual cost of SMA type 1 ranges from US$75,047 to US$196,429 [23]. Another review estimated the cost of SMA type 1 in the United States to be even greater, with annual expenses of US$324,410 [96].

The availability of disease-modifying treatments for SMA substantially reduces direct and indirect expenses, but the costs of treatments carry their own concerns regarding benefit and cost effectiveness. Long-term benefits and future savings should be compared with out-of-pocket expenses to evaluate costs [99]. While some treatments require ongoing use, gene therapies, such as onasemnogene abeparvovec, can potentially provide a lifetime of benefit with a single treatment, although this is very difficult to ascertain today. Onasemnogene abeparvovec has a list price of US$2.125 million, and the estimated lifetime cost for patients with SMA type 1 treated with onasemnogene abeparvovec is approximately US$4.0 million [98,100]. However, therapies for other chronic illnesses may cost much more over patients' lifetimes. For example, bypassing agents for hemophilia A may exceed US$15–18 million and lifetime cost of care for these patients may exceed US$103 million [99].

Because disease-modifying treatments have been available for only a few years, considerable uncertainty persists regarding the true lifetime costs of SMA care in the era of disease-modifying treatments. Gene therapies have challenged models for conventional drug pricing and concerns for health budgets are increasing as new therapies are anticipated. The increased survival of patients who would have died and the substantial upfront costs of gene therapies may impose a strain on the pricing and economics of drug development [23]. As such, innovative approaches to costs and reimbursement models are being considered, including pay-over-time and pay-for-performance models, manufacturing changes [101,102], and expanded risk pools [103].

Several factors should be considered when assessing the long-term societal value compared with the price of gene therapy, including life expectancy (and, therefore, future costs unrelated to the disease), caregiver burden (including lost productivity and time spent providing care), health-related quality of life, and budget impact for health care payors [104,105]. Health equity and overall family well-being are also important [106,107], and one analysis proposed that hope and knowledge are substantial components of the value of gene therapies [99]. As we continue to learn more about the long-term impact that onasemnogene abeparvovec and other gene therapies will have for patients and caregivers, financial decisions will need to be made with input from health care providers, policymakers, and insurance providers, as well as patients and caregivers [103]. New models of care may further enhance health outcomes and cost effectiveness for gene therapy technologies. Specifically, NBS combined with gene therapy will likely improve health outcomes and cost effectiveness [108].

6. Future directions and forthcoming data

Disease-modifying treatments, including onasemnogene abeparvovec, have demonstrated dramatic benefits with respect to survival and motor function for patients with SMA. However, these novel treatments have been available for only a few years and, as such, we are not yet able to describe the benefits of these therapies over patients' expected lifetimes. To date, published clinical trials of onasemnogene abeparvovec have included infants with clinically diagnosed SMA type 1 and two *SMN2* gene copies, or presymptomatic children with two or three *SMN2* copies. More research in a greater range of SMA patient types, reflecting real-world use scenarios and the broader indications of onasemnogene abeparvovec, is needed. Human gene therapy is still a growing field, and ongoing research and development will almost certainly define the use of this technology in future clinical practice.

6.1. Onasemnogene abeparvovec in expanded patient populations

Clinical trials of onasemnogene abeparvovec have included only young infants who weighed 8.5 kg or less, but future and ongoing studies will assess intravenous onasemnogene abeparvovec for 'heavier' patients (≥8.5 kg to ≤21 kg [Phase IIIb SMART trial; NCT04851873]) [109]. An intrathecal formulation was assessed for patients aged 6 to 50 months (Phase I STRONG; NCT03381729) [110,111] and will be assessed for patients with SMA type 2 and two to four copies of *SMN2* (Phase III STEER trial; NCT05089656) [112] (Table 2).

6.2. Use of multiple disease-modifying treatments

Clarification regarding treatment with multiple disease-modifying treatments for SMA and switching between disease-modifying treatments is needed [113,114]. Currently no evidence of additive benefit for combination or sequential therapy has been reported, but this is an active area of investigation [115,116].

RESPOND (NCT04488133) is a single-arm Phase IV study that will evaluate the safety and efficacy of nusinersen for patients aged 2 to 36 months who have already received onasemnogene abeparvovec. RESPOND is expected to enroll 60 patients who, in the opinion of the investigator, may achieve additional benefit from nusinersen after first-line treatment with onasemnogene abeparvovec. This study has an expected completion date of September 2024 [117].

6.3. Multidisciplinary care

Ongoing multidisciplinary care is critical for patients with SMA. Disease-modifying treatments greatly improve the prognosis for the disease, but these treatments are not curative, and care is still required over the patient's lifetime. Multidisciplinary care is tailored to the clinical and

functional status of the patient to address ongoing pulmonary, orthopedic, nutritional, and neuromuscular complications resulting from the underlying disease, and the care required will likely change during the patient's lifetime [12,13,118].

New treatments suggest an evolving paradigm for supporting patients with SMA. With the evolution of phenotypes, an approach focused on clinical status and current needs is necessary to optimize outcomes. A patient's baseline motor function and age at initiation of therapy are important parameters to consider when communicating with families regarding informed decision-making and establishing expectations for potential treatment options.

6.4. Vector technology

Vector technology may also evolve and change gene therapy for SMA. Specifically, the development and use of capsids with reduced hepatotropism, and changes to vector design, particularly for promoter-enhancer specificity, could improve the theoretical safety profile [119]. In addition, vectors that are more efficient than the AAV9 capsid at crossing the blood-brain barrier could improve systemic administration of gene therapies for CNS diseases [120–122].

Recent work in barcoding capsid variants (i.e. linking a specific nucleotide sequence to each capsid for visualization and improved efficiency of transduction [123]) resulted in improved muscle targeting with AAV-based gene therapy products. An AAV9 mutant was directed to target skeletal muscle, including the heart and diaphragm, and demonstrated superior efficiency and specificity compared with non-barcoded vectors [124]. A similar study of muscle-targeting AAV9 capsids allowed for administration of doses that were up to 250 times less than those used in preclinical and clinical trials [125]. However, the peptide used for improved efficiency of transduction would potentially impose immunologic concerns, and barcoding technologies do not necessarily lead to avoidance of AAV pre-existing immunity. In addition, the importance of the difference between mouse models used for barcoding work and humans is yet to be determined. Results obtained with specific AAV capsids in mice likely do not reliably predict performance in equivalent human tissues and cell types, but the development of predictive preclinical models of the blood-brain barrier and human CNS remained challenging. Still, this work will likely help optimize AAV vectors for gene therapy and may have applications in gene therapies that target the CNS.

7. Conclusions

The therapeutic landscape for SMA has changed dramatically over the past few years. Although most cases were essentially untreatable and fatal within the first years of life, disease-modifying treatments, including onasemnogene abeparvovec, are improving survival and permitting many patients to thrive. Prognoses for most patients with SMA are greatly improved, and the importance of ongoing multidisciplinary care remains undiminished. While onasemnogene abeparvovec for SMA represents a significant milestone in human gene therapy, this field is still in its infancy, and challenges and uncertainties, such as patient and disease selection, need to be clarified. The safety and efficacy of gene therapy may be affected by many factors, including patient age, weight, and disease severity, as well as delivery mechanisms and targets of the gene therapy vector.

8. Expert opinion

8.1. Prioritizing early diagnosis and intervention

Onasemnogene abeparvovec is the only one-time disease-modifying treatment for SMA. Outcomes in presymptomatic children highlight the need for universal NBS, and NBS needs to be linked with best practice. Today, only approximately 3% of the world population is screened at birth for SMA [22]. NBS has demonstrated that approximately 30% of infants with two copies of *SMN2* have symptoms around the time of diagnosis and exhibit early, rapid decline in motor nerve function [126]. Facilitating timely access to therapy is critical to optimize outcomes. A multidisciplinary team and strong partnerships among stakeholders are key to implementation of NBS and subsequent health care [127].

Onasemnogene abeparvovec represents an attractive option for parents of presymptomatic children identified by NBS. The possibility of further treatment in case of transgene loss of expression should be considered for inclusion in therapeutic options and health economic models. Further investigation is necessary to determine if these approaches are safe and beneficial.

8.2. Overcoming barriers to gene therapy administration

The implementation and integration of gene therapy into health practice requires establishment of appropriate infrastructure, timely delivery, and multidisciplinary medical management. Delays in administration of onasemnogene abeparvovec, such as confirmation of diagnosis, testing of AAV9 serology, navigation of coverage issues, and drug shipment, must be minimized.

Barriers to administration of onasemnogene abeparvovec that deserve attention include facility-specific challenges such as discomfort with or being ill-equipped to facilitate administration of gene therapy, reimbursement decisions, and patient/family willingness to accept gene therapy. Specifically, the rare occurrence of a serious adverse effect is likely more difficult for families to accept in presymptomatic children. More research is needed to identify potential predispositions to adverse reactions and options for minimizing or mitigating these risks. In addition, a better understanding of individual serious adverse reactions, as the mechanisms of adverse events related to gene therapy are not fully understood, is needed.

8.3. Enhancing AAV9 technology

AAV9 therapy is an evolving technology. Manufacturing bottlenecks for the research and development of new gene therapies also present a barrier to innovation. In the future, we anticipate that gene therapies will become safer and more efficient and

Table 2. Future and ongoing clinical trials of onasemnogene abeparvovec.

	SMART (NCT04851873) [106]	STRONG (NCT03381729) [107,108]	STEER (NCT05089656) [109]
Purpose	To assess safety and tolerability over 12 months after administration of intravenous onasemnogene abeparvovec	To evaluate safety and tolerability of an intrathecal formulation of onasemnogene abeparvovec	To evaluate efficacy, safety, and tolerability of an intrathecal formulation of onasemnogene abeparvovec
Population	Patients who weigh ≥8.5 kg to ≤21 kg	Patients aged 6–50 months with three copies of *SMN2*	Patients with SMA type 2 and two to four copies of *SMN2* who are aged 2–18 years
Estimated enrollment	24 patients	32 patients enrolled as of December 2019 interim analysis	125 patients
Design	Phase IIIb, open-label, single-arm multicenter study Motor-milestone achievement and function will be used to assess efficacy	Phase I, open-label, dose-comparison study The trial assessed three doses of onasemnogene abeparvovec administered via one-time intraspinal injection (Dose A: 6.0×10^{13}; Dose B: 1.2×10^{14}; Dose C: 2.4×10^{14} vg).	Phase III, multicenter, randomized, sham-controlled, double-blind study
Preliminary results/ notable findings to date		The US Food and Drug Administration halted the trial in October 2019 for safety concerns prompted by an animal study of intrathecal onasemnogene abeparvovec. The hold was lifted in August 2021. As of an interim analysis in December 2019, patients achieved clinically meaningful motor milestones in all dose groups and age groups. No deaths were reported, and no new safety signals were identified.	
Expected completion	August 2023	No longer recruiting	October 2024

SMA: spinal muscular atrophy; *SMN2: survival motor neuron 2* gene.

use less expensive manufacturing technology through advances in AAV manufacturing, as well as advances in other gene transfer and editing technologies. Specifically, future AAV technologies will likely exhibit decreased hepatotropism, which will enhance efficiency and potentially reduce adverse events, ultimately improving future outcomes and successes.

In addition, AAV technology will potentially expand to deliver CRISPR/Cas9 nucleases [126,127]. This approach has shown promise in preclinical trials by demonstrating greater concentrations of AAV integration with high target specificity [128–130] and no evidence of genome-wide genotoxicity [128]. Importantly, a report of CRISPR/Cpf1-mediated correction of patient-specific induced pluripotent stem cells from a patient with SMA demonstrated successful expression of SMN. This provides support for future investigations into therapeutic approaches using CRISPR technology for patients with SMA [131132]

To date, CRISPR/Cas systems have been investigated in animal and cell models of human diseases, including cancer; cardiovascular, pulmonary, and metabolic diseases; hemophilia; and monogenic diseases such as muscular dystrophy [133–136]. CRISPR/Cas systems have also been combined with induced pluripotent stem cells to investigate cell replacement therapy and precision medicine for human diseases. Further, CRISPR has been applied to diagnostic testing, demonstrating high sensitivity and specificity in a fast and inexpensive system [134].

Currently, CRISPR technology is being investigated in several early stage clinical trials. Studies are investigating its use in the treatment of viral diseases, including human papillomavirus-related cervical neoplasia, refractory viral keratitis, human immunodeficiency virus, and coronavirus disease; solid tumors, including esophageal cancer, T- and B-cell malignancies, gastrointestinal malignancies, renal carcinoma, and tumors of the central nervous system; blood disorders, including leukemia and lymphoma, multiple myeloma, sickle cell disease, and β-thalassemia; and rare genetic disorders, including Kabuki Syndrome [137]. CRISPR technology has the opportunity to revolutionize treatment for countless patients and though its use in humans is limited, early results are promising.

8.4. Implementing personalized care for SMA

The discovery of biomarkers to identify individual capacity for response will improve the use of onasemnogene abeparvovec and allow targeted treatment to more specific populations and assessment of meaningful prognostic endpoints. Several genetic, epigenetic, proteomic, electrophysiologic, and imaging biomarkers have been considered for SMA, but their reproducibility and applicability should be confirmed [138]. Once identified and confirmed, these biomarkers will allow for a personalized approach to SMA treatment. Similarly, identifying factors that predispose patients to TMA or severe hepatotoxicity could help to appropriately select patients for gene therapy treatment rather than treatment by another disease-modifying approach.

Drug name (generic)	Onasemnogene abeparvovec
Phase (for indication under discussion)	Approved in the United States in 2019
Indication (specific to discussion)	Onasemnogene abeparvovec is an adeno-associated virus vector-based gene therapy indicated for the treatment of pediatric patients less than 2 years of age with spinal muscular atrophy (SMA) with biallelic mutations in the *survival motor neuron 1* gene.
Pharmacology description/ mechanism of action	Onasemnogene abeparvovec is a recombinant AAV9-based gene therapy designed to deliver a copy of the gene encoding the human SMN protein. SMA is caused by a biallelic mutation in the *SMN1* gene, which results in insufficient SMN protein expression.
Chemical structure	Not available
Pivotal trial(s)	START, STR1VE-US, STR1VE-EU

8.5. Clarifying the role of combination or sequential treatments

The goal of providing treatment to patients with SMA has shifted to optimizing their function and health-related quality of life to ensure that they 'thrive' instead of only 'survive.' This has provoked discussion about a potential role for combination or sequential treatments, including combination SMN-directed therapies or the addition of non-SMN directed therapies such as an anti-myostatin therapy [138]. Clinical trials will be important to answer this question because no evidence that combination or sequential therapy offers meaningful clinical benefit for patients with SMA currently exists. We predict that the future of SMA treatment may involve using complementary mechanisms of action or different targets along the motor unit [36].

8.6. Establishing the durability of clinical benefit of gene therapy

The duration of gene expression is still largely unknown, and it is likely that evaluation of onasemnogene abeparvovec administration in presymptomatic children will help describe median gene expression duration. The stability of episomes in post-mitotic cells (e.g., motor neurons) is unclear. While no evidence of phenomena that could affect long-term expression (e.g., promoter/enhancer methylation) exists, longer follow-up studies are needed.

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Declaration of interest

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ORCID

Hugh J. McMillan http://orcid.org/0000-0001-8927-2018
Laurent Servais http://orcid.org/0000-0001-9270-4061

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